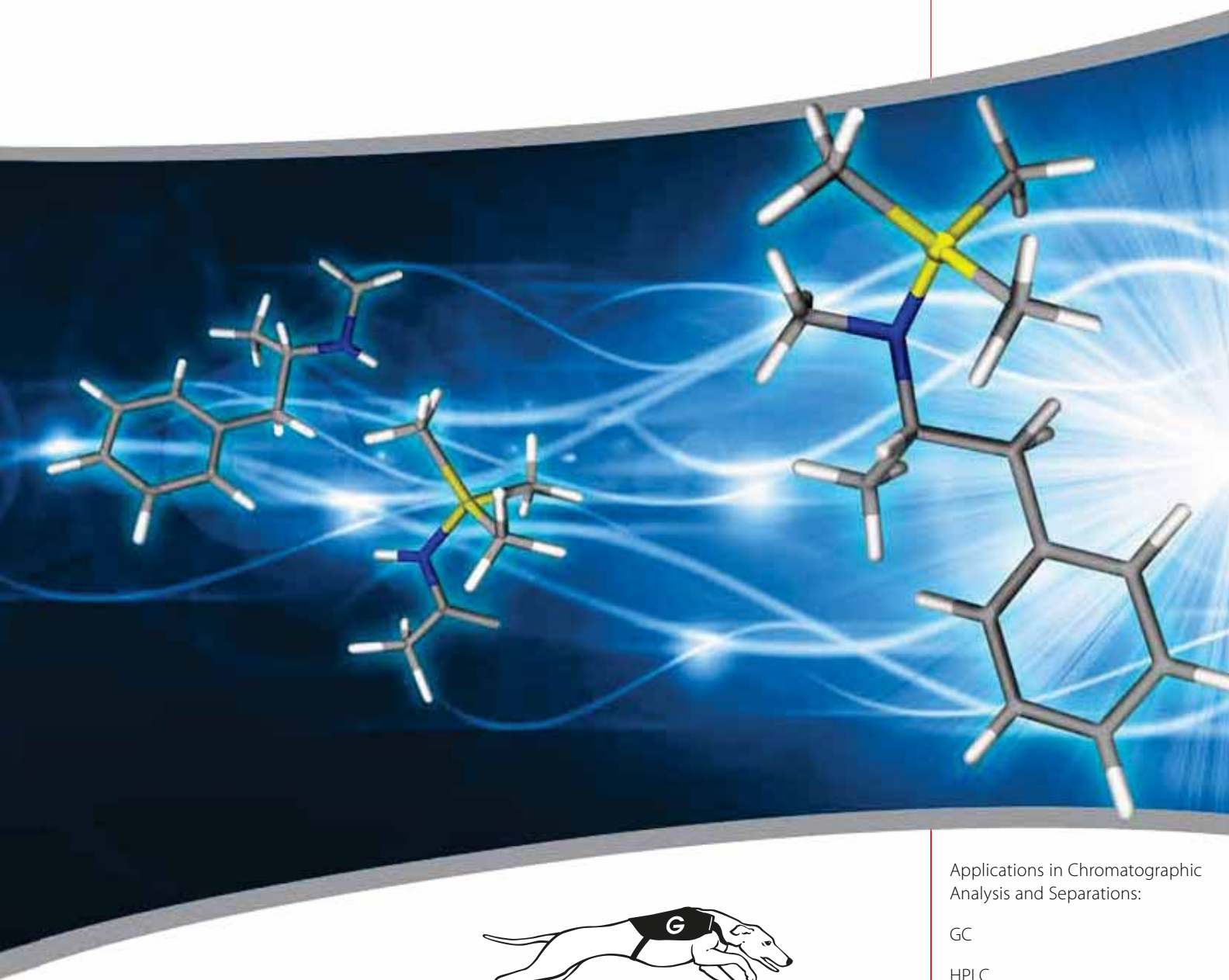
Derivatization Reagents

For Selective Response and Detection in Complex Matrices

Fluka Analytical

SUPELCO Analytical



Greyhound CHROMATOGRAPHY AND ALLIED CHEMICALS

Applications in Chromatographic Analysis and Separations:

GC

HPLC

Chiral

TLC

Serving the Analytical World …..

through Innovation, Quality and Leadership

Derivatization is a commonly used technique to augment chromatography analysis. It requires efficient and effective reagents that can modify the behavior of complex compounds and allow their detection in chromatographic analysis.

Since the release of the last Derivatization guide in 2009, several innovative derivatization reagents have been introduced for various detection methods, and many other products and pack sizes have been modified or eliminated. This new version of the derivatization guide includes up-to-date information on the available products. We strongly believe that this will serve as a handy reference guide for finding suitable derivatization reagents and methods for your chromatographic analysis. The "Application" tables listed for each chromatographic technique offer a convenient resource for selecting a reagent suitable for derivatizing a specific functional group of interest.

This guide offers a comprehensive list of over 450 derivatization reagents, suitable methods and a new section on 'Troubleshooting in Derivatization". We hope it will serve as a convenient and reference-ready resource for selecting derivatization reagents for your daily needs in chromatographic analysis.

In addition to the basic information about derivatization, this monograph covers the following major topics:

- Reagents and methods for four types of derivatization reactions preceding a GC analysis: silylation, acylation, alkylation and esterification
- Derivatization reagents and methods for HPLC analysis based on suitable detection schemes
- Derivatization reagents for chiral separations
- A variety of derivatizing reagents for TLC applications
- Troubleshooting and accessories for derivatization

For your convenience, the back cover presents the information about how you can order these products, obtain technical help or request additional information.

We hope that the information and the products presented here will be of immense help in your chromatographic analysis of routine and other complex matrices.

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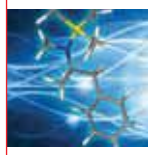
The Value in Derivatization for Separation Science



Derivatization is an integral part of chemical analysis in many areas of chemistry such as medical, forensic, food science, and environmental disciplines. Derivatization enables the analyst to analyze a wide variety of compounds by GC, GC-MS, HPLC and LC-MS that were otherwise less volatile and unstable for these techniques. In general, analytical derivatization is employed for two reasons:

1. To permit analysis of compounds with inadequate volatility or stability
2. To improve chromatographic behavior or detectability

The primary use of derivatization is the chemical modification of a compound by derivatizing its functional group (e.g., O-H, COOH, N-H, and S-H) to promote the use of chromatographic analysis. The underivatized compounds can demonstrate poor chromatographic behavior, insufficient volatility, and poor thermal stability or have inadequate detector response. By chemically modifying these compounds into derivatives they have properties amendable for chromatographic separation and accurate analysis.

"...derivatized sample increases volatility, improves selectivity and stability, and enhances detectability."

Introduction

Many polar compounds and samples are not suitable for chromatographic analysis due to their physical and chemical properties. These compounds are either silylated, acylated, or alkylated in order to render them more volatile. Organic acids, amides, hydroxy compounds, amino acids are examples of polar compounds that need to be derivatized. The functional group (e.g., O-H, COOH, N-H, and S-H) on these polar compounds determines the bonding and shape, type and strength of intermolecular forces, physical properties, and chemical reactivity of a molecule.

Functional groups are less stable than the carbon backbone and are likely to participate in chemical reactions. For example, amine groups decrease compound volatility and are not suited for GC analysis since they do not vaporize rapidly. GC analysis requires a compound to be volatile at temperatures below 350-400 ºC. The compound must be able to withstand high temperatures and rapidly transformed into vapor state without degradation or reaction with other compounds. If the compound does not meet these criteria, it needs to be chemically modified (derivatized) for chromatographic analysis. A derivatized sample increases sample volatility, improves selectivity, increases stability and enhances detectability.

Volatility

Volatility enhancement is an important consideration in derivatization of polar compounds for chromatographic analysis. Derivatizing the polar functional groups (O, S, N and P) can yield dramatic increases in volatility.

Since GC analysis is dependent on the volatility of the compound, compounds with multiple polar groups that show poor volatility due to intermolecular interactions caused by hydrogen bonding must be derivatized. For example, carbohydrates must undergo derivatization prior to GC analysis. Carbohydrate derivatives must be formed to increase volatility and decrease polarity for analysis. The derivative enables the analyst to quantify a sample at trace levels.

Many drugs have a high molecular weight and contain polar functional groups that are not amendable to be assayed by GC. A thermally stable and volatile derivative can be prepared that exhibits minimal tailing for GC analysis.

For small and volatile compounds excessive volatility may also pose problems during analysis. Chemical derivatization increases the molecular weight of very volatile compounds which can minimize losses in sample handling and help separate the gas chromatographic sample peak(s) from the solvent front.

Selectivity

Derivatization may be employed to improve chromatographic performance and peak shape. The derivative should give a single symmetrical peak corresponding to the parent compound. The functional group of polar compounds do not chromatograph well as they tend to adsorb on the active surfaces of the column wall causing peak tailing, poor response or no response. This makes identification and integration difficult. Use of derivatization leads to improved peak shape.

Closely related compounds that are poorly separated by chromatography yield improved separations by use of an appropriate derivative. Optical isomers may be separated by gas chromatography as diastereomeric derivatives. Derivatization may also be employed to separate complex mixtures. In all these cases, derivatization serves to accentuate the differences in the sample compounds to facilitate the chromatographic separation and detection.

Derivatization helps in improving detectability of a compound either by increasing the yield or by introducing groups that are easily captured by a detector. For example, the introduction of ECD (Electron Capture Detector) detectable groups, such as halogenated acyl groups, via derivatization allows detection of previously undetectable compounds. The determination of urea and pesticides by gas chromatography is difficult because they undergo thermal decomposition at standard chromatographic conditions. Detection of these compounds is made possible by derivatizing with HFBA (Heptaflurobutyric anhydride) and analyzing by ECD.

Stability

Some sensitive compounds, although able to be volatilized, undergo partial thermal decomposition in the gas chromatograph and they need to be made more stable. Thermal decomposition is a chemical reaction where a single compound breaks up into two or more simpler compounds when heated. Drugs, for example, are not chromatographed well because they lack the chemical stability for routine chromatography applications. However, derivatization improves chemical stability of these compounds and their metabolites.

In summary, derivatization improves sample volatility, selectivity, detectability and thermal stability in chromatographic applications. If a sample does not possess these characteristics, a chromatographic analysis becomes highly unproductive. In order to fit these parameters imposed by chromatography, an impressive variety of derivatization techniques have been developed that allow the modification of sample properties.

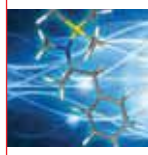
The ideal derivatization procedure will:

- accomplish the desired chemical modification. 95-100% of the sample should be derivatized.
- proceed quantitatively, or at least reproducibly.
- produce products that are readily distinguishable and separable from starting materials.

In developing a method, the following considerations are recommended:

- Make sure the reaction is complete.
- Use more than one reaction temperature and time.
- Watch out for possible side reactions with additional functional groups on the compound or sample matrix.
- Determine the thermal stability of the derivative.
- The reaction should proceed rapidly with simple and easy methodology.
- Use reagents and reactions that present no unusual hazards.
- The derivatized form of the analyte should be readily extractable.
- The derivative can be chromatographed accurately for trace analysis or separated from interfering compounds.

Derivatization for GC



Gas chromatography, a technique for separation of volatile compounds that are thermally stable does not necessarily apply to compounds of biomedical and environmental interest, particularly for those compounds containing functional groups with active hydrogen atoms (-COOH, -OH, -NH, and –SH). These groups are difficult to analyze by GC because they are not sufficiently volatile, show excessive tailing, can be too strongly attracted to the stationary phase or are thermally unstable.

The majority of derivatization reactions commonly used for gas chromatography applications are categorized into three types: Silylation, Acylation, and Alkylation & Esterification.

GC samples are derivatized prior to analysis to:

- increase the volatility and decrease the polarity of the compound
- reduce thermal degradation of samples by increasing their thermal stability
- increase detector response by incorporating functional groups which lead to higher detector signals, e.g. CF_3 groups for electron capture
- improve separation and reduce tailing
- enlarge substrate spectrum

Silylation

Silylation is used to enhance GC performance. The silyl reagents have two desirable results: increase analyte volatility and decrease surface adsorption.

Silyl derivatives are formed by displacement of active hydrogen on -OH, -SH, and -NH groups. Compounds containing active hydrogen atoms amendable to silylation are acids, alcohols, thiols, amines, amides, and enolizable ketones and aldehydes. Their silyl derivatives generally are more volatile, less polar, and thermally more stable.

"...silyl derivatives generally are more volatile, less polar, and thermally more stable."

The choice of a silyl reagent is based on its reactivity and selectivity toward the compound, the intended application, the stability of the derivative, and the abundance and nature of reaction byproducts. Sterically crowded reagents with bulkier R groups are generally less reactive, but give more stable derivatives after silylation.

Reaction

Sample-OH + R_3Si – X ⟶ Sample-O-Si-R_3 + HX

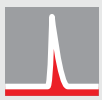
The general reaction for silylation is shown in the above equation. It involves nucleophilic attack upon the silicon atom of the silyl donor, producing a bimolecular transition state. The ideal silyl compound leaving group (X) must be such that it is readily lost from the transition state during the reaction, but possesses sufficient chemical stability in combination with the alkyl silyl group to allow long term storage of the derivatizing agent for use as required. As the formation of the transition state is reversible, the derivatization will only proceed to completion if the basicity of the leaving group 'X' exceeds that of the group it replaced (1).

Features

TMS (Trimethylsilyl) derivatives are the most common for GC analysis. The TMS group contributes both chemical and thermal stability as well as increases analyte volatility for GC and GC-MS applications. BSTFA (N, O-Bis(trimethylsilyl) trifluoroacetamide) and BSA (N,O-Bis(trimethylsilyl)-acetamide) are widely used reagents to introduce the TMS (Trimethylsilyl) group. They are used as such or in the presence of a catalyst, such as, TMCS (Trimethylchlorosilane), TFA (Trifluoroacetamide), hydrochloric acid, potassium acetate, piperidine or pyridine. TMCS is often added to reagents to increase the silyl donor strength. Basic catalysts such as potassium acetate can be used to promote silyl enol ether formation.

Silylation is also valuable for mass spectrometry applications where introduction of the silyl group either produces more interesting diagnostic fragments or particular characteristic ions used for SIM (Selected Ion Monitoring).

MSTFA (N-Methyl-N-trimethylsilylfluoroacetamide) is also an important TMS reagent. It has similar reactivity as BSA and BSTFA. However, because the reaction byproducts are more volatile, MSTFA is useful for GC analysis of early-eluting compounds that would otherwise be obscured in the chromatogram. Other trimethylsilylating reagents include: TMSDEA (Trimethylsilyl diethylamine), TMSI (N-Trimethylsilyimidazole), TMCS and HMDS (Hexamethyldisilazane). TMSI reacts readily with hydroxyl groups but not with aliphatic amines.



Related Information

Bulletin 909 contains detailed information on selecting a suitable derivatization reagent for most applications. Request a free copy of Bulletin 909 by phone or visit our website: *sigma-aldrich.com/literature*

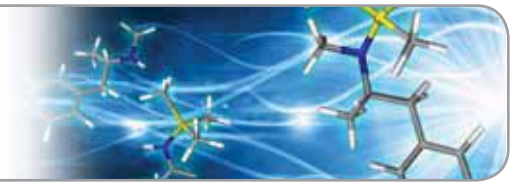
No.	Subject
T196909	Derivatization Reagents

Sensitive Factors

1. Silyl derivatives tend to be moisture sensitive. Moisture will decompose both TMS reagents and derivatives. The use of excess derivatization reagent and solvent can help minimize problems of moisture interference or other sample impurities. The water reacts with the reagent and is chemically removed from the reaction. Suitable solvents may not contain active hydrogen atoms.
2. Silylation may require heating. Therefore, glassware should be chosen to withstand temperatures of at least 100 °C or higher. In case of heating, thermal stability of the compounds and silylation reagents must be considered.
3. Derivatization time varies widely depending upon the specific compound(s) being derivatized. Many compounds are completely derivatized upon dissolution in the reagent. Compounds with poor solubility may require warming and some compounds may require heating. Under extreme conditions, compounds may require heating for up to 16 hours to react completely. Sterically unhindered primary alcohols are usually completely derivatized at room temperature within minutes.
4. The silyl reagent often serves as the solvent. Nonpolar organic solvents, such as hexane, ether, benzene, and toluene are excellent solvents for the reagent since their reaction byproducts do not accelerate the rate of reaction. Polar solvents such as pyridine, dimethylformamide (DMF), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), and acetonitrile are used more often because they can facilitate the reaction. Pyridine is often used as a solvent; it is both non-protic and a catalyst because it can act as a HCl scavenger in organosilane reactions.
5. Silyl derivatives have the undesirable property of fouling flame ionization detectors with silica deposits, especially when samples are injected in excess reagent. These deposits are reduced by use of fluorine containing reagents such as BSTFA.
6. Use a glass injection port liner or direct on-column injection when working with silylating reagents. Erratic and irreproducible results are common when stainless steel injection ports are used.
7. TMS derivatives and silylating reagents react with and are sensitive to active hydrogen atoms. Stationary phases containing these functional groups should be avoided. Siloxane-based phases are the most useful for TMS derivatives – they combine inertness and stability with excellent separating characteristics for these derivatives. Nonpolar siloxane phases include Equity®-1, Equity-5 and SLB™-5ms. Normal hydrocarbons (carbon-hydrogen analytes with single bonds) are separated by these phases. More polar phases, Equity-1701 and SP™-2250, separate carbon-hydrogen analytes that also contain Br, Cl, F, N, O, P, or S atoms or groups. A highly polar cyanopropylphenylsiloxane phase, SP-2330, is useful for separating fatty acid methyl esters or aromatics.
8. Multiple peaks (artifacts) may also arise due to derivatization conditions employed. To remove artifacts the silylation method may need to be optimized. Factors to consider in optimization would be solvent, derivatization reagent, catalyst, temperature and the reaction time. Aldehydes and ketones can form artifacts. Using an excess of a reagent or substituting another silyl reagent can reduce the artifacts. Multiple peaks may indicate that the silyl reaction did not go to completion.

For additional details on key silylation reagents or to order, visit ***sigma-aldrich.com/silylation***

Also refer to the Reference section on page 15.

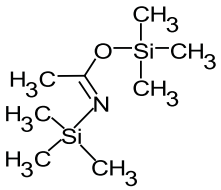


Key Silylation Reagents

Product listing on page 14.

BSA

N,O-Bis(trimethylsilyl)acetamide



Molecular Formula: $CH_3C[=NSi(CH_3)_3]OSi(CH_3)_3$
CAS Number: 10416-59-8
Formula Weight: 203.43
bp: 71-73°/35mm
Flash Point: 53°F (11°C)
d: 0.823
nD: 1.4170 at 20°C
Appearance: Clear, colorless liquid, moisture sensitive

Features/Benefits

- Mild reaction conditions form highly stable products with most organic functional groups. TMS derivatives are thermally stable but more susceptible to hydrolysis than their parent compounds.
- Reactions are generally fast and quantitative.
- Will silylate unhindered hydroxyl groups.
- BSA and its byproducts are more volatile than many other silylating reagents, causing less chromatographic interference.
- BSA has good solvent properties and usually can function as an efficient silylating reagent without additional solvents (DMF, dimethyl formate, is the solvent most frequently used to improve silylation efficiency).

Common Reactive Functional Groups

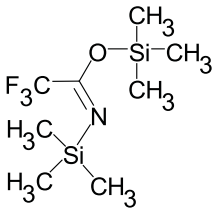
Alcohols, phenols, carboxylic acids, amides, amines and acid anhydrides.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If required, dissolve in solvent.
2. Add excess silylating reagent (minimum a 2:1 molar ratio of BSA to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is complete.

BSTFA

N,O-Bis(trimethylsilyl)trifluoroacetamide



Molecular Formula: $CF_3C=NSi(CH_3)_3OSi(CH_3)_3$
CAS Number: 25561-30-2
Formula Weight: 257.40
bp: 45-55°/14mm
Flash Point: 75°F (23°C)
d: 0.969
n_D: 1.384 at 20°C
Appearance: Clear, colorless to very light yellow liquid, moisture sensitive

Features/Benefits

- Very versatile. Reacts with a range of polar organic compounds by replacing active hydrogens with a TMS group.
- TMS derivatives are thermally stable but more susceptible to hydrolysis than their parent compounds.
- Reacts rapidly and more completely than BSA.
- BSTFA and its by-products (trimethylsilyltrifluoroacetamide and trifluoroacetamide) are more volatile than many other silylating reagents, causing less chromatographic interference.
- Hydrogen fluoride, a by-product of silylation with BSTFA (see Mechanism), reduces detector (FID) fouling.
- Very soluble in most commonly used silylation solvents. Has a good solvent property and can function as a silylation reagent without additional solvents.

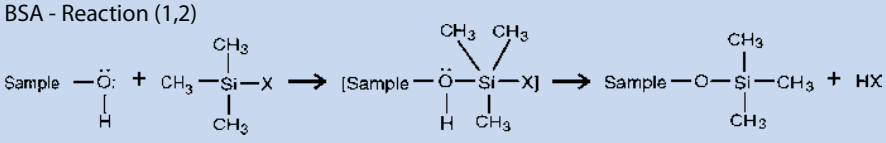
Common Reactive Functional Groups

Alcohols, phenols, carboxylic acids, carbohydrates, amides, amines and acid anhydrides, and sulfonamides.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If required, dissolve in solvent.
2. Add excess silylating reagent (minimum a 2:1 molar ratio of BSTFA to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

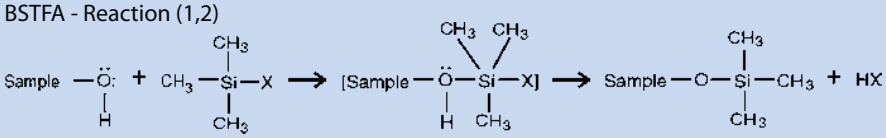
BSA - Reaction (1,2)



For BSA: X = $CH_3-C(=O)-N-Si(CH_3)_3$

Adapted from Knapp (2).

BSTFA - Reaction (1,2)

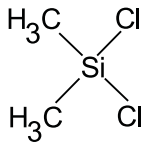


For BSTFA: X = $CF_3-C(=O)-N-Si(CH_3)_3$

Adapted from Knapp (2).

DMDCS

Dimethyldichlorosilane



Molecular Formula: $(CH_3)_2SiCl_2$
CAS Number: 75-78-5
Formula Weight: 129.06
bp: 70 °C
Flash Point: 47 °F (8 °C)
d: 1.064
nD: 1.4010 at 20 °C
Appearance: Clear, colorless liquid, moisture sensitive

Features/Benefits

- Chemically binds a thin, water-repellent film to glass, quartz, silica, and ceramics.
- Coated surfaces are neutral, hydrophobic, and non-sticky, offer increased electrical resistivity, not affected by solvents, and are not readily hydrolyzed. Can be wiped on or applied by immersion.

Common Applications

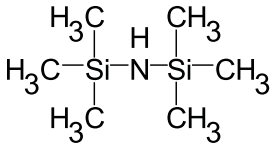
Treatment of laboratory glassware, inlet liners.

Procedure

1. Coat glass surface by rinsing with reagent for 10 to 15 seconds and discard the reagent.
2. Rinse the surface two times with toluene.
3. Rinse the surface three times (or until rinsing is neutral) with methanol.
4. Dry the surface using pure nitrogen.

HMDS

Hexamethyldisilazane



Molecular Formula: $(CH_3)_3SiNHSi(CH_3)_3$
CAS Number: 999-97-3
Formula Weight: 161.39
bp: 125 °C
Flash Point: 48 °F (8 °C)
d: 0.765
n_D: 1.4079 at 20 °C
Appearance: Clear, colorless liquid with characteristic odor, moisture sensitive

Features/Benefits

- HMDS is inexpensive and has a relatively low boiling point (124-127 °C). It can be used without solvent but its silylating power can be increased by various (mostly acidic) catalysts. The only reaction byproduct, ammonia, can leave the reaction mixture as the reaction goes to completion.

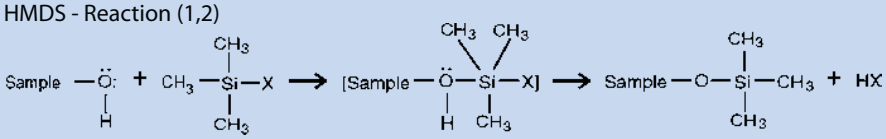
Common Relative Functional Groups

- Alcohols, phenols, carboxylic acids, and amines.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If appropriate, dissolve in solvent.
2. Add excess silylating reagent (at least a 2:1 molar ratio of HMDS to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

HMDS - Reaction (1,2)



For HMDS: X = $NHSi(CH_3)_3$

Adapted from Knapp (2).

Rejuv-8™

BSA + HMDS + TMSI

(Refer to individual compounds on pages 7, 8 and 10 for structure and properties of each component)

Features/Benefits

- Contains no chlorosilanes. Minimizes active sites that cause peak tailing and sample loss.

Common Application

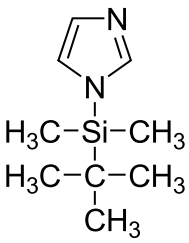
Active sites in packed GC columns.

Procedure

1. Disconnect column from detector.
2. Set injection port temperature, oven temperature, and carrier gas flow according to recommended column conditions.
3. Make four consecutive injections of 25 µL of Rejuv-8, approximately 30 minutes apart. Allow at least three hours for final injection to elute from column.
4. With carrier gas flowing through column, allow injection port and oven to cool.
5. Shut-off carrier gas and allow column pressure to fall.
6. Install new septum and reconnect column to detector.

TBDMSIM

N-t-Butyldimethylsilylimidazole



Molecular Formula:	$C(CH_3)_3$-$Si(CH_3)_2$–NCH=NCH=CH
CAS Number:	54925-64-3
Formula Weight:	182.34
bp:	53 °C/0.2mm
Flash Point:	211 °F (99 °C)
d:	0.939
n_D:	1.480 at 20 °C
Appearance:	Clear, pale yellow liquid, moisture sensitive

Features/Benefits

- t-Butyldimethylsilyl (TBDMS) derivatives are more stable to hydrolysis than the corresponding trimethylsilyl (TMS) ethers (the tert-butyldimethylsilyl group is larger than the TMS group).
- Stability of TBDMS-enol ethers is an advantage in the isolation of ketone enolates from aqueous solution.
- Does not release HCl.
- Useful for mass spectrometry (tends to provide high-mass ions).

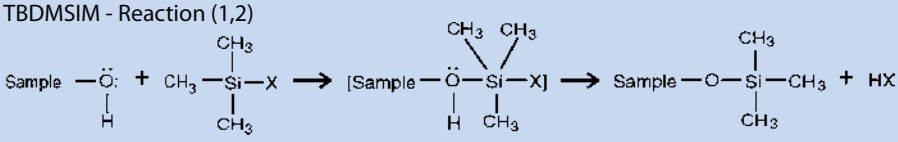
Common Reactive Functional Groups

Sterically unhindered alcohols, phenols.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If required, dissolve in solvent.
2. Add excess silylating reagent. (minimum a 2:1 molar ratio of reagent to achieve hydrogen)
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

TBDMSIM - Reaction (1,2)

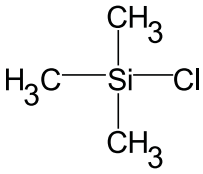


For TBDMSIM: X = NCH=NCH=CH

Adapted from Knapp (2).

TMCS

Trimethylchlorosilane



Molecular Formula: $ClSi(CH_3)_3$
CAS Number: 75-77-4
Formula Weight: 108.64
bp: 57 °C
Flash Point: -18 °F (-27 °C)
d: 0.856
n_D: 1.3870 at 20 °C
Appearance: Clear, colorless liquid with a pungent odor, moisture sensitive

Features/Benefits

- TMCS increases the reactivity of other silylation reagents. Amides and many secondary amines and hindered hydroxyl groups, incompletely derivatized by BSTFA alone, can be derivatized by adding 1-20% TMCS to BSTFA.

Common Applications

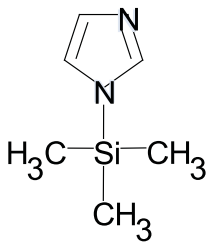
Silylation catalyst, typically used with other silylating reagents.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If required, dissolve in solvent.
2. Add excess silylating reagent (minimum a 2:1 molar ratio of TMCS to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

TMSI

N-Trimethylsilylimidazole (or TMSIM)



Molecular Formula: $(CH_3)_3SiNCH{=}NCH{=}CH$
CAS Number: 18156-74-6
Formula Weight: 140.26
bp: 93-94°/14mm
Flash Point: 42°F (5°C)
d: 0.956
n_D: 1.4750 at 20°C
Appearance: Clear, colorless to light yellow liquid, moisture sensitive

Features/Benefits

- Useful for derivatizing alcohols, hormones, fatty and other organic acids, phenols, prostaglandins, steroids, sulfonic acids, and thiols.
- Useful in multiderivatization schemes containing hydroxyl and amine groups.
- Does not react with aliphatic amines. Less basic amines and amides may react with TMSI.
- Derivatizes sugars in the presence of small amounts of water.
- TMS derivatives are thermally stable but more susceptible to hydrolysis than their parent compounds.

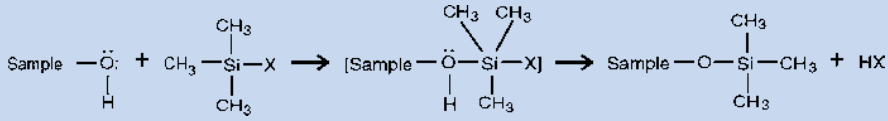
Common Reactive Functional Groups

Alcohols, fatty/organic acids, carbohydrates, carboxylic acids, sulfonic acids, phenols, and thiols.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If required, dissolve in solvent.
2. Add excess silylating reagent (minimum a 2:1 molar ratio of TMSI to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

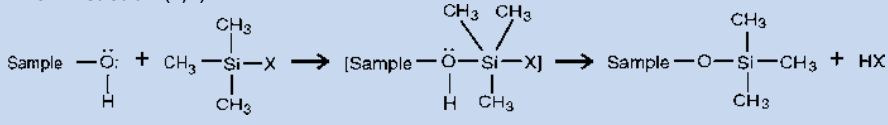
TMCS - Reaction (1,2)



For TMCS: X = Cl

Adapted from Knapp (2).

TMSI - Reaction (1,2)



For TMSI: X = NCH=NCH=CH

Adapted from Knapp (2).

BSA+TMCS

(5:1) *N,O*-Bis(trimethylsilyl)acetamide and Trimethylchlorosilane

Molecular Formula:

BSA: $CH_3C{=}NSi(CH_3)_3OSi(CH_3)_3$
TMCS: $ClSi(CH_3)_3$

(Refer to individual compounds on pages 7 and 10 for structure and properties of each component)

Features/Benefits

- Under mild reaction conditions, BSA forms highly stable products with most organic functional groups. BSA will silylate unhindered hydroxyl groups. Reactions are generally fast and quantitative.
- BSA and its byproducts are volatile, causing less chromatographic interference than many other silylating reagents.
- BSA has good solvent properties and usually can function as an efficient silylating reagent without additional solvents. (DMF is the solvent most frequently used to improve efficiency).
- TMCS increases the reactivity of BSA (or other silylation reagents). Amides and many secondary amines and hindered hydroxyls, incompletely derivatized by BSA alone, can be derivatized by adding 1-20% TMCS to BSA.
- BSA+TMCS has good solvent properties and can function as a silylation reagent without additional solvents. Alternatively, the mixture is very soluble in most commonly used silylation solvents.

Common Reactive Functional Groups

Alcohols, alkaloids, amines, biogenic amines, carbohydrates, carboxylic acids, phenols, and steroids.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If appropriate, dissolve in solvent.
2. Add excess silylating reagent (at least a 2:1 molar ratio of BSA + TMCS reagent to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

BSA+TMCS+TMSI

(3:2:3) *N,O*-Bis(trimethylsilyl) acetamide, Trimethylchlorosilane and N-Trimethylsilyimidazole

Molecular Formula:

BSA: $CH_3C{=}NSi(CH_3)_3OSi(CH_3)_3$
TMCS: $ClSi(CH_3)_3$
TMSI: $(CH_3)_3SiNCH{=}NCH{=}CH$

(Refer to individual compounds on pages 7 and 10 for structure and properties of each component)

Features/Benefits

- Will derivatize all hydroxyl groups in any position. Useful in multi derivatization schemes involving hydroxyl or amine groups.
- TMS derivatives are thermally stable but more susceptible to hydrolysis than their parent compounds.

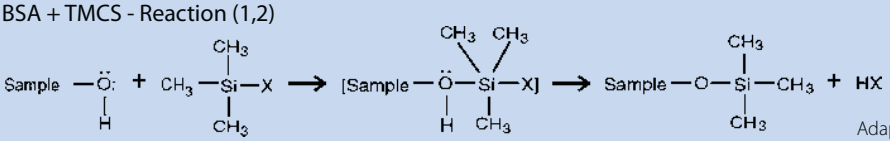
Common Reactive Functional Groups

Alcohols, amines, amides, amino acids, carboxylic acids, phenols, wet sugars and steroids.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If appropriate, dissolve in solvent
2. Add excess silylating reagent (at least a 2:1 molar ratio of reagent to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

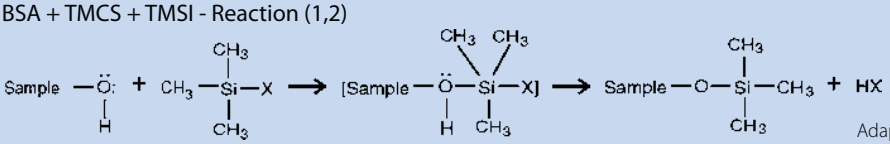
BSA + TMCS - Reaction (1,2)



For BSA: X = $CH_3—C(=O)—N—Si(CH_3)_3$
For TMCS: X = Cl

Adapted from Knapp (2).

BSA + TMCS + TMSI - Reaction (1,2)



For BSA: X = $CH_3—C(=O)—N—Si(CH_3)_3$
For TMCS: X = Cl

Adapted from Knapp (2).

BSTFA+TMCS

(99:1) *N,O*-bis(trimethylsilyl) trifluoroacetamide and TMCS (Trimethylchlorosilane)

Molecular Formula:

BSTFA: $CF_3C=NSi(CH_3)_3OSi(CH_3)_3$
TMCS: $ClSi(CH_3)_3$

(Refer to individual compounds on pages 7 and 10 for structure and properties of each component)

Features/Benefits

- BSTFA is very versatile, reacting with a range of polar organic compounds and replacing active hydrogens with a $-Si(CH_3)_3$ (trimethylsilyl) group. Reacts rapidly and more completely than BSA.
- BSTFA and its by-products (trimethylsilyltrifluoroacetamide and trifluoroacetamide) are more volatile than many other silylating reagents, causing less chromatographic interference.
- Hydrogen fluoride, a by-product of silylation with BSTFA (see mechanism), reduces detector (FID) fouling.
- TMCS increases the reactivity of BSTFA (or other silylation reagents).
- Amides and many secondary amines and hindered hydroxyls, incompletely derivatized by BSTFA alone, can be derivatized by adding 1-20% TMCS to BSTFA.
- Has good solvent properties and can function as a silylation reagent without additional solvents. Alternatively, the mixture is very soluble in most commonly used silylation solvents.

Common Reactive Functional Groups

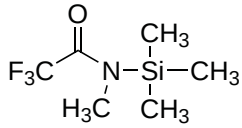
Alcohols, alkaloids, amides, amines, biogenic amines, carboxylic acids, phenols and steroids.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If appropriate, dissolve in solvent.
2. Add excess silylating reagent (at least a 2:1 molar ratio of reagent to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

MSTFA

N-Methyl-*N*-(trimethylsilyl)trifluoroacetamide



Molecular Formula: $CF_3CON(CH_3)Si(CH_3)_3$
CAS Number: 24589-78-4
Formula Weight: 199.25
bp: 130-132 °C
Specific gravity: 0.075
nD: 1.38 at 20 °C
Appearance: Clear, colorless to pale yellow liquid

Features/Benefits

- Very volatile of TMS-acetamides.
- More volatile than BSA or BSTFA but with similar silylation strength.
- Useful in the analysis of volatile trace materials.
- Used in preparation of volatile and thermally stable derivatives for GC and MS analysis.

Common Relative Functional Groups

- Amides, secondary amines, hydroxyls and hydrogen on polar compounds.

Procedure

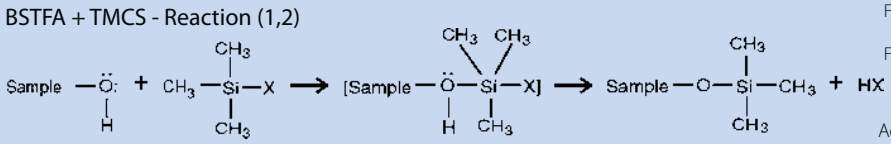
Without solvent:

1. Weigh 1-10 mg of sample into 5 mL reaction vessel and add 0.1-0.5 mL of MSTFA.
2. Cap vial, mix well and let the solution stand at room temperature until the sample has dissolved (5-10 min.) or heat at 60 °C for 15 minutes.
3. Cool to room temperature and analyze the sample.

With solvent:

1. Dissolve 1-10 mg of sample in pyridine, dimethyl sulfoxide, dimethyl formamide plus hydrofluoric acid, or acetonitrile.
2. Add 0.1-0.5 mL of MSTFA .
3. Cap vial, mix well and let the solution stand at room temperature until the sample has dissolved (5-10 min.) or heat at 60 °C for 15 minutes.
4. Cool to room temperature and analyze the sample.

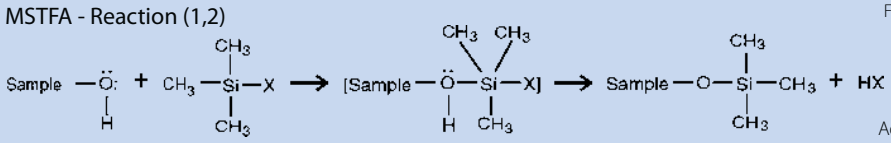
BSTFA + TMCS - Reaction (1,2)



For BSTFA: X = $CF_3-C(=O)-N=Si(CH_3)_3$
For TMCS: X = Cl

Adapted from Knapp (2).

MSTFA - Reaction (1,2)



For MSTFA: X = CF_3-CO-N(CH_3)

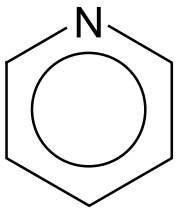
Adapted from Knapp (2).

HMDS+TMCS+Pyridine

(3:1:9) Hexamethyldisilazane, Trimethylchlorosilane and Pyridine

Molecular Formula:

HMDS: $(CH_3)_3SiNHSi(CH_3)_3$
TMCS: $ClSi(CH_3)_3$
Pyridine: C_5H_5N



CAS Number: 110-86-1
Molecular Formula: C_5H_5N
Formula Weight: 79.10
bp: 115-117 °C
Flash Point: 68 °F (20 °C)
d: 0.978
nD: 1.5100 at 20 °C
Appearance: Clear, colorless liquid which has an odor\

(For HMDS and TMCS, refer to individual compounds on pages 8 and 10 for structure and properties)

Features/Benefits

- HMDS+TMCS+pyridine has greater silylating potential than HMDS alone – it will derivatize alcohols, bile acids, phenols, steroids (except 3-ketosteroids), sterols, and sugars which will not be completely derivatized by HMDS.
- The reagent is versatile, fast and easy to use, and can be used without solvent.
- TMS derivatives are thermally stable but more susceptible to hydrolysis than their parent compounds.

Common Reactive Functional Groups

Alcohols, bile acids, carbohydrates, phenols, steroids, sterols, and sugars.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If appropriate, dissolve in solvent.
2. Add excess silylating reagent (at least a 2:1 molar ratio of reagent to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

TMSI+Pyridine

(1:4) *N*-Trimethylsilylimidazole and Pyridine

Molecular Formula:

TMSI: $(CH_3)_3SiNCH{=}NCH{=}CH$
Pyridine: C_5H_5N

(Refer to individual compounds on pages 10 and 13 for structures and properties of components)

Commercial Name: Sylon™ TP

Features/Benefits

- TMSI is useful for derivatizing alcohols, hormones, fatty and other organic acids, phenols, prostaglandins, steroids, sulfonic acids, and thiols. It derivatizes sugars in the presence of small amounts of water.
- It also is useful in multiderivatization schemes containing hydroxyl and amine groups.
- TMSI does not react with amines or amides.
- TMS derivatives are thermally stable but more susceptible to hydrolysis than their parent compounds.

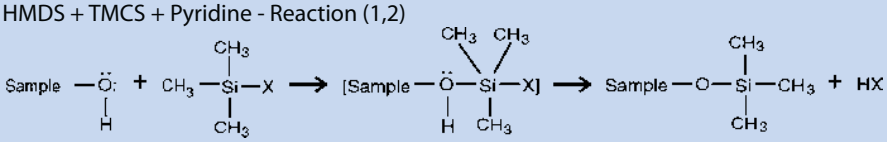
Common Reactive Functional Groups

Carbonyls, steroids, wet sugars.

Procedure

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If appropriate, dissolve in solvent.
2. Add excess silylating reagent (at least a 2:1 molar ratio of reagent to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed which indicates that the reaction is completed.

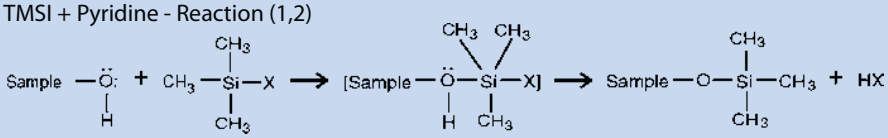
HMDS + TMCS + Pyridine - Reaction (1,2)



For HMDS: X = $NHSi(CH_3)_3$
For TMCS: X = Cl
For Pyridine: X = C_5H_5N

Adapted from Knapp (2).

TMSI + Pyridine - Reaction (1,2)



For TMSI: X = NCH=NCH=CH
For Pyridine: X = C_5H_5N

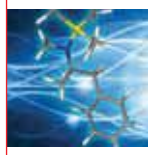
Adapted from Knapp (2).

Product Listing for Key Silylation Reagents

Cat. No.	Pkg. Size
BSA *N,O*-Bis(trimethylsilyl)acetamide	
33036	20 x 1 mL
33037-U	25 mL
15269	5, 25 mL
33035-U	144 x 0.1 mL
BSTFA *N,O*-bis(trimethylsilyl)trifluoroacetamide	
15222	144 x 0.1 mL, 10 x 1 mL, 1, 5, 25 mL, 20 x 1 mL
DMDCS Dimethyldichlorosilane	
33009	100 mL
HMDS Hexamethyldisilazane	
33011	100 mL
33350-U	30 mL
52619	50, 250 mL
Rejuv-8™ BSA + HMDS + TMSI	
33059-U	25 mL
TBDMSIM N-t-Butyldimethylsilylimidazole	
33092-U	10 x 1 mL
TMCS Trimethylchlorosilane	
33014	100 mL
TMSI *N*-Trimethylsilylimidazole (or TMSIM)	
33068-U	25 mL
MSTFA *N*-Methyl-N-(trimethylsilyl) trifluoroacetamide	
69479	10 x 1 mL, 5, 25 mL

Cat. No.	Pkg. Size
BSA+TMCS (5:1) *N,O*-Bis(trimethylsilyl)acetamide and Trimethylchlorosilane	
33018	20 x 1 mL
33019-U	25 mL
BSA+TMCS+TMSI (3:2:3) *N,O*-Bis(trimethylsilyl) acetamide, Trimethylchlorosilane and N-Trimethylsilyimidazole	
33151	144 x 0.1 mL
33030	20 mL
33031-U	25 mL
BSTFA+TMCS (99:1) *N,O*-bis(trimethylsilyl) trifluoroacetamide and TMCS (Trimethylchlorosilane)	
33154-U	144 x 0.1 mL
33148	20 x 1 mL
33155-U	25 mL
33149-U	50 mL
HMDS+TMCS (3:1) Hexamethyldisilazane and Trimethylchlorosilane	
33046	20 x 1 mL
HMDS+TMCS+Pyridine (3:1:9) Hexamethyldisilazane, Trimethylchlorosilane and Pyridine	
33038	20 x 1 mL
33039	25 mL
TMSI+Pyridine (1:4) *N*-Trimethylsilylimidazole and Pyridine	
33159-U	20 x 1 mL
33156-U	25 mL

For a complete list of Silylation Reagents for GC Derivatization see pages 17-19.



References

For additional details on reagents and derivatizing procedure check the website: *sigma-aldrich.com/derivatization*, or request an electronic copy of the listed publications (Reagent Type): T496017A (BSA); T496020B (BSTFA); T496022A (DMDCS); T496024A (HMDS); T496066 (Rejuv-8); T496065A (TBDMSIM); T496028A (TMCS); T496029A (TMSI); T496018A (BSA + TMCS);T496019A (BSA + TMCS + TMSI); T496021A (BSTFA + TMCS); T496025A (HMDS + TMCS); T496026A (HMDS + TMCS + Pyridine); T496030A (TMSI + Pyridine).

1. K. Blau and J. Halket Handbook of Derivatives for Chromatography (2nd ed.) John Wiley & Sons, New York, 1993.
2. D.R. Knapp Handbook of Analytical Derivatization Reactions John Wiley & Sons, New York, 1979.

Additional Reading

BSA

L.W. Woo and S.D. Lee, Capillary Gas-Chromatographic Determination of Proteins and Biological Amino-Acids J. Chromatography., B, Biomed. Appl., 665 (1), 15-25 (1995).

C.F. Poole in Handbook of Derivatives for Chromatography K. Blau and G.S. King, Eds., Heyden & Son, Ltd. p 152 (1977).

J.D. Nicholson, Analyst, 103: 193 (1978).

J. Drozd, Chemical Derivatisations in Gas Chromatography Elsevier (1981).

C.E. Kientz and A. Verweij, J. Chromatogr., 355: 229 (1986).

P. Englmaier, Fresenius' Z. Anal. Chem., 324: 338 (1986).

L.A. Cole, et al., Analyst, 116: 1287 (1991).

N. Hirota, et al., J. Chromatogr., 425: 237 (1988).

BSTFA

M.S.F. Ross, J. Chromatogr. 141: 107 (1977).

A.A.Gallo, Y. Liang, F.H. Walters Volatile BSTFA Derivatives of Amino Acid Hydroxamates Anal.-Lett., 28 (4): 697-701 (Feb. 1995).

A. Pena and P. Sandra Chemotaxonomic Characterization of Yeast Cells J. Chromatogr. Sci., 33 (3): 116-122. (1995)

B.W. Wenclawiak, T.E. Jenson, J.F.O. Reichert GC-MS-FID Analysis of BSTFA Derivatized Polar Components of Diesel Particulate Matter (NBS SRM 1650) Extract. Fresenius'-J. Anal. Chem, Jun-Jul, 346 (6-9), 808-812. 1993

K. Molever Quantitative Determination of Sodium Lauroyl Sarcosinate By Gas Chromatography J. Am. Oil Chem. Soc., 70 (1): 101-103 (1993).

Y. Iijima, K. Saegusa, T. Ito, T. Anjo, Y. Matsuki, T. Nambara Simultaneous Determination of Anabolic Compounds in Beef by Capillary GC-MS Hatano Res. Inst., Food and Drug Saf. Centre, Kanagawa 257, (1992).

A.D. Fraser, W. Bryan, A. Fisher Urinary Screening Formidazolam and its Major Metabolites with the Abbott ADx and TDx Analyzers and the EMIT d.a.u. Benzodiazepine Assay with Confirmation by GC-MS J. Anal. Toxicol., 15 (1): 8-12 (1991).

DMDCS

K. Blau and J. Halket Handbook of Derivatives for Chromatography (2nd ed.)John Wiley & Sons, New York, 1993.

D.R. Knapp Handbook of Analytical Derivatization Reactions John Wiley & Sons, New York, 1979.

S. Wachholz, et al., J. Chromatogr. A, 693 (1): 89-99 (1995).

S.K. Sahoo, Bull. Chem. Soc. Japan, 64: 2484-2487 (1991).

K. Lekova and N. Ivanova, J. Chromatogr., 552: 353-363 (1991).

B. Boe, J. Organomet. Chem., 43: 275 (1972).

T.H. Chan, et al., J. Chem. Soc., Chem Commun. 325 (1987).

M.G. Voronkov and Y.P. Romadane, Khim. Geterotsikl. Soedin 879 (1966) CA 67, 32755f (1967).

J.-C. Pommier, et al., Bull. Soc. Chim. Fr. 1475 (1968).

R.W. Kelly, Tetrahedron Lett. 967 (1969).

R.W. Kelly, Steroids 13: 507 (1969).

R.H. Cragg and R.D. Lane, J. Organomet. Chem. 289: 23 (1985).

L.W. Jenneskens, et al., J. Chem. Soc. Perkin I, 2119 (1985).

A.W. Hanson, et al., Can. J. Chem., 64: 1450 (1986).

R.H. Cragg and R.D. Lane, J. Organomet. Chem., 270: 25 (1984).

D. Brandes, J. Organomet. Chem., 136: 25 (1977).

K. Barlos, et al., J. Org. Chem., 47: 1324 (1982).

M. Wieber and M. Schmidt, Chem. Ber., 96: 1561 (1963).

J. Lukasiak, et al., Rocz. Chem., 48: 891 (1974).

R.M. Ismail and E. Bressler, J. Organomet. Chem., 13: 253 (1968).

D.R. Knapp Handbook of Analytical Derivatization Reactions John Wiley & Sons, New York, 1979.

N.J. Carvajal and R.H. Zienius J. Chromatogr., 355 (1): 107-116 (1986).

Y. Kita, et al., Tetrahedron Lett., 25: 1813 (1984).

J.A. Cella and T.D. Mitchell, J. Organomet. Chem., 244: C5 (1983).

Z.A. Fataftah, et al., Tetrahedron Lett., 27: 4067 (1986).

G.S. Gol'din, et al., Zh. Obshch. Khim., 44: 566 (1974).

J. Barluenga, et al., Synthesis, 489 (1987).

D.J. Cram, et al., J. Am. Chem. Soc., 107: 2574 (1985).

J.A. Soderquist and E.I. Miranda, Tetrahedron Lett., 27: 6305 (1986).

R.H. Cragg and R.D Lane, J. Organomet. Chem., 212: 301 (1981).

P. Rupani, et al., J. Organomet. Chem., 162: 17 (1978).

M. Bols, Tetrahedron 49: 10049 (1993).

HMDS

W.H. Tallent and R. Kleiman, J. Lipid Res., 9: 146 (1968).

L.W. Woo and S.D. Lee Capillary Gas Chromatographic Determination of Proteins and Biological Amino Acids J. Chromatogr., Biomed. Appl., 665 (1): 15-25 (1995).

TBDMSIM

E.J. Corey and T.A. Vendateswarlu, J. Am. Chem. Soc., 94: 6190 (1972).

C. Norsten-Hoog and T. Cronholm, Anal. Biochem., 189: 131-137 (1990).

K. Satouchi and K. Saito, Bio Med.-Mass Spectrom., 6: 396-402 (1979).

TMCS

K. Grob, et al., Recognition of Adulterated Oils by Direct Analysis of the Minor Components Fett-wiss.-Technol., 96 (8), 286-290 (1994).

W.G. Taylor, et al., Rapid Identification of 4-hydroxy-2-alkylquinolines Produced by Pseudomonas aeruginosa, Using Gas Chromatography J. Chromatogr. B, Biomed. Appl., 664 (2), 458-462 (1995).

J. M. Bennett, et al., Tiglyglycine Excreted in Urine in Disorders of Isoleucine Metabolism and the Respiratory Chain Measured by Stable Isotope Dilution GC-MS Clin. Chem., 40 (10), 1879-1883 (1994).

C. Cocito and C. Delfino Simultaneous Determination By GC of Free and Combined Fatty Acids and Sterols in Grape Musts and Yeasts as Silanized Compounds Food-Chem., 50 (3), 297-305 (1994).

D.A. Black, et al., Analysis of Urinary Benzodiazepines, Using Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry J. Anal. Toxicol., 18 (4), 185-188 (1994).

M.J. Bennett, et al., Population Screening for Medium-chain Acyl-CoA Dehydrogenase Deficiency: Analysis of Medium-chain Fatty Acids and Acylglycines in Blood Spots Ann. Clin. Biochem., 31 (1), 72-77 (1994).

TMSI

A.E. Pierce Silylation of Organic Compounds Pierce Chemical Co. (1979).

J. Drozd Chemical Derivatisation in Gas Chromatography Eiseviers (1981).

R. Piekos, et. al., J. Chromatogr. 116: 315 (1976).

Y. Maruyama and A.E. Takemori, Biochem. Pharmacol. 20: 1833 (1971).

O.A. Mamer and B.F. Gibbs, Clin. Chem. 19: 1006 (1973).

T. Aoyama and T. Yashiro, J. Chromatogr. 265: 57 (1983).

K.E. Rasmussen, J. Chromatogr., 120: 491 (1976).

M. Osman, J. Chromatogr., 186: 273 (1979).

E.M. Chambaz and E.C. Horning, Anal. Biochem., 30: 7 (1969).

T.V. Pokushalova, et al., Zh. Anal. Khim., 41: 721 (1986).

C.E. Kientz and A. Verweij, J. Chromatogr., 355: 229 (1986).

A.F. Rizzo, et al., J. Chromatogr., 368: 381 (1986).

J.P. Thenot and E.C. Horning, Anal. Lett., 5: 21 (1972).

A.B. Benkö and V. Mann, Anal. Lett., 13: 735 (1980).

K. Uobe, J. Chromatogr., 214: 177 (1981).

N. Sakauchi and E.C. Horning, Anal. Lett., 4: 41 (1971).

M. Donike, Chromatographia, 9: 440 (1976).

E. Martinez and E. Gelpi, J. Chromatogr., 167: 77 (1978).

R.S. Glass, J. Organomet. Chem., 61: 83 (1973).

K.R. Williams and R.A. Anderson, Anal. Chim. Acta, 275: 163 (1993).

BSA + TMCS + TMSI

H. Gangopadhyay, et al., Capillary Gas Chromatography for the Assessment of Cholesterol Oxides in the Heart J. Pharm. Biomed.-Anal., 9 (10-12): 1003-1008 (1991).

BSTFA + TMCS

K. Grob, et al., Recognition of Adulterated Oils by Direct Analysis of the Minor Components Fett-wiss.-Technol., 96 (8) 286-290 (1994).

W.G. Taylor, et al., Rapid Identification of 4-hydroxy-2-alkylquinolines Produced by Pseudomonas aeruginosa, Using Gas Chromatography J. Chromatogr. B, Biomed. Appl., 664 (2), 458-462 (1995).

J.M. Bennett, et al., Tiglyglycine Excreted in Urine in Disorders of Isoleucine Metabolism and the Respiratory Chain Measured by Stable Isotope Dilution GC-MS Clin. Chem., 40 (10), 1879-1883 (1994).

C. Cocito and C. Delfino Simultaneous Determination By GC of Free and Combined Fatty Acids and Sterols in Grape Musts and Yeasts as Silanized Compounds Food- Chem., 50 (3) 297-305 (1994).

D.A. Black, et al., Analysis of Urinary Benzodiazepines Using Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry J. Anal. Toxicol., 18 (4), 185-188 (1994).

M.J. Bennett, et al., Population Screening for Medium-Chain Acyl-CoA Dehydrogenase Deficiency: Analysis of Medium-chain Fatty Acids and Acylglycines in Blood Spots Ann. Clin. Biochem., 31 (1), 72-77 (1994).

M.S.F. Ross J. Chromatogr. 141, 107 (1977).

A.A. Gallo, et al., Volatile BSTFA Derivatives of Amino Acid Hydroxamates Anal.-Lett., 28 (4), 697-701 (1995).

A. Pena and P. Sandra Chemotaxonomic Characterization of Yeast Cells J. Chromatogr. Sci., 33 (3), 116-122 (1995).

B.W. Wenclawiak, et al., GC-MS-FID Analysis of BSTFA Derivatized Polar Components of Diesel Particulate Matter (NBS SRM 1650) Extract Fresenius'J. Anal. Chem., (6-9), 808-812 (1993).

K. Molever Quantitative Determination of Sodium Lauroyl Sarcosinate By Gas Chromatography J. Am. Oil Chem. Soc., 70 (1), 101-103 (1993).

Y. Iijima, et al., Simultaneous Determination of Anabolic Compounds in Beef by Capillary GC-MS Hatano Res. Inst., Food and Drug Saf. Centre, Kanagawa 257 (1992).

A.D. Fraser, et al., Urinary Screening Formidazolam and its Major Metabolites with the Abbott ADx and TDx Analyzers and the EMIT d.a.u. Benzodiazepine Assay with Confirmation by GC- MS J. Anal. Toxicol., 15 (1), 8-12 (1991).

MSTFA

M. Donike, Chromatographia 9, 440, (1976)

M. Donike, J. Chromatogr. 115, 591, (1975)

M. Donike et al., J. Chromatogr. 134, 385, (1977) Abstract

Husek P, Liebich H.M., J Chromatography 656, 37 (1994)

W. Schunzer, J. Chromatography, B687, 93 (1996).

HMDS+TMCS

L.W. Woo and S.D. Lee Capillary Gas Chromatographic Determination of Proteins and Biological Amino Acids J. Chromatogr., Biomed. Appl., 665 (1): 15-25 (1995).

A.E. Pierce Silylation of Organic Compounds Pierce Chemical Co., Rockford, IL.

HMDS+TMCS+Pyridine

W.H. Tallent and R. Kleiman, J. Lipid Res., 9: 146 (1968).

E.B. Cahoon and D.V. Lynch Analysis of Glucocerebrosids of Rye Dept. Agronomy, Cornell Univ., Ithaca, N.Y. 14853.

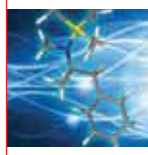
P. Englmaier Identification and Quantitative Estimation of Plant Cyclitols and Polyols by Gas Chromatography Fresenius' Z. Anal. Chem., 324 (3-4): 338-339 (1986).

F. Saura-Calixto, J. Canellas, A. Garcia-Raso Gas Chromatographic Analysis of Sugars and Sugar Alcohols in the Mesocarp, Endocarp and Kernel of Almond Fruit J. Agric. Food Chem., 32 (5): 1018-1020 (1984).

S. Tisza, P. Sass, I. Molnar-Peri Optimization of the Simultaneous Determination of Acids and Sugars as their Trimethylsilyl(oxime) Derivatives by Gas Chromatography- Mass Spectrometry and Determination of the Composition of Six Apple

Varieties J.Chromatogr. A, 676 (2): 461-468 (1994).

A.E. Pierce Silylation of Organic Compounds Pierce Chemical Co., Rockford, IL.



Silylation Reagents for GC Derivatization

Product Name	Description	CAS Number	Pkg Size	Cat. No.
1-(Trimethylsilyl)imidazole	≥98.0% (T). Powerful silylating agent, particularly for alcohols; Synthesis of acyl imidazolides	18156-74-6	10 x 1, 5, 25 mL	394874
N-(Trimethylsilyl)acetamide	for GC derivatization, 98% (GC)	13435-12-6	25 g	91566
4-Trimethylsiloxy-3-penten-2-one	for GC derivatization, 98% (GC)	13257-81-3	25 g	69649
1-(Trimethylsilyl)imidazole-Pyridine mixture 1:4 (v/v)	for GC, Silylating mixture for the mild silylation of alcohols. It is often called Tri-Sil Z	8077-35-8	10 mL	92718
Chlorotrimethylsilane	≥99%	75-77-4	100 mL, 1 L	386529
Bromotrimethylsilane	97%; Used with In Cl3 to catalyze the direct allylation of a variety of alcohols with allyltrimethylsilane	2857-97-8	5, 25 100 g	194409
Triisopropylsilyl chloride	≥97.0% (GC), Selective silylating agent, useful in nucleotide synthesis (the TIPS group is more stable than the tert-butyldimethylsilyl group against hydrolysis)	13154-24-0	10, 50 g	241725
Triisopropylsilyl trifluoro-methanesulfonate	97%, Used to prepare silyloxy acetylenes from lithium acetylides; the silyloxy acetylenes were then employed in protic acid-promoted cyclizations of arenes, alkynes and olefins	80522-42-5	10, 50 g	248460
Triethylsilyl trifluoromethane-sulfonate	99%; Triethylsilylating agent.	79271-56-0	10, 50 g	279471
Chlorotriethylsilane	≥97.0% (GC), Silylating agent with some advantage over the trimethylsilylating agents for synthetic and analytical applications	994-30-9	50 mL	90383
N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-Butyldimethylchlorosilane	≥95%; Preparation of N-tert-butyldimethylsilyl ethanolamines resulting from hydrolysis of nitrogen mustards. Selective O-silylation of N,O-diacylhydroxyl amines	77377-52-7	5, 10, 25, 10 x 1 mL	375934
Silylating mixture Fluka II according to Horning	for GC, One of the most powerful, general silylating mixture	101660-05-3	10 x 1 mL 10 mL	85435 85432
Silylating mixture Fluka I according to Sweeley	for GC, Ready to use solution in pyridine (10 vol parts). Useful silylating mixture for the silylation of hydroxy-compounds	318974-69-5	10 x 1 mL 10 mL	85434 85431
Silylating mixture Fluka III	for GC, Powerful and general silylating mixture		10 mL	85433
Chloro-dimethyl (pentafluorophenyl)silane	≥95.0% (GC), Extremely sensitive derivatizing agent for the analysis of sterols and lower aliphatic alcohols by electron capture gas chromatography	20082-71-7	5 mL	76750
N-Methyl-N-trimethylsilyl-heptafluorobutyramide	≥90% (GC), Silylating agent for use in GC analyses. It interferes least with flame-ionization detectors	53296-64-3	1, 5 mL	69484
N-Methyl-N-trimethyl-silylacetamide	≥97.0% (GC), Powerful silylating agent for polar compounds	7449-74-3	10 mL	69480
N-Methyl-N-(trimethylsilyl)-trifluoroacetamide	for GC, ≥98.5% (GC), Powerful silylating agent	24589-78-4	10 x 1 mL, 5, 25 mL 10 x 1 mL, 5, 25 mL	69479 394866
N-Methyl-N-(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane	for GC, suitable for the silylation of testosterone, tested by GC-MS	24589-78-4	1, 5 mL	69478
Hexamethyldisiloxane	for GC derivatization, 98% (GC)	107-46-0	5, 100, 500 mL	01565
Hexamethyldisilazane	for GC, ≥99.0% (GC), Important silylating agent	999-97-3	50, 250 mL	52619
N-Methyl-N-trimethylsilyl-trifluoroacetamide activated I	for GC, activated with ethanethiol and ammonium iodide, suitable for the silylation of testosterone, This mixture is equivalent to a 1000:2 mixture of MSTFA and iodotrimethylsilane, Derivatizing agent for the trimethylsilylation of -OH and -NH groups for GC and GC-MS		5, 25 mL	50992
N-Methyl-N-trimethylsilyl-trifluoroacetamide activated II	for GC, activated with trimethylsily-ethanethiol. This mixture is equivalent to a 1000:2 mixture of MSTFA and iodotrimethylsilane. Derivatizing agent for the trimethylsilylation of -OH and -NH groups for GC and GC-MS. Suitable for the silylation of testosterone, tested by GC-MS		5, 100 mL	44156
1,1,3,3-Tetramethyl-1,3-diphenyldisilazane	≥97.0% (GC), For the deactivation of glass capillary columns by persilylation; The lithium amide is used for the selective formation of (Z)-enolates from ketones.	3449-26-1	10, 50 mL	43340
N,N-Dimethyltrimethylsilylamine	97%; Reagent employed in the preparation of iminium salts and amides, as well as for the silylation of polymers.	2083-91-2	10, 50 g	226289
1,3-Dimethyl-1,1,3,3-tetraphenyldisilazane	≥98.0% (NT), For the deactivation of glass capillary columns by persilylation.	7453-26-1	10 g	41663

Silylation Reagents for GC Derivatization (Contd.)

Product Name	Description	CAS Number	Pkg Size	Cat. No.
HMDS, Derivatization Grade	Silylation reagent		30 mL 100 mL	33350-U 33011
TMSI+Pyridine, 1:4 (Sylon TP)	Silylation mixture	8077-35-8	20 x 1 mL 25 mL	33159-U 33156-U
BSTFA + TMCS, 99:1	Silylation reagent (Sylon BFT)		25 mL 144 x 0.1 mL	33155-U 33154-U
BSA+TMCS+TMSI, 3:2:3	Silylation reagent 3:2:3 (Sylon BTZ)		144 x 0.1 mL	33151
BSTFA + TMCS, 99:1	(Sylon BFT)		50 mL 20 x 1 mL	33149-U 33148
TBDMSIM, tert-Butyl-dimethylsilylimidazole solution	Silylation reagent TBDMSIM in DMF, for GC derivatization	54925-64-3	10 x 1 mL	33092-U
TMSI, Derivatization Grade	derivatization grade, silylation reagent		25 mL	33068-U
Sylon-CT™	5% dimethyldichlorosilane in toluene; Silyl reagent; For deactivating glassware. It deactivates the tubing for use up to 350 °C to 400 °C.	75-78-5	400 mL	33065-U
Rejuv-8™	Silylating reagent to improve deteriorating chromatographic results, salvage a tired column, or minimize peak tailing and sample loss when working with submicrogram samples? Simply inject 10- 50 µL of Rejuv-8 silylating agent directly onto your column. Contains no chlorosilanes.		25 mL	33059-U
HMDS+TMCS	silylation reagent		20 x 1 mL	33046
HMDS+TMCS+Pyridine, 3:1:9 (Sylon HTP)	3:1:9 (Sylon HTP), composition: 1,1,3,3,3-Hexamethyldisilazane, 22.9%, Pyridine, 69.4%, Trimethylchlorosilane, 7.7 % (w/v)		25 mL 20 x 1 mL	33039 33038
BSA+TMCS+TMSI, 3:2:3 (Sylon BTZ)	3:2:3 (Sylon BTZ)		25 mL	33031-U
BSA+TMCS 5:1	5:1 (Sylon BT)		25 mL	33019-U
BSA+TMCS 5:1	5:1 (Sylon BT)		20 x 1 mL	33018
TMCS, Chlorotrimethysilane	derivatization grade;silylation reagent		100 mL	33014
N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide	≥97%; Silylating agent reportedly much less moisture-sensitive than other silylating reagents used for GLC derivatization. Preparation of N-tert-butyldimethylsilyl ethanolamines resulting from hydrolysis of nitrogen mustards. Selective O-silylation of N,O-diacylhydroxyylamines.	77377-52-7	10 x 1 mL, 5, 25 100 mL	394882
N,O-Bis(trimethylsilyl)acetamide	≥97.0% (GC); Powerful silylating agent	10416-59-8	5, 25 mL	15269
BSA+TMCS, with 5% trimethylchlorosilane	for GC derivatization; Powerful silylation mixture with wide applicability		10, 50 mL	15256
N,N′-Bis(trimethylsilyl)urea	≥98.0%; Silylating agent	18297-63-7	250 g	15248
N,O-Bis(trimethylsilyl)-trifluoroacetamide	≥99%; Derivatization reagent employed in a GC-MS analysis of furfural content in foods	25561-30-2	5, 25, 100 g	155195
N,O-Bis(trimethylsilyl)-trifluoroacetamide with trimethylchlorosilane	for GC derivatization; The position of the silyl groups has not been unequivocally established. It has been referred to as both N,N and N,O. Silylating mixture used for the silylation of various compounds.	25561-30-2	5, 25, 100 mL	15238
N,O-Bis(trimethylsilyl)carbamate	≥98.0%; Extremely suitable reagent for the silylation of alcohols, phenols and carboxylic acids. The only by-products are CO_2 and NH_3; Silyloxycarbonylation of amines; Acylisocyanates from carboxylic acid chlorides.	35342-88-2	10 g	15236
N,N-Bis(trimethylsilyl) methylamine	≥97.0% (GC); Reagent for methylamination of acid halides, e.g. a phosphorus fluoride.	920-68-3	50 mL	15235
N,O-Bis(trimethylsilyl)-trifluoroacetamide	for GC derivatization, p.a., ≥99.0% (GC);	25561-30-2	1, 5, 25 mL, 10 x 1 mL, 20 x 1 mL, 144 x 0.1 mL	15222

Silylation Reagents for GC Derivatization

Product Name	Description	CAS Number	Pkg Size	Cat. No.
N,O-Bis(trimethylsilyl) trifluoroacetamide with trimethylchlorosilane	for GC; The position of the silyl groups has not been unequivocally established. It has been referred to as both N,N and N,O.	25561-30-2	5 mL	15209
Bis(dimethylamino)dimethylsilane	≥95.0% (GC); Derivatization reagent for steroids: the (dimethylamino)dimethylsilyl derivatives can be analyzed by GC using a nitrogen-phosphorus detector; Protection of diols, diamines, etc.	3768-58-9	100 mL	14755
N-Methyl-N-trimethylsilyl-trifluoroacetamide activated I	for GC, activated with ethanethiol and ammonium iodide, suitable for the silylation of testosterone, tested by GC-MS		10x1 mL	12245
N-Methyl-N-trimethylsilyl-trifluoroacetamide activated III	for GC; activated with imidazole, Derivatizing agent for the per(trimethylsilylation) of analytes for GC and GC-MS; suitable for the silylation of testosterone.		10x1 mL, 5 mL	12124
Chlorotriethylsilane Solution, ~1.0 M in tetrahydrofuran	for GC derivatization	994-30-9	100 mL	372943
BSA+TMCS+TMSI	3:2:3		20x1 mL	33030
Trimethylsilyl methallylsulfinate	for GC derivatization	72336-86-5	5 mL	79271
Triethylsilyl methallylsulfinate	for GC derivatization	850418-19-8	5 mL	79264
tert-Butyldimethylsilyl methallylsulfinate	for GC derivatization	850418-20-1	5 mL	79262
Silylation Sampler Kit	BSA, 3 x 1 mL BSTFA, 3 x 1 mL BSTFA + TMCS, 99:1 (Sylon BFT), 3 x 1 mL HMDS + TMCS, 3:1 (Sylon HT), 3 x 1 mL TMSI, 3 x 1 mL		Kit	505846

Selected Silylation Applications

The Derivatization and Analysis of Amino Acids by GC-MS

Katherine K. Stenerson

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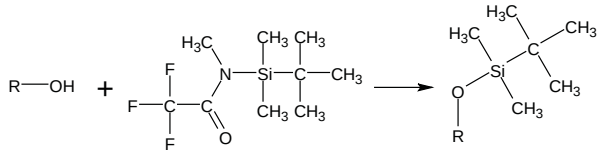
The polar nature of amino acids requires derivatization prior to GC analysis. The goal of derivatization is to make an analyte more volatile, less reactive, and thus improve its chromatographic behavior. In the case of amino acids, derivatization replaces active hydrogens on OH, NH_2, and SH polar functional groups with a nonpolar moiety.

For this study, we evaluated the use of the silylation reagent N-tert-butyldimethylsilyl- N-methyltrifluoroacetamide (MTBSTFA) for the derivatization of amino acids. MTBSTFA, forms tert-butyl dimethylsilyl (TBDMS) derivatives when reacted with polar functional groups containing an active hydrogen. MTBSTFA derivatives are more stable and less moisture sensitive (1).

Figure 1. Structure of MTBSTFA



A 50 µL aliquot of a solution containing a mix of L-amino acids at 91 µg/mL in 0.1 N HCl was dried, and 100 µL of neat MTBSTFA, followed by 100 µL of acetonitrile, were added. The mixture was heated at 100 °C for 4 hours. The sample was then neutralized with sodium bicarbonate and subjected to GC-MS analysis on a 20 m x 0.18 mm I.D. x 0.18 µm SLB™-5ms capillary column.

A chromatogram of the TBDMS derivatives of the amino acids is presented in **Figure 2**.

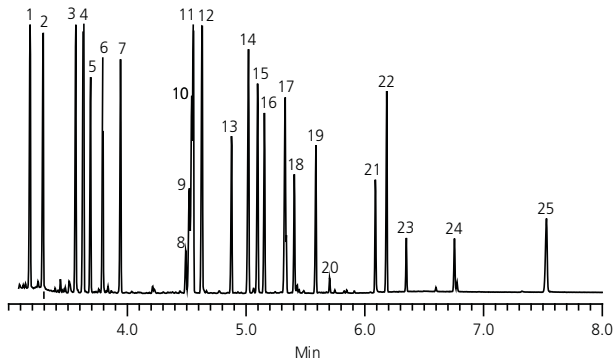
Spectral data obtained from the peaks helped in identification of the amino acid derivatives. Replacement of an active hydrogen with a TBDMS group adds 114 to the molecular weight. Electron impact spectra (2) of these derivatives contains typical fragments corresponding to the molecular weight of the derivative less CH_3 (M-15), C_4H_9 (M-57), C_4H_9 + CO (M-85), and CO-O-TBDMS (M-159). **Figure 3** (page 16) shows an example of this fragmentation pattern in the spectrum of TBDMS-valine.

Under the reaction conditions used, the majority of the amino acids produced one derivative, with active hydrogens on hydroxyl, amine, and thiol groups (in the case of cysteine) replaced by TBDMS. Some amino acids produced multiple derivatives, specifically asparagine, glutamine, and tryptophan. While TBDMS derivatives are more stable than traditional TMS derivatives, their higher molecular weights result in longer elution times during GC analysis. To balance this, the separation was done on a short, narrow bore capillary column. A starting temperature no higher than 100 °C was necessary to maintain resolution of the glycine derivative peak from the solvent. A quick ramp to 360 °C after the elution of the cystine derivative was performed to ensure the column was clean for subsequent analyses.

Figure 2. GC-MS Analysis of Amino Acid Derivatives on the SLB-5ms

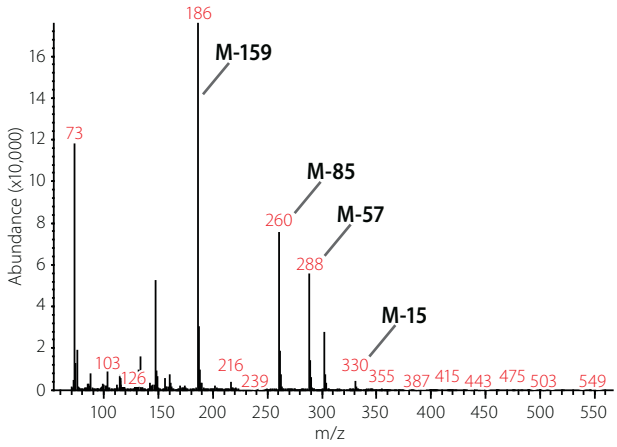
column:	SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
oven:	100 °C (1 min.), 35 °C/min. to 290 °C (3 min.), 40 °C/min. to 360 °C
inj.:	250 °C
MSD interface:	325 °C
scan range:	m/z = 40-450
carrier gas:	helium, 1 mL/min., constant
injection:	0.5 µL, splitless (1.0 min.)
liner:	2 mm I.D., straight
sample:	TBDMS derivatives of amino acids, each approximately 23 µg/mL

1. Alanine; m/z=260, 232, 158
2. Glycine; m/z=246, 218
3. Valine; m/z= 288, 260, 186
4. artifact from derivatization
5. Leucine; m/z=302, 274, 200
6. Isoleucine; m/z=302, 274, 200
7. Proline; m/z= 286, 258, 184
8. Asparagine, extra derivative; m/z= 327, 285, 243
9. Glutamine, extra derivative; m/z= 342, 300, 272
10. Methionine; m/z= 320, 292, 218
11. Serine; m/z= 390, 362
12. Threonine; m/z= 404, 376, 303
13. Phenylalanine; m/z= 336, 302, 234
14. Aspartic acid; m/z= 418, 390, 316
15. Hydroxyproline; m/z= 416, 388, 314
16. Cysteine; m/z= 406, 378
17. Glutamic acid; m/z= 432, 330, 272
18. Asparagine; m/z= 417, 302
19. Lysine; m/z= 431, 329, 300
20. Glutamine; m/z= 431, 357, 329, 299
21. Histidine; m/z= 459, 440, 338, 196
22. Tyrosine; m/z= 466, 438, 364, 302
23. Tryptophan, extra derivative m/z= 417, 375, 347, 302, 273
24. Tryptophan; m/z= 489, 302, 244
25. Cystine; m/z=639, 589, 537, 348



* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Figure 3. Mass Spectrum of TBDMS Derivative of Valine (MW of the derivative = 345)



Conclusions

This study demonstrates that with the proper use of derivatization reagents such as MTBSTFA, amino acids can be analyzed by GC-MS. The reaction conditions may have to be "tweaked" to produce maximum response of the derivatives of interest. The derivatives produce characteristic fragments, allowing for easy identification by MS. To reduce the overall GC analysis time of these derivatives, a short, narrow bore column such as the 20 m x 0.18 mm I.D. x 0.18 µm SLB-5ms is recommended.

References

1. T. G. Sobolevsky, A. I. Revelsky; Barbara Miller, Vincent Oriedo, E. S Chernetsova, I.A Revelsky, J. Sep. Sci. 2003, 26, 1474-1478.
2. F. G. Kitson, B. S. Larsen, C. N. McEwen, Gas Chromatography and Mass Spectrometry, A Practical Guide; Academic Press: San Diego, 1996; Chapter 9.
3. I. Molnar-Perl, Zs. F. Katona,. GC-MS of amino acids as their trimethylsilyl/t-butyldimethylsilyl derivatives: in model solutions III. Chromatographia Supplement, 51, 2000, S228-S236.

GC Analysis of Estrogenic Compounds and Lysergic Acid Amide (LSD)

Jay Jones, Katherine K. Stenerson

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Without derivatization, estrogenic compounds and lysergic acid amide (LSD) show little or no response by GC analysis. With the addition of the TMS group, these analytes show great peak shape and response. It is important to optimize and ensure that the derivatization reactions go to completion. Of the four estrogenic compounds, three were fully derivatized within 30 minutes at 75 °C. However, estriol was derivatized a second time at 75 °C. Additionally, the reaction time was increased to 45 minutes. After being allowed to sit overnight at room temperature, GC-MS analysis then confirmed that all three active hydrogens had been replaced with TMS groups. Figure below shows the final chromatogram.

GC Analysis of Estrogenic Compounds

Lysergic acid amide (LSD) was derivatized under similar conditions as the estrogenic compounds but at 68 °C. At this temperature only 60% derivatization was achieved in 5 hours. At 75 °C the reaction was approximately 95% complete. In 3 hours reaction time, both the LSD and the LSD (TMS) peak are still detected by GC analysis, which means that the reaction has not gone to 100% completion and need further optimization. This chromatogram is shown in **Figure 1**.

Conclusion

In these examples, both temperature and time were found to be critical for completion of reaction. It is, therefore, important that each derivatization reaction must be optimized to achieve a high derivatization completion percentage. This will result in good peak shape and detector response.

Along with time and temperature, the concentration of the reagent is important. For example, an alcohol may fully derivatize in just a matter of minutes at room temperature. However, for an amide or a sterically hindered carboxylic acid, it may take hours at an elevated temperature to complete the reaction. It is recommended to add the silylating reagent in excess. As a general rule, add at least a 2:1 molar ratio of BSTFA to active hydrogens.

Most derivatization reactions are sensitive to water that may slow or completely stop the reaction. Moisture may also decompose the TMS reagent or the derivatives that are formed. It is recommended that derivatization reagents be stored in a secondary container that contains desiccant. Additionally, moisture should be removed from the extract that is to be derivatized.

Figure 1. GC Analysis of LSD-TMS

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven: 175 °C (0.5 min.), 15 °C/min. to 300 °C (5 min.)
inj.: 250 °C
MSD interface: 330 °C
scan range: m/z 45-525
carrier gas: helium, 1 mL/min., constant
injection: 0.5 µL, pulsed splitless (30 psi until 0.5 min., splitter open at 1.5 min.)
liner: 2 mm I.D., straight
sample: Estrogenic compounds, as methylated derivatives, 10 ppm in ethyl acetate

1. 17 α-Estradiol
2. Estrone
3. 17 β-Estradiol
4. Estriol

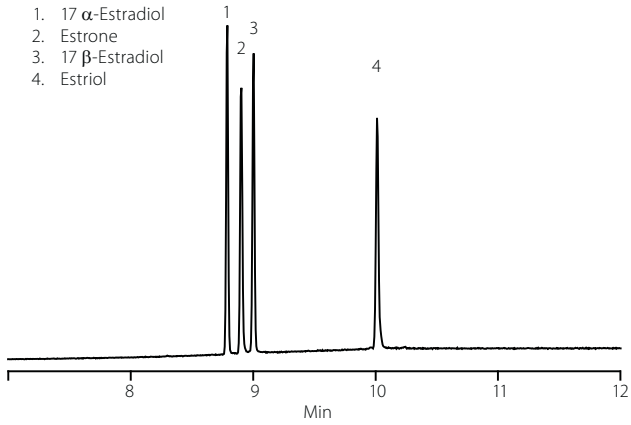
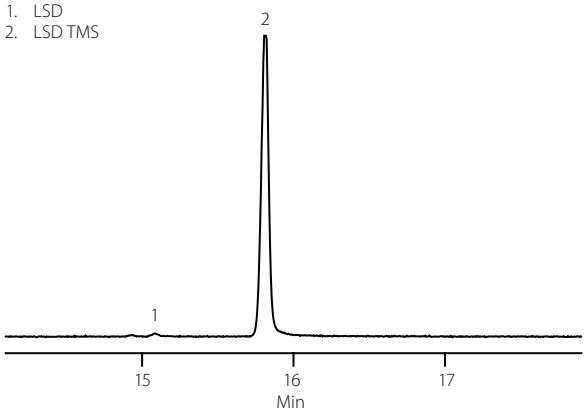


Figure 2. GC Analysis of LSD-TMS

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven: 150 °C (1.5 min.), 20 °C/min. to 300 °C (20 min.)
inj.: 250 °C
MSD interface: 330 °C
scan range: m/z 45-525
carrier gas: helium, 0.7 mL/min. constant
injection: 0.5 µL, pulsed splitless, 30 psi. (0.20 min.), purge on (1.5 min.), purge flow (50 mL/min.)
liner: 2 mm I.D., straight
sample: derivatized LSD using BSTFA

1. LSD
2. LSD TMS



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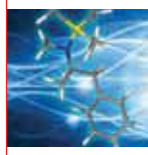

Improved Silylation of Drug Substances for GC/MS Analysis using Activated MSTFA Reagents

Ingrid Hayenga

This article originally published in Analytix 4, 2005*

GC or GC/MS analysis can be accomplished in most instances by converting these compounds to a molecular form that has a boiling point below its decomposition point. Such derivatization facilitates GC analysis by:

- Reducing the polarity and enhancing the volatility of polar drug substances
- Increasing the thermal stability of the compound to prevent decomposition

Common Derivatization Reagents for GC and GC/MS

There are many GC derivatization reagents suitable for drug substances. A few drug classes and a summary of the types of derivatives that have been employed to enhance their GC analysis along with a recent citation are listed in **Table 1**.

The fluorine-containing acylation reagents, including TFAA and PFAA, and the silylation reagent combination BSTFA/TMCS are commonly used, but are corrosive and can damage the capillary GC column. An ideal derivatization reagent provides fast, complete reactions for sensitive GC and GC/MS detection without damaging by-products.

Of the myriad trimethylsilylation reagents available, MSTFA is one of the most important. A benefit of MSTFA is that the by-products of MSTFA silylation, primarily N-methyltrifluoroacetamide, are more volatile than BSA and BSTFA, making MSTFA valuable to identify compounds that would otherwise go undetected or obscured in the GC analysis. Additionally, MSTFA reactions do not produce corrosive by-products that can damage the capillary GC column.

The silylation power of MSTFA can be increased by the use of catalysts or additives that scavenge reaction by-products. MSTFA reacts in situ with ammonium iodide (NH4I) to produce trimethyliodosilane (TMSI), which has been reported to be the most powerful trimethylsilyl donor available. TMSI reacts with adequate speed to produce both trimethylsilyl (TMS) ether and trimethylsilyl enol (TMS enol) ether derivatives. Ethanethiol is added to reduce the formed iodine to hydrogen iodide in order to prevent iodine incorporation into the product. As a result, diethyl disulfide is produced during the derivatization reaction. Diethyl disulfide formation depends on the amount of ammonium iodide and ethanethiol added to the extract and the chosen experimental conditions such as reaction time and temperature. Imidazole acts as a base catalyst in the MSTFA silylation reaction.

In this short communication, we report the results of the efficacy of BSTFA/TMCS and three activated MSTFA reagents (**Table 2**, next page) to form GC-MS-compatible trimethylsilyl derivatives of several important drug classes: cannabinoids, amphetamines and opiates.

As summarized in **Table 3**, next page, although results varied with drug substance tested, one or more of the three activated MSTFA reagents was as effective as or more effective than the BSTFA/TMCS reagent, without the generation of system-damaging corrosive byproducts. Therefore, the choice of silylation reagent depends on what drug classes are to be derivatized.

Table 1. Example of Different Derivatization Reagents for Drug Substances

Drug Group	Derivatization Reagents	Literature
Amphetamines	MSTFA + 1% TMCS TFAA BSTFA	Rood, H. J. and Knitter, J.A., *Capillary Chromatography* 115-120 (1991)
Barbiturates	BSTFA	Kananen, G. *et al., J. Chromatogr. Sci.,* 10, 283-287 (1972)
Marijuana	BSTFA + 1% TMCS BSTFA MSTFA MSTFA + 1% TMCS MTBSTFA	Nelson, C. C. and Foltz, R. L., *Anyl. Chem.,* 64, 1578-1585 (1992)
LSD	BSA	Harkey, M. R., *et al., J. Anal. Toxicol,* 15, 260-265 (1992)
	BSTFA MSTFA TFAA	
Opiates	BSTFA + 1 % TMCS	Chen, B.H. *et al., J. Anal. Toxicol,* 14, 12-17 (1990)
	MBTFA PFAA TFAA BSTFA	
PCP	BSTFA + 1% TMCS	Woodworth, J.R. *et al., J. Anl. Toxicol.,* 8, 2-6 (1984)

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Table 2. Derivatization (silylation) reagents

Cat. No.	Description	Remarks	Pkg. Size
50992	MSTFA I (N-Methyl-N-trimethylsilyltrifluoroacetamide activated I)	Activated with ethanethiol and amonium iodide**	5 mL, 25 mL
44156	MSTFA II (N-Methyl-N-trimethylsilyltrifluoroacetamide activated II)	Activated with trimethylsilyl-ethanethiol**	5 mL, 25 mL
12124	MSTFA III (N-Methyl-N-trimethylsilyltrifluoroacetamide activated III)		5 mL, 25 mL
15238	BSTFA + 1 % TMCS (N, O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane		10 x 1 mL, 5, 25, 100 mL

** Equivalent silylation power to a 1000:2 iodotrimethylsilane:MSTFA:iodotrimethylsilane:mixture.

Table 3. Results of Derivatization Experiments

Test Compounds	Group	BSTFA + 1 % TMCS Cat. No. 15238	MSTFA I Cat. No. 50992	MSTFA II Cat. No. 44156	MSTFA III Cat. No. 12124
11-Nor-Δ^9-tetrahydrocanna-binol-9-carboxylic acid	Cannabinoids	Complete silylation	Complete silylation	Complete silylation	Complete silylation
Codeine	Opiates	Complete silylation	Not measured silylation	Not measured	Complete
Hydrocodone (+)-bitartrate salt	Opiates	50:50 mixture of silyated and non-silylated product	Complete silylation	Incomplete silylation	Complete silylation
Ethylmorphine	Opiates	Complete silylation + minor by-product	Complete silylation + minor by-product	Complete silylation + minor by-product	Complete silylation +
Oxycodone Hydrochloride	Opiates	Not measured	Complete silylation (double silyated)	Complete silylation (double silyated)	Double silylated main product and mono-silylated as by-product
Morphine Sulfate	Opiates	Complete silylation	Complete silylation	Complete silylation	Complete silylation
(-)-Deoxyephedrine	Amphetamines	Complete silylation	Complete silylation	Incomplete silylation	Complete silylation
D-Amphetamine Sulfate salt	Amphetamines	Not measured	Complete silylation	Complete silylation	Complete silylation
(+)-3-4-Methylenedioxy methamphetamine hydrochloride (Ecstasy)	Amphetamines	Not measured	Complete silylation	Complete silylation	Complete silylation

For detailed information on this topic, including methods of analysis and chromatograms, please ask for Analytix Notes: *Silylation of Drug Substances with MSTFA* (IEF)

Derivatization of Non-Steroidal Anti-Inflammatory Drugs with Different Activated Silylating Reagents

Luis-Alberto Martin & Ingrid Hayenga

This article originally published in Analytix 2, 2010*

NSAID residue analysis in water samples has usually been performed by a gas chromatograph coupled to a mass spectrometer.

Silylation is the most widely used technique for the derivatization of functional groups present in these drugs. Among the various silylating reagents, MSTFA is one of the most important, because the by-products of MSTFA silylation, primarily N-methyltrifluoroacetamide, are more volatile than BSA and BSTFA. This characteristic enables MSTFA to be valuable in identifying compounds that would otherwise go undetected or obscured in the GC analysis. In addition, MSTFA reactions do not produce by-products that can damage the capillary GC column. The silylation power of MSTFA can be enhanced by the use of catalysts or additives that scavenge reaction by-products. MSTFA reacts in situ with ammonium iodide (NH4I) to produce trimethyliodosilane (TMSI), a powerful trimethylsilyl donor.

In order to prevent iodine incorporation into the product, ethanethiol is added to reduce the formed iodine to hydrogen iodide. As a result, diethyl disulphide is produced during the derivatization reaction. Imidazole acts as a base catalyst in the MSTFA silylation reaction.

Silylation of the ten NSAIDs with the three silylating mixtures, MSTFA and BSTFA

Five silylating reagents (Silylating mixture I, II, III, MSTFA and BSTFA) were able to form GC-MS-compatible trimethylsilylmethyl derivatives of ten important non-steroidal anti-inflammatory drugs (NSAIDs). It is concluded that, for these targeted compound classes, the activated Silylating mixture I appears to be the most effective choice.

Figure1. Main product and by-product of the acetaminophen silylation

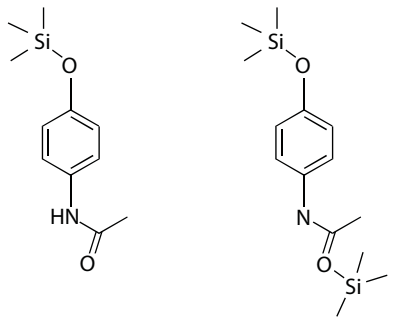
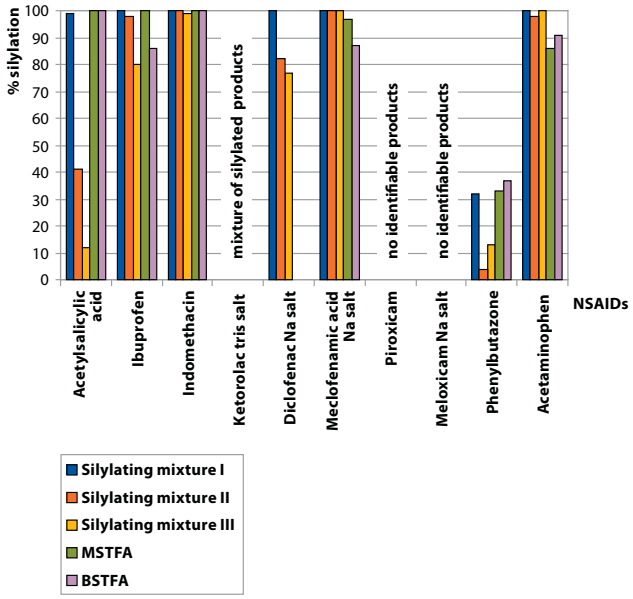


Figure 2. Results of derivatization experiments



All derivatization reagents and NSAID substances are available from Sigma-Aldrich.

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Silylation for Resveratrol Analysis

Katherine K. Stenerson

This article originally published in Reporter 27.4*

Resveratrol is a phytoallexin produced by grapes and other plants to increase resistance to fungal infection. Red wine, which is produced by fermentation on the crushed grapes, has been found to contain resveratrol. Recent research (1) suggests that consumption of resveratrol may reduce the risk of certain cancers, heart disease, and other age-related disorders.

In this application, the extraction and analysis of resveratrol from red wine is demonstrated using SPME and GC-MS. The presence of 3 –OH groups make it necessary to derivatize this compound prior to GC analysis. Derivatization was conducted, after extraction, directly on the SPME fiber by exposing it to the vapors of a silyating reagent. The TMS derivative of resveratrol was then analyzed directly from the fiber using GC-MS.

The extraction conditions and the fiber type were chosen based on recently published findings (2,3). Few modifications were made to extraction and desorption times to decrease matrix interference from the wine sample.

sample/Matrix:	3 mL of Red wine (California merlot) diluted 3:1 in 12% ethanol:water
SPME fiber:	Polyacrylate, 85 µm (57304, 57305, 57294-U)
extraction:	immersion at room temperature, 15 min., with stirring at 400 rpm
derivatization:	20 min. in 4 mL vial containing 5 µL of Sylon-BFT
desorption temp.:	280 °C, 2 min.
column:	SLB-5ms; 30 m x 0.25 mm I.D. x 0.25 µm (28471-U)
oven:	100 °C (1 min.), 10 °C/min. to 325 °C (3 min.)
MSD interface:	325 °C
scan range:	m/z 40-450
carrier gas:	helium, 1 mL/min constant flow
liner:	0.75 mm I.D. SPME

The vial containing the Sylon-BFT was allowed to equilibrate for 60 – 90 minutes prior to use. A new Sylon-BFT vial was used for each extraction. After extracton, the SPME fiber was gently blotted with a kimwipe to remove excess water prior to exposure to the Sylon-BFT.

Calibration standards were prepared in 12% ethanol:water using trans-resveratrol, Sigma cat. no. R5010. A small amount of cis was detected upon SPME GC-MS analysis of the standard (Figure 1). For simplicity, the calculations for this work were based on the standards using the trans isomer only.

Figure 1. SPME/GC-MS Analysis of Resveratrol Standard; 500 ppb

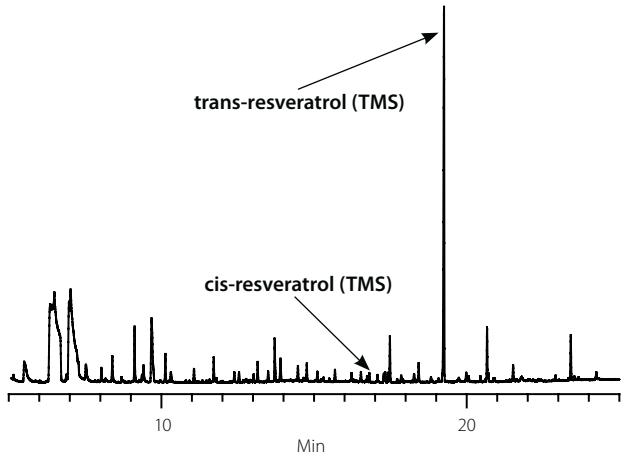


Figure 2. SPME on-fiber derivatization analysis of resveratrol in unspiked red wine sample

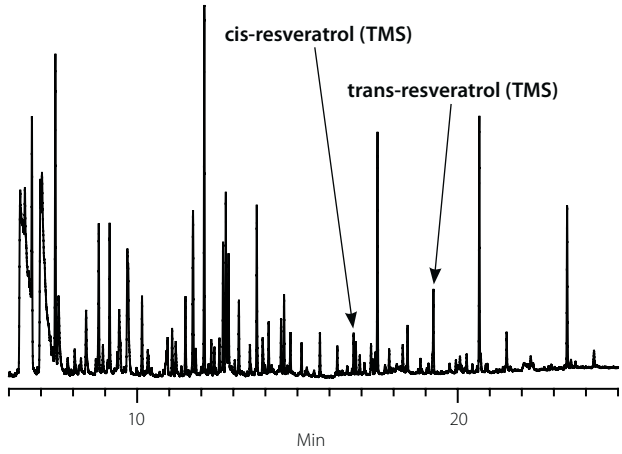
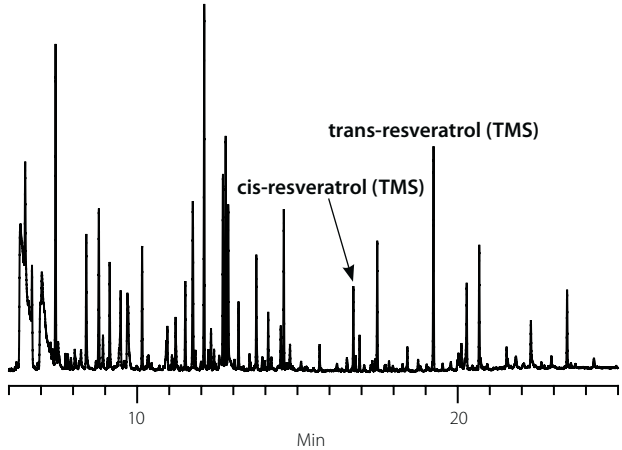


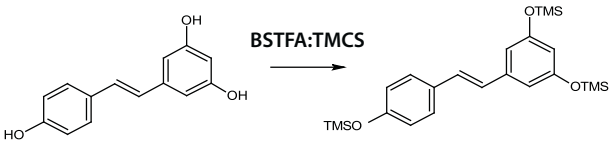
Figure 3. SPME on-fiber derivatization analysis of resveratrol in spiked red wine sample.



* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Results

The derivatization procedure using Sylon-BFT (BSTFA + 1% TMCS) resulted in silylation of all three –OH groups present in resveratrol:



The resulting derivative has a molecular weight of 444, and subsequent GC-MS analysis showed a predominance of the molecular ion, which was used for quantification.

A calibration curve, ranging from 10 – 500 µg/L extracted using on-fiber derivatization SPME showed some leveling off in response at > 300 µg/L. Unspiked and spiked (100 µg/L) red wine samples were extracted. Chromatograms of each are presented in **Figures 2 and 3**. Using the average response factor from the calibration, the levels of trans-resveratrol in each were calculated along with a percent recovery for the spiked wine sample:

	Unspiked red wine	Spiked red wine (100 µg/L)
Conc. of trans-resveratrol (µg/L)	22.6	134.7
% recovery	---	110%

In addition to the trans form a sizable cis-resveratrol peak was detected in the wine samples. The cis form is not naturally found in grapes, however it has been theorized that it can be formed from the trans form during the analysis, or the production and/or aging of wine (2).

References

1. Red Wine Compound Resveratrol Demonstrates Significant Health Benefits. ScienceDaily. Retrieved June 18, 2009, from http://www.sciencedaily.com /releases/2009/06/090611174052.htm
2. C.Lingshuang, Koziel, Jacek A., D. Murli, van Leeuwen, J. (Hans), Rapid Determination of trans-resveratrol in red wine by solid-phase microextraction with on-fiber derivatization and multidimensional gas chromatography-mass spectrometry. J. Chrom. A (2009), 1216, 281-287.
3. V, Pilar, C. Natalia, Martinez-Castillo, N.H. Manuel, Solid-phase microextraction on-fiber derivatization for the analysis of some polyphenols in wine and grapes using gas chromatography-mass spectrometry. J. Chrom A (2009), 1216, 1279-1284.

Silylation for Analysis of Aliphatic Alcohols in Olive Oil

Katherine K. Stenerson

Aliphatic alcohol content is used to analyze the composition and determine the grade and authenticity of olive oil. Test results are compared against standards such as those established by the International Olive Oil Council (IOC) and US Dept. of Agriculture.

In olive oil, long chain aliphatic alcohols (also referred to as "fatty alcohols") exist in the free form, or esterified to fatty acids. The IOC method COI/T.20/Doc. No. 26 or EN 1991R2568 for aliphatic alcohol content in olive oil calls for saponification followed by fractionation of the unsaponifiables using TLC. The recovered fraction is then silylated and analyzed by GC-FID (1). The liberated C22-C28 alcohols are identified by comparison to a standard. The levels present in the sample are calculated using an internal standard (eicosanol) added to the sample and carried through the saponification/fractionation/silylation process.

Olive leaves and drupes were subjected to saponification and derivatization using microwave assisted heating. Fractionation of the unsaponifiable matter was done by SPE (not TLC as recommended) using amino-propyl functionalized silica. The final extract was then analyzed by GC-MS in place of GC-FID. SPE allowed for removal of a large portion of the fatty acids in the unsaponifiable fraction. GC-MS enabled the clean detection and identification of the aliphatic alcohols by mass, and identification of several sterols. The procedure used was adopted from a study reported earlier (2).

Experimental

Material Specifications:

Olive oil: All purpose olive oil (Wegman's brand), listed as a product of Italy and a blend or extra-virgin or virgin oils.

SPE tube: DSC-NH_2, 6mL/1g (52640-U)

Sample Preparation:

The following samples were prepared for analysis:

1. olive oil + 100 µL aliphatic alcohol spike* (to be SPE cleaned)
2. olive oil, no spike (to be SPE cleaned)
3. olive oil, no spike (no SPE)
4. control (100 µL aliphatic alcohol spike*, no oil; SPE cleaned)

*aliphatic alcohol spike contained C23, C24, C26, C27, and C28 alcohols at 100 µg/mL

Saponification to liberate esterified fatty alcohols

1. Weigh 1 g of olive oil into a 40 mL vial. Add 100 µL of 100 µg/mL solution of fatty alcohols in $CHCl_3$ to samples 1 and 4. Add 10 µL of 1000 µg/mL solution of eicosanol internal standard in $CHCl_3$ to all samples
2. Add 1 mL of 20 M KOH (in water) and 9 mL of ethanol
3. Heat above solution at 85 °C for 30 min. in water bath (hot plate setting of 4.5, 125 mL water in 250 mL beaker)
4. Cool, and add 10 mL of water and 10 mL of diethyl ether.

5. Shake and draw off diethyl ether layer (top) into a conical centrifuge tube
6. Repeat with an additional 10 mL of diethyl ether and combine extracts in the centrifuge tube.
7. Add 5 mL of 0.5 M KOH, shake, draw off and discard aqueous layer. Repeat
8. Add 10 mL of water, shake, draw off and discard aqueous layer. Repeat until water is neutral. (3-4 washes). If an emulsion forms, centrifuge to break.
9. Evaporate ethyl ether extract to dryness.

Extract clean-up using SPE

1. Reconstitute extract in 0.5 mL of chloroform
2. Condition NH_2 tube with 2 x 5 mL of hexane
3. Add reconstituted sample to SPE tube. Rinse sample container with 0.5 mL chloroform and add rinse to SPE tube.
4. Dry tube briefly with vacuum
5. Elute with 2 x10 mL 1:1 hexane-ethyl ether

Derivatization with Sylon BFT

1. Evaporate cleaned extract to dryness
2. Add 125 µL of pyridine and 125 µL Sylon BFT
3. Heat at 70 °C for 30 min.

After derivatization, add $CHCl_3$ to bring extract to FV=1 and analyze by GC-MS

GC-MS Analysis

column:	SLB-5ms, 20 m x 0.18 mm I.D. x 0.18 µm
oven:	125 °C (1 min.), 10 °C/min. to 330 °C (20 min.)
inj. temp.:	250 °C
MSD interface:	330 °C
scan range: m/z =	50 – 600
carrier gas:	helium, 0.6 mL/min constant flow
injection:	1 µL, splitless
liner:	4 mm ID, single taper

Results

Chromatograms (TICs) of the control sample (spiked solvent/ run through sample prep. process) and olive oil samples are presented in Figures 1-4. While there appears to be a good deal of matrix in the olive oil extracts, the use of extracted ion chromatograms (EICs) allowed for easy detection and quantification of each alcohol peak. As an example, the EIC for m/z =439, an ion in the spectrum of the silylated C26 alcohol, is shown in Figure 5. Based on the masses calculated for the silylated derivatives, C22 – C28 alcohols can be identified in the olive oil extracts.

SPE cleanup. In place of TLC proposed in the EN method, SPE was used to clean the extract. LC-NH_2 was chosen as the SPE packing, in that it was expected to retain alcohols and sterols (2). Chromatograms of olive oil extracts that were SPE cleaned and analyzed without SPE are presented in Figures 3 and 4. The SPE clean-up primarily removed fatty acids, and did not adversely affect recovery of the aliphatic alcohols. Several sterols were also detected (by mass) as their silylated derivatives, indicating that they were retained on the LC-NH_2 cartridge along with the alcohols.

Calculated results. Per the EN method, the concentrations of the C22-C28 aliphatic alcohols are calculated using the internal standard (1-eicosanol/C20) added to the sample. Using this procedure, the calculated concentrations (and % recoveries) in the olive oil samples are shown in Table 1.

Conclusions

- SPE using LC-NH2 cartridges removed fatty acids and provided a cleaner extract than not using SPE.
- SPE clean-up did not adversely affect recovery of the aliphatic alcohols.
- GC-MS using extracted ion chromatograms allowed for clean detection of the aliphatic alcohol peaks.
- GC-MS allowed the identification of aliphatic alcohols not identified by direct comparison with a standard.
- GC-MS allowed for identification of sterols in the olive oil extracts.
- The accuracy of the method could not be determined by direct spiking of samples with free alcohols.

Table 1. Calculated Concentrations of C22-C28 Aliphatic Alcohols

Results in mg/Kg	Extracted control	Olive oil, spiked* SPE cleaned	Olive oil, unspiked SPE cleaned	Olive oil, unspiked No SPE
C22 aliphatic alcohol		54.7	53.8	50.5
C23 aliphatic alcohol	7.2 (72%)	10.2 (72%)	3.0	2.9
C24 aliphatic alcohol	5.9 (59%)	71.6 (39%)	67.7	58.5
C25 aliphatic alcohol		3.0	3.0	2.3
C26 aliphatic alcohol	2.6 (26%)	46.5 (25%)	44.0	33.3
C27 aliphatic alcohol	1.8 (18%)	2.6 (18%)	0.8	0.6
C28 aliphatic alcohol	1.0 (1%)	6.5 (6%)	5.9	4.4
Total		**204.9**	**178.2**	**152.5**

*spike containing C23, C24, C26, C27, C28 at 10 mg/kg

References

1. Determination of Aliphatic Alcohols Content by Capillary Gas Chromatography; European Economic Community (EEC) Commission Regulation No. 2568/91; Document 1991R2568 – EN – 06.09.1991 – 000.001 – 13; Annex IV.
2. Orozco-Solano, M.; Ruiz-Jimenez, J.; Luque de Castro, M.D. Ultasound-assisted extraction and derivatization of sterols and fatty alcohols from olive leaves and drupes prior to determination by gas chromatography-tandem mass spectrometry. J. Chromatogr., A 2010, 1217, 1227-1235.
3. Characteristics of Olive Oil; European Economic Community (EEC) Commission Regulation No. 2568/91; Document 1991R2568 – EN – 06.09.1991 – 000.001 –7; Annex I.

Figure 1. Extracted and derivatized aliphatic alcohol control sample

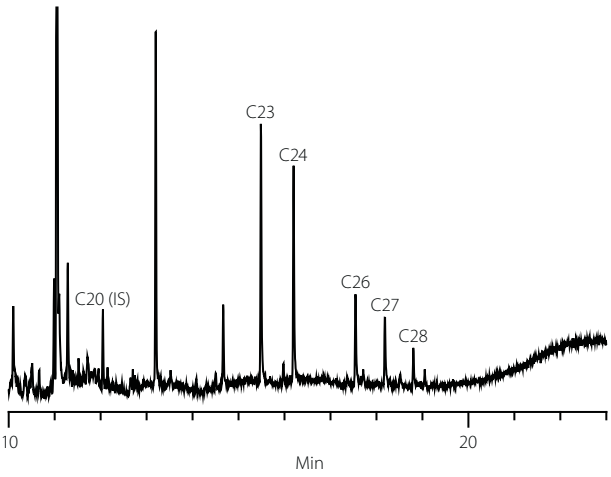


Figure 2. Extracted Olive oil spiked with aliphatic alcohol standard; SPE cleaned

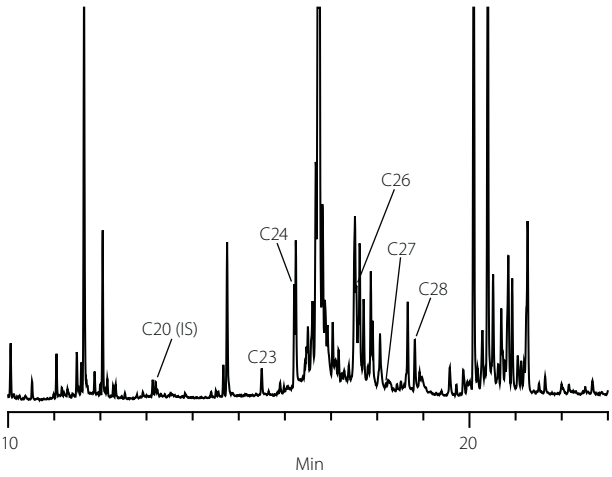


Figure 3. Olive oil extract; SPE cleaned

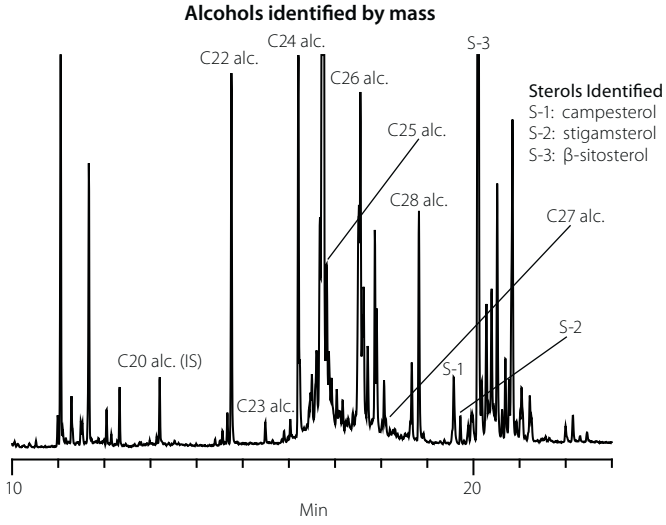


Figure 4. Olive oil extract; not SPE cleaned

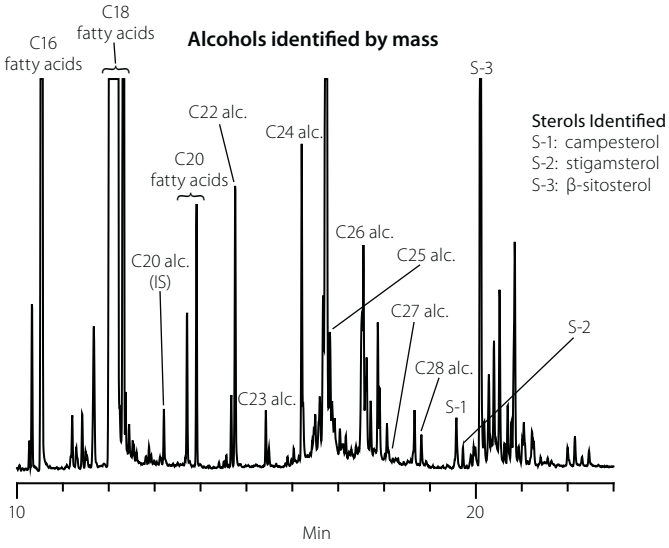
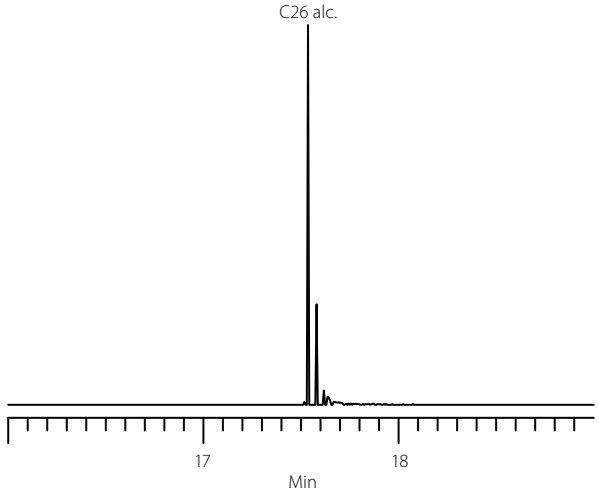


Figure 5. EIC, m/z=439 for unspiked olive oil extract, SPE cleaned



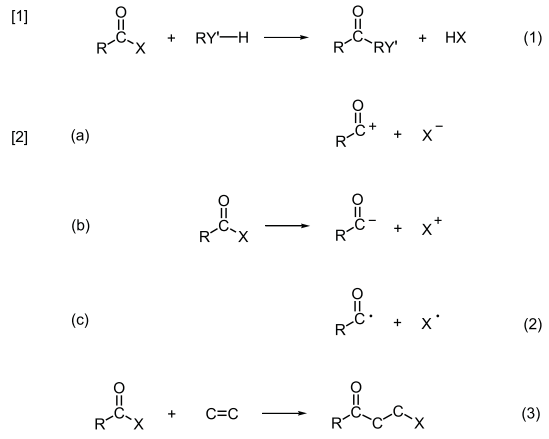
Acylation

Acylation, an alternative to silylation, is the conversion of compounds with active hydrogen such as –OH, -SH, and –NH into esters, thioesters and amides. Acylation is an important derivatization procedure because so many compounds of interest have amine and/or hydroxy groups.

Acylation enhances GC performance as the analyte volatility is increased and peak shape improved because of reduced surface adsorption. Acylation can reduce the polarity of a molecule, making it more retentive in reversed-phase HPLC applications.

These derivatives are both stable and highly volatile. A very popular example of this method is the insertion of perfluoroacyl groups into a substance to enable electron capture detection (ECD). Carbonyl groups adjacent to halogenated carbons enhance the response of ECD. A further benefit of acylation is the formation of fragmentation-directing derivatives for GC-MS analysis. Acylation reagents can be classified into two main groups: fluoro acid anhydrides and fluoracylimidazoles. Fluoracylimidazoles react readily with hydroxyl groups and secondary or tertiary amines to form acyl derivatives. The imidazoles produced as a by-product are relatively inert.

Reaction



The general reaction for acylation is shown in the above equations. Acylation involves the introduction of an acyl group into a molecule with a replaceable hydrogen atom. In the above equation R-C(:O)-X is the acylation reagent and R'Y-H is the target analyte, equation (1). The acylating agent R-C(:O)-X can lose the group –X by (a) electrophilic, (b) nucleophilic or (c) free radical mechanisms as represented in equation (2). Less commonly, an acyl group may be added across a double bond, equation (3). Direct electrophilic acylations of the first of these types are the most common mode of acylation (1).

Features

Acylation reactions for chromatography are carried out with three main types of acylation reagents: acid anhydrides, acid halides and reactive acyl derivatives such as acylated imidazoles and acylated amides of acylated phenols. The different types of these reagents are chosen for different reasons.

The principal use of acylation in chromatographic applications is the conversion of compounds containing active hydrogen atoms into derivatives that are more easily chromatographed, or offer a greater response to the chromatographic detection system than the parent compound. As mentioned earlier, an important example of this application is the insertion of perfluoroacyl groups. The presence of a carbonyl group adjacent to the halogenated carbons enhances the ECD response. In addition, acyl derivatives tend to direct the fragmentation patterns of compounds in MS applications, and provide useful information on the structure of these materials.

Acylation reduces the polarity of amino, hydroxy and thio groups which helps in reducing non-specific adsorption effects, and improves chromatographic properties. Acylation helps chromatographic separations which might not be possible with the underivatized compounds.

Acylation may improve stability of compounds by protecting unstable groupings, for example, the neighboring phenolic groups in the catecholamines that are very susceptible to oxidation. Acylation may confer volatility on substances such as carbohydrates or amino acids which have so many polar groupings that they are involatile and normally decompose on heating, and thus makes it possible to analyze these classes of compounds by gas chromatography. Acylation may be a preferable derivatization technique when the acylated compound is more stable than the silylated one, such as with primary amines.

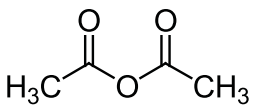
Acylation can be used to introduce detector-oriented groupings, such as the various halogen-containing acyl groups used to make compounds detectable at very low levels with ECD in gas chromatography. ECD technique is very sensitive and useful in measuring compounds in biological fluids, tissues and water samples at low concentrations.

Excess acyl halides must be removed before chromatographic analysis because of their reactivity. These reagents can produce interfering artifacts and damage the chromatographic column and system. It is more advantageous to use the acid anhydrides. The acid anhydrides are more volatile and can be easily evaporated.

Key Acylation Reagents

Product information for the following select group of Acylation Reagents can be found on pages 34-36.

Acetic Anhydride



Molecular Formula: $(CH_3CO)_2O$
CAS Number: 108-24-7
Formula Weight: 102.09
d: 1.080-1.085
n_D: 1.3901
Appearance: Clear, colorless liquid

Features/Benefits

- Acylation is an alternative to silylation, producing stable, volatile derivatives of alcohols, phenols, and amines for analysis by GC/FID.
- Acylated compounds are more stable than corresponding silylated compounds.

Common Reactive Functional Groups

Alcohols, phenols, carbohydrates and amines.

Procedure

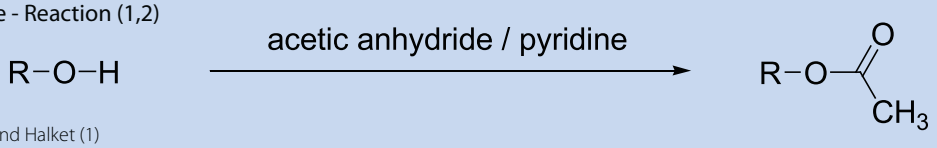
General

1. Dissolve 5 mg of sample in 5 mL of chloroform.
2. Add 0.5 mL acetic anhydride and 1 mL of acetic acid. Heat at 50 °C for 2-16 hours.
3. Remove excess reagent by evaporating the mixture to dryness and redissolve the residue in chloroform for GC analysis.

Alditol Formation

1. Dissolve 5 mg of sample in 5 mL of chloroform.
2. Add 1mL acetic anhydride:pyridine, 1:1. Heat at 100 °C for 20 minutes.
3. Remove excess reagent by evaporating the mixture to dryness and redissolve the residue in ethyl acetate for GC analysis.

Acetic Anhydride - Reaction (1,2)



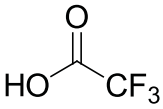
Adapted from Blau and Halket (1)

For additional details on key acylation reagents and derivatizing procedures, visit *sigma-aldrich.com/acylation*

Also refer to the Reference section on page 34.

TFA

Trifluoroacetic Acid



Molecular Formula:	CF_3COOH
CAS Number:	76-05-1
Formula Weight:	114.02
bp:	72.4 °C
d:	1.480
Appearance:	Clear, colorless liquid moisture sensitive

Features/Benefits

- Silyl Catalyst - Addition of a small amount of acidic catalyst usually increases the rate or degree of silylation. In acid catalysis, protonation of the silyl donor weakens the Si-X bond (X is the leaving group).
- TFA derivatives are stable and volatile. Use of TFA in combination with HMDS avoids the formation of ammonium chloride.
- Ion Pair Reagent - Use of TFA as an ion pair reagent in reversed-phase ion-pair chromatography on a nonionic column can maximize polarity differences between proteins or peptides in a sample mixture and improve chromatographic separation (to avoid protein denaturation, use 0.1% or less TFA).
- Mobile Phase Additive - In reversed-phase HPLC, inclusion of TFA in the aqueous mobile phase provides an ion with detectable properties and with affinity for the solid phase. Analyte injection into the system gives rise to equilibrium disturbances and influences the distribution of the detectable ionic component.

Common Reactive Functional Groups

Amides, amines, carbonyl, hydroxyls, sulfonamides, silyl catalyst for carbohydrate derivatization, reagent for large peptide purification, ion-pair reagent, and mobile phase additive for HPLC.

Procedure

Carbohydrates in syrups

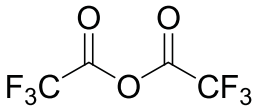
1. Place 60-70 mg of 80% soluble syrup in a vial and dissolve with pyridine.
2. Add 900 µL HMDS, then 100 µL TFA. Shake 30 seconds, then let stand 15 minutes with occasional shaking. Inject aliquot on to GC column for analysis.
3. To determine when derivatization is complete, analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed showing reaction completion.

Aflatoxins

1. Place extracted sample containing aflatoxins in screw cap vial. Evaporate to dryness using clean nitrogen.
2. Add 200 µL of hexane to re-dissolve aflatoxins.
3. Add 50 µL of TFA, cap, and vortex for 30 sec. Let stand for 5 min.
4. Add 2.0 mL deionized water:acetonitrile (9:1). Vortex for 30 seconds, then allow layers to separate.
5. Remove aqueous layer containing aflatoxins. Filter through 0.45 µm syringe-tip filter and inject aliquot into LC column.

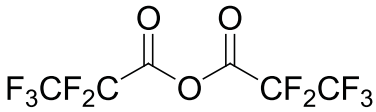
Perfluoro Acid Anhydrides

Trifluoroacetic acid anhydride (TFAA)



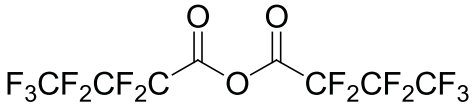
Molecular Formula:	$(CF_3CO)_2O$
CAS Number:	407-25-0
Formula Weight:	210.03
bp:	39.5-40°C
d:	1.487
nD:	<1.300
Appearance:	Clear, colorless liquid

Pentafluoropropionic acid anhydride (PFPA)



Molecular Formula:	$(CF_3CF_2CO)_2O$
CAS Number:	356-42-3
Formula Weight:	310.05
bp:	69-70°C
d:	1.571
nD:	<1.300
Appearance:	Clear, colorless liquid

Heptafluorobutyric acid anhydride (HFBA)



Molecular Formula:	$(CF_3CF_2CF_2CO)_2O$
CAS Number:	336-59-4
Formula Weight:	410.06
mp:	-43°
d:	1.665
nD:	1.2870
Appearance:	Clear, colorless liquid

Features/Benefits

- Produce stable, volatile derivatives of alcohols, amines, and phenols for electron capture or flame ionization detection.
- Frequently used in confirmation testing for drugs of abuse by GC-MS. (TFAA is used to identify methamphetamine, PFPA is used to identify opiates and benzoylecgonine, HFBA is used to identify amphetamines and phencyclidine).

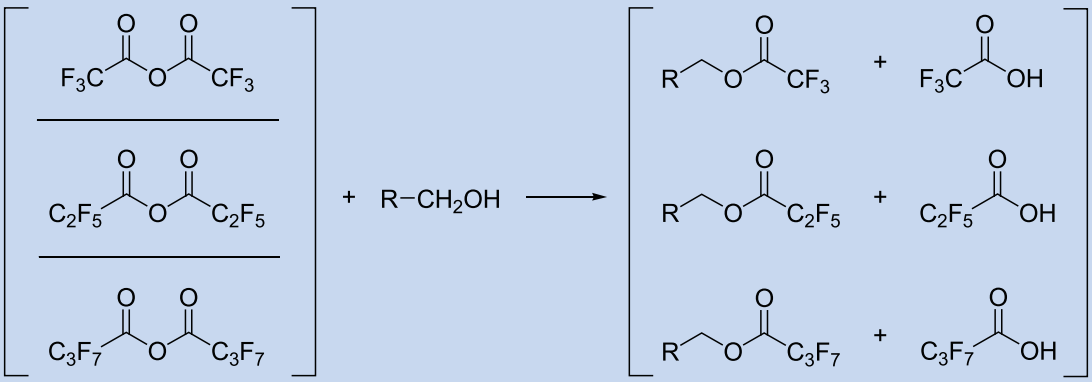
Common Reactive Functional Groups

Alcohols, amino acids, amides, amines, phenols, and steroids.

Procedure

1. Dissolve 50 µg of sample (250 µg for FID) in 0.5 mL of benzene.
2. Add 0.1 mL of 0.05 M trimethylamine (acid scavenger) in benzene followed by 10 µL of PFPA, HFBA, or TFAA.
3. Cap the vial and heat at 50 °C for 15 minutes.
4. Cool the mix and add 1 mL of 5 % ammonia in water.
5. Shake for 5 minutes, allow layers to separate, and inject an aliquot of the benzene (top) layer into the chromatograph

TFAA + PFPA + HFBA - Reaction (1,2)



Product Listing for Key Acylation Reagents

Cat. No.	Pkg. Size
Acetic Anhydride	
33085	10 x 2 mL
TFA	
33164	25 mL
33140	25 mL
91719	10 x 1 mL, 10, 50 mL

Cat. No.	Pkg. Size
TFAA	
33165-U	10 x 1 mL
33164	25 mL
PFPA	
33167	10 x 1 mL
33168	25 mL
HFBA	
77253	10 x 1, 10, 50 mL

To see a complete listing of Acylation Reagents for GC Derivatization, go to page 35.

References

For additional details on reagents and derivatizing procedure check the website: *sigma-aldrich.com/derivatization* or request an electronic copy of publication (Reagent Type): T497121 (Acetic anhydride); T496027A (Trifluoroacetic acid); T497104 (Perfluoro acid anhydride).

1. K. Blau and J. Halket Handbook of Derivatives for Chromatography (2nd ed.) John Wiley & Sons, New York, 1993.
2. D.R. Knapp Handbook of Analytical Derivatization Reactions John Wiley & Sons, New York, 1979.

Additional Reading

Acetic Anhydride

C.J.W. Brooks and E.C. Horning, Anal. Chem. 36, 1540 (1964).

E.C. Horning, et al., Anal. Chem. 36, 1546 (1964).

Trifluoroacetic Acid

K. Blau and J. Halket Handbook of Derivatives for Chromatography (2nd ed.) John Wiley & Sons, New York, 1993.

D.R. Knapp Handbook of Analytical Derivatization Reactions John Wiley & Sons, New York, 1979.

M. Morvai, P. I. Monlnar Simultaneous Gas Chromatographic Quantitation of Sugars and Acids in Citrus Fruits, Pears, Bananas, Grapes, Apples and Tomatoes Chromatographia, 34 (9-10): 502-504 (1992).

G.W. Chapman, R.J. Horvat Determination of Non-volatile (Organic) Acids and Sugars from Fruits and Sweet Potato Extracts by Capillary GLC and GLC-MS, J. Agric. Food Chem., 37 (4): 947-950 (1989).

P. Englmaier High Resolution GLC of Carbohydrates as Their Dithioacetal-Trimethylsilylates and Trifluoroacetates J. High Res. Chromatogr., 13 (2): 121-125 (1990).

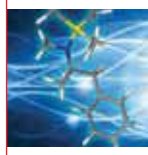
Perfluoro Acid Anhydrides

F.F. Lawrence and J.J. Ryan, J. Chromatogr. 130, 97 (1977).

D.E. Coffin, J. Assoc. Off. Anal. Chem. 52, 1044 (1969).

N.P. Sen, J. Food Sci. 34, 22 (1969).

D.D. Clarke, et al., J. Gas Chromatogr. 5, 307 (1967).



Acylation Reagents for GC Derivatization

Product Name	Description	CAS Number	Pkg Size	Cat. No.
Methyl trifluoromethanesulfonate	for GC derivatization; ≥98%, Shown to be the most effective monomethylating agent in reactions with potassium enolates, Useful methylation reagent for the conversion of amines to methyl ammonium triflates	333-27-7	1, 5 g	18503
Ethyl trifluoromethanesulfonate	≥99.0% (GC), powerful ethylating agent	425-75-2	5 mL	91734
Trifluoroacetic anhydride	derivatization grade (for GC), ≥99.0% (GC); for the protection of prim. and sec. amines, derivatives are more volatile	407-25-0	10 x 1 mL, 10, 50 mL	91719
1-(Trifluoroacetyl)imidazole	for GC derivatization	1546-79-8	5, 10 x 1 mL	394920
Acetic anhydride	for GC derivatization, ≥99.0% (GC)	108-24-7	10 x 1 mL	91204
Trichloroacetyl chloride	for GC derivatization, 99% (GC)	76-02-8	25, 100, 500 g	80521
2-Thenoyltrifluoroacetone	≥99.0% (GC), reagent for the extraction and spectrophotometric determination of metals	326-91-0	5 g	88300
2,2,6,6-Tetramethyl-3,5-heptanedione	≥98.0% (GC)	1118-71-4	5, 25 mL	87851
N-Methyl-bis-heptafluorobutyramide	≥96.0% (GC), derivatization reagent for GC-MS; derivatizing agent for amines, alcohols and thiols, forming neutral butyramide	73980-71-9	1 mL	78268
Pentafluoropropionic anhydride	≥97.0% (GC), PFPA acylates amines, amino acids and other compounds; PFPA derivatives are highly volatile, used in GC separation	356-42-3	5, 25 mL	77292
2 Bromoacetophenone	≥99.0% (GC), preparation of crystalline esters from acids	70-11-1	10, 50 g	77450
Pentafluoropropionic anhydride	Acylation reagent	356-42-3	10 x 1 mL	33167
Heptafluorobutyric anhydride	puriss., ≥99.0% (GC), HFAA acylates amines, amino acids and other compounds; HFBA derivatives are highly volatile: used in GC separation.	336-59-4	10 x 1 mL, 10, 50 mL 10 x 1 mL, 5, 25 mL	77253 394912
2,3,4,5,6-Pentafluorobenzoyl chloride	99%, derivatizing agent for GC	2251-50-5	1, 5, 25 g	103772
N,N'-Diisopropyl-O-methylisourea	97%	54648-79-2	25 g	226408
(±)-α-Methoxy-α-trifluoromethylphenylacetic acid	≥97.0% (T)	81655-41-6	5 g	65371
6,6,7,7,8,8,8-Heptafluoro-2,2-dimethyl-3,5-octanedione	98%	17587-22-3	5 g	175161
4-Bromophenacyl trifluoromethanesulfonate	≥95% (H-NMR/C-NMR); Assay of free and 'total' carnitine after derivatization with 4'-bromophenacyl triflate and HPLC, Detn. of carnitine capillary electrophoresis	93128-04-2	50 mg	41392
2,3-Diaminotoluene	97%	2687-25-4	5 g	272361
Esterate M	25% in pyridine	4637-24-5	25 mL	33140
Acetic anhydride		108-24-7	10 x 2 mL	33085
2,6-Diamino-4-pyrimidinone	96%	56-06-4	25, 100 g	D19206
Benzoic acid	for calorimetrical determination (approx. 26460 J/g)	65-85-0	100 g	33045
tert-Butyl methyl ether	≥99% (GC), pertains only in Germany: für Deutschland: Mineralölerzeugnis, steuerbegünstigt! Darf nicht als Treib-, Heiz-, Schmierstoff oder zur Herstellung solcher Stoffe verwendet werden!	1634-04-4	1, 2.5 L	20256
Ethyl acetoacetate	≥99.0% (GC)	141-97-9	100 mL, 1 L	00410
(1S)-(+)-Menthyl chloroformate	optical purity 97% (GLC), chiral auxiliary in the asymmetric synthesis of quaternary carbon centers	7635-54-3	5, 25 mL	378712
Butylboronic acid	for GC, ≥96.0% (T), derivatizing agent for gas chromatography	4426-47-5	1, 5 g	19667
1-(Pentafluoropropionyl) imidazole	≥98.5% (GC), derivatization agent for alcohols and carboxylic acids, etc. in GC	71735-32-5	1 mL	17281
1-(Heptafluorobutyryl)imidazole	97%	32477-35-3	1, 5 g	556645
Boron trifluoride-methanol Solution	~10% (~1.3 M)	373-57-9	50, 250 mL, 1 L	15715
1-Acetylimidazole	98%, relatively specific reagent for tyrosyl residue acetylation; reagent used in the synthesis of annulated imidazole derivatives.	2466-76-4	25, 100 g	157864
Hexafluoroacetylacetone	98%	1522-22-1	5, 25 g	238309
N-Methyl-bis-trifluoroacetamide	≥99.0% (GC), reagent for trifluoroacetylations under mild conditions	685-27-8	5, 25 mL	65943
N-Methyl-bis-trifluoroacetamide	~98% (GC), complies for derivatization; Reagent for introducing trifluoroacetyl group	685-27-8	10 x 1 mL, 5 mL	M0789
2,3,4,5,6-Pentafluorobenzaldehyde	98%, reagent for derivatizing primary amines for GC	653-37-2	2.5, 10 g	103748
Pentafluorobenzenesulfonyl chloride	99%	832-53-1	5, 25 g	103764

Acylation Reagents for GC Derivatization (Contd.)

Product Name	Description	CAS Number	Pkg Size	Cat. No.
1,1,1-Trifluoro-2,4-pentanedione	98%	367-57-7	10, 25 g	235970
1,1,1-Trifluoroacetone	97%, used in a synthesis of 2-trifluoromethyl-7-azaindoles starting with 2,6-dihalopyridines; the derived chiral imine was used to prepare enantiopure α-trifluoromethyl alanines and diamines via a Strecker reaction followed by either nitrile hydrolysis or reduction	421-50-1	5, 25, 100 g	T62804
4-(Trifluoromethyl)benzoyl chloride	97%	329-15-7	1, 5, 25 g	249475
8-Quinolinesulfonyl chloride	≥96.0% (AT), coupling reagent in oligonucleotide synthesis; formation of olefins from secondary esters at moderate temperature	18704-37-5	5, 25 g	22695
(1R)-(-)-Menthyl chloroformate	optical purity ee: 99% (GLC), chiral derivatizing agent used for the resolution of alcohols and amines by GC, HPLC, or crystallization	14602-86-9	25, 100 g	245305
Phenylboronic acid	95%, Boronic acid component in Suzuki bi-aryl cross-coupling studies; employed in Stille and Suzuki cross-coupling reactions	98-80-6	10, 50, 250 g	P20009
Methylboronic acid	97%, reagent for derivatizing many carbohydrates and biologically active compounds for GLC analysis	13061-96-6	1, 5 g	165336
2,3,4,5,6-Pentafluorobenzoic Anhydride	≥98.0% (GC); Derivatizing reagent for lipids	15989-99-8	5 g	02379
4-(Dimethylamino) benzoyl chloride	≥99 % Material is reported to be very moisture sensitive. Handle under an inert atmosphere	4755-50-4	1 g	67954
Acylation Sampler Kit				505862
Lab kit	for the evaluation of FA status in blood (n-3 + n-6 PUFA)			05904

Selected Acylation Applications

Analysis of 2,4,6-Trichloroanisole and Precursors in Red Wine

Katherine K. Stenerson

This article originally published in Reporter 29.1*

"Cork taint" or a musty odor sometimes detected in wine results from 2,4,6-trichloroanisole (TCA). The source of TCA is thought to be the fungal methylation of chorophenols present in the wine, with these compounds emanating from the cork or other sources such as biocides, fungicides, and exposure of processing equipment to antiseptic cleaning products containing halophenols (1).

This application demonstrates the use of solid phase microextraction (SPME) for the analysis of TCA and several chlorophenolic precursors from wine. The chlorophenols were derivatized in matrix using acetic anhydride, and the acylated derivatives extracted from the headspace. The TCA, which is not derivatized, was simultaneously extracted with the halophenols. Final analysis was performed by GC-ECD on the SLB-5ms capillary column.

Experimental

The 100 µm PDSM fiber and extraction conditions were based on published findings for derivatized halophenols (1), with adjustments made to sample volume, and corresponding amounts of reagents. The wine sample used for extraction was a California shiraz in a wax-lined carton-type container with a plastic closure. The extraction and GC analysis conditions were as follows:

sample:	1.5 mL sample + 600 µL 5% K_2CO_3 + 240 mg NaCl + 60 µL acetic anhydride;
SPME fiber:	metal fiber coated with 100 µm PDMS (57928-U)
extraction:	headspace, 50 °C, 30 min., with stirring
desorption temp.:	250 °C, 3 min.
column:	SLB-5ms; 30 m x 0.25 mm I.D. x 0.25 µm (28471-U)
oven:	50 °C (1 min.), 25 °C/min. to 280 °C
detector:	ECD, 290 °C
carrier gas:	helium, 1.5 mL/min constant flow
liner:	0.75 mm I.D. SPME

Calibration standards were prepared in 12% ethanol:water using a mixture of TCA and the halophenols. The wine sample was analyzed as is, and spiked at 100 ng/L.

Results

Derivatization and Extraction. The halophenols were acylated with acetic anhydride prior to extraction. Acetic anhydride will hydrolyze in the presence of water, however the phenolic groups present on the analytes are more reactive, making it possible to conduct derivatization in an aqueous matrix (2). The addition of K_2CO_3 drives the reaction by removing the acetic acid that is formed (Figure 1):

The resulting derivatives demonstrated good peak shape and response by ECD (Figure 2), allowing for easy and consistent integration. The use of headspace extraction and selective detection (ECD) resulted in minimal background interference.

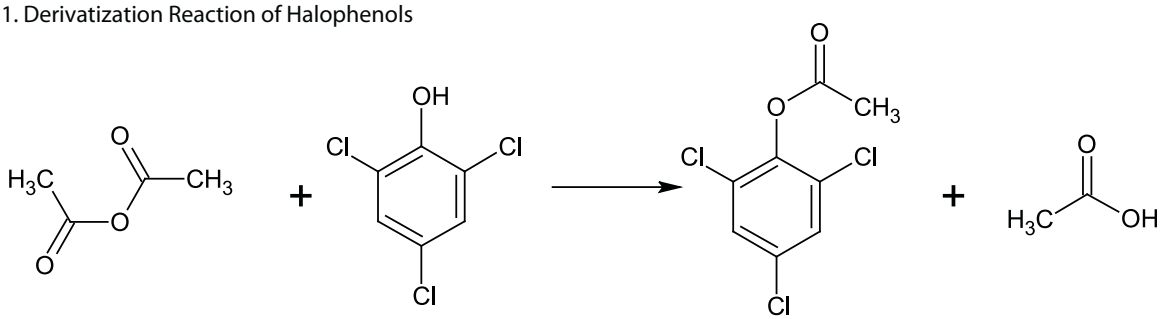
Linearity. Standards from 10-300 ng/L prepared in 12% ethanol in water were extracted. A linear response was observed over this concentration range, with correlation coefficients of >0.990 for all analytes except pentachlorophenol. Above 10 ng/L, the linearity for pentachlorophenol improved (correlation coefficient of 0.998).

Recovery. Immediately after calibration, unspiked and spiked (100 ng/L) red wine samples were extracted. Chromatograms of each are presented in Figures 3 and 4. Using the calibration curves, the levels of TCA and the halophenols in each were calculated, along with a percent recovery for the spiked wine sample. These results are summarized in Table 1. All four analytes were recovered, however it appears that the wine matrix may have interfered with accuracy to some extent, as indicated by the % recovery values.

Table 1. Recovery of spiked red wine sample (100 ng/L)

Spiked with 100 ng/L of:	Unspiked wine (ng/L)	Spiked wine (ng/L)	%Rec.
2,4,6-Trichloroanisole	ND	60.7	61
2,4,6-Trichlorophenol	22.7	96.3	74
2,3,4,6-Tetrachlorophenol	ND	55.7	56
Pentachlorophenol	3.3	33.5	30

Figure 1. Derivatization Reaction of Halophenols



* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Figure 2. Headspace-SPME analysis of standard spiked at 100 ng/L

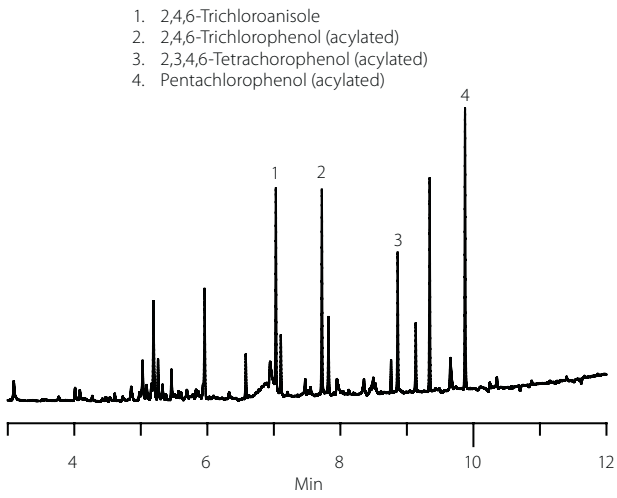


Figure 3. Headspace-SPME analysis of red wine sample, unspiked

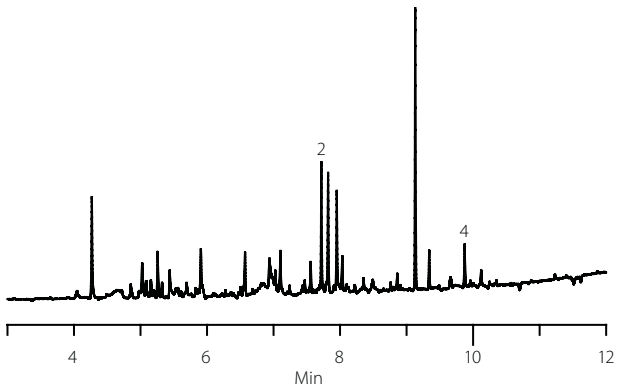
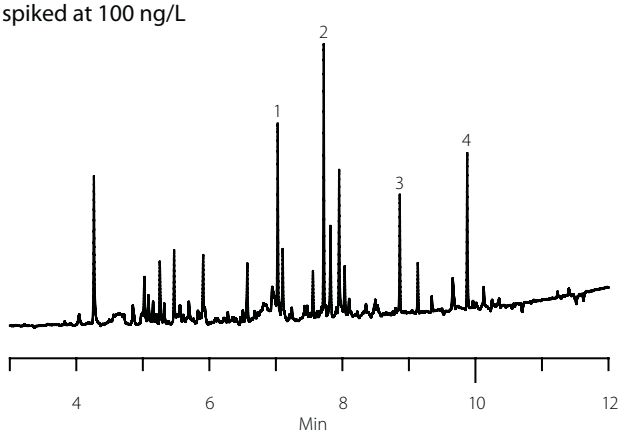


Figure 4. Headspace-SPME analysis of red wine sample, spiked at 100 ng/L



Reproducibility. A check of reproducibility was performed by doing extractions of sets of three spikes prepared in the 12% ethanol in water and red wine standards. Reproducibility was good, with %RSD values of <10%.

Conclusions

SPME can be used for the extraction of 2,4,6-trichloroanisole and its halophenolic precursors from wine. In the case of the later, derivatization makes the analytes easier to extract and analyze by GC. Headspace extraction in combination with ECD can be used to reduce background interference. The method appears to be quantitative, although further work would be necessary to optimize extraction efficiency from wine matrix.

References

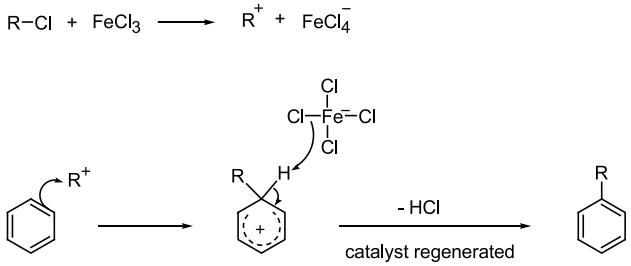
1. Insa, S., Salvado, V., Antico, E., Development of solid-phase extraction and solid-phase microextraction methods for the determination of chlorophenols in cork macerate and wine samples. J. Chromatogr. A, 2004, 1047: 15-20.
2. K. Blau; J. Halket, Handbook of Derivatives for Chromatography, Second Edition, John Wiley & Sons, New York, 1993. pp

Alkylation and Esterification

Alkylation

Alkylation refers to the small scale reaction of organic substances that contain a reactive hydrogen, R-COOH, R-OH, -SH, R-SH, R-NH-R, R-NH_2, R-$CONH_2$, R-CONH-R' and R-$COCH_2$-CO-R', with a derivatizing agent. Replacement of such a hydrogen with an alkyl group is important in chromatographic analysis because of the decreased polarity of the derivative as compared with the parent substance, facilitating analysis by chromatographic techniques. The decrease in polarity and intermolecular association is particularly important for analysis by gas chromatography and mass spectrometry. Mixtures of closely related compounds that show poor separation before derivatization may often be resolved by use of a proper derivative.

Alkylation of weak acidic groups like alcohols requires strong basic catalysts (sodium methoxide, potassium methoxide). More acidic groups like phenols and carboxylic acids require less basic catalysts (boron trifluoride).



Reaction

The general reaction for alkylation is shown in the equation above. This is a substitution reaction catalyzed by aluminum chloride in which an alkyl (R) or an acyl (RCO) group replaces a hydrogen atom of an aromatic nucleus to produce a hydrocarbon or a ketone.

Features

Alkylation represents the replacement of an active hydrogen by an aliphatic or aliphatic-aromatic (benzyl) group. This technique is used to modify compounds containing acidic hydrogens, such as carboxylic acids and phenols. The principal chromatographic use of this reaction is the conversion of organic acids into esters that produce better chromatograms than the free acids. Alkylation reactions can also be used to prepare ethers, thioethers, N-alkylamines, amides and sulphonamides. As the acidity of the active hydrogen decreases, a stronger alkylating reagent must be used. As the reagents and conditions become harsher, the selectivity and applicability of the method become more limited.

The products of alkylation are less polar than the starting materials because an active hydrogen has been replaced by an alkyl group. The most common application of alkylation for analytical derivatization is the conversion of organic acids into esters, especially methyl esters. Although TMS derivatives of carboxylic acids are easily formed, these compounds suffer from limited stability. The alkyl esters, on the other hand afford excellent stability and can be isolated and stored for extended periods if necessary.

BF_3-Methanol is an excellent first choice for derivatization of a compound for which there is no published method available.

Esterification is the most popular alkylation method. The largest applications of alkylation are the derivatization of organic acids into methyl esters. Another important area of chromatography where alkylation has been applied concerns carbohydrates. Methyl derivatives have been of the utmost value in structural determination as a means of labeling free hydroxyl groups.

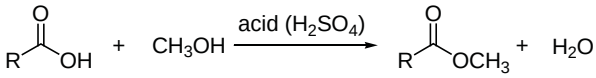
DMF-Dialkyl acetals derivatize acids to methyl esters, but not hydroxyl groups. A reagent like DMF-DMA is not only useful for derivatizing carboxylic acids, but also primary amines and amino acids.

Esterification

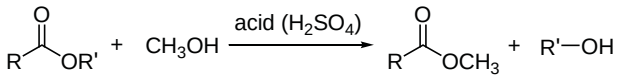
Esterification is the first choice for derivatization of acids for gas chromatography. Esterification involves the condensation of the carboxyl group of an acid and the hydroxyl group of an alcohol, with the elimination of water.

Reaction

Esterification[1]



Transesterification[2]



The general reactions for esterification and transesterification are shown in the above equations (1,2).

In the esterification of a carboxylic acid with an alcohol, reaction occurs by acyl-oxygen or alkyl-oxygen heterolysis. Acid-catalyzed esterification involves three possible mechanisms according to Ingold (3). These mechanisms are described in the following paragraphs.

The first mechanism involves acyl-oxygen fission. An esterification reaction is mainly bimolecular where the rate-limiting step is the attack of the alcohol on the protonated carboxylic acid. The carbon atom becomes tetrahedrally bonded, and for this reason retardation of the reaction rate may occur due to steric hindrance if –R and –R' are bulky groups.

The second possible mechanism is common for tertiary alcohols, and explains the formation of racemized esters when esterifying with an optically active alcohol. This reaction is slow for primary alcohols. The alcohol is first protonated and then loses water to form a carbonium ion, which reacts rapidly with the acid.

The third mechanism occurs with sterically hindered acids, e.g. 2,4,6-trimethylbenzoic acid, using sulphuric acid catalysis, where the acylium ion intermediate reacts with the alcohol.

Esterification is a reversible reaction. Water must be removed to drive the reaction to the right and obtain a high ester yield. A chemical reagent can be used to remove water as it is formed or, if the reaction is conducted at a temperature above 100 °C, water may distill off as it is formed.

In transesterification, the alcohol is displaced from the ester by another alcohol (e.g., methanol) in a process similar to hydrolysis (the second alcohol is used instead of water) forming a new ester. Transesterification is also an equilibrium reaction. To shift the reaction to the right, it is necessary to use a large excess of the second alcohol or to remove one of the products from the reaction mixture.

Conversion is maximized if excess alcohol is used. The reaction temperature also influences the conversion rate – the reaction is generally conducted near the boiling point of the alcohol.

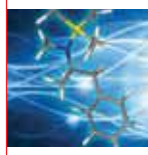
Features

For analytical work, esterification is best done in the presence of a volatile catalyst such as hydrogen chloride or thionyl chloride, which can be removed along with excess alcohol. Hydrogen chloride is the favored catalyst because of its acid strength and because it is readily removed. Sulphuric acid is less easily removed and has other drawbacks, such as, the risk of charring and other dehydrating reactions. Other acids, both conventional and 'Lewis-type", including trifluoroacetic and dichloroacetic acids, benzene- and p-toluene-sulphonic acids, sulphuryl and thionyl chlorides, phosphorus trichloride and oxychloride, polyphosphoric acids, and strongly acidic cation exchange resins have been used as esterification catalyst.

A good way of driving esterification reactions is to remove the water as it is formed. 2,2-Dimethoxypropane is a water scavenger. It reacts with acid solution to yield acetone. Other water scavengers are anhydrous sulfuric acid and graphite bisulfate.

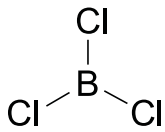
For additional details on key alkylation/esterification or to order, visit *sigma-aldrich.com/derivatization*

Product information on page 46.



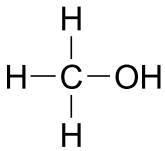
Boron trichloride-Methanol, 12% w/w

Boron trichloride



Molecular Formula: BCl_3
CAS Number: 10294-34-5
Formula Weight: 117.17
bp: 12.5 °C
Appearance: Colorless gas

Methanol



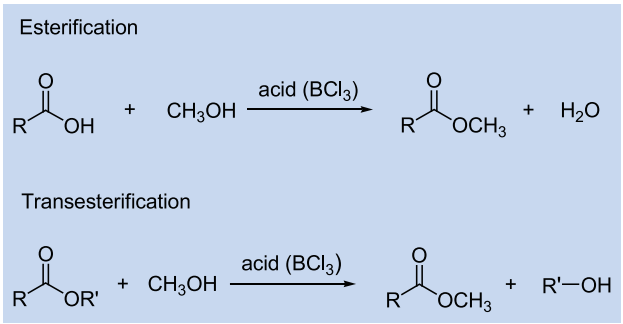
Molecular Formula: CH_3OH
CAS Number: 67-56-1
Formula Weight: 32.04
bp: 64.7 °C
Flash Point: 52 °F (11°C)
d: 0.791
n_D: 1.3290 at 20 °C
Appearance: Clear colorless liquid

Applications/Benefits

- Used for derivatizing carboxylic acids and transesterifying esters.
- Particularly useful for derivatizing carboxylic acids in bacterial lipids and seed oils.
- Provides convenient, fast, quantitative esterification/ transesterification.
- Clean reaction (no side reactions) with volatile by-products.
- More stable than BF_3 -methanol at room temperature (BCl_3 is not as volatile as BF_3).

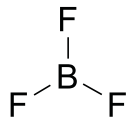
Procedure

1. Weigh 1-25 mg of sample (acid) into 5 mL reaction vessel
2. Add 2 mL BCl_3-methanol, 12 % w/w.
3. Heat at 60 °C for 5-10 minutes. Cool and then add 1 mL of water and 1 mL of hexane.
4. Shake the reaction vessel.
5. Carefully remove the upper (organic) layer and dry it over anhydrous sodium sulfate.
6. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed indicating reaction completion.



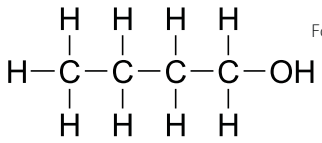
Boron trifluoride-Butanol, 10% w/w

Boron trifluoride



Molecular Formula: BF_3
CAS Number: 7637-07-2
Formula Weight: 67.81
bp: -100.4 °C

n-Butanol



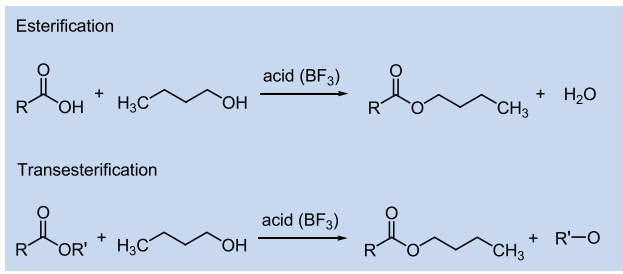
Molecular Formula: $CH_3(CH_2)_3OH$
CAS Number: 71-36-3
Formula Weight: 74.12
bp: 117.7 °C
Flash Point: 95 °F (35 °C)
d: 0.810
n_D: 1.3990 at 20 °C
Appearance: Clear, colorless liquid

Applications/Benefits

- Used for preparing short chain (C1-C10) monocarboxylic and dicarboxylic acids for GC
- Provides convenient, fast, quantitative esterification of fatty acids or transesterification of esters
- Clean reaction (no side reactions) with volatile by-products
- Resulting n-butyl esters are stable, volatile, and water soluble

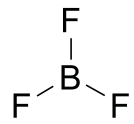
Procedure

1. Weigh 25-100 mg of sample (acid) into 5 mL reaction vessel
2. Add 2 mL BF_3-butanol, 10% w/w.
3. Heat at 60 °C for 5-10 minutes. Cool and then add 1 mL of water and 1 mL of hexane.
4. Neutralize and then remove excess butanol by adding saturated solution of sodium chloride.
5. Shake the reaction vessel.
6. Carefully remove the upper (organic) layer and dry it over anhydrous sodium sulfate.
7. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed indicating reaction completion.



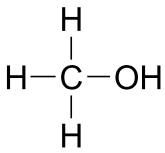
Boron trifluoride-Methanol, 10% w/w

Boron trifluoride



Molecular Formula: BF_3
CAS Number: 7637-07-2
Formula Weight: 67.81
bp: -100.4 °C

Methanol



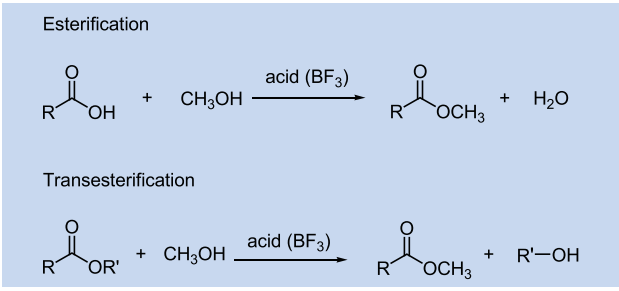
Molecular Formula: CH_3OH
CAS Number: 67-56-1
Formula Weight: 32.04
bp: 64.7 °C
Flash Point: 52 °F (11°C)
d: 0.791
n_D: 1.3290 at 20 °C
Appearance: Clear colorless liquid

Applications/Benefits

- Used for derivatizing C8-C24 carboxylic acids and transesterifying esters.
- Convenient, fast, quantitative esterification/ transesterification.
- Clean reaction (no side reactions) with volatile by-products.
- Derivatives are easily and quantitatively isolated.

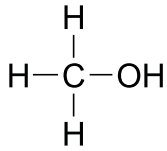
Procedure

1. Weigh 1-25 mg of sample (acid) into 5 mL reaction vessel
2. Add 2 mL BF_3-methanol, 10% w/w.
3. Heat at 60 °C for 5-10 minutes. Cool and then add 1 mL of water and 1 mL of hexane.
4. Shake the reaction vessel.
5. Carefully remove the upper (organic) layer and dry it over anhydrous sodium sulfate.
6. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed indicating reaction completion.



Methanolic H_2SO_4 ,10% v/v

Methanol



Molecular Formula: CH_3OH
CAS Number: 67-56-1
Formula Weight: 32.04
bp: 64.7 °C
Flash Point: 52 °F (11°C)
d: 0.791
n_D: 1.3290 at 20 °C
Appearance: Clear colorless liquid

Sulfuric acid

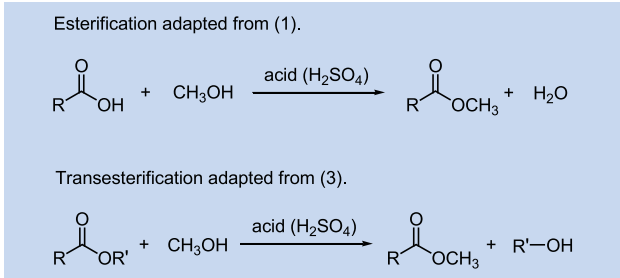
Molecular Formula: H_2SO_4
CAS Number: 7664-93-9
Formula Weight: 98.08
bp: ~290 °C (at 340 °C decomposes into $SO_3 + H_2O$
d: ~1.84
Appearance: Colorless, odorless (but highly pungent), oily liquid

Applications/Benefits

- Methanolic H_2SO_4, 10% v/v, is useful for esterifying acids and transesterifying esters.
- Clean reaction (no side reactions) w/volatile byproducts. Colorless, odorless (but highly pungent), oily liquid

Procedure

1. Weigh 1-25 mg of sample (acid) into 5 mL reaction vessel.
2. Add 2 mL Methanolic H_2SO_4, 10% v/v.
3. Heat at 60 °C for 30 minutes. Allow mixture to cool, add 1 mL saturated sodium bicarbonate solution and 1 mL of hexane.
4. Shake the reaction vessel.
5. Carefully remove the upper (organic) layer and dry it over anhydrous sodium sulfate.
6. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed indicating reaction completion.



Methanolic Base, 0.5N

Metallic sodium in methanol

Sodium

Molecular Formula: Na
CAS Number: 7440-23-5
Formula Weight: 22.99
Appearance: light silver-white metal (tarnishes to dull gray on exposure to air)

Methanol

Molecular Formula: CH_3OH
CAS Number: 67-56-1

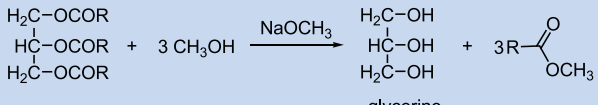
(Refer to page 42 for structure and properties)

Applications/Benefits

- Transesterification of triglycerides, cholesteryl esters, phospholipids. Sequence of esterification is triglycerides, diglycerides, monoglycerides, methyl esters.
- Provides convenient, fast, quantitative derivatization.

Procedure

1. Weigh 10-30 mg of sample into a reaction vessel containing 1 mL of organic solvent.
2. Add 2 mL Methanolic Base, 0.5 N, and mix.
3. Heat at 70-80 °C for 15-20 minutes. Allow mixture to cool, then add 1 mL of water and 1 mL of hexane or heptane
4. Carefully remove the upper (organic) layer and dry it over anhydrous sodium sulfate.
5. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed indicating reaction completion.



Adapted from (3).

Methanolic HCl, 0.5N and 3N

Hydrochloric acid in methanol

Methanol

Molecular Formula: CH_3OH
CAS Number: 67-56-1

(Refer to page 42 for structure and properties)

Hydrochloric acid

Molecular Formula: HCl
CAS Number: 7647-01-0
Molecular Formula: HCl
Formula Weight: 36.46
bp: -85 °C
Appearance: Colorless to slightly yellow,

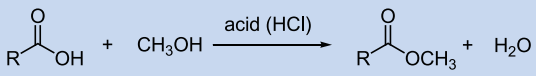
Applications/Benefits

- Derivatization of fatty acids, particularly volatile (short chain) fatty acids.
- Clean reaction (no side reactions) with volatile by-products.
- Provides convenient, fast, quantitative derivatization.

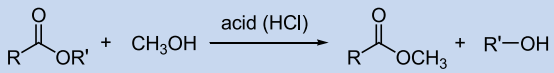
Procedure

1. Weigh 1-25 mg of sample into a 5 mL reaction vessel.
2. Add 2 mL Methanolic HCl, and mix.
3. Heat at 50 °C for 5-10 minutes. Allow mixture to cool.
4. Analyze aliquots of the upper (organic) level at selected time intervals until no further increase in product peak(s) is observed indicating reaction completion.

Esterification adapted from (1).

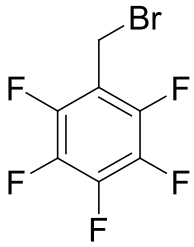


Transesterification adapted from (3).



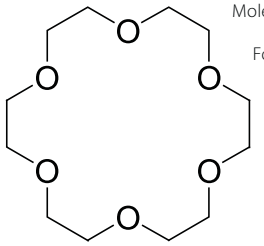
Pentafluorobenzyl Bromide, Hexaoxacyclooctadecane

Pentafluorobenzyl bromide (PFBBr)



Molecular Formula: $CH_2F_5CH_2Br$
CAS Number: 1765-40-8
Formula Weight: 260.09
bp: 174-175 °C
d: 1.728
n_D: 1.4720
Appearance: Clear, colorless liquid

1,4,7,10,13,16-Hexaoxacyclooctadecane



Molecular Formula: $C_{12}H_{24}O_6$
CAS Number: 17455-13-9
Formula Weight: 264.32
mp: 42-45 °C
Appearance: White solid

Common Applications

Halogenated derivatives of carboxylic acids, mercaptans, phenols, and sulfonamides.

Procedure

Acids

1. Combine 0.8 mg of acid and 100 mL acetone. Add 250 mg PFBBr and 50 mg potassium bicarbonate.
2. Reflux for 3 hours
3. Add 500 mL ethyl ether and 20 mL ethyl acetate.
4. Wash briefly with water, then dry over sodium sulfate and evaporate to dryness.
5. Dissolve residue in hexane containing 1% acetone and 1% ethanol.
6. Analyze 1 µL aliquot by GC.

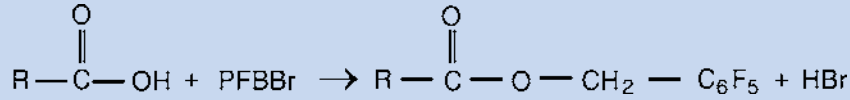
Extractive Alkylation

1. Combine 0.2 mg sample and 1 mL methylene chloride in a reaction vessel.
2. Add 1 mL 0.1 M tetrabutylammonium hydrogen sulfate, 1 mL 0.2 M sodium hydroxide, and 25 µL PFBBr and cap.
3. Shake for 20-30 minutes at 25 °C.
4. Analyze aliquots by GC/FID.

Reagent for Preparing Pentafluorobenzyl-Phenol Derivatives:

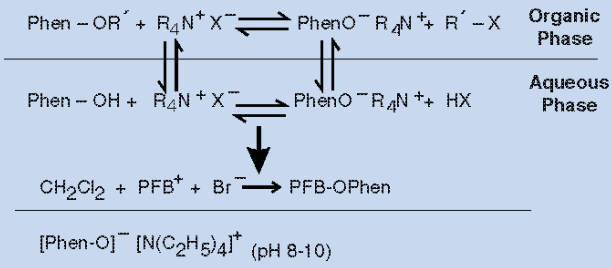
1. Combine 1 mL PFBBr and 1 g 18 Crown 6. Dilute with 50 mL 2-propanol.

Pentafluorobenzyl Bromide



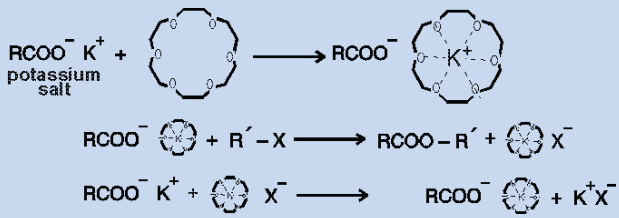
Adapted from Blau and Halket

Extractive Alkylation (Phenols)



Adapted from Knapp (2)

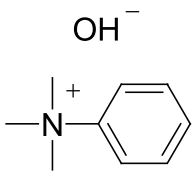
1,4,7,10,13,16-Hexaoxacyclooctadecane



Adapted from Knapp (2)

TMAH

Trimethylanilinium hydroxide



Elemental Formula: Mixture of trimethylphenyl-ammonium iodine, silver oxide, methanol
bp: 65 °C at 760 mm Hg
d: 1.10
n_D: 0.790 at 20 °C
Appearance: Clear, colorless liquid

Applications/Benefits

- Provides convenient, fast, quantitative derivatization of nitrogen-bearing molecules
- Preferred reagent for derivatizing barbiturates (except meprobamate, which is analyzed as the free base)

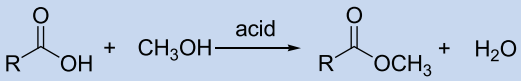
Procedure

1. Extract sample with appropriate solvent, or weigh 1-10 mg in a reaction vessel
2. Add TMAH reagent. Begin with equal amounts of TMAH and sample; up to 1000-fold excess reagent
3. Analyze 1 µL aliquot by GC (Direct injection)

Flash Alkylation

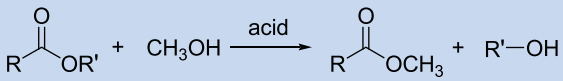
Draw 1 µL TMAH, then 1 µL sample, then 1 µL TMAH into a 10 µL syringe. Inject into a heated GC injection port

Esterification
General Mechanism



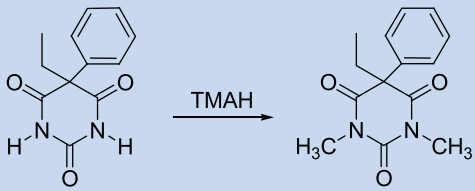
Adapted from (1).

Transesterification adapted from (3).



Adapted from (3).

Action of TMAH



phenobarbital methyl derivative

Adapted from (1).

Product Listing for Key Alkylation and Esterification Reagents

Cat. No.	Pkg. Size
Boron trichloride-Methanol, 12% w/w	
33353	20 x 1 mL
33089-U	20 x 2 mL
33033	400 mL
Boron trifluoride-Butanol, 10% w/w	
33126-U	10 x 5 mL
33125-U	100 mL
Boron trifluoride-Methanol, 10% w/w	
33356	20 x 1 mL
33020-U	19 x 2 mL
33040-U	10 x 5 mL
33021	400 mL
Methanolic H_2SO_4 ,10% v/v	
506516	6 x 5 mL
Methanolic Base, 0.5N	
33352	30 mL
33080	100 mL

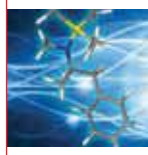
Cat. No.	Pkg. Size
Methanolic HCl, 0.5N	
33354	20 x 1 mL
33095	10 x 5 mL
Methanolic HCl, 3N	
33355	20 x 1 mL
33051	10 x 3 mL
33050-U	400 mL
40104-U	20 x 2 L
Pentafluorobenzyl bromide (PFBBr)	
33001	5 g
Hexaoxacyclooctadecane (18-Crown-6)	
33003-U	25 g
TMAH (0.2M in methanol)	
33358-U	10 x 1 mL
33097-U	10 mL

To see a complete listing of Alkylation/Esterification Reagents for GC Derivatization, go to page 48.

References

For additional details on reagents and derivatizing procedure check the website: *sigma-aldrich.com/derivatization* **or request an electronic copy of publication (Reagent Type):** T496123A (BCl_3-Methanol 12% w/w); T496124A (BF_3-Butanol, 10% w/w); T496125B (BF_3-Methanol, 10% w/w); T497018B (Methanolic H_2SO_4 10% v/v); T497007 (Methanolic Base, 0.5N); T497099B (Methanolic HCl, 0.5N and 3N); T497103 (Pentafluorobenzyl Bromide Hexaoxacyclooctadecane); T496180 (TMAH).

1. D.R. Knapp Handbook of Analytical Derivatization Reactions John Wiley & Sons, New York, 1979.
2. Bailey's Industrial Oil and Fat Products, fifth edition, Vol. 5, John Wiley & Sons, New York (1995).
3. C. K. Ingold, Structure and Mechanism in Organic Chemistry, Cornell University Press, Ithaca, p 1128, 1969.



Additional Reading

BCl_3-Methanol, 12% w/w

H.E. Nordby and G. Yelenosky Analysis of Triacylglycerols in Leaves of Citrus by HPLC JAOCS, 61: 1028-1031 (1984).

J. Herranen, A. Huhtikangas, H. Tirronen, T. Halonen, M. Huuskonen, K. Reinidainen, P. Riekkinen Quantification of a Number of Biogenic Amines and Their Metabolites in Brain Homogenates by Gas Chromatography – Mass Spectrometry J. Chromatogr., Biomed. Appl, 32 (2) (J. Chromatogr., 307), 241- 249 (1984).

BF_3-Butanol, 10% w/w

F. Sakaguchi and K. Kawamura Identification of 4-Oxoheptanedioic Acid in the Marine Atmosphere by Capillary Gas Chromatography-Mass Spectrometry J. Chromatogr., 687: 315-321 (1994).

K. Kawamura Identification of C2-C10 omega-Oxocarboxylic Acids, Pyruvic Acid and C2-C3 alpha-Dicarbonyls in Wet Precipitation and Aerosol Samples by Capillary GC and GC-MS Anal. Chem., 65: 3505-3511 (1993).

J.L. Iverson and A.J. Sheppard Determination of Fatty Acids in Butter Fat, Using Temperature-Programmed Gas Chromatography of the Butyl Esters Food Chem., 21: 223-234 (1986).

J.K. Khandelwal, T. Kline, J.P. Green Measurement of Imidazolylacetic Acid in Urine by Gas Chromatography-Mass Spectrometry J. Chromatogr., Biomed. Appl., 44 (2) (J. Chromatogr., 343), 249-257 (1985).

BF_3-Methanol, 10% w/w

R. Kleiman, G.F. Spencer, F.R. Earle Boron Trifluoride as Catalyst to Prepare Methyl Esters From Oils Containing Unusual Acyl Groups Lipids, 4 (2): 118-122 (1968).

E.S. Woodbury, P.R. Evershed, J.B. Rossell, R.E. Griffith, P. Farnell Detection of Vegetable Oil Adulteration Using Gas Chromatography Combustion-Isotope Ratio Mass Spectrometry Anal. Chem., 67: 2685-2690 (1995).

R.M. Le-Lacheur, L.B. Sonnenberg, P.C. Singer, R.F. Christman, M.J. Charles Identification of Carbonyl Compounds in Environmental Samples Environ. Sci. Technol., 27: 2745-2753 (1993).

X. Yan, P.J. Barlow, C. Craven Discrimination in Recovery During Capillary GLC Analysis of Fish Oil: The Use of a Recovery Correction Factor Food Chem., 40 (1): 93-99 (1991).

Methanolic H_2SO_4 ,10% v/v

R. Kleiman, G.F. Spencer, F.R. Earle Boron Trifluoride as Catalyst to Prepare Methyl Esters from Oils Containing Unusual Acyl Groups Lipids, 4 (2): 118-122 (1968).

E.S. Woodbury, P.R. Evershed, J.B. Rossell, R.E. Griffith, P. Farnell Detection of Vegetable Oil Adulteration Using Gas Chromatography Combustion-Isotope Ratio Mass Spectrometry Anal. Chem., 67: 2685-2690 (1995).

R.M. Le-Lacheur, L.B. Sonnenberg, P.C. Singer, R.F. Christman, M.J. Charles Identification of Carbonyl Compounds in Environmental Samples Environ. Sci. Technol., 27: 2745-2753 (1993).

X. Yan, P.J. Barlow, C. Craven Discrimination in Recovery During Capillary GLC Analysis of Fish Oil: The Use of a Recovery Correction Factor Food Chem., 40: 93-99 (1991).

Methanolic Base, 0.5N

G. Marquez-Ruiz, M. Tasioula-Margari, C.M. Dobarganes Quantitation and Distribution of Altered Fatty Acids in Frying Fats JAOCS, 72: 1171-1176 (1995).

G.F. Dunlap, J.P. White, M.L. Pollak, J.T. Brumm Fatty Acid Composition of Oil from Adapted, Elite Corn Breeding Materials JAOCS, 72: 981-987 (1995).

C. Wesen, L.H. Mu, P. Sundin, P. Froyen, J. Skramstad, G. Odham Gas- Chromatographic-Mass-Spectrometric Identification of Chlorinated Octadecanoic Acids in Eel Lipids J. Mass Spectrom., 30: 959-968 (1995).

P.M. Yurawecz, A.A. Molina, M. Mossoba, Y. Ku Estimation of Conjugated Octadecatriens in Edible Fats and Oils JAOCS, 70: 1093-1099 (1993).

Methanolic HCl, 0.5N and 3N

R.N.M. Carballeira, F. Shalabi The Rare Caribbean Sponge Leucosolenia canariensis: Phospholipid Fatty Acids and Sterols Lipids, 30 (5): 467-470 (1995).

J. Miralles, G. Barnathan, R. Galonnier, T. Sall, A. Samb, E.M. Gaydou, J.M. Kornprobst New Branched-Chain Fatty Acids from the Senegalese Gorgonian Leptogorgia piccola (white and yellow morphs) Lipids, 30 (5): 459-466 (1995).

S. Joron, H. Robert Simultaneous Determination of Antidepressant Drugs and Metabolites by HPLC. Design and Validation of a Simple and Reliable Analytical Procedure. Biomed. Chromatogr., 8 (4): 158-164 (1994).

Pentafluorobenzyl Bromide, Hexaoxacyclooctadecane

F.K. Kawahara, Anal. Chem. 40, 2073 (1968).

O. Gyllenhaal, et al., J. Chromatogr. 129, 295 (1976).

H.D. Durst, et al., Anal. Chem. 47, 1797 (1975).

P.T.-S. Pei, et al., Lipids 11, 814 (1976).

B. Davis, Anal. Chem. 49, 832 (1977).

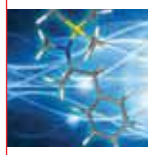
TMAH

P. Lillsunde, L. Michelson, T. Forsstrom, T. Korte, E. Schultz, K. Ariniemi, M. Portman, M.L. Sihvonen, T. Seppala Comprehensive Drug Screening In Blood for Detecting Abused Drugs or Drugs Potentially Hazardous for Traffic Safety Forensic Sci. Int., 77 (3): 191-210 (1996).

I. Brondz, I. Olsen Intra-Injector Formation of Methyl Esters from Phenoxy Acid Pesticides J. Chromatogr., 598: 309-312 (1992).

Alkylation/Esterification Reagents for GC Derivatization

Product Name	Description	CAS Number	Pkg Size	Cat. No.
1,1,1,3,3,3-Hexafluoro-2-propanol	for GC, ≥99.8% (GC); Size-exclusion chromatography in 1,1,1,3,3,3-hexafluoro-2-propanol.	920-66-1	10, 50 mL	52517
2,2,3,3,3-Pentafluoro-1-propanol	97%, generates fluorinated alpha-keto ethers with alkenes and has wide applications in the expanding fluorous research area; preparation of trifluoromethyl ynamines which, in turn, converted aldehydes to α-trifluoromethyl-α,β-unsaturated amides	422-05-9	5, 25 g	257478
2,3,4,5,6-Pentafluorobenzyl bromide	≥99.0%; Derivatizing agent for GC analysis of polyfunctional thiols. For the preparation of pentafluorobenzyl esters of organic acids for determination by capillary and GC. Also used to derivatize N-7-substituted guanine adducts of DNA for determination by GC-electron capture-MS	1765-40-8	1, 5, 25 g	101052
2,3,5,6-Tetrafluoro-4-ethanol (trifluoromethyl)benzyl bromide	98%	76437-40-6	1 g	406406
2,3-Dihydroxy-biphenyl	≥98.0% (HPLC)	1133-63-7	100 mg	17403
Boron trifluoride-ethanol	~10% (~1.3 M) in ethanol		10, 100 mL	05576
Boron trifluoride-methanol solution	~10% (~1.3 M); Reagent for esterifying of fatty acids for gas chromatographic analysis	373-57-9	10, 100 mL	15716
Boron trifluoride -1-butanol solution	puriss, ~10% in 1-butanol (~1.3 M); Reagent for the esterification of carboxylic acids for GC analysis	7637-07-2	100 mL	83253
Alcohols with hydrogen chloride	derivatizing agents, set for GC		1 SET	72558
Hydrogen chloride – 2-propanol solution	for GC, ~1.25 M (T); Derivatizing agent for GC		250 mL	17933
Hydrogen chloride – ethanol solution	for GC, ~1.25 M (T); Derivatizing agent for GC	123864-74-4	50, 250 mL	17934
Hydrogen chloride – methanol solution	for GC, ~1.25 M (T); Derivatizing agent for GC	132228-87-6	100 x 1 mL, 50, 250 mL	17935
O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride	derivatization grade (for GC), ≥99.0% (AT); Sensitive derivatizing agent for electron capture gas chromatographic analysis of carbonyl-containing compounds: keto steroids and carbohydrates	57981-02-9	1 g	76735
Pentafluoroiodoethane	97%	354-64-3	25, 300 g	331015
Tetrabutylammonium tetrabutylborate	97%	23231-91-6	5 g	477230
Trimethylboroxine	99%, Derivatizing agent for GLC analysis. Used in a diverse array of areas, including as a polymerization additive. Also used in the preparation of CBS catalysts for asymmetric reductions	823-96-1	5, 25 g	323136
Trimethylphenylammonium hydroxide solution	for GC, ~0.5 M in methanol	1899-02-1	10, 50 mL	79266
Trimethylsulfonium hydroxide solution	~0.25 M in methanol, derivatization reagent for GC; Reagent for the methylation of nucleophiles, e.g. carboxylic acids, alcohols, thiols, N-heterocycles; Rapid derivatization of acids for GC by pre-column transesterification of glycerides	17287-03-5	10 mL	92732
O-Ethylhydroxylamine hydrochloride	≥97.0%	3332-29-4	1, 5 g	274992
Boron trifluoride - 1-Propanol Solution	14wt. %, in excess 1-propanol	762-48-1	100 g	156825
4-Bromobenzyl bromide	≥98.0%	589-15-1	25, 100 g	112186
Hydrogen chloride Butanol Solution	for GC, puriss. p.a., ~3 M in 1-butanol; Reagent for the esterification of amino acids and carnites for GC-MS analysis	7647-01-0	50, 250 mL	87472
N,N-Dimethylformamide diethyl acetal	≥95.0% (GC); Reagent for the esterification of fatty acids.	1188-33-6	25, 100 mL	40252
N,N-Dimethylformamide dibutyl acetal	≥98.0% (NT), Reagent for the esterification of fatty acids	18503-90-7	10 mL	40262
N,N-Dimethylformamide diisopropyl acetal	95%	18503-89-4	25 g	178535
N,N-Dimethylformamide dineopentyl acetal	99%	4909-78-8	10, 50 g	140244



Product Name	Description	CAS Number	Pkg Size	Cat. No.
N,N-Dimethylformamide dipropyl acetal	97%	6006-65-1	25 g	178527
N,N-Dimethylformamide di-tert-butyl acetal	derivatization grade; Reagent used for the preparation of indolizines via intermolecular cyclization of picolinium salts	36805-97-7	5, 25 mL, 10 x 1mL	395005
N,N-Dimethylformamide diethyl acetal	derivatization grade	1188-33-6	5, 25 mL, 10 x 1mL	394971
N,N-Dimethylformamide dimethyl acetal	derivatization grade; Used in the preparation of formamidine derivatives, which are synthetic intermediates. Used to catalyze the coupling of epoxides with carbon dioxide under solvent free conditions leading to cyclic carbonates	4637-24-5	5, 25 mL, 10 x 1mL	394963
N,N-Dimethylformamide dipropyl acetal	derivatization grade	6006-65-1	10 x 1 mL, 25 mL	394998
O-Methylhydroxylamine hydrochloride	98%, Reagent for the preparation of O-methyl oximes. Reagent used to make O-methyl oximes from aldehydes or ketones	593-56-6	1, 5, 25, 100 g	226904
4-Nitrobenzyl bromide	99%	100-11-8	25, 100 g	N13054
Pentafluorophenylhydrazine	97%	828-73-9	10 g	156388
Nonafluoro-1-iodobutane	98%	423-39-2	25, 100 g	317845
Triethyloxonium tetrafluoroborate; stab. with 1-3% ether	purum, ≥97.0% (T); Powerful ethylating agent; Esterification of acids; Modifies carboxyl residues in proteins	368-39-8	25, 100 g	90520
2,2,2-Trichloroethanol	≥99%	115-20-8	100, 500 g	T54801
Triethyloxonium hexafluorophosphate	contains ~10% diethyl ether as stabilizer	17950-40-2	5, 25 g	164682
2,2,2-Trifluoroethanol	ReagentPlus®, ≥99.0%;	75-89-8	25, 100, 500 g	T63002
Trimethyloxonium tetrafluoroborate	95%; Reagent for the methylation of hydroxyl groups recently used in a complex, multistep synthesis directed towards spirastrellolide, a marine natural product	420-37-1	1, 10 g	281077
2,2-Dimethoxypropane	Alkylation reagent	77-76-9	25 g	33053
Esterate M (N,N-Dimethyl-formamide dimethyl acetal)	25% in pyridine	4637-24-5	25 mL	33140
Methoxyamine hydrochloride	Alkylation reagent	593-56-6	5 g	33045-U
Diazald® 99%	99%; Nitrosylating agent for transition metal complexes, amines, and carbanions, as well as a precursor to diazomethane	80-11-5	25, 100 g	D28000
18-Crown	Alkylation reagent for GC derivatization	17455-13-9	25 g	33003-U
Pentafluorobenzyl bromide	Alkylation reagent	1765-40-8	5 g	33001
BCl_3-2-Chloroethanol 10% (w/w)	Esterification reagent		10 x 1 mL	33056-U
BF_3-Butanol solution 10% (w/w)	Esterification reagent	7637-07-2	10 x 5 mL	33126-U
			100 mL	33125-U
BF_3-Methanol 10% (w/w)	Esterification reagent		10 x 5 mL	33040-U
			19 x 2 mL	33020-U
			20 x 1 mL	33356
			400 mL	33021
BF_3-Methanol 50% (w/w) in methanol	Reagent used for the mild deacetylation of amines.		4 x 25 mL, 50, 250 mL, 1L	134821
Boron trichloride 12% (w/w)	Esterification reagent		20 x 1 mL	33353
			20 x 2 mL	33089-U
			400 mL	33033
Methanolic Base, 0.5N	Esterification reagent	124-41-4	100 mL	33080
		124-41-4	30 mL	33352
Methanolic H_2SO_4, 10% in methanol	Esterification reagent	7664-93-9	6 x 5 mL	506516
Methanolic HCl, 0.5N	Esterification reagent	7647-01-0	10 x 5 mL	33095
		7647-01-0	20 x 1 mL	33354
Methanolic HCl, 3N	Esterification reagent	7647-01-0	10 x 3 mL	33051
		7647-01-0	20 x 1 mL	33355
		7647-01-0	400 mL	33050-U
TMAH, 0.2M in methanol	Esterification reagent	1899-02-1	10 x 1 mL	33358-U
		1899-02-1	10 mL	33097-U
FID Alkylation Sampler Kit				505854

References

1. N. E. Hoffman et al., Anal. Biochem. 32, 386 (1969)
2. N. E. Hoffman and K. M. Gooding, Anal. Biochem. 31, 471 (1969)
3. A. J. Poole et al., Clin. Chim. Acta 73, 527 (1976)
4. M. R. Guerin et al., Anal. Chem. 46, 761 (1974)
5. T. J. Sprinkle et al., Clin. Chim. Acta 25, 409 (1969)
6. S. Addanki et al., Clin. Chem. 22, 310 (1976)
7. L. J. Walter et al., J. Pharm. Sci. 63, 1754 (1974)
8. M. Rowlands and S. Riegelman, Anal. Biochem. 20, 463 (1967)
9. M. Rowlands and S. Riegelman, J. Pharm. Sci. 56, 717 (1964)
10. E. R. Blakley, Anal. Biochem. 15, 350 (1966)
11. E. Pierce, Silylation of Organic Compounds, Pierce Chemical Co. Rockford, IL., 1968, p72
12. M. Donike, J. Chromatogr. 42, 103 (1969)
13. P. F. G. Boon and W. Sudds, J. Phar. Pharmacol. 19, 88S (1967)
14. W. J. A. Vandenheuval, J. Chromatogr. 36, 354 (1968)
15. N. Narasimhachari et al., J. Chromatogr. Sci. 9, 502 (1971)
16. N. Narasimhachari and P. Vouros, Anal. Biochem. 45, 154 (1972)
17. N. Narasimhachari and K. Leiner, J. Chromat. Sci. 15, 181 (1977)
18. N. P. Sen and P. L. McGeer, Biochem. Biophys. Res. Commun. 14, 227 (1964)
19. M. Ervik et al., Acta Pharm. Suecica 7, 625 (1972)
20. T. Walle et al., Acta Pharm. Suecica 9, 509 (1972)
21. K. Matsubayashi et al., J. Chromatogr. 143, 571 (1977)
22. J. A. Corbin and L. B. Rogers, Anal. Chem. 42, 974 (1970)
23. T. Walle and H. Ehrsson, Acta Pharm. Suecica 8, 27 (1971)
24. T. Walle and H. Ehrsson, Acta Pharm. Suecica 7, 389 (1970)
25. M. Scoggins, J. Chromatogr. Sci. 13, 146 (1975)
26. ASTM D6584
27. EN 14105

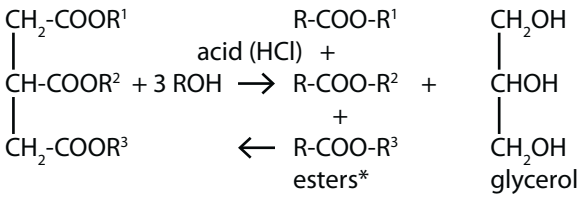
Note: Also refer to silylation, acylation and alkylation sections for additional readings

Selected Alkylation Applications

Derivatization of Corn Oil for Analysis by GC

K. Kiefer, K. Herwehe

Before the fatty acid composition of a lipid can be analyzed by gas chromatography, the lipid must be converted to low molecular weight, volatile, nonpolar derivatives (e.g., fatty acid methyl esters). This conversion usually is through a transesterification – the glycerol (alcohol) portion of the triglyceride (ester) is displaced by another alcohol, in the presence of an acid. The reaction is represented by the general equation:



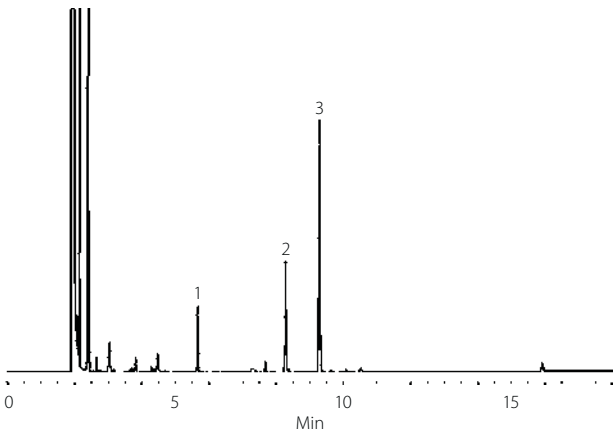
Transesterification is best done in the presence of a volatile, acidic catalysta which can be removed, along with excess alcohol,when the reaction is completed.

The chromatogram in Figure 1 is a representative derivatization of corn oil, using methanolic HCl, 3N, 250 µL 2,2-Dimethoxypropane (2,2-DMP), and 50 µL dimethylsulfoxide (DMSO). This study showed that using 2,2-DMP in preparing methyl esters of fatty acids increased the methyl ester yield. Addition of DMSO to the transesterification reaction mixture inhibits byproduct formation. DMSO, however, may interfere with the chromatography of early eluting fatty acid methyl esters

Figure 1. Fatty Acid Methyl Esters from Corn Oil Derivatized with 1 mL Methanolic HCl, 3N, 250 µL 2,2-DMP, 50 µL DMSO

column: SP-2380, 30 m x 0.25 mm I.D., 0.20 µm film (24110-U)
oven: 150 °C to 250 °C at 4 °C/min
carrier: helium, 20 cm/sec, set at 150 °C
det.: FID, 300 °C
inj.: 3 µL, split 100:1

1. C16:0
2. C18:1
3. C18:2



Flash Alkylation with TMAH for Derivatizing Barbiturates

K. Kiefer and K. Herwehe

This article originally published in The Reporter, Vol 16, No. 1, 1997*

Methylation with TMAH is a simple, rapid, quantitative procedure for derivatizing barbiturates.

Many drugs have a relatively high molecular weight and contain relatively polar functional groups, which are not well suited for gas chromatography (GC) analyses. Derivatizating drugs can result in a thermally stable, volatile compound that exhibits minimal tailing and improves chromatographic separation.

Methylation is the most widely used means of derivatizing drugs for GC sample preparation. Trimethylanilinium hydroxide (TMAH) commonly is employed as a reagent to form the 1,3-dimethyl derivatives of barbituratess, which do not form stable trimethylsilyl derivatives. The application of TMAH for the formation of derivatives provides a simple, rapid, and quantitative procedure for the analysis of barbiturates, and for sedatives, xanthine bases, phenolic alkaloids, dilantin, anticonvulsants, and fatty acids.

A particularly useful methylation technique is referred to as flash alkylation. Flash alkylation is an on-column reaction — the methylating agent, along with the drug, is injected into the GC, whose temperatures initiate and complete the derivatization. The temperature is critical in obtaining symmetrical peaks, reproducible retention times, and maintaining good separation efficiency. Some acids may require a higher temperature for maximum yield.

To determine the best injection port temperature for flash alkylation of 5-p-methylphenyl-5-phenylhydantoin using TMAH, we ran analyses at four injection port temperatures, between 80 °C and 250 °C. The sample consisted of a mixture of equal volumes of TMAH (0.2M) and 5-p-methylphenyl-5 phenylhydantoin (1000 µg/mL in methanol), prepared fresh before each analysis and immediately analyzed to prevent degradation of the barbiturate and its n-methyl derivative. The 80 °C injection port temperature provided insufficient heat, resulting in poor resolution and peak symmetry (Figure A). At higher temperatures (Figure 1, 200 °C and 250 °C), responses were poor, peaks tailed, and artifacts started to appear. At the injection port temperature of 150 °C, the derivative was sharp and response was best (Figure 1).

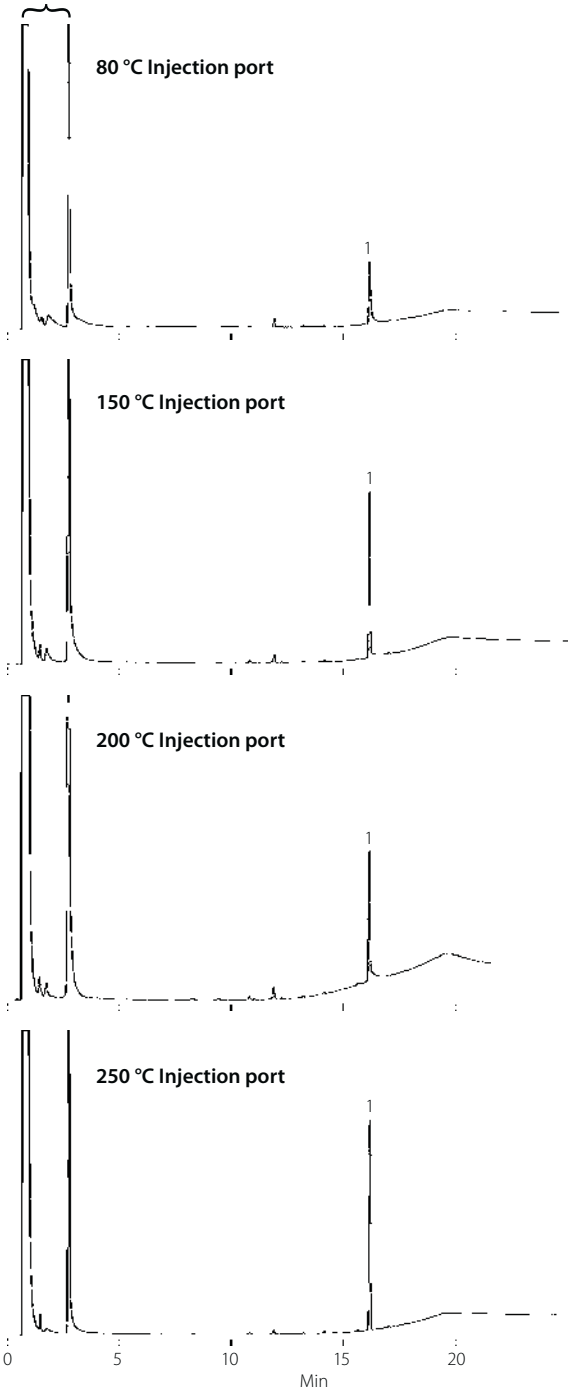
To prevent poor resolution and peak tailing, and to improve response, the injection port temperature should be slightly higher than the column temperature, sufficient to vaporize the analyte. However, higher injection port temperatures should be avoided because they can strip or decompose the phase at the inlet of the column, and they can be detrimental to heat-liable compounds. Indications that injection port temperature may be too high include breakdown of compounds, ghost peaks, poor symmetry, and baseline drift. Do not exceed the stationary phase temperature limit recommended by the column manufacturer. Injection port temperature is critical in achieving optimum results in flash alkylation/GC analyses. In this example an injection port temperature of 150 °C provided the best results for flash alkylation of 5-p-methylphenyl-5-phenylhydantoin.

* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Figure 1. Analysis of 5-p-Methylphenyl-5-phenylhydantoin Using Flash Alkylation

column: SUPELCOWAX™ 10, 30 m x 0.53 mm ID, 1.0 µm film
oven: 120°C (2 min) to 290°C at 10°C/min
carrier: helium, 10 mL/min
det.: FID, 300 °C
inj.: 1µL (equal proportions of TMAH and sample combined in reaction vessel and analyzed immediately)
Reagent: TMAH

Reagent 1. 5-p-Methylphenyl-5-phenylhydantoin derivative



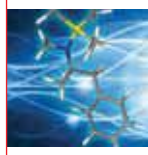
Derivatization Reagent Sampler Kits

Our derivatization reagent sampler kits enable you to determine the best reagent for a specific application, without the cost of purchasing, storing, and ultimately disposing of large volumes of individual reagents. Because of our purity specifications and reaction efficiency checks, we can guarantee consistently high reactivity from every lot of each reagent. Documentation detailing the chemistry, a tested derivatization procedure, and handling and storing recommendations is available for most reagents. Each of our four kits incorporates a group of related reagents.

Description	Concentration		Cat. No.	Qty.
Acylation Sampler Kit			505862	1
	Acetic anhydride, 3 x 2 mL	Pentafluoropropionic anhydride, 3 x 1 mL		
	Heptafluorobutyric anhydride, 3 x 1 mL	Trifluoroacetic anhydride, 3 x 1 mL		
FID Alkyation Sampler Kit			505854	1
	BF_3-Methanol, 3 x 1 mL	Methanolic HCl (3N), 3 x 1 mL		
	Methanolic Base, 3 x 1 mL	TMAH, 0.2 Min Methanol, 3 x 1 mL		
	Methanolic HCl (0.5N), 3 x 1 mL			
Silylation Sampler Kit			505846	1
	BSA, 3 x 1 mL	HMDS + TMCS, 3:1 (Sylon HT), 3 x 1 mL		
	BSTFA, 3 x 1 mL	TMSI, 3 x 1 mL		
	BSTFA + TMCS, 99:1 (Sylon BFT), 3 x 1 mL			

GC Derivatization Reagents by Industrial Applications

Functional Method	Derivatization Group	Reagent	Cat. No.	Reference
PHARMACEUTICALS, FORENSICS, AND DRUGS OF ABUSE				
Silylation	Alcohols	BSA	33035-U 33036 33037-U	13
		BSA + TMCS	33018 33019-U	
		BSTFA	15222	
		BSTFA + TMCS	33148 33149-U 33154-U 33155-U	
		HMDS	33350-U 33011	
	Amides	BSA	33035-U 33036 33037-U	11
		BSTFA	15222	
		MTBSTFA	394882	
	Amines	BSA	33035-U 33036 33037-U	
		BSA + TMCS	33018 33019-U	14
		BSTFA	15222	
		BSTFA + TMCS	33148 33149-U 33154-U 33155-U	15-17
	HMDS		33350-U 33011	
	Carboxylic Acid	BSA	33035-U 33036 33037-U	1,2
		BSA + TMCS	33018	3
		BSTFA	15222	4
		BSTFA + TMCS	33148 33149-U 33154-U 33155-U	5,6
		HMDS	33350-U 33011	7-10
	Steroids	BSA	33035-U 33036 33037-U	
		BSTFA	15222	
		BSTFA + TMCS	33148 33149-U 33154-U 33155-U	
		TMSI	33068-U	



GC Derivatization Reagents by Industrial Applications (Contd.)

Functional Method	Derivatization Group	Reagent	Cat. No.	Reference
PHARMACEUTICALS, FORENSICS, AND DRUGS OF ABUSE (Contd.)				
Acylation	Amines	Acetic Anhydride	33085	18
		HFBA		23, 24
		PFPA	33167 33168	22
		TFAA	33165-U 33164	19-21
	Amides	HFBA	33170-U	23, 24
		PFPA	33167 33168	
		TFAA	33165-U 33164	
Alkylation	Amines	DMF-DBA	395005	
		DMF-DEA	394971	
		DMF-DMA	394963	
		DMF-DPA	394998	
		PFBBr	33001	
		TMAH	33358-U 33097-U	
	Amides	DMF-DBA	395005	
		DMF-DEA	394971	
		DMF-DMA	394963	
		DMF-DPA	394998	
		TMAH	33358-U 33097-U	
	Steroids	BCl_3-Methanol	33353 33089-U 33033	
		DMF-DBA	395005	
		DMF-DEA	394971	
		DMF-DMA	394963	
		DMF-DPA	394998	
		TMAH	33358-U 33097-U	
ENVIRONMENTAL				
Silylation	Steroids	BCl_3-Methanol	33353 33089-U 33033	
		DMF-DBA	395005	
		DMF-DEA	394971	
		DMF-DMA	394963	
		DMF-DPA	394998	
		TMAH	33358-U 33097-U	
FOOD AND BEVERAGE				
Silylation	Carbohydrate	BSA	33035-U 33036 33037-U	1, 2
		BSA + TMCS	33018 33019-U	3
		BSTFA	15222	4
		BSTFA + TMCS	33148 33149-U 33154-U 33155-U	5, 6
BIOFUELS				
Silylation		MSTFA	394866 69479	26, 27

Analyte Derivatization in HPLC Analyses

Liquid chromatography can be used for a majority of known organic compounds. It is, therefore, considered as a very useful technique for food/beverage, pharmaceutical, environmental, and DNA analyses. Derivatization is often required to alter retention characteristics, increase response to various detection techniques and/or provide selective response for analytes in complex matrices. The derivatization reaction can be performed either pre- or post-column.

HPLC Pre-column Derivatization

The technique of derivatizing analytes prior to introduction into a HPLC system is termed pre-column derivatization. This technique is often used to promote improved chromatographic response of the analyte(s) under investigation. Important aspects of this technique include completeness of reaction, stability of the resultant derivatives and ease of preparation.

According to Blau (1), the selection of HPLC system is governed, to some extent, by the pre-column derivatization though it is not limited to compatibility with the mobile phase. The reagent can be color or UV-adsorbing material provided it is removed or separated by the system.

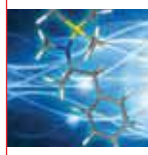
HPLC Post-column Derivatization

Post-column derivatization is accomplished by introducing a derivatizing reagent between the column and the detector. The post-column technique is typically utilized for compounds with low or no response to the desired detection scheme or when a particular analyte or set of analytes can be made to selectively respond through chemical alteration. Post-column derivatization often improves sensitivity and selectivity in HPLC analyses. In the case of post-column derivatization, it is desirable that the mobile phase must be compatible with the reaction mixture and not result in any precipitation or interference (1).

To perform post-column derivatization, the HPLC system must be modified with the addition of a secondary fluid delivery system. Typically this consists of a pump, tubing and fittings and a reaction coil. Derivatization reagent(s) is added after the column. The post-column reaction system mixes the stream of eluant from the HPLC column with a stream of reagent solution. The mixture flows through a reactor to allow enough time for the chemical reaction to complete. Optionally, the reactor can be heated, if needed, to accelerate the derivatization reaction. The well mixed stream is then passed through the detector.

Requirements for post-column derivatization in liquid chromatographic systems:

- Reagent solution must be miscible with column effluent
- Reagent must be stable
- Mixing of reagent with effluent must be rapid
- Mixing must be efficient to prevent generation of noise
- Reagent and effluent flow rates must be matched
- The background signal must be minimal
- Reaction must be fast enough to minimize loss of resolution
- The design of the reactor should minimize dispersion
- Reaction must be complete
- Reproducibility for maximum sensitivity



Detection Methods

Reagents for Colored and UV Absorbing Derivatives

UV detection is the most commonly used technique in HPLC but it sometimes lacks sensitivity or selectivity for trace analysis of compounds. Chemical derivatization modifies substances with a low UV absorption into highly sensitive products.

The chromatographic detectivity of the compounds which do not posses a chromophore or fluorophore can be improved by derivatization to produce colored and UV absorbing derivatives. Derivatization can also improve chromatographic retention of polar compounds and resolution of closely eluted compounds because the derivatives are typically more hydrophobic than the underivatized analyte.

Usually, highly conjugated aromatic compounds are used in UV-Visible derivatization. These compounds react with the analyte to produce derivatives that show high absorptivity and allow highly sensitive detection.

Reagents for Amines and Amino Acids

Amino groups are common to many biological compounds and many amines, such as, amphetamine, are used in pharmaceuticals. Most of these compounds show week absorption in direct HPLC analysis with UN-VIS detection. However, strongly absorbing derivatives of amines can be easily detected by HPLC with high sensitivity even when present at low concentrations.

Primary and secondary amino groups are usually derivatized as aromatic derivatives by nucleophilic substitution reactions. The common labeling reagents are acyl chlorides, arylsulphonyl chlorides, isothiocyanates, and nitrobenzenes. Tertiary amines are difficult to detect and their determination involves quaternization.

Acyle chlorides readily react with amino groups to form amides. In some cases the derivatization reaction can be free of by-products or the derivatives are water miscible and cn be easily separated. Arylsiphonyl chlorides react with primary and secondary amines. Nitrobenzeness are commonly used for derivatizing amino compounds. Isocyanates react aliphatic and aromatic rimary and secondary amines while isothyocynates is used for derivatizing primary amines (1).

Description	Cat. No.
Acyl chlorides	
m-Toluoyl chloride	122254
p-Nitrobenzoyl chloride	73120
Benzoyl chloride	12930
Arylsulphonyl chlorides	
p-Toluenesulphonyl chloride (TSCl)	240877
Benzenesulphonyl chloride (BSCl)	108138
Dimethylaminoazobenzenesulphonyl chloride (DABSCl)	39068
Nitrobenzenes	
1-Fluoro-2,4-dinitrobenzene (FDNB)	42085
Picrylsulfonic acid	92822
Isocyanates and Isothiocyanates	
Phenyl isocyanate (PIC)	78750
1-Naphthyl isocyanate (NIC)	170518
Phenyl isothiocyanates	P1034
Naphthyl isothiocyanates	N4525
4-N,N'-Dimethylaminoazobenzene-4'-isothiocanate (DABITC)	317802

Reagents for Carboxylic Acids

Carboxylic acids are a large group of naturally occurring compounds, and include fatty acids, prostaglandins, bile acids and other organic acids. Majority of such compounds do not show strong UV or visible absorption. Carboxylic acids are reacted with aromatic halides to produce UV-VIS absorbing ester derivatives. Phenacyl bromide (PBr), naphthacyl bromide (NBr) and their analogues are most often used as labels. Carboxylic acids can also form amides with aromatic amines after being first converted to the corresponding acyl chloride.

Fatty acids can be derivatized with phenacyl, napthacyl, p-bromo-phenacyl or p-nitrophenacyl bromide Methylphthalimides react quantitatively with fatty acids, dicarboxylic acids and barbiturates. O-p-Nitrobenzyl-N,N'-diisopropylisourea (p-NBDI) is another alkylation agent for derivatization (1).

Description	Cat. No.
Phenacyl bromide	77450
Methylphthalimide	407992
O-p-Nitrobenzyl-N,N'-diisopropylisourea (p-NBDI)	38434

Reagents for Hydroxy Compounds

Hydroxy compounds include alcohols, carbohydrates, steroids and phenols. For these compounds derivatization prior to HPLC analysis is necessary. Hydroxyl groups are converted to esters by reaction with acyl chlorides, and can be derivatized with methylsilyl chlorides or phenylisocyanate.

For derivatizing hydroxyl groups, estrification with benzoyl chloride and its analogues is widly used for introducing a strong UV-absorbing chromophore. Benzoyl chloride has been used for derivatization and HPLC analysis of benzoate derivatives of steroids. P-Methoxybenzoyl chloride has been used as a derivatizing agent for pharmaceutical products, such as, hexachlorophene and pentaaerythritol. P-Nitrobenzoyle chloride and 3,5-Dinitrobenzoyl chloride are ther derivatization reagents for benzoylation for hydroxyl functional groups. Phenyl isocyanate reacts with hydroxyl compounds similar to amines to produce alkylpheylurethane (1).

Description	Cat. No.
Acyl chlorides	
Benzoyl chloride	12930
p-Nitrobenzoyl chloride	73120
3,5-Dinitrobenzoyl chloride	42030
Phenyl isocyanate (PIC)	78750

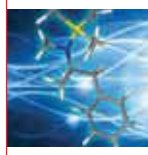
Reagents for Carbonyl Compounds

Aldehydes, ketones, ketosteroids and sugars contain the carbonyl group. Studies (1) have shown the use of 2,4-dinitrophenylhydrazine (2,4-DNPH) as a derivatization reagent to enhance detection of compounds containing carboyl functional group. 2,4-DNPH reacts with carbonyl group to form 2,4-dinitrophenylhydrazone. p-nitrobenzylhydroxylamine with keto functional groups in same way as 2,4-DNPH. 3-methyle-1-phenyl-2-pyrazoline (PMP) quantitatively reacts with reducing carbohydrates via their hydroxyl groups and produces derivatives with strong UV-absorbance.

Description	Cat. No.
2,4-Dinitrophenylhydrazine (2,4-DNPH)	42210
p-Nitrobenzylhydroxylamine	73200
3-methyl-1-Phenyl-2-pyrazoline-5-one (PMP)	M70800

HPLC Derivatization Reagents for UV/VIS Detection

Product Name	Description	CAS Number	Pkg Size	Cat. No.
N-Acetyl-D-penicillamine	>99.0% (T) , chiral reagent for precolumn derivatization of amino acids or amino alcohols; the diastereoisomers formed can be efficiently resolved by RP-HPLC	15537-71-0	1, 5 g	01423
Benzoin	98%, for quantification of endogenous guanidino compounds in uremic patients with pre- or post-column derivatization	119-53-9	100, 500 g	B8681
Benzoyl chloride	ACS, > 99.5% (T), derivatize di-and polyamines, affords stable reaction products	98-88-4	100, 500 mL, 1 L	12930
Benzylamine	derivatization reagent 99.0% (GC), reacts with 5-hydroxyindoles such as serotonin and 5-hydroxyindol-3-ylacetic acid	100-46-9	100, 500 mL	13180
(9-Carbazolyl)acetic acid	>99.0% (T), for pre-column derivatization of amino acids	524-80-1	500 mg	17925
(+)-Camphor-10-sulfonic acid	>99% (T), used for the resolution of bases; as a catalyst for coupling dipeptides	3144-16-9	5, 100, 500 g	C2107
(1S)-(+)-10-Camphorsulfonyl chloride	97%, determination of enantiomeric purity of amines by derivatization; of alcohols and amines by derivatization and H-NMR; Reagent used for the simultaneous resolution and activation of alcohols	21286-54-4	5, 25, 100 g	219576
(-)-Camphor-10-sulfonyl chloride	puriss.; >99.0% (AT), determination of enantiomeric purity of amines	39262-22-1	5, 25 g	21382
9-Fluorenylmethyl chloroformate	>99.0% (HPLC), used as a derivatization reagent to determine biogenic amines, derivatization of aminoglycosides, such as gentamycine and neomycine	28920-43-6	1, 5 g	23186
(+)-(1S)-Menthyl chloroformate	Optical purity 97% (GLC)	7635-54-3	5, 25 mL	378712
(-)-(1R)-Menthyl chloroformate	99% (GLC); chiral derivatizing agent used for the resolution of alcohols and amines by GC, HPLC, or crystallization	14602-86-9	25, 100 g	245305
R(-)-1-Cyclohexylethylamine	98%, for derivatization of racemic amides	5913-13-3	5 g	336505
(+)-O,O′-Diacetyl-L-tartaric anhydride	>97.0% (NT), reagent for the chiral derivatization of amino alcohols, react with alkanoamines in aprotic medium containing trichloroacetic acid and produce tartric acid monoesters	6283-74-5	10, 50 g	358924
N,N′- Diisopropyl-O-(4-nitrobenzyl)isourea	≥90% (CHN), for derivatization of carboxylic acids by HPLC	2978-11-2	500 mg	38434
4-(Dimethylamino)azobenzene-4′-sulfonyl chloride	≥97.5% (AT), for the determination of N-terminal amino acids, also used in the determination of primary and secondary amines by HPLC	56512-49-3	250 mg, 1, 5 g	39068
4-(Dimethylamino)benzaldehyde	> 99.0% (HPLC), for determination of amino acids and peptides, amines, indoles, hydrazines and hydrogen peroxides and tryptophan in proteins and hydroxyproline; spray reagent for tryptophan	100-10-7	50, 250 g	39070
Dimethylaminopyridine on Polystyrene	~3.0 mmol/g resin, for improved derivatization of chiral and achiral aliphatic amines, amino alcohols and amino acids		5, 25 g	39410
1-Fluoro-2,4-dinitrobenzene	> 98.0% (GC), for pre-column derivatization of amino-glycosides	70-34-8	50, 250 g	42085
N(a)-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide	powder; for derivatization and resolution of amino acids; derivatized D- and L-amino acids can be resolved and quantitated by HPLC	95713-52-3	25, 100 mg	D7906
N,N′- Disuccinimidyl carbonate	for the HPLC determination of amino compounds, used to prepare a heterobifunctional, cleavable linker-SVEC-(succinimide vinylsufonylethyl carbonate)-for thiol modified-DNA; also useful reagent for the synthesis of carbamates	74124-79-1	1, 5, 25 g	225827



Product Name	Description	CAS Number	Pkg Size	Cat. No.
Acetic anhydride	> 99.0% (NT), for post-column derivatization of tertiary aliphatic amines; for the unspecific acetylation of functional groups (NH_2, OH and SH) in proteins; for the specific blocking of side chain amino groups	108-24-7	250 mL, 1, 2.5 L 500 mL, 1, 2.5 L, 6 x 1 L, 4 x 2.5 L	45830 33214
9-Fluorenylmethyl carbazate	>99.0% (HPLC), for the sensitive derivatization of carbohydrates	35661-51-9	250 mg	46917
(R)-(–)-O-Formylmandeloyl chloride	97%	29169-64-0	5 g	479284
Ninhydrin	used in the post-column derivatization of amino-glycosides (streptomycine and dihydrostreptomycine); detection and assay of peptides, amino acids, amines, and amino sugars yielding highly fluorescent ternary compounds with aldehydes and primary amines; reaction with sarcosine or proline gives azomethine ylides	485-47-2	10, 25, 100 g	151173
4-Nitrobenzoyl chloride	derivatization grade (for HPLC), >99.0% (GC), derivatization of hydroxy groups	122-04-3	25, 100, 500 g	73120
4-Nitrobenzyl bromide	99%	100-11-8	25, 100 g	N13054
4-Nitrophenylhydrazine	97%, for colorimetric det. of aldehydes and ketones	100-16-3	5, 25 g	642983
(-)-Noe's Reagent	for introducing the optically active MBF group; the MBF derivatives are used for resolution and for asymmetric inductions	108031-79-4	1 g	74153
(R)-5-Oxotetrahydrofuran-2-carboxylic acid	98%	53558-93-3	1, 5 g	310476
Phenyl isocyanate	puriss. p.a., >99.0% (GC), for the detection of alcohols, primary and secondary aromatic amines. UV detection at 255 nm	103-71-9	25, 100 mL	78750
Phenyl isothiocyanate	99% (HPLC), Sigma Grade, 8.36 M, derivatizing reagent for primary and secondary amines, used in sequencing peptides by Edman degradation and in amino acid analyses by HPLC	103-72-0	1, 10 mL, 10 x 1 mL	P1034
R(+)-1-Phenyl-1-propanol	99%	1565-74-8	1 mL	256331
S(-)-1-Phenyl-1-propanol	99%	613-87-6	1 mL	256323
1-Pyrenebutyric hydrazide	>97.0% (T), fluorescent labeling reagent for carbonyl compounds, e.g. cyclobutanones formed on food irradiation	55486-13-0	100, 500 mg	82669
(S)-5-Oxotetrahydrofuran-2-carboxylic acid	98%	21461-84-7	1, 5 g	301469
DiaZald	99%, Diazald, nitrosylating agent for transition metal complexes, amines, and carbanions, as well as a precursor to diazomethane	80-11-5	25, 100 g	D28000
4-Methoxybenzoyl chlorid	99%	100-07-2	5, 25, 100 g	A88476
m-Toluoyl chloride	99%	1711-06-4	5, 100, 500 g	122254
p-Toluenesulfonyl chloride	≥99.0% (AT)	98-59-9	100, 500 g	89730
p-Toluenesulfonyl chloride	ReagentPlus, ≥99%, derivatize di- and polyamines	98-59-9	5, 100, 500 g	240877
Picrylsulfonic acid solution	1 M in water, reacts with primary amines, and in particular with amino acids and peptides	2508-19-2	1, 5 mL	92822
9-Oxo-10(9H)-acridineacetic acid	≥99.0% (T), for pre-column derivatization of amino acids	38609-97-1	250 mg	17927
(-)-Camphor-10-sulfonic acid	98%), used for the resolution of bases	35963-20-3	25, 100 g	282146
(R)-(–)-N-(3,5-Dinitrobenzoyl)-α-phenylglycine	>99.0% (HPLC, sum of enantiomers), for chiral chromatography	74927-72-3	1, 5 g	250031
2,4-Dinitrophenylhydrazine, moist. with water (~50%)	moistened with water (~50%), ≥99.0% (HPLC), for the determination of α-keto groups after the transamination of α-amino groups in proteins with glyoxylic acid; reagent for the colorimetric determination of aldehydes and ketones; and ketosteroids; reagent for UV-visible absorption derivatization of carbonyl compounds in liquid chromatography	119-26-6	25, 100, 500 g	42210
Benzenesulfonyl chloride	99%	98-09-9	5, 100, 500 g, 1 Kg	108138
1-Naphthyl isocyanate	98%	86-84-0	5 g	170518
1-Naphthyl isothiocyanate	95%	551-06-4	10 g	N4525

HPLC Derivatization Reagents for UV/VIS Detection (Contd.)

Product Name	Description	CAS Number	Pkg Size	Cat. No.
4-(4-Isothiocyanatophenylazo)-N,N-dimethylaniline	≥97% (NT), chromophoric reagent for high-sensitivity protein sequence analysis	7612-98-8	250 mg, 1 g	317802
2-Bromoacetophenone	puriss., ≥99.0% (GC)	70-11-1	10, 50 g	77450
N-Methylphthalimide	98%	550-44-7	5 g	407992
3,5-Dinitrobenzoyl chloride	for fluorescence, ≥98.0% (AT), reagent for the UV-visible absorption derivatization of alcohols in liquid chromatography	99-33-2	10, 50, 250 g	42030
O-(4-Nitrobenzyl)-hydroxylamine hydrochloride	≥98.5% (AT)	2086-26-2	1, 5 g	73200
3-Methyl-1-phenyl-2-pyrazoline-5-one	≥99% (NT)	89-25-8	5, 100, 500 g	M70800
6-Amino-1-phenalenone	≥97.0% (HPLC)	70402-14-1	100 mg	09117
Hydrindantin dihydrate	for Stein-Moore-Chromatography	5950-69-6	10, 50 g	53940
Hydroxylamine hydrochloride	≥99.0%	5470-11-1	50, 250 g	55459
(R)-(–)-1-[7-(Dimethylamino-sulfonyl) benzofurazan-4-yl] pyrrolidin-3-yl isothiocyanate	≥98.0% (sum of enantiomers, HPLC); for flourescence	163927-31-9	10 mg	60252
3-Methyl-1-phenyl-4-trifluoro-acetyl-2-pyrazolin-5-one	≥98.0% (T)	1691-93-6	1, 5 g	68752
4-Isothiocyanato-2,2,6,6-tetramethylpiperidine 1-oxyl	≥97%, for ESR-spectroscopy	36410-81-8	250 mg	76381
Dabsyl chloride		56512-49-3	500 mg	502219
4-(Dimethylamino) benzoyl chloride	≥99 % Material is reported to be very moisture sensitive. Handle under an inert atmosphere	4755-50-4	1 g	67954

Reagents for Fluorescent Derivatives

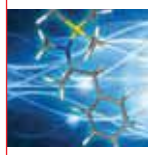
Fluorescence occurs when a molecule absorbs a photon, usually in the UV range, which triggers the emission of another photon with longer wavelength. Many compounds have natural fluorescence, but it can be induced into a naturally non-fluorescing molecule by creating a derivative that possesses a fluorescing ligand. Fluorescence of an analyte, whether natural or induced by derivatization, can be leveraged to increase the sensitivity (detect lower levels) of the analysis. High fluorescence efficiency of the derivatives is necessary. In addition, the uniqueness of fluorescent character can allow for the selective identification of a molecule in a complex mixture. Fluorescence is quantifiable at lower concentrations and usually has a wider linear range of response vs. concentration compared to optical (UV-VIS) absorbance.

Reagents for forming fluorescent derivatives should have the following characteristics:

- React rapidly and quantitatively under mild conditions
- High specificity for a functional group
- Form relatively non-polar derivatives
- Derivatives show high fluorescence efficiency
- Excess reagent easily separable

Many fluorigenic reagents are available. Densyl Chloride (Dns-Cl) has been used for preparing: fluorescent conjugates of proteins for studying their structure, fluorescent antibodies, and for the study of active centers of enzymes. Halogennitrobenzofuranes, such as NBD-Cl is a known fluorescent label. In aqueous solution it reacts with primary and secondary amino acids, it react less readily with phenolic OH and thiol groups under alkaline conditions (1). Isocyanates and iso thiocyanates are useful derivatizing reagents for primary amines and also in analysis of amino acids. With primary amines they react to produce urea and thiourea derivatives. Fluorescamine is a specific reagent for derivatizing compounds with primary amino groups as primary amines form fluorescent derivatives upon reaction.

Description	Cat. No.
Sulphonyl Chlorides	
5-Dimethylaminonaphthalene-1-sulphonyl chloride (Dns-Cl)	39220
Halogenonitrobenzofurazans	
4-Chloro-7-nitrobenzofurazan (NBD-Cl)	25455
4-Fluoro-7-nitrobenzofurazan (NBD-F)	47140
Isocyanates and Isothiocyanates	
Fluorescein isothiocyanate	46950
Fluorescamine	
4-Phenylspiro-[furon-2(3H),1-phthalan]-3, 3′-dione	F9015
2-Methoxy-2,4-diphenyl-3(2H)-furanone (MDPF)	64958



Pyridoxal and pyridoxal 5-phosphate are the naturally occurring compounds that have been used as fluorescent label. These compounds form Schiff bases with primary amino groups. The reduction of the C=N bond with sodium borohydride leads to the formation of a fluorescent derivative. 2-Fluorencarboxaldehyde and 1-pyrenecarboxaldehyde form strongly fluorescent Schiff bases with primary amines. O-phthaldialdehyde (OPA) forms fluorescent derivatives for a number of compounds such as, glutathione, arginine, agmatine and 5-hydroxy- and 5-methoxy indol, and histidine containing peptides. w-Formyl-o-hydroxyacetophenone and benzopyrone Benzoin react with primary and secondary aliphatic and aromatic amines to form derivatives with improved detection.

Since aldehydes and ketones readily react with hydrazines, they can be used for the fluorescence labeling of carbonyl compounds. Dansylhydrazine is a suitable fluorescent reagent for carbonyl compounds (1). It forms derivatives with steroids and sugars as well. 2-Aminopyridine reacts with reducing sugars to form Schiff bases that can be reduced to fluorescent 2-aminopridyl derivatives. 1,2-Diphenylethylenediamine (DPE) convert catecholamines into highly fluorescent products. 2-Cynoacetamide reacts with reducing carbohydrates to form fluorescent products. This reaction can be used post-column for the determination of sugars and catecholamines, as well as precolumn derivatization (1).

Description	Cat. No.
Schiff Base-Forming and Related Reagents	
Pyridoxal	93759
Pyridoxal 5-phosphate	82870
2-Fluorenecarboxaldehyde	150142
1-Pyrenecarboxaldehyde	144037
o-Phthaldialdehyde/ alkylthiol (OPA/R-SH) reagents	79760
2-Acetylbenzaldehyde (OAB)	562912
w-Formyl-o-hydroxyacetophenone and benzopyrone Benzoin	B8681
Carbonyl Compounds	
Dansylhydrazine	30434
Semicarbazide	363634
2-Aminopyridine	A77997
1,2-Diphenylethylenediamine (DPE)	364002
Cyanoacetamide	108448

Reagents for Electrochemical Derivatives

Reagents for forming fluorescent derivatives should have the following characteristics:

- React rapidly and quantitatively under mild conditions
- High specificity for a functional group
- Form relatively non-polar derivatives
- Derivatives show high fluorescence efficiency
- Excess reagent easily separable

Many fluorigenic reagents are available. Densyl Chloride (Dns-Cl) has been used for preparing: fluorescent conjugates of proteins for studying their structure, fluorescent antibodies, and for the study of active centers of enzymes. Halogennitrobenzofuranes, such as NBD-Cl is a know fluorescent label. In aqueous solution it reacts with primary and secondary amino acids, it react less readily with phenolic OH and thiol groups under alkaline conditions (1). Isocyanates and iso thiocyanates are useful derivatizing reagents for primary amines and also in analysis of amino acids. With primary amines they react to produce urea and thiourea derivatives. Fluorescamine is a specific reagent for derivatizing compounds with primary amino groups as primary amines form fluorescent derivatives upon reaction.

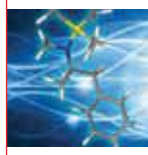
Description	Cat. No.
Primary and secondary amines	
o-Phthalaldehyde (OPA)	79760
1-Fluoro-ferrocene	F 408
2,4-Dinitrobenzene	D1529

References

1. Karl Blau and John Halket, Handbook of Derivatives for Chromatography, Willey & Sons, (1993, Second Edition).
2. Mrs. Laurence Coppex, Derivatives for HPLC Analysis, Faculty of Chemistry and Pharmacy University of Genf, Nov 1999 –Feb. 2000.

HPLC Derivatization Reagents for Fluorimetric Detection

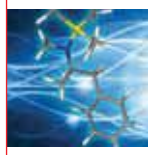
Product Name	Description	CAS Number	Pkg Size	Cat. No.
Benzylamine	> 99.0% (GC), for post-column derivatization of 5-Hydroxyindoles in urine, plasma and rat brain as well as tryptophane hydroxylase. Pre-column derivatization of indoles	100-46-9	100, 500 mL	13180
NIR-797 isothiocyanate	for fluorescence; ≥98.0% (HPCE), Near-infrared cyanine dyes for labelling of proteins	152111-91-6	25 mg	15167
3-Bromomethyl-7-methoxy-1,4-benzoxazin-2-one	for fluorescence; >98.0% (HPLC), sensitive fluorescent derivatizing agent for carboxylic acids; λ_{em} 440 nm, λ_{ex} 345 nm	124522-09-4	25 mg	17631
2,4′-Dibromoacetophenone	>98%, undergoes condensation reactions with aldehydes in the presence of $SnCl_2$ to afford α,β-unsaturated ketones; also useful in the esterification of carboxylic acids; modifies histidine residues in proteins"	99-73-0	10, 50, 100 g	D38308
Fmoc chloride	derivatization grade (for HPLC), ≥99.0% (HPLC), derivatization of aminoglycosides, such as gentamycine and neomycine, (λ_{ex} 265 nm ; λ_{em}315 nm); reagent for precolumn derivatization of amines for HPLC and fluorescent detection; also used for capillary electrophoresis"	28920-43-6	1, 5 g	23186
4-Chloro-7-nitrobenzofurazan	>99.0% (TLC), for fluorescence; react with both primary and secondary amines at 50-60 °C under alkaline conditions (pH 8-9), λ_{ex} 420 nm; λ_{em} 540 nm in ethanol (after derivatization with glycine)	10199-89-0	1, 5, 25 g	25455
Dansylhydrazine	for fluorescence; ≥95.0% (HPLC), marker for carbonyl compounds, λ_{ex} 340 nm; λ_{em} 520 nm in ethanol	33008-06-9	250 mg, 1, 5 g	30434
Dansyl chloride	for fluorescence; >99.0% (HPLC), for amino acid and peptide detection by RP-HPLC, λ_{ex} 337 nm; λ_{em} 4 81 nm in chloroform (after derivatization with hexylamine)	605-65-2	1, 5, 50 g	39220
3,5-Dinitrobenzoyl chloride	for fluorescence; >98.0% (AT), reagent for the UV-visible absorption derivatization of alcohols in liquid chromatography	99-33-2	10, 50, 250 g	42030
7-Fluorobenzofurazane-4-sulfonic acid Ammonium salt	≥98.5% (HPLC), pre-column derivatization of biological thiols, λ_{ex} 385 nm; λ_{em} 524 nm in 0.1 M borate pH 9.5 (after derivatization with glutathione)	84806-27-9	5, 25 mg	46640
Fluorescein isothiocyanate	for fluorescence; mixture of 2 components; ≥90% (HPLC), for derivatization of proteins, λ_{ex} 492 nm; λ_{em} 518 nm in 0.1 M phosphate pH 8.0	27072-45-3	50, 250 mg, 1 g	46950
4-Fluoro-7-nitrobenzofurazan	for fluorescence; ≥98.0% (HPLC), for pre-column derivatization of amines and amino acids, λ_{ex} 440 nm, λ_{em} 550 nm	29270-56-2	10, 50 mg	47140
Fluram®	for fluorescence; >99.0% (UV), derivatize sulfonamides and fumonisines; non-fluorescent reagent that reacts readily under mild conditions with primary amines in amino acids and peptides to form stable, highly fluorescent compounds. Post-column derivatization of ampicilline in biological fluids, λ_{ex} 395 nm, λ_{em} 485 nm	38183-12-9	25, 100 mg, 1 g	47614
2-Methoxy-2,4-diphenyl-3(2H)-furanone	for fluorescence, > 98.0% (HPLC), reagent for the derivatization of primary and secondary amines for HPLC; highly fluorescent derivatives are formed; Pre-column derivatization of amines, λ_{ex} 384 nm; λ_{em} 472 nm in acetonitrile (after derivatization with hexylamine) ; Fluorescent labeling of proteins before SDS-PAGE	50632-57-0	25, 100 mg	64958
4-Methoxybenzamidine, for fluorescence	for fluorescence; >97.0% (NT), for the determination of reducing carbohydrates and glycoproteins by post-column derivatization, λ_{ex} 393 nm; λ_{em} 478 nm (after derivatization with glucose)	22265-37-8	100 mg	64785
4-(6-Methyl-2-benzothiazolyl)-phenylisocyanate	for fluorescence; >98.0% (HPLC), for pre-column derivatization of primary, secondary and tertiary -hydroxy compounds and primary and secondary amines, λ_{ex} 327 nm; λ_{em} 382 nm in dichloromethane (after derivatization with hexylamine)	67229-93-0	100, 500 mg	65877
4,5-Methylenedioxy-1,2-phenylenediaminedi-hydrochloride	for fluorescence; >99.0% (HPLC), for detection of α-keto acids, λ_{ex} 367 nm; λ_{em} 445 nm in 0.1 M phosphate pH 7.0 (after derivatization with pyruvate)	81864-15-5	10, 50 mg	66807
Naphthalene-2,3-dicarboxaldehyde	for fluorescence, reacts in presence of cyanide ions with primary amines to produce a cyano[f]benzoisoindole (CBI) derivative which fluoresces in a similar way as the derivative produced with OPA, λ_{ex} 420 nm; λ_{em} ~480 nm in 0.1 M borate pH 9.3 (after derivatization with glycine)	7149-49-7	100, 500 mg	70215
1,2-Naphthoquinone-4-sulfonic acid sodium salt	97% (T), reacts with the guanidino moieties of certain amino-glycosides; detection of streptomycine in foods by using post-column derivatization, λ_{ex} 347 nm, λ_{em} 418 nm	521-24-4	10 g	226017



Product Name	Description	CAS Number	Pkg Size	Cat. No.
1-Naphthaleneacetic anhydride	96%, for the determination of airborne polyamines; N,N-Diisopropyl-naphthylacetamide (P/N 38432) serves as standard λ_{ex} 392 nm; λ_{em} 578 nm in 0.1 M phosphate pH 7.0	5415-58-7	1 g	438952
o-Phenylenediamine	solid, determination of urinary oxalate and pyruvate	95-54-5	50, 100 g	P9029
Phthaldialdehyde	for fluorescence; >99.0% (HPLC), pre-column derivatization of amino acids for HPLC separation; for flow cytometric measurements of protein thiol groups, λ_{ex} 340 nm; λ_{em} 445 nm in reaction buffer; glycine	643-79-8	1, 5, 50 g	79760
Rhodamine B isothiocyanate	mixture of isomers, red fluorescent marker of proteins, λ_{ex} 540 nm; λ_{em} 573 nm in ethanol	36877-69-7	100, 500 mg, 1 g	R1755
Tetramethylrhodamine B isothiocyanate	for fluorescence; mixture of isomers, for labelling antibodies, λ_{ex} 529 nm; λ_{em} 596 nm in DMSO	95197-95-8	10, 50 mg	87918
2-Methoxy-5-(N-phthalimidinyl) benzenesulfonyl chloride	purum, for fluorescence, >97.0% (CHN), reacts with both primary and secondary amines, λ_{ex} 330 nm; λ_{em} 427 nm in chloroform after derivatization with hexylamine	126565-42-2	50, 250 mg	91587
4-(N,N-Dimethylsulfamoyl)-7-piperazino-benzofurazan	≥ 99.0% (HPLC), for fluorescence, derivatization of natriuretic hormones like LLU-alpha, λ_{ex} 408 nm; λ_{em} 585 nm in 0.1 M phosphate pH 7.0	139332-64-2	50 mg	93087
4-(2-Aminoethylamino)-7-(N,N-dimethylsulfamoyl)-benzofurazan	for fluorescence, ≥ 99..0% (HPLC), for primary and secondary amines, λ_{ex} 406 nm; λ_{em} 581 nm in 0.1 M phosphate pH 7.0	189373-41-9	25 mg	93088
4-Fluoro-7-sulfamoylbenzofurazan	Reagent for fluorimetric assay of thiols	91366-65-3	10, 50 mg	F3639
4-(1-Methylhydrazino)-7-nitrobenzofurazan	≥97.0% (HPLC)		50 mg	93524
4-Bromomethyl-6,7-dimethoxycoumarin	97%, fluorescent labeling reagent for the reversed-phase HPLC separation and detection of carboxylic acids; labeling of S-1 ATPase	88404-25-5	1 g	301450
1-(2-Naphthoyl)imidazole	for fluorescence; ≥95.0% (N), derivatizing agent for alcohols and amines	141903-34-6	500 mg	70684
S(+)-1-[7-(Dimethylamino-sulfonyl)benzofurazan-4-yl] pyrrolidin-3-yl isothiocyanate	for fluorescence, ≥98.0% HPLC, sum of enantiomers), λ_{ex} 443 nm; λ_{em} 595 nm in 0.1 M phosphate pH 7.0	163927-32-0	10 mg	91609
Pyridoxal hydrochloride	≥99.5% (AT), for the labeling of amino acids and their detection in picomolar amounts	65-22-5	5, 25, 100 g	93759
Pyridoxal 5′-phosphate monohydrate	≥97.0% (NT)	41468-25-1	1, 5, 25 g	82870
Fluorene-2-carboxaldehyde	99%	30084-90-3	5 g	150142
1-Pyrenecarboxaldehyde	for fluorescence, ≥99.0% (HPLC)	3029-19-4	10, 50 g	144037
2-Acetylbenzaldehyde	95%	24257-93-0	1 g	562912
Benzoin	≥99.0% (GC)	119-53-9	5, 100, 500 g	B8681
Semicarbazide	6 wt. % (on silica gel)	57-56-7	25, 100 g	363634
2-Aminopyridine	99%	504-29-0	5, 100, 500 g	A77997
(1S,2S)-(–)-1,2-Diphenylethylenediamine	≥97.0% (HPLC)	29841-69-8	100, 500 mg	364002
Cyanoacetamide	≥98.0% (GC), for the spectrofluorimetric determination of catecholamines	107-91-5	5, 100, 500 g	108448
Ferrocene	≥98.0% (Fe)	102-54-5	5, 100, 500 g	F408
1-Fluoro-2,4-dinitrobenzene	≥99.0% (GC), puriss	70-34-8	100 mL	D1529
Ferrocenecarboxaldehyde	≥98.0% (HPLC); for HPLC derivatisation	12093-10-6	100 mg	95159
Ferroceneboronic acid	≥97.0% (HPLC); Derivatization reagent for the determination of brassinosteroids; Contains varying amounts of anhydride	121-52-94-2	100 mg	56257
N-Ferrocenyl-maleimide	≥97.0% (HPLC)	96482-68-0	100 mg	89111
3-Ferrocenylpropionic anhydride	≥98.0% (HPLC)	132098-76-1	100 mg	76737
Ferrocenoyl azide	≥98.0% (HPLC)	1273-85-4	100 mg	50203
2,6-Dimethyl-4-quinoline-carboxylic acid N-hydroxy-succinimide ester	≥98.0% (HPLC); Fluorescent probe DMQCOSu for analysis of aliphatic primary amines and diamines in water (tap water and river water), human urine and sera without solvent extraction. The detection limit in real samples is between 0.07–4.00 nmol/L.	569355-30-2	100 mg	49558
Dibenzyl chloromethyl phosphate	≥97.0%; Synthesis of water-soluble prodrugs of lipophilic alcohols, phenols and amines. This compound is an improved derivative of the well known Di-tert-butyl chloromethyl phosphate. So it can be detected more easily by HPLC-UV, because of higher stability, higher yields and better UV-activity.	258516-84-6	1, 5 g	86546

HPLC Derivatization Reagents for Fluorimetric Detection (Contd.)

Product Name	Description	CAS Number	Pkg. Size	Cat. No.
N-Methyl-N-(trimethyl-d_9-silyl trifluoroacetamide	≥94.0% (GC)	945623-67-6	500 µL	68768
Fluorescamine	≥98 %, Non-fluorescent reagent that reacts readily under mild conditions with primary amines in amino acids and peptides to form stable, highly fluorescent compounds.Low background due to hydrolysis. Useful for the fluorometric assay of amino acids, protein, and proteolytic enzymes. Effectively blocks newly generated amino termini in protein sequence analyses.	38183-12-9	100, 250 mg, 1g	F9015
4-Nitro-7-piperazinobenzofurazan	≥99.0%; suitable as derivatizing reagent for isocyanate; Derivatizing agent for the determination of mono- & diisocyanates by LC and MS, UV or fluorescent detection	139332-66-4	100 mg	92614
N-(7-Nitro-4-benzofurazanyl)-D-prolyl chloride	Fluorescent reagent for the resolution of alcohols and amines by HPLC	159717-69-8	50 mg	88823
2-[N-(7-Nitro-4-benzofurazanyl) methylamino]acethydrazide	≥97%; Derivatizing agent for fluorescence detection of carbonyl compounds with HPLC	221263-97-4	50 mg	89464
N-[7-(N,N-Dimethylsulfamoyl)-4-benzofurazanyl]methylamino-acetyl chloride	≥97%; Reagent for the fluorometric detection of alcohols, amines and thiols	156153-43-4	50 mg	96799
N-(7-Nitro-4-benzofurazanyl)-L-prolyl chloride	for fluorescence	159717-68-7	50 mg	84999



HPLC Derivatization – Application Overview

Applications	Special Applications	Reagent	Description	Reaction Type	Derv.(V), Post-column Derv.(N), Both(B)	Wavelength	Cat. No.
Primary amines, amino acids, secondary amines (via Hypochlorite)	Aminoglycosides, Gentamicin, Neomycin, biogenic Amines, amino acids	Phthaldialdehyde	for fluorescence, ≥ 99.0% (HPLC)	Isoindole-reaction with OPA, boric acid, mercaptoethanol	B	340 nm Ex. 440 nm Em.	79760
Primary Amine, amino acids	Sulfonamides, Amoxicillin, Ampicillin, Gentamicin	Fluram	for fluorescence, ≥ 99.0% (UV)	Condensation to fluorescent pyrrolidone	B	395 nm Ex. 495 nm Em.	47614
Primary and secondary Amines, biogenic, Amines, amino acids	Secondary amines, Erythromycin, Gentamicin, Neomycin, Pirlimycin	Fmoc chloride	for fluorescence, ≥ 99.0% (HPLC)	Nucleophilic substitu-ation to fluorescent chloroformiat-derivative	V	265 nm Ex. 315 nm Em.	23184
Primary and secondary amines, biogenicamines, amino acids, phenoles	Biogenic amines, Stilbene derivatives, Gentamicin	Dansyl chloride	for fluorescence, ≥ 99.0% (HPLC)	Nucleophilic substitu-ation to fluorescent dansyl-derivative	V	360 nm Ex. 420 nm Em.	39220
Primary aromatic amines, hydrazines	Sulfonamides	4-(Dimethyl-amino)benzal-dehyde solution (Ehrlich's reagent)	for the determination of hydroxyproline, ≥ 99.0% (HPLC)	Condensation with amines to schiff base	B	450 nm	39070
Primary aromatic amines	Sulfonamides, Clen-buterol, Mabuterol, Cimaterol, Brombu-terol, Chloramphen-icol (after reduction with zinc)	Bratton-Marshall (N-(1-naphthyl) ethylene-diamine-dihydrochloride	ACS reagent, >98%	Diazotation of aromatic amines	B	550 nm	222488
Polyether antibiotics in general	Monensin, Semduramicin, Narasin, Salinomycin	Vanillin (4-Hy-droxy-3-meth-oxybenzaldehyde)	ReagentPlus, 99%	Decomposition reaction to the dye	N	520 nm	V1104
α-Aminocarboxylic acids	Paromomycin	1-Fluoro-2,4-dinitrobenzene (Sanger Reagent)	≥ 99%	Nucleophilc aromatic substitution	V	350 nm	D1529
Amino acids	amino acids, (Streptomycin)	Ninhydrin	ACS reagent	Condensation of primary amines to Aza-Oxonol	N	440 nm	151173
Steroides, Makrolid-Antibiotics	Josamicin, Rokitamycin, Progesterone	Dansylhydrazine	for fluorescence, ≥ 95.0% (HPLC)	Forming of a dansylhydrazone with an aldehyde	V	350 nm Ex. 540 nm Em.	30434
Amprolium	Amprolium	Potassium hexa-cyanoferrate(III)	BioUltra, ≥ 99.0% (RT)	Redox-reaction to fluorescent complex	N	370 nm Ex. 470 nm Em.	60299
Penicillines	Amoxicilline, Ampicilline, Penicilline G, Cloxacilline, Penicilline V, Oxacilline, Dicloxacilline, Cephalexine, Cephradine	Mercury (II) chloride	puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., ≥ 99.5% (calc. to the dried substance)	Nucleophilic addition of imidazol	V	325 nm	31005
Penicillines	Amoxicilline, Ampicilline	Formaldehyde solution	ACS reagent, ≥ 36.5%	Fluorescent Pyrazine-derivative	V	350 nm Ex. 420 nm Em.	33220
Avermectine	Ivermectine, Moxidectine	Trifluoroacetic anhydride	derivatization grade (for GC), ≥ 99.0% (GC)	Aromatization by elimination	GC	365 nm Ex. 465 nm Em.	91719

Selected Applications

Derivatization and Fast HPLC Analysis of Aliphatic Amines

Hugh Cramer and Shyam Verma

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The aliphatic amines, generally, do not show ultraviolet absorption or emit fluorescent light that makes their detection difficult. Huang et al. (1) reported that these amines can be conveniently separated by HPLC following a pre-column derivatization that improves sensitivity and selectivity.

The derivatization reagent allows quantitative conversion of an analyte to a single detectable derivative, with minimal side reactions under mild conditions. Huang et al(1) reported derivatization of aliphatic amines with 2,6-dimethyl-4-quinolinecarboxylic acid N-hydroxysuccinimide ester (DMQCOSu). This reagent readily reacts with primary and diamines exhibiting several advantages, such as, good selectivity in aqueous solution, fewer byproducts, mild reaction conditions and excellent derivative stability.

In this work, a mixed aqueous amine solution was used that contained a mixture of 8 different aliphatic amines (methyl-,ethyl-, propyl-, ethylenedi-, butyl-, amyl-, hexyl- and heptylamines). The solution was derivatized following the method optimized for maximum derivatization yield. A 20 µL sample of amine solution was mixed with 20 µL of derivatization reagent solution (2 mmole DMQC-OSu in acetonitrile) and 200 µL of 0.2 M boric acid titrate buffered to pH 7.5 with sodium hydroxide at 50 °C for 40 minutes. The mixture was then cooled to room temperature. A sample of 20 µL of this derivatized solution was then injected for HPLC analysis.

Ascentis Express column of 10 cm length and 2.7 µm particle size was used for separation. The derivatized sample was run by HPLC-FL and experiments were performed by varying gradient time for ACD LC simulator. Simulations were run to develop this test method and simulation conditions were confirmed. The chromatogram is shown below.

The run time on Ascentis Express column was achieved in 7 minutes. The Ascents Express column show excellent peak resolution, especially early in the chromatogram, and improved peak shapes. These results demonstrate the suitability of the derivatization reagent, 2,6-Dimethyl-4-quinolonecarboxylic acid N-hydroxy succinimide ester for complete derivatization of aliphatic amines for HPLC analysis. The Ascentis Express C18 column allowed efficient separation of derivatized primary and secondary aliphatic amines after pre-column derivatization.

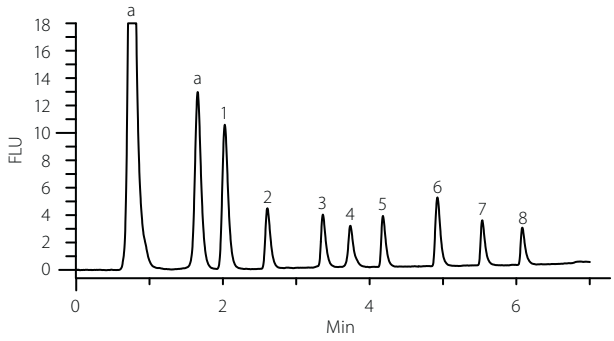
Figure 1. DMQC-OSu Derivatives of Amines using Ascentis Express C18 Column

column: Ascentis Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase A: water
mobile phase B: methanol
gradient:

min	%A	%B
0	50	50
5	10	90
7	10	90

flow rate: 1.0 mL/min
temp.: 35 °C
det.: fluorescence, ex=326 nm, em=409 nm
injection: 10 µL

a. Hydrolysate
1. DMQC-methylamine
2. DMQC-ethylamine
3. DMQC-propylamine
4. DMQC-ethylenediamine
5. DMQC-butylamine
6. DMQC-amylamine
7. DMQC-hexylamine
8. DMQC-heptylamine



Reference

1. Huang, K. et al., Chromatographia, 2009; 70.

* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Analysis of Glutathione on Ascentis RP-Amide with MS Detection

Hillel Brandes

This article originally published in Reporter, Vol. 23.2*

Glutathione is the tripeptide g-glutamylcysteinylglycine that serves as an important affecter of cellular redox status. Redox status is modulated by the relative ratio of the peptide in its reduced (GSH) and oxidized (GSSG) forms. As a key cellular antioxidant, researchers need to be able to assay the two forms in biological samples. Reversed-phase liquid chromatographic (RPLC) retention can be a challenge because the peptides are small and polar. A polar moiety in a bonded phase could provide advantages for retention and permit the use of a highly aqueous mobile. Ascentis RP-Amide provides such a solution by being a polar embedded phase that is aqueous compatible and well-suited for mass spectral detection.

Artifactual oxidation of reduced glutathione that can take place during sample handling poses a serious challenge in analyzing glutathione levels, both oxidized and reduced, from biological samples. Sample was prepared by adding to a mixture of GSH and GSSG a sub-molar concentration (relative to GSH) of iodoacetic acid (IAA) in order to derivatize some of the GSH. The sample was incubated overnight and quenched by dilution into a low-pH buffer. Figure below shows the resolution of all three components on Ascentis RP-Amide, made possible by the highly aqueous mobile phase.

Auto-oxidation of GSH in biological samples leads to an artifactual formation of GSSG. Therefore it is necessary to include a derivatizing reagent in an extraction buffer in order to trap all GSH, precluding oxidation to GSSG. In order to ascertain the efficacy of GSH derivatization by inclusion of IAA in a reaction mixture, alkylation of GSH was performed in the presence of an exogenous oxidant. This would mimic what would spontaneously occur in a biological extract. Therefore incubation of GSH with an oxidant [5-5′-Dithiobis-(2-nitrobenzoic acid)] was performed in the presence or absence of a molar excess (relative to GSH) of IAA. The oxidant was present at a 0.5% level relative to GSH (on a molar basis).

Figure below demonstrates that even in the presence of an oxidant, the alkylating agent IAA prevents formation of any GSSG. All GSH is trapped in the carboxymethylated form. In the absence of IAA, the included oxidant is effective in generating GSSG. These results demonstrate the viability of this strategy for processing of biological samples.

Conclusion

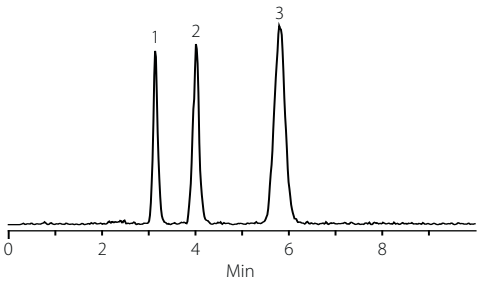
Alkylation of GSH by IAA is an effective strategy to prevent auto-oxidation, thus precluding artifactual generation of GSSG that would occur in processing biological samples. True levels of GSSG could then be obtained. Ascentis RP-Amide provides an effective solution for analysis of GSH and GSSG by mass spectral detection.

* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Resolution of All Glutathione Forms on Ascentis RP-Amide

column: Ascentis RP-Amide, 15 cm x 1.0 mm I.D., 5 µm particles (custom)
mobile phase: 98:2, 25 mM formic acid titrated with ammonium hydroxide (pH 3.0):methanol
flow rate: 45 µL/min
temp.: ambient
det.: ESI (+), XIC m/z = 308.4, 366.5, 613.0
injection: 1 µL
sample: 30 mg/L GSH, 12 mg/L GSSG in 50 mM formic acid, 0.1 mM ethylenediaminetetraacetic acid

1. GSH; $(M+H)^+$ = 308.4
2. Carboxymethylated GSH; $(M+H)^+$ = 366.5
3. GSSH; $(M+H)^+$ = 613.0

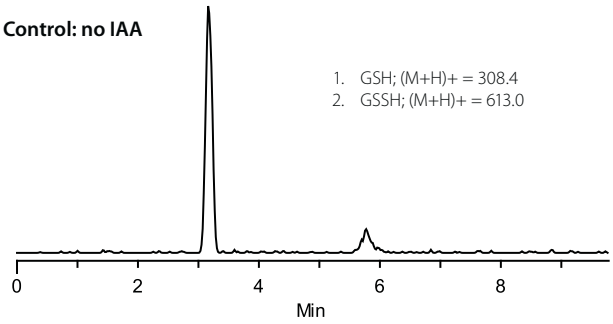


Efficacy of Trapping GSH in Alkylated Form

column: Ascentis RP-Amide, 15 cm x 1.0 mm I.D., 5 µm particles
mobile phase: 98:2, 25 mM formic acid titrated with ammonium hydroxide (pH 3.0):methanol
flow rate: 45 µL/min
temp.: ambient
det.: ESI (+), XIC m/z = 308.4, 366.5, 613.0
injection: 1 µL
sample: 20 mg/L GSH in 50 mM formic acid, 0.1 mM ethylenediaminetetraacetic acid

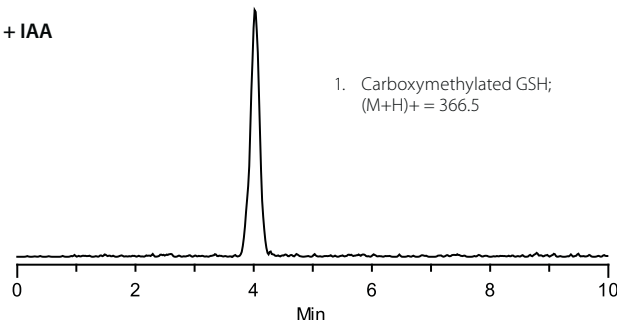
Control: no IAA

1. GSH; (M+H)+ = 308.4
2. GSSH; (M+H)+ = 613.0



+ IAA

1. Carboxymethylated GSH; (M+H)+ = 366.5



Derivatization Agents for LC/MS – An Improved Detection of Estradiol with ESI-MS

Rudolf Köhling

This article originally published in Analytix 4, 2011*

Steroid hormones are derivatives of cholesterol and play an important role in a large variety of organisms, as they can have a direct control on the gene expression. 17β-Estradiol (E2) controls the growth and the function of female secondary sexual characteristics. High blood concentrations inhibit the formation of further regulatory factors responsible for ovulation and pregnancy. E2 and its derivatives, e. g. ethinyl estradiol are part of combined contraceptive pharmaceuticals, which have become wide spread and common in use thus leading so far to an unconsidered environmental problem: increased concentrations of estradiol and its metabolites in waste water (1-2). Now both, clinical and environmental laboratories have a vital interest in finding the most sensitive method for analysis of E2 and other steroid hormones mostly in matrices, which are difficult to remove. E2 is a very unpolar compound and hardly to detect by ESI. Fortunately, the analyte can be extracted very efficiently with solvents like methylene chloride or acetone. This procedure additionally reduces negative effects of the matrix, e. g.signal suppression by alkali salts. But only the introduction of ionizable moieties by derivatization can enhance the detection limits significantly. Dansyl chloride is the most common agent and reacts selectively and quantitatively with E2, testosteron and their derivatives (3-4). The detection is limited to APCI and APPI sources, which have some disadvantages regarding availability, dopant usage and lower sensitivity of the APCI source (Figure 1). Only a short pre-column is necessary to separate the analyte from excessive reagent and byproducts (BPC, magenta). The MS/MS spectra result a large number fragments and a lower sensitivity on the quantifier.

A more sensitive and versatile derivatization agent for ESI sources is 4-(Dimethylamino) benzoyl chloride (67954-1G, DMABC). The reagent can be dissolved in acetone and applied on the dried residue of the sample extract. An adjustment of the pH is not necessary, only an anhydrous reaction medium is needed. The high purity of DMABC guarantees a good solubility, very selective and quantitative reaction at a moderate temperature between 55–60 °C (5 min). At a high E2 level of 5 ppm only 0.2 % (rel. area fraction) of DAMBC react with the 2nd hydroxyl moiety (2:1 adduct). At 5 ppb E2 concentration the 2:1 adduct is below the detection limit.

The reagent and possible byproducts can be separated from the analytes by a standard reversed-phase HPLC column and detected down to very low concentrations (Figure 3). The MS/MS spectrum (inset) shows only 4 major peaks, which is ideal for the quantification and identification using triple quadrupole mass spectrometer.

References

1. Jason W. Birkett, John Norman Lester (eds.), Endocrine disrupters in wastewater and sludge treatment processes, CRC Press, 2003.
2. M. Metzler (ed.), The handbook of environmental chemistry: Endocrine Disrupters, Vol. 3, Part 1, Springer Verlag, Heidelberg, 2001.
3. R. E. Nelson, S. K. Grebe, D. J. O'Kane, R. J. Singh, Clinical Chemistry 50: 373-384, 2004.
4. F. Zhang et al, Rapid Commun. Mass Spectrom. 2009; 23: 3637–3646.

* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Figure 1. Separation and detection of 55 pg E2 as dansyl derivative (EIC, peak 1). The inset shows the MS/MS spectrum of [M+H]+=506.235 Da (APCI+).

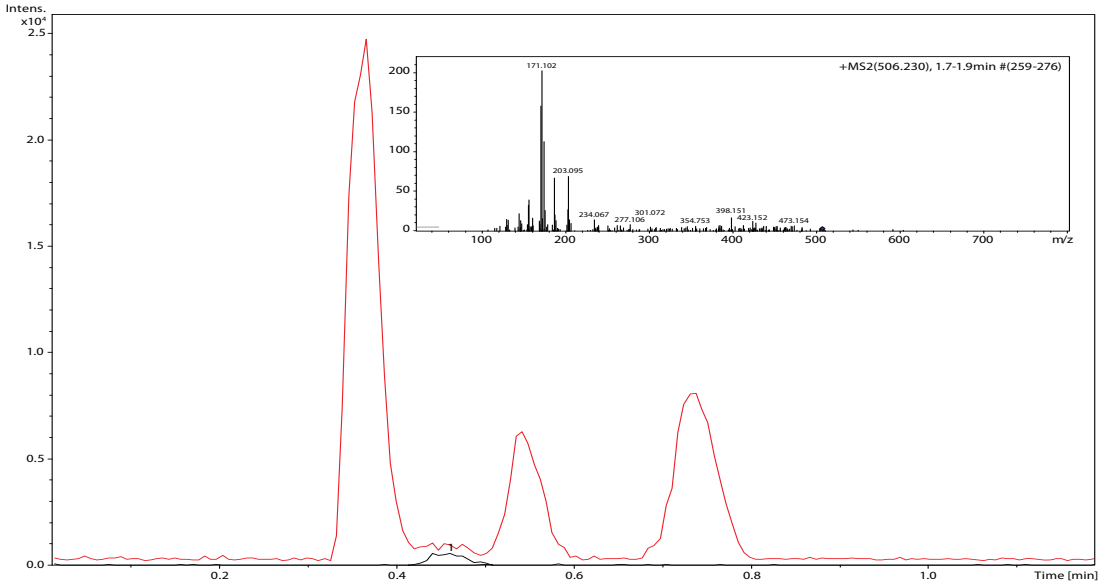


Figure 2. Derivatization reaction of E2 a nd DMABC.

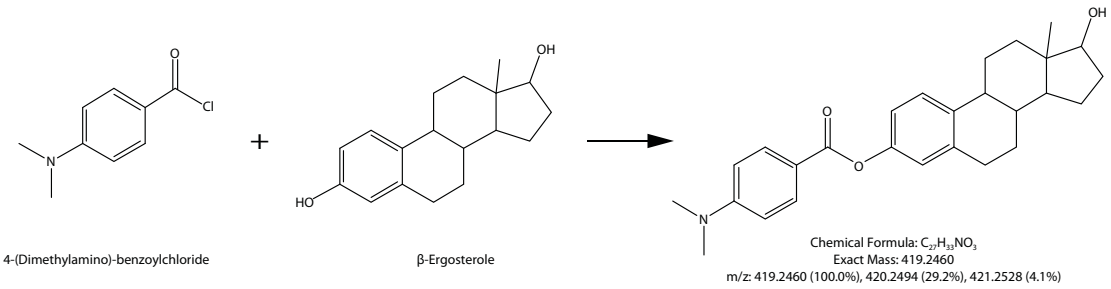
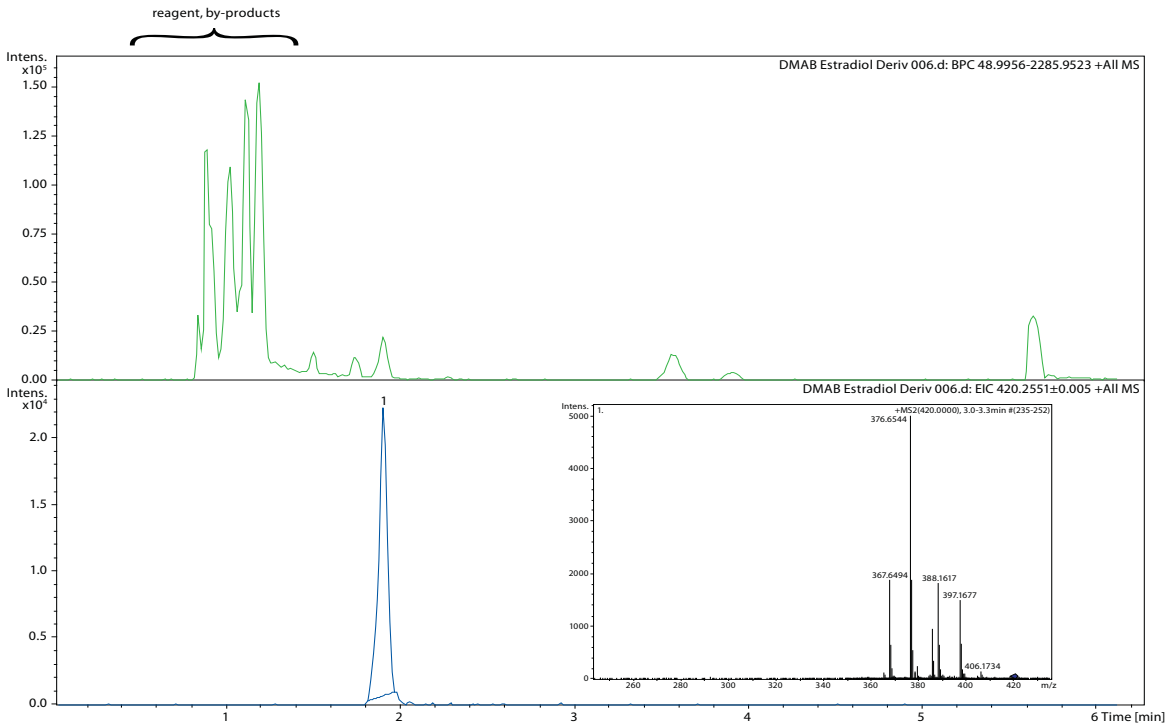


Figure 3. Injection of 5 pg DMAB-E2 derivative and separation on a UHPLC system

column: Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm (53822-U)
mobile phase: water/0.01% formic acid/acetonitrile (30/70)
flow rate: 0.4 mL/min.



Derivatization for Chiral GC/HPLC

When measuring a sample of chiral material in a chiral environment (chiral solvent or additive or chiral shift reagent) is not possible, it can be derivatized with a chiral, optically pure reagent to form a pair of diastereoisomers which in principal exhibit different spectrums (1,2). This indirect method of derivatization of enantiomers with an optically pure reagent allows separation of the resulting diastereomers on conventional achiral phases.

Derivatization reagents with high optical purity furnish the most accurate results. The reaction conditions should be sufficiently mild to prevent racemization or epimerization of the chiral components. Also, the derivatization by-products, if any, should not interfere with the analysis. Greater care is needed, especially when the analyte has more than one functional group that is capable of reacting with the derivatization reagent (e.g. amino alcohol). The chromatographic methods are commonly used as they have the advantage of high accuracy, selectivity and efficiency.

Chiral Derivatization Reagents - HPLC

The close relationship between biological (physiological) activities and the absolute configuration of chiral molecules has been well established and is one of the major reasons for their synthesis in enantiomerically pure form. Frequently, only one of the enantiomers in a racemic mixture displays the desired pharmacological activity, while its antipode is inactive, shows undesirable side effects or is even toxic. Inactive enantiomers are frequently referred to as *Xenobiotics*, which have to be metabolized as ballast by the organism. As a consequence, practically all newly developed chiral drugs are synthesized and tested as single enantiomers.

Scientists involved in the synthesis of bioactive natural products have to prepare single enantiomers, frequently, the only way in equivocally securing the absolute configuration of a newly discovered natural molecule. Numerous research groups are engaged in the syntheses of pure enantiomers using biocatalysts (enzymes, microorganisms), requiring the determination of enantiomeric purities.

Various chiral compounds are known for their illicit use in doping, as narcotics, psychotropic agents, and illegal use in foods and drugs.

Clearly, in all of the above areas, the determination of enantiomeric ratios (purities) both of final products and employed synthetic building blocks is of prime importance. Typical examples are:

i) monitoring of enantioselective syntheses

ii) quality control in the manufacturing of drugs

iii) testing the stability and metabolic fate of drugs in biological systems (e.g. blood serum)

iv) analysis of illicit drugs and narcotics in various body fluids (e.g. doping/body building/athletic sports).

In summary, the determination of enantiomeric purities is of ever increasing importance, for which facile, rapid, efficient and inexpensive methods are highly desirable.

Determination of Enantiomeric Purities – Methodologies

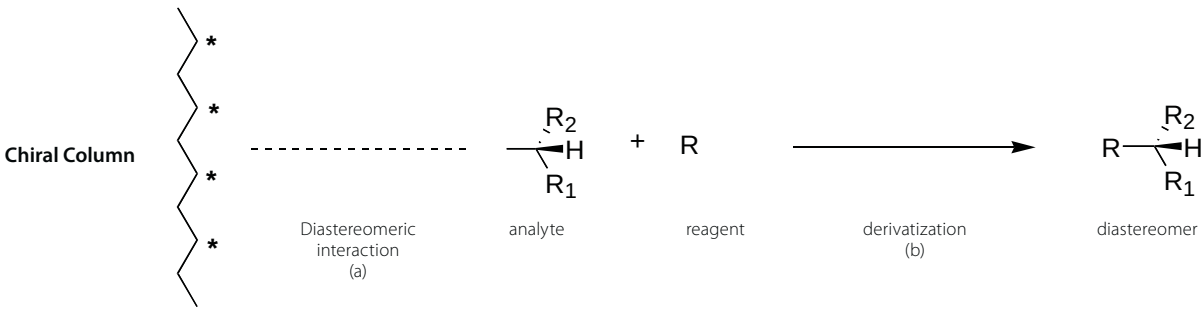
For the analysis of single enantiomers (and mixtures thereof, e.g. racemates) interactions with a defined chiral environment are imperative. In other words, we need and a priori regardless of the molecular structure of the molecule in question the establishment of a diastereomeric relationship or the chemical formation of diastereoisomers.

This can be achieved principally in two different ways, using a direct methodology employing chiral stationary phases (so-called chiral columns) or by chemical reaction with chiral, enantiomerically pure, derivatization reagents (Figure 1).

While both methods have demonstrated merits, the chemical preparation of diastereoisomers (derivatization) frequently has distinctive advantages, listed below, over the direct method:

- the separation of diastereoisomers is usually simpler to perform and provides better resolutions
- the choice of chromatographic conditions is much broader and these can be more easily optimized
- the employed reagents can be made to contain chromophores (fluorophores) for highly sensitive UV- and fluorescence detections.
- the separation of diastereoisomers usually requires only typical reverse-phase columns.

Figure 1. Determination of enantiomeric purities: a) direct using chiral columns and b) via derivatizations



Requirements of the Analytes

To undergo derivatization, the analyte molecules must carry suitable functionalities that react readily with the reagents to be employed. Thus, alcohols and hydroxy functional groups in general can be converted into diastereomeric esters by reaction with the reagent enantiomers of carboxylic acids, acid chlorides, and anhydrides. Amino groups are converted into amides and ureas (thioureas) by reaction with acid chlorides or isocyanates (isothiocyanates), respectively. In a similar way, carboxylic acids can be converted into the corresponding esters or amides, and so forth. The method is further illustrated in using types of reagents listed in this brochure (Figure 2).

Requirements of the Derivatization Reagents

The reagents to be employed for the derivatization of enantiomers into diastereoisomers should have the following properties:

- high enantiomeric purity
- react rapidly, quantitatively or at least reproducibly with the molecules to be analyzed
- carry chromophores for sensitive UV or Fluorescence detection
- lead to products which are well separated from the reagents
- readily decomposable if used in excess by simple nonchiral reagents
- readily and commercially available

Methodology

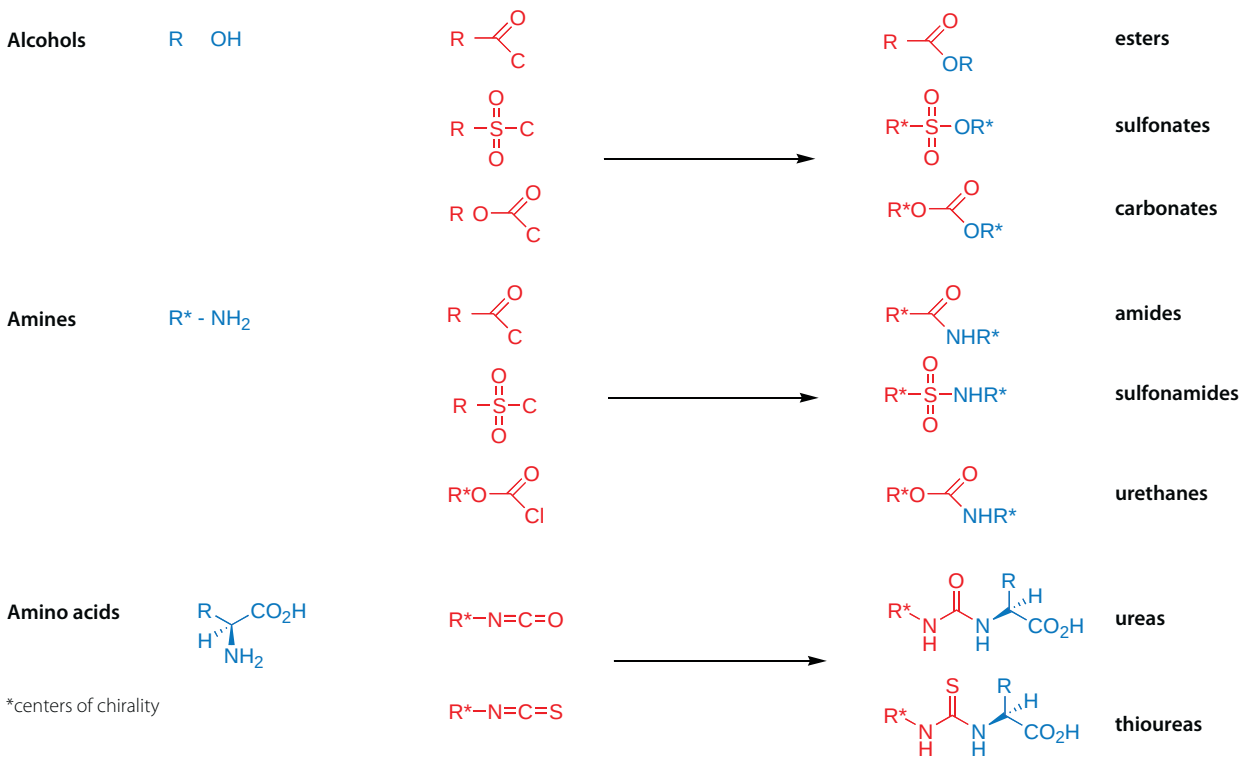
For the determination of enantiomeric purities via diastereomeric derivatives, analytes should have suitable functional groups for derivatizations. The derivatization reaction should be rapid and quantitative. The derivatizing reagents should be commercially available, and should not pose a toxic hazard.

The derivatives should be separated from excess reagent or other byproducts, and analyzed directly, that is, injected onto the HPLC columns without isolation or additional purification. The reagents should carry chromophores for sensitive UV or fluorescence detection that allows the detection of trace amounts of materials, especially, in body fluids. The methodology can usually be adapted for automation. In view of the availability of numerous, commercially available derivatization reagents of high enantiomeric purity (see this brochure) a wide variety of such separations can now be achieved economically with high efficiency.

References

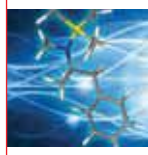
1. R.W. Souter, "Chromatographic Separations of Stereoisomers" CRC Press Boca Raton, FL 1985).
2. I. Wainer and Drayer (Eds.), "Drug Streochemistry: Analytical methods and pharmacology", Dekker, New York, NY (1987).

Figure 2. Formation of diastereoisomers using chiral derivatization reagents



Chiral Derivatization Reagents (ChiraSelect) - HPLC

Product Name	Description	CAS Number	Pkg Size	Cat. No.
BOC-L-cysteine	ChiraSelect, optical purity enantiomeric ratio: ≥99.5:0.5 (HPLC), chiral derivatizing agent used together with OPA for assaying the enantiomeric purity of amino acids	20887-95-0	250, 1000 mg	15411
(+)-Camphanic chloride	ChiraSelect; ≥97.0% (AT), chiral derivatizing reagent used for the determination of enantiomeric purity of alcohols and amines by HPLC	104530-16-7	250 mg	21286
(-)-Camphanic chloride	ChiraSelect; ≥98.0% (AT),chiral derivatizing reagent used for the determination of enantiomeric purity of alcohols and amines by HPLC	39637-74-6	5, 25 g	21287
(+)-1-(9-Fluorenyl)ethyl chloroformate solution	ChiraSelect; ≥18 mM in acetone, chiral derivatizing agent for primary and secondary amino acids and amines; the derivatives are separated by reversed-phase LC with fluorescence detection for determining the enantiomeric purity by reversed-phase LC with fluorescence detection for determining the enantiomeric purity	107474-79-3	10 x 1 mL 10 mL	23182
(–)-1-(9-Fluorenyl)ethyl chloroformate solution	ChiraSelect; 18 mM in acetone, chiral derivatizing agent for primary and secondary amino acids and amines; the derivatives are separated by reversed-phase LC with fluorescence detection for determining the enantiomeric purity by reversed-phase LC with fluorescence detection for determining the enantiomeric purity	154479-90-0	1 mL	338710
(R)-2,8-Dimethyl-5,11-methano-dibenzo(b,f)(1,5)diazocine	ChiraSelect; ≥99.0% (HPLC, sum of enantiomers), Chiral diamine with hindered inversion at the nitrogen atoms	21451-74-1	100 mg	40764
(S)-2,8-Dimethyl-5,11-methano-dibenzo(b,f)(1,5)diazocine	ChiraSelect; >99.0% (HPLC, sum of enantiomers)	14645-24-0	100 mg	40765
Nα-(2,4-Dinitro-5-fluorophenyl)-D-valinamide	ChiraSelect; ≥98.0% (HPLC, sum of enantiomers)	210529-62-7	500 mg	42100
Nα-(2,4-Dinitro-5-fluorophenyl)-L-valinamide	ChiraSelect; ≥98.0% (HPLC, sum of enantiomers), chiral derivatizing agent (chiral Sanger's Reagent) for determining the enantiomeric purity of amino acids with HPLC; this analogue of Marfey's reagent can also be used with amines	132679-61-9	100 mg	42102
R(-)-3,5-Dinitro-N-(1-phenylethyl)benzamide	98%	69632-32-2	1 g	296902
S(+)-3,5-Dinitro-N-(1-phenylethyl)benzamide	98%	69632-31-1	1 g	296910
N-Isobutyryl-D-cysteine	ChiraSelect; ≥97.0% (RT)	124529-07-3	250 mg	58689
N-Isobutyryl-L-cysteine	ChiraSelect; ≥97.0% (RT), chiral derivatizing agent employed in the assay of the enantiomeric purity of amino acids with OPA	124529-02-8	250, 1000 mg	58698
(-)-1-(9-Fluorenyl)ethyl chloroformate solution	18 mM in acetone	154479-90-0	1 mL	338710
(+)-1-(9-Fluorenyl)ethyl chloroformate solution	ChiraSelect; >18 mM in acetone; optical purity enantiomeric ratio:≥99.5:0.5 (HPLC)	107474-79-3	10 x 1 mL	23182
S(+)-α-Methoxyphenylacetic acid	ChiraSelect; ≥99.0% (T)	26164-26-1	250 mg	65208
R(-)-α-Methoxyphenylacetic acid	ChiraSelect, ≥99.0% (T), chiral reagent for the det. of enantiomeric purity and absolute configuration of sec-alcohols and α-chiral amines by NMR, analysis of amines using the ONSu ester	3966-32-3	1 g	65209
S(-)-1-(1-Naphthyl)ethylamine	≥99%	10420-89-0	1, 5 g	237450
S(+)-1-(1-Naphthyl)ethylisocyanate	99%, contains stabilizer	73671-79-1	1000 mg	295957
R(+)-1-(1-Naphthyl)ethylamine	ChiraSelect; ≥99.5% (sum of enantiomers, GC), reagent used in the determination of the enantiomeric purity of acids as amides by HPLC or NMR	3886-70-2	1 mL	70710
Nα-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide	ChiraSelect; ≥99.0% (sum of enantiomers, TLC), derivatization reagent for the assay of unusual chiral α-amino acid analogs	95713-52-3	50 mg	71478



Product Name	Description	CAS Number	Pkg Size	Cat. No.
S(+)-2-Octanol	99%	6169-06-8	5 g	147982
R(-)-2-Octanol	99%	5978-70-1	5, 10 g	147990
2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate	Chiral reagent for resolution of amino acid derivatives	14152-97-7	100, 1000 mg	T5783
2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate	99%	132413-50-4	100* mg	335622
2,3,4,6-Tetra-O-pivaloyl-β-D-galactopyranosyl isothiocyanate	ChiraSelect; ≥98%	147948-52-5	100 mg	44891
2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate	ChiraSelect; ≥98.0% (sum of enantiomers, HPLC), chiral derivatizing agent for amines, amino acids and amino alcohols; det. of enantiomeric purity by HPLC	62414-75-9	100 mg	90245
(R) -Trolox™ methyl ether	ChiraSelect; ≥99.0% (sum of enantiomers, HPLC)	139658-04-1	50 mg	93509
(S) -Trolox™ methyl ether	ChiraSelect; ≥98.0% (sum of enantiomers, HPLC), chiral derivatizing agent for alcohols; some methyl substituted primary alcohols which are difficult to separate otherwise, can also be separated	135806-59-6	50 mg	93510
N-(7-Nitro-4-benzofurazanyl)-L-prolyl chloride	Chiral fluorescent derivatizing reagent for the resolution of enantiomeric alcohols and amines by HPLC	159717-68-7	50 mg	84999
(–)-α-Methylbenzyl isothiocyanate	purum, ≥99.0% (sum of enantiomers, GC), very deep yellow; Derivatizing agent for HPLC; assay of enantiomeric purity of amines	24277-44-9	250 mg	89568
(R)-(–)-α-(Trifluoromethyl) benzyl alcohol	puriss., ≥99.0% (sum of enantiomers, GC; Chiral solvent for the determination of enantiomeric composition by NMR; Chiral auxiliary	10531-50-7	1 mL	79231
(S)-(+)-α-Methylbenzyl isothiocyanate	purum, ≥99.0% (sum of enantiomers, GC), very deep yellow	24277-43-8	1 g	75491
(S)-5-Allyl-2-oxabicyclo [3.3.0]oct-8-ene	purum p.a., chiral derivatization reagent for HPLC, ≥97.0% (GC)		1, 5 g	53835
2,3,4,6-Tetra-O-(2-naphthoyl)-β-D-galactopyranosyl isothiocyanate	~90% (HPLC), for derivatization		25, 100 mg	04466
2,3,4,6-Tetra-O-pivaloyl-β-D-glucopyranosyl isothiocyanate	ChiraSelect, ≥95.0% (HPLC)		100 mg	44891
L-(+)-2,3-Butanediol	purum, ≥97.0% (sum of enantiomers, GC)	19132-06-0	1, 5 mL	18967
Boc-Cys-OH	ChiraSelect, optical purity enantiomeric ratio: ≥99.5:0.5; Chiral derivatizing agent used together with OPA for assaying the enantiomeric purity of amino acids	20887-95-0	250 mg, 1 g	15411
Quaternary β-cyclodextrin			100 mg	33805
Sulphated β-cyclodextrin			100 mg	33806
(R)-(–)-2-Octanol		5978-70-1	5 g	147990
(S)-(+)-2-Octanol		6169-06-8	5 g	147982
(S)-(+)-α-(Trifluoromethyl) benzyl alcohol		340-06-7	1 g	411140
(S)-(+)-O-Acetylmandelic acid	The (R)- and (S)-isomers are chiral derivatizing agents for NMR determination of enantiomeric purity of α-deuterated carboxylic acids, alcohols, and amines	7322-88-5	5 g	253022
(S)-(+)-2-Phenylpropionic acid	97%	7782-24-3	250 mg, 1 g	279900
α-Cyano-4-hydroxycinnamic acid	99%	28166-41-8	2, 10 g	476870

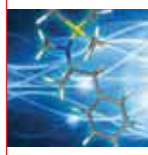
* Non-European Pack Size

Chiral Derivatization Reagents (ChiraSelect) - GC

Product Name	Description	CAS Number	Pkg Size	Cat. No.
(+)-Diisopropyl O,O'-bis (trimethylsilyl)-L-tartrate	ChiraSelect; 99.0% (GC, sum of enantiomers)	130678-42-1	1000 mg	420131
R(-)-α-Methoxy-α-trifluoro-methyl-phenylacetic acid Cl	ChiraSelect, ≥99.0% (AT), ready-to-use reagent for the determination of the enantiomeric purity of alcohols and amines after derivatization	39637-99-5	100, 500 mg	65363
S(+)-α-Methoxy-α-trifluoro-methylphenylacetic acid-Cl	ChiraSelect; ≥99.0% (AT)	20445-33-4	100, 500 mg	65365
S(-)-α-Methoxy-α-trifluoro-methylphenylacetic acid	ChiraSelect; ≥99.0% (T)	17257-71-5	250 mg	65369
R(-)-1-(1-Naphthyl)ethylisocyanate	ChiraSelect; ≥99.0% (GC, sum of enantiomers), chiral derivatizing agent for the indirect resolution of alcohols, thiols and amines by chromatographic separation of diastereomeric derivatives	42340-98-7	1 mL	70725
(R)-(+)-α-Methyl-2,3,4,5,6-(Pentafluorophenyl)ethanol	ChiraSelect; ≥99.0% (sum of enantiomers, GC), chiral derivatizing agent for GC; derivatives can be detected in high sensitivity by electron capture detection, GC-ECD, or negative ion MS, NI/CI-MS	104371-21-3	1 g	76744
(S)-(–)-α-Methyl-2,3,4,5,6-pentafluorobenzyl alcohol	ChiraSelect; ≥99.0% (sum of enantiomers, GC)	104371-20-2	1 g	76746
R(+)-1-Phenylethanol	ChiraSelect; ≥99.0% (sum of enantiomers, GC), chiral reagent used for the determination of enantiomeric purity and for resolutions of acids; asymmetric opening of cyclic anhydrides and of epoxides	1517-69-7	1, 5 mL	77848
S(-) -1-Phenylethanol	ChiraSelect;≥99.0% (sum of enantiomers, GC)	1445-91-6	1, 5 mL	77849
(S) -(–) -α-Methylbenzylamine	ChiraSelect; ≥99.0% (sum of enantiomers, GC)	2627-86-3	5, 25 mL	77869
(R) -(+) -α-Methylbenzylamine	ChiraSelect; ≥99.0% (sum of enantiomers, GC), chiral amine used for the determination of the enantiomeric purity of acids	3886-69-9	5, 25 mL	77879
(R) -(+) -α-Methylbenzyl isocyanate	ChiraSelect, ≥99.0% (sum of enantiomers, GC), reagent used for the determination of the enantiomeric purity of alcohols and amines	33375-06-3	1, 5 mL	77968
(S) -(–) -α-Methylbenzyl isocyanate	ChiraSelect; ≥99.0% (sum of enantiomers, GC)	14649-03-7	1, 5 mL	77970
R(+)-α-Methoxy-α-trifluoro-methylphenylacetic acid	ChiraSelect; ≥99.0% (T), reagent used for the determination of the enantiomeric purity and the absolute configuration of alcohols and amines by NMR	20445-31-2	250, 1000 mg	65361
(S)-(–)-2-Acetoxypropionyl chloride	puriss., ≥99.0% (AT); Chiral derivatizing agent for the det. of enantiomeric purity of alcohols by GC or NMR	36394-75-9	500 mg	00877
(S)-2-Hydroxybutyric acid	≥97.0% (T)	3347-90-8	1 g	54918
(R)-(–)-2-Octanol	99%	5978-70-0	5, 10 g	147990
(S)-(+)-2-Octanol	99%	6169-06-8	5 g	147982

Chiral Derivatization Reagents (ChiraSelect) - CE

Product Name	Description	CAS Number	Pkg Size	Cat. No.
(1R,4aS,10aR)-7-Isopropyl-1-isothiocyanato-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydro-phenanthrene	Chiral derivatizing reagent for the enantioseparation of racemic amino acids by CE	784213-51-0	100 mg	89394



Chiral Derivatization Reagents by Application

Compound class	Analytical Method	Detection Method	Reagents	Cat. No.
Amino acids	HPLC		BOC-L-cysteine	15411
	HPLC	Fluorescence	(+)-1-(9-Fluorenyl)ethyl chloroformate solution	23182
	HPLC	Fluorescence	(-)-1-(9-Fluorenyl)ethyl chloroformate solution	338710
	HPLC		Nα-(2,4-Dinitro-5-fluorophenyl)-L-valinamide	42102
	HPLC		N-Isobutyryl-L-cysteine	58698
	HPLC		Nα-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide	71478
	HPLC		2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate	T57833
	HPLC		2,3,4-Tri-O-acetyl-α-D- arabinopyranosyl isothiocyanate	90245
	CE		(1R,4aS,10aR)-7-Isopropyl-1-isothiocyanato-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	89394
Alcohols	HPLC		(+)-Camphanic chloride	21286
	HPLC		(-)-Camphanic chloride	21287
	HPLC		(S) -Trolox™ methyl ether	93510
	HPLC		N-(7-Nitro-4-benzofurazanyl)-L-prolyl chloride	84999
	GC		(+)-Camphanic chloride	21286
	GC		(-)-Camphanic chloride	21287
	GC		R(-)-α-Methoxy-α-trifluoro-methyl-phenylacetic acid Cl	65363
	GC		R(-)-1-(1-Naphthyl)ethylisocyanate	70725
	GC		(R) -(+) -α-Methylbenzyl isocyanate	77968
	GC		R(+)-α-Methoxy-α-trifluoro-methylphenylacetic acid	65361
Amines	GC		(+)-Camphanic chloride	21286
	GC		(-)-Camphanic chloride	21287
	HPLC	Fluorescence	(+)-1-(9-Fluorenyl)ethyl chloroformate solution	23182
	HPLC	Fluorescence	(-)-1-(9-Fluorenyl)ethyl chloroformate solution	338710
	HPLC		Nα-(2,4-Dinitro-5-fluorophenyl)-L-valinamide	42102
	NMR	NMR	R(-)-α-Methoxyphenylacetic acid	65209
	HPLC		2,3,4-Tri-O-acetyl-α-D- arabinopyranosyl isothiocyanate	90245
	HPLC		N-(7-Nitro-4-benzofurazanyl)-L-prolyl chloride	84999
	GC		(+)-Camphanic chloride	21286
	GC		(-)-Camphanic chloride	21287
	GC		R(-)-α-Methoxy-α-trifluoro-methyl-phenylacetic acid Cl	65363
	GC		R(-)-1-(1-Naphthyl)ethylisocyanate	70725
	GC		(R) -(+) -α-Methylbenzyl isocyanate	77968
	GC		R(+)-α-Methoxy-α-trifluoro-methylphenylacetic acid	65361
Diamines	HPLC		(R)-2,8-Dimethyl-5,11-methano-dibenzo(b,f)(1,5)diazocine	40764
Thiols	GC		R(-)-1-(1-Naphthyl)ethylisocyanate	70725
Acids	HPLC, NMR		R(+)-1-(1-Naphthyl)ethylamine	70710
	GC		R(+)-1-Phenylethanol	77848
	GC		(R) -(+) -α-Methylbenzylamine	77879
Anhydrides	GC		R(+)-1-Phenylethanol	77848

Selected Applications

A Novel Chiral Derivatizing Reagent for Enantiomeric CE Separation

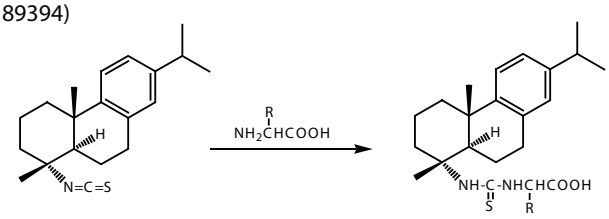
Yi-Ming Liu, PhD

This article originally published in Analytix Issues 1-3, 2010*

Various approaches have been developed to achieve chiral separations by CE. These include direct approaches where a chiral additive is added into the running buffer and indirect avenues that are based on diastereomer formation through pre-column derivatization using a chiral derivatizing reagent. Pre-column derivatization often improves detection sensitivity as well. To date, several chiral derivatizing reagents, including Marfey's reagent and (+) or (-)-1-(9- fluorenyl)ethyl chloroformate, are widely used for pre-column derivatization of amino compounds. An optimal chiral derivatizing reagent reacts readily with the targeted analytes and produces highly stable, detectable, and separable diastereomers in addition to a high optical purity of itself. In this work, degradingdehydroabietylisothiocyanate (DDHAIC) prepared from dehydroabietic acid is evaluated as a chiral derivatizing reagent for enantiomeric CE separation of amino compounds, using amino acids as the model analytes.

The reaction between DDHAIC and amino acids is illustrated in Figure 1. Isothiocyanate-type reagents require basic reaction media for the derivatization of amino acids. Best reaction yields are obtained with triethylamine (TEA) solution. Heating facilitates the derivatization reaction. The molar ratio of DDHAIC to analytes ranging from 0.1 to 50 is investigated. It is found that the derivatization yield becomes almost constant at a molar ratio greater than 10.

Figure 1. Derivatization of amino acids with DDHAIC (Fluka® 89394)



Procedure for amino acids derivatization with DDHAIC:

- Mix 10 µL amino acid solution (5 mM prepared in 0.1 M HCl) with 100 µL of 5% TEA (in acetonitrile);
- Add DDHAIC (50 µL of 30 mM in acetonitrile); the mixture is vortexed and then heated at 70 °C for 60 min.
- After cooling, the derivative solution is injected into the CE system for separation.

Under the selected reaction conditions, no racemization of any stereogenic carbon centers, neither those in DDHAIC nor that in amino acid, is observed during the derivatization. Figure 2 shows the three electropherograms after derivatizing three Ala solutions: enantiomeric L-Ala, racemic Ala (i.e. 50% D-Ala in a D-/L-Ala mixture), and a D-/L-Ala mixture containing 1%. No DDHAIC-D-Ala peak is seen (Figure 2A) from derivatizing enantiomeric L-Ala. Figure 2B indicates that 1% D-Ala present in a D-/L-Ala mixture can be unambiguously detected. It is also safe to say that there is no significant difference in derivatization kinetics towards the two enantiomers (D- and L-Ala) based on the peak areas corresponding to D-Ala and L-Ala as shown in Figure 2C.

The indirect chiral CE separation is performed by the mode of micellar electrokinetic chromatography (MEKC). The composition is as following: 50 mM Na_2HPO_4 (at pH 9.0), 18 mM contains sodium dodecyl sulfate (SDS), and 25% (v/v) acetonitrile.

Figure 2. Electropherograms obtained from (A) derivatization of L-Ala; (B) derivatization of a D- /L-Ala mixture containing 1% D-Ala; and (C) derivatization of racemic Ala. CE conditions (on a HP3D CE system): running buffer, 50 mM phosphate at pH 9.0 containing 18 mM SDS and25% acetonitrile; capillary, 50 mm id x 55 cm in length; applied voltage, 20 kV; capillary temperature, 20 °C; UV detection at 202 nm

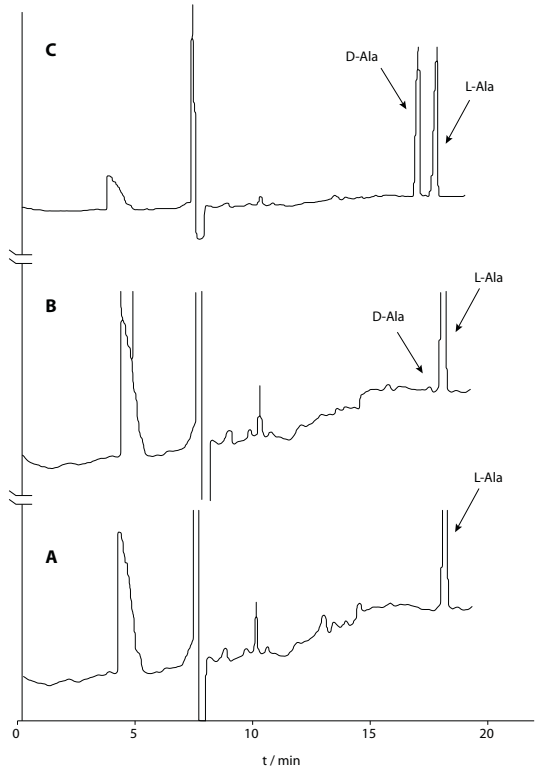


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Figure 3 shows an electropherogram obtained from separating a mixture of 12 DDHAIC derivatized D/L-amino acids. It is worth noting that UV absorption from the derivatizing reagent, DDHAIC, is small compared with the absorption from DDHAICamino acid derivatives.

CE conditions are as presented in Figure 2.

Figure 3. Electropherogram obtained from separating a mixture of 12 DHAIC-D/L-amino acids.

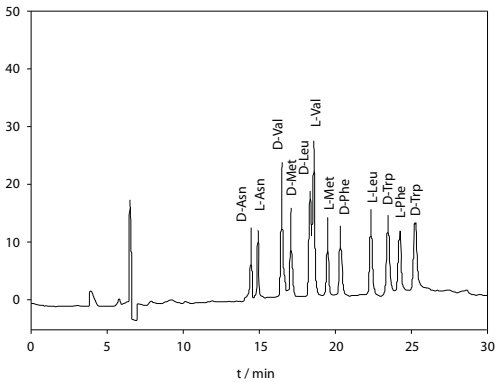


Table 1 lists chiral resolution values (Rs) for 10 pairs of DDHAIC-amino acid diastereomers. These results indicate that amino acid diastereomers derived from DDHAIC are very separable by MEKC. DDHAIC reacts readily with amino acid enantiomers at an elevated temperature, forming diastereomers stable for at least 72 hours at room temperature. No racemization occurs during derivatization. Diastereomers formed from 10 pairs of amino acid enantiomers tested in this work are all base-line resolved. These results indicate that DDHAIC is a useful addition to the family of chiral derivatizing reagents.

Table 1. MEKC migration times and resolution values of DDHAIC-amino acid diastereomers

	Migration Time (min)		
	D	L	RS
Asn	14.45	14.90	1.7
Val	16.47	18.55	4.8
Met	17.06	19.48	5.9
Leu	18.28	22.32	8.9
Phe	20.31	24.20	7.9
Trp	23.43	25.23	2.5
Ala	17.59	18.29	1.9
Thr	16.43	17.61	3.2
Ser	16.07	16.33	0.95
Vigabatrin	19.34 (R)	20.88 (S)	5.0

Description	Abbr.	Pkg. Size	Cat. No.
(1R,4aS,10aR)-7-Isopropyl-1-isothiocyanato-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	DDHAIC	100 mg	89394
Triethanolamine for amino acid analysis, ≥99.5% (GC)	TEA	5 mL, 25 mL, 100 mL	90337
Sodium dodecyl sulfate for ion pair chromatography, ≥99.0%	SDS	10 g, 50 g	71726

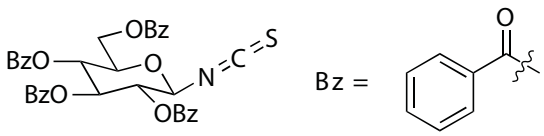
Simple, rapid and inexpensive determinations of enantiomeric purities: Oxiranes, Amino Acids and Pharmaceuticals

Ref: Manfred P.Schneider (FB C – Bergische Universitat Wuppertal, D-42097 Wuppertal, Germany), Analytix Issues 1,2 & 3, 2010*

An attractive alternative to high-cost chiral columns (chiral stationary phases) is use of a suitable derivatization reagent in combination with a relatively inexpensive reversed-phase (RP) column. This applications demonstrates the methodology that involves suitably substituted monosaccharide-based isothiocyanates for the determination of enantiomeric purities of the title compounds.

The derivatization is based on simple transformation of these reagents into the corresponding diastereomeric thiourea by reaction with primary and secondary amino groups. As derivatives of natural mono-saccharides, these reagents are optically pure, and the ratios of diastereoisomers thus produced directly reflect the enantiomeric composition of the amino compound in question.

Figure 1. Structure formula of monosaccharide-based isothiocyanate

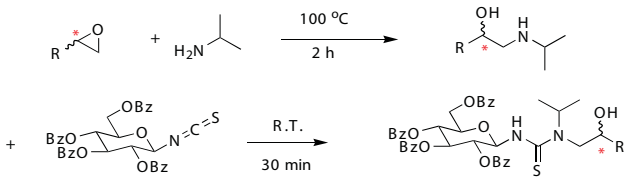


Oxiranes

This methodology allows separation using a two-step strategy that involves a) regioselective ring opening of the oxirane moiety using a simple sterically demanding amine leading to the corresponding s-amino alcohols, followed by b) derivatizations of the resulting s-amino alcohols with the above reagents.

Owing to their different retention times, it was possible to separate a mixture of 10 oxirane-derived s-amino alcohols (derived from 5 racemic oxiranes of different substitution patterns) in one single experiment.

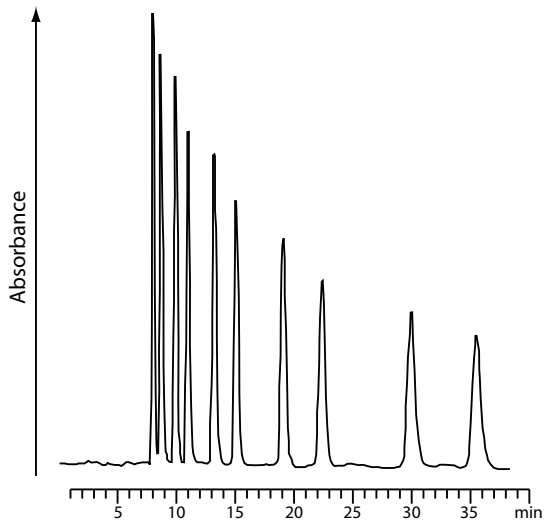
Figure 2. Conversion of alkyl oxiranes into diastereomeric thioureas using a two-step strategy [* denotes centre of chirality]



R = -$(CH_2)_nCH_3$; n= 0-9; -$C(CH_3)_3$; -$CH=CH_2$; -$(CH_2)_2$-$CH=CH_2$; -$(CH_2)_4$-$CH=CH_2$

* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Figure 3. Separation of ten oxirane–derived s-amino alcohols as diastereomeric thiourea derivatives in a single experiment. Mobile phase, methanol-water (90:10); flow rate, 0.5 mL/min-1; 0.7 nmol of each derivative are injected. Components were eluted in the following order: always (R) - before (S) for R = C2, C4, C6, C8, C10.



Amino Acids

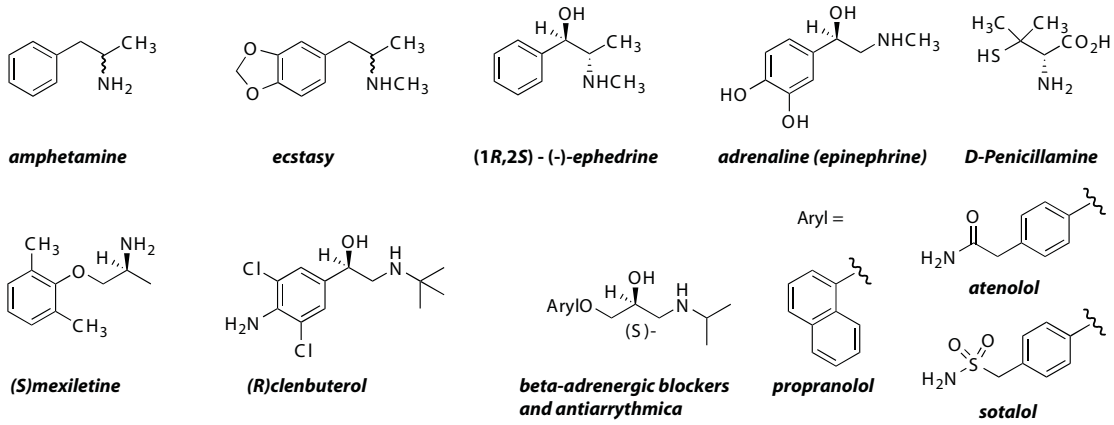
In view of the range of novel derivatization reagents which recently became available (BGITC, PGITC, PGalITC, NGalITC), the method is an interesting alternative and can also substitute for the frequently employed Marfay's reagent.

These reagents are highly suitable for the enantiomeric analysis of amino acids of widely varying structures such as proteinogenic-, nonproteinogenic (non-coded)-, and non-natural- (1,2) amino acids as well as α,α'-disubstituted and s-amino acids. To their advantage, native (underivatized) amino acids ("straight out of the bottle" or the reaction medium) react under mild conditions and at a rapid rate (at room temperature) with these reagents, leading to the corresponding diastereomeric thioureas that can then be injected directly into the HPLC without the need for further purification. In the majority of cases, baseline separations are achieved. By using a suitable derivatization reagent, baseline separations are observed in nearly all cases.

Ethanolamine reacts with any excess of BGITC and the resulting thiourea derivative is eluted well behind any of the amino acid derivatives. The mixture is then diluted to a final volume of 1 mL and a 10 µL aliquot is injected into the HPLC. (RP-18, mobile phase MeOH:H2O [67 mM phosphate buffer (pH 7) = 65:27:8 up to 70:25:5 and 80:15:5], depending on the case, flow rate of 0.5 mL/min).

The method is quite general and applicable to a) detection of trace amounts of amino acids in biological samples; b) check for racemisations, and c) monitoring asymmetric syntheses of amino acids.

Figure 4. Pharmacologically active molecules with functional amino groups



Pharmaceuticals

The carbohydrate-based isothiocyanates have also been shown to be highly suitable for the enantiomeric analysis of neurotransmitters (e.g. adrenaline and related molecules), numerous pharmaceuticals carrying functional amino

groups, such as s-adrenergic blockers, various pharmaceuticals such as penicillamine and mexiletine, and fine chemicals such as 1-phenyl-2-aminoethanol. Representative examples of these classes of molecules are shown in Figure 4.

Several of these compounds are known for their illicit use in doping, as narcotics or psychotropic agents, and for their illegal use in food and feed. It is well established that in so-called s-adrenergic blockers, the pharmacological activity resides in the (S)-enantiomers, while the (R)-enantiomer of penicillamine is highly toxic. On the other hand, the neurotransmitter activity of adrenaline resides largely in the (R)-enantiomer. Many more similar examples can be found in the literature. All of these compounds can be analyzed without any prior manipulation ("straight out of the bottle" or the reaction medium, e.g. biological fluids). They react under mild conditions and at a rapid rate (at room temperature) with the mono-saccharide isothiocyanates, leading to the corresponding diastereomeric thioureas. These, in turn, can be injected – without the need for further purification – directly into the HPLC.

As derivatives of natural mono-saccharides (Figure 1), all of the employed reagents are enantiomerically pure by definition, and the ratios of thus produced diastereomers directly reflect the enantiomeric composition of the chiral amino compound in question. This requires, of course, that both enantiomers of a racemic mixture react rapidly and quantitatively, and with the same rate in order to avoid a diastereoselectivity during the derivatization process. For new target molecules, this must be ascertained in every case by calibration with the corresponding racemate. The described strategy frequently has distinct advantages over the so-called direct method employing chiral stationary phases in that (a) the separation of diastereomers is usually more simple to perform and often provides better resolutions, (b) the choice of chromatographic conditions is much greater and thus can be more easily optimized, and (c) the reagents contain chromophores (fluorophores) for convenient UV- or fluorescence detection. In view of the range of novel derivatization reagents which recently became available (PGITC, PGalITC, NGalITC) [5], the method is an interesting alternative to so-called chiral columns. In principle, all of the above reagents can be employed for the above pharmaceuticals. Thus, Nimura et al. [1] achieved base-line separations in the analysis of adrenaline (epinephrine) and noradrenaline (norepinephrine) using GITC (Aldrich T5783) and AITC (Fluka 90245). Adrenaline is only present in minute quantities in lidocain local anesthetics; nevertheless this method allowed the quantitative determination of the enantiomeric ratio in more than 250 commercially available anesthetics [2]. Using the same reagents, a series of differently substituted amphetamines were analyzed [3].

While simple RP-18 columns are generally employed, the separation conditions can be varied widely in order to achieve the best separating conditions. Various different mobile phases have been used ranging from MeOH:phosphate buffer (pH 2.8) [1] over MeOH:H_2O:phosphate buffer (pH 7) to acetonitrile:water:0.1% trifluoroacetic acid in order to optimize the separation conditions. In certain cases the reagent may interfere with the separation, having the same or similar retention time. The addition of small amounts of thanolamine or hydrazine is sufficient to destroy excess reagent by formation of the corresponding thioureas, which elute at different retention times.

This method allows the rapid and inexpensive determination of enantiomeric purities in a wide variety of structurally varied alkyl oxiranes, amino acids, pharmaceuticals and fine chemicals. The method is clearly adaptable to automation using reaction batteries and auto-samplers and can be applied on a laboratory scale or in on-line quality control. It is highly suitable for monitoring asymmetric syntheses including enzyme-catalyzed transformations.

References

1. Nimura, N., Kasahara, Y., Kinoshita, T [1] ., J. Chromatogr.213 (1981) 327–330.
2. Allgire, J.F., Juenge, E.C., Adamo, C.P., Sullivan, G.M., Kirchhoefer, R.D., J. Chromatogr. 325 (1985) 249–254.
3. Miller, K.J., Gal, J.,Ames, M.M., J. Chromatogr. 307 (1984) 335 –342.

Enentiomeric purities of pharmaceutical using cabohydrate-based isothiocynates as derivatizing reagents.

Ref: Manfred P.Schneider (FB C – Bergische Universitat Wuppertal, D-42097 Wuppertal, Germany), Analytix, issue 3, 2010*

The carbohydrate-based isothiocyanates have also been shown to be highly suitable for the enantiomeric analysis of neurotransmitters (e.g. adrenaline and related molecules), numerous pharmaceuticals carrying functional amino groups, such as s-adrenergic blockers, various pharmaceuticals such as penicillamine and mexiletine, and fine chemicals such as 1-phenyl-2-aminoethanol. Representative examples of these classes of molecules are shown in Figure 1.

Several of these compounds are known for their illicit use in doping, as narcotics or psychotropic agents, and for their illegal use in food and feed. It is well established that in so-called s-adrenergic blockers, the pharmacological activity resides in the (S)-enantiomers, while the (R)-enantiomer of penicillamine is highly toxic. On the other hand, the neurotransmitter activity of adrenaline resides largely in the (R)-enantiomer. Many more similar examples can be found in the literature. All of these compounds can be analyzed without any prior manipulation ("straight out of the bottle" or the reaction medium, e.g. biological fluids). They react under mild conditions and at a rapid rate (at room temperature) with the mono-saccharide isothiocyanates, leading to the corresponding diastereomeric thioureas. These, in turn, can be injected – without the need for further purification – directly into the HPLC.

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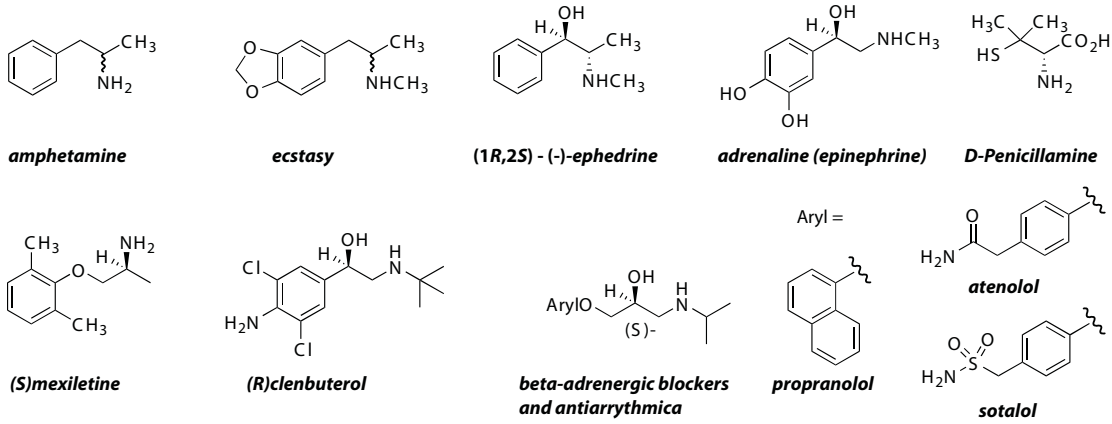
The method described above allows the rapid, efficient and inexpensive determination of enantiomeric purities in a wide variety of structurally varied pharmaceuticals and fine chemicals. By using a suitable derivatization reagent, base-line separations are observed in nearly all cases. The procedure is

Figure 1. Pharmacologically active molecules with functional amino groups



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quite general and applicable to (a) detecting enantiomeric ratios of pharmaceuticals, in addition to biological samples; (b) determining racemizations and differences in metabolic degradation; (c) monitoring asymmetric syntheses; and (d) detecting molecules in illicit drug abuse and doping. The method is clearly adaptable to automation using reaction batteries and auto-samplers. The technique is applicable both on a laboratory scale and in on-line quality control. It is thus highly suitable for monitoring asymmetric syntheses including enzyme-catalyzed transformations.

References

1. Nimura, N., Kasahara, Y., Kinoshita, T [1] ., J. Chromatogr.213 (1981) 327–330.
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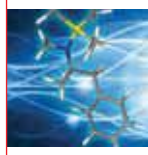


Proline Derivatization and Enantioresolution by Chiral GC

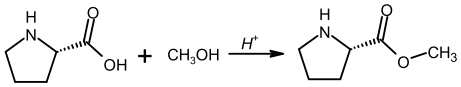
Katherine K. Stenerson and Jauh-Tzuoh Lee

This article originally published in Reporter 26.3*

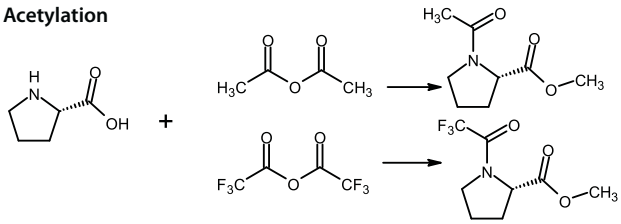
This application demonstrates achiral derivatization of an amino acid, proline followed by Chiral GC analysis. An amino acid molecule contains both amine and carboxyl functional groups. Before GC analysis, the carboxyl group of the analyte must be esterified, and subsequently the amino group needs to be blocked in order to obtain good peak shape and selectivity. The carboxyl group can be esterified using methanolic HCl (methylated), trimethyl chlorosilane (TMCS), or N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA). The reagents used for the amino group's derivatization typically are trifluoroacetic anhydride (TFAA), acetic anhydride and chloroacetic anhydride.

A sample mixture of D and L proline was methylated and then acetylated using a two step reaction.

Methylation



Acetylation



Chromatograms showing the GC analyses of the proline mixture are presented below. An Astec CHIRALDEX ™ G-TA , a unique chiral GC column made using a trifluoroacetyl derivatized cyclodextrin, was used. The achiral derivatization did not affect the chiral center of the molecule, as evidenced by the presence of two peaks in the chromatograms. However, the two different acetylation reagents resulted in an elution order reversal of the D and L enantiomers (enantioreversal). Trifluoroacetic anhydride also produced more volatile derivatives than the acetic anhydride, resulting in a shorter analysis.

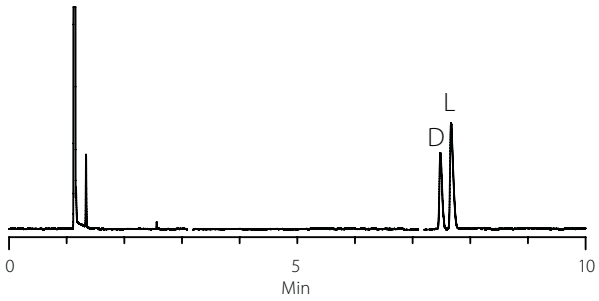
* Available at ***sigma-aldrich.com/analytical*** or contact your local Sigma-Aldrich Technical Service.

Conclusions

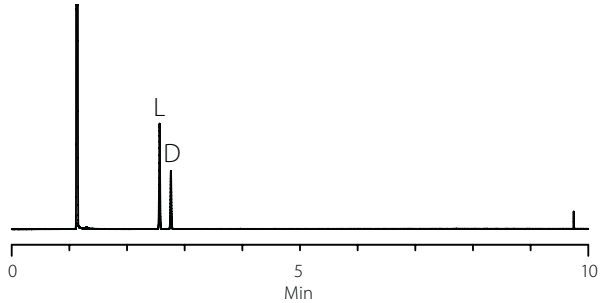
A two-step derivatization process (methylation followed by acetylation) can be used to selectively replace active hydrogens on the amino acid proline. This process will not cause racemization of the compound and, therefore, allows a successful separation of the enantiomers on the CHIRALDEX G-TA column. Using different acetylation reagents, such as acetic anhydride and trifluoroacetic anhydride, enantioreversal of the D and L enantiomers can be achieved.

Chiral GC Analysis of D and L Proline on the CHIRALDEX G-TA after Methylation with Methanolic HCl and Acetylation with Acetic Anhydride

column: CHIRALDEX G-TA, 30 m x 0.25 mm I.D. x 0.12 µm (73033AST)
oven: 160 °C
inj.: 200 °C
det.: FID, 200 °C
carrier gas: helium, 50 cm/sec
injection: 1 µL, 50:1 split



Chiral GC Analysis of D and L Proline on the CHIRALDEX G-TA after Methylation with Methanolic HCl and Acetylation with Trifluoroacetic Anhydride



Derivatization in TLC Analyses

Introduction

Thin Layer Chromatography (TLC) is a commonly used chromatographic method for qualitative determination of the purity of chemical substances and identification of components in a reaction mixture (1). It allows rapid and simple monitoring of the progress of an organic reaction and helps to qualitatively verify the purity of products. TLC provides qualitative information about a reaction between the reacting species and the number of products generated as a result of this reaction. This technique is advantageous because it is quick, uses inexpensive accessories, and requires only a small amount of the analyte. It is, therefore, an effective tool in rapid analysis of simple mixtures, large number of samples, and analysis of species with poor detection characteristic that require post-chromatographic treatment for detection (2). The repeatability and precision of this method make it a useful tool in daily laboratory work.

TLC method is widely used in laboratories, for example, for determining active components in forensic chemistry, clinical chemistry, biochemistry and in many pharmaceutical products. Often, TLC is preferred for large sample screening programs, such as screening of biowaste and drugs of abuse (3-5), therapeutic monitoring of drugs in biological fluids (6,7), characterization of plant extracts (8,9), detection of aflatoxins in agricultural products (3), and characterization of lipid extracts (6,10,11). TLC technique is also suitable for detection of pesticides or insecticides in food and drinking water and for estimating the purity of reaction mixture in synthetic chemistry. Besides these applications, TLC can also be succesfully utilized in inorganic chemistry for both detection and separation of cations and anions in the usual systematic qualitative analysis.

An organic solvent or a mixture of polar and non-polar solvents is used as the mobile phase. The mobile phase migrates up through the plate and provides a carrier for the components of the analyzed sample. The sample, a liquid or a solid dissolved in a volatile solvent, is spotted on the TLC plate. The solvent moves up the plate together with the sample via capillary action. The various components in the mixture move up the plate at various rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase (12, 13).

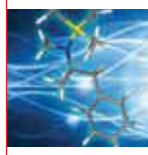
Visualization Methods

Visualization reaction enables detection of colorless compounds. A visualization reagent shows selectivity for a specific functional group or compound type. Many of these reactions are qualitative in nature. However, for quantitative scanning densitometry, these reactions are adopted as pre- or post-chromatographic treatment. The visualization reagent is applied by uniformly spraying it on the plate or by dipping the plate in a dilute reagent solution.

Since both pre- and post-chromatographic methods enhance selectivity and sensitivity of detection, their selection depends upon the involved chemistry, the matrix and reagent interferences and ease of optimization. Pre-chromatographic derivatization is usually preferred when the reaction is desired to enhance stability and chromatographic resolution (2, 14-16). Post-chromatographic methods are either selectivly based on the specificity of the reaction or may become reversible or destructive based on the interactions between the derivatizing reagent and the separated compound.

The most common reversible detection methods that are also qualitative in nature include exposure to iodine vapor, water, fluorescein, bromine or pH indicators visualizing reagents (17). Compounds containing unsaturated hydrocarbon or phenolic groups may irreversibly react with iodine, and mercaptans are oxidized to disulfides. Spraying a TLC plate with water allows detection of hydrophobic compounds as white spots on a translucent background when the water-moistened plate is held against the light. When a TLC plate is sprayed with a fluorescein solution, followed by exposure to bromine vapor while still damp, the plate becomes uniformly colored except at the sites where the components to be detected are located. Solutions of pH indicators, such as bromophenol blue and bromocresol green are widely used for the detection of acidic and basic compounds.

Destructive visualization reagents are used in quantitation techniques. Most common destructive reagents are solutions of sulfuric acid, and mixture of sulfuric acid-acetic acid and sulfuric acid-sodium dichromate. After spraying a TLC plate with these reagents, it is heated in an oven at 100-115 °C for some time. The reaction with the sprayed reagent converts the organic compounds to carbon which appear as black spots on a white-grey background. Accurate quantitative data can be obtained when degradative reactions are carried under controlled conditions. Examples are the determination of monosaccharides by treatment with Hansen's reagent (a mixture of diphenylamine and aniline in phosphoric acid), and the determination of lipids by reaction with phosphoric acid and copper sulfate (13,18,19). Reagents that are functional group specific or compound type selective can be used for determination of low levels of compounds in biological fluid and environmental extract matrices.



Visualization of the Plates

If the spots of the separated materials are visible under normal, ambient light they should be carefully outlined with a pencil. The following methods are applicable to visualize the colorless components on a TLC plate.

- Application of the TLC plates with a fluorescent indicator and observation of the results under ultraviolet illumination
- Color reactions by using appropriate spray reagents
- Visualization of the spots by placing the plate in iodine vapor

The spray reagents are mixtures of chemicals that are sprayed on the TLC plate and allowed to react. Upon reaction these reagents will cause colorless compounds to change to colored species for easy detection. Some of the visualization mixtures must be prepared at the time of using. Examples are listed below with product information.

Marquis Reagent (20)

A mixture of formaldehyde and concentrated sulphuric acid, 1:5 for the detection of alkaloids (e.g. amphetamines).

Description	Cat. No.
Formaldehyde, ACS reagent, 36.5% in H_2O	47629
Sulphuric acid, ACS reagent, 95.0-98.0%	84719

Simon's Reagent (20)

Two solutions for detection of amphetamines:

(1) 2% aqueous solution of sodium carbonate, and

(2) 6% sodium nitroferricyanide in 10% acetaldehyde.

Description	Cat. No.
Sodium carbonate, anhydrous, ≥99.0%	71351
Sodium nitroferricyanide, ACS reagent, ≥99.0%	71778
Acetaldehyde, anhydrous ≥99.5% (GC)	00070

Spraying Reagent for Iodine-Azide Reaction (20,21)

It is used for the detection of amino acids and biogenic amines. The method is based on the derivatization reaction with phenyl isothiocyanate. Derivatization solution consisting of a mixture of phenyl isothiocyanate (20 µL) and 2-propanol (200 µL) mixed with distilled water (20 µL) and phosphate buffer pH 12 is directly sprayed on the TLC plate prior to analysis.

The derivative containing sulphur(II) induces the following iodine-azide reaction.

$$I_2 + 2N_3^- \xrightarrow{\text{C=S inductor}} 2I^- + 3N_2$$

The chromatogram is sprayed with a freshly prepared mixture of sodium azide (2 g) in distilled water (20 mL) and starch solution (0.5%, 25 mL) and then exposed to iodine vapor. The visualization procedure provides white spots on violet-grey background. Phosphate buffer was obtained by dissolving 3.58 g of sodium phosphate in 80 mL of water and adjustment of the pH to 12 by addition of 1 mol/L of sodium hydroxide solution.

Description	Cat. No.
Sodium azide, purum p.a., ≥ 99.0%	71290
Starch solution, BioChemika, indicator, 1% in H_2O	85655
Hydrochloric acid, puriss. p.a., ACS reagent, ≥ 37%	84422
Phenyl isothiocyanate, purum, ≥ 98.0%	78782
2-Propanol, puriss. p.a., ACS reagent, ≥ 99.8%	59300
Sodium phosphate dibasic, anhydrous, ≥ 99.5%	71639
Sodium hydroxide solution, ~1.0 M NaOH	72082

Spray Solution for Detection of Sugars, Steroids and Terpenes (22)

Mixture of anisaldehyde, ethanol, concentrated sulphuric acid and glacial acetic acid, 5:90:5:1. To complete the visualization, after treatment with spray solution, the TLC plate should be heated for 5 minutes at 90-100 °C.

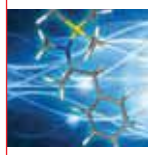
Description	Cat. No.
Anisaldehyde, purum, ≥ 98.0% (GC)	10440
Ethanol, puriss. p.a., ACS reagent, absolute alcohol, ≥ 99.8%	02860
Sulphuric acid, puriss. p.a., ACS reagent, 95.0-98.0% (T)	84719
Glacial acetic acid, puriss. p.a., ACS reagent, ≥ 99.8% (GC/T)	45731

References

1. A.I. Vogel, A.R. Tatchell, B.S. Furnis, A.J. Hannaford, and P.W.G. Smith Vogel's Textbook of Practical Organic Chemistry (5th ed.) Prentice Hall (5th ed.) 1996.
2. C.F. Poole, S.K. Poole, T.A.Dean, and N.M.Chirco, J. Planar Chromatography., 2, 180 (1989).
3. C.F. Poole, and S.K. Poole, J. Chromatogarphy, 492, 539 (1989)
4. B.R. Hepler, C.A. Sutheimer, and I. Sunshine, Clinical Toxicology, 22, 503 (1985).
5. A.K. Singh, K. Granley, M. Ashraf and U. Mishra, J. Planar Chromatography, 2 41, (1989)
6. A.M. Siouffi, E. Mincsovics, and E. Tyihak, J. Chromatography, 492, 471 (1989).
7. G.C. Fenimore, C.M. Davis, and C.J. Meyer, Clinical Chemistry, 24, 1386 (1978).
8. F.A.A. Dallas, H. Read, R.J. Ruane, and I.D. Wilson (Eds.), "Recent Advances in Thin-Layer Chromatography" Plenum, London, UK (1988).
9. L.R. Treiber (Ed.), "Quantitative Thin-Layer Chromatography and its Industrial Applications", Dekker, New york, NY (1987).
10. J. Sherma and B. Fried (Eds.), "Handbook of Thin-Layer Chromatography" Dekker, New York, NY (1990).
11. B. Fried and J. Sharma, "Thin-Layer Chromatography. Techniques and Applications", 2nd Edn., Dekker, New York, NY (1986).
12. J. Sherma and B. Fried Handbook of Thin-Layer Chromatography (3rd ed.) Marcel Dekker, 2003.
13. J.G. Kirchner Thin-Layer Chromatography: Techniques of Chemistry Vol. XIV (2nd edn.) John Wiley & Sons (1978).
14. W. Funk, Fresenius Z. Anal. Chemistry, 318, 228 (1984).
15. W.R.G. Baeyens and B.L. Ling, J. Planar Chromatography, 1, 198 (1988).
16. H. Jork, W. Funk, W. Fischer, and H. Wimmer, J. Planar Chromatography, 1, 280 (1988).

Derivatization Reagents for TLC

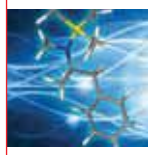
Product Name	Description	CAS Number	Pkg. Size	Cat. No.
Aluminum chloride - ethanol solution	for thin layer chromatography, ~11% in ethanol/water	7371-55-3	100 mL	28672
2-Aminoethyl diphenylborinate	≥97.0% (NT), for detection of flavones	524-95-8	5, 25 g	42810
Antimony(III) chloride reagent (free of CHC)	for thin layer chromatography, ~30% Sb, for detection of vitamins		100 mL	21623
2,2′-Bipyridyl	≥99.0% (NT), for detection of Fe, high-affinity chelator of iron	366-18-7	5, 25, 100 g	14454
Bromocresol Green solution	ready-to-use, for the detection of flavanoids and foodstuff	76-60-8	500 mL	02559
Bromothymol Blue	ACS reagent, Dye content 95%	76-59-5	5, 25 g	114413
Chloramine T trihydrate	≥98.0% (RT), for detection of halogens and bromate, oxidizing agent for the preparation of 131I-labeled material with high specific radioactivity; reagent for the selective oxidation of methionine	7080-50-4	50, 250, 1000 g	23270
4-Chloro-7-nitrobenzofurazan	for fluorescence, ≥99.0% (HPLC), for detection of glycine	10199-89-0	1, 5, 25 g	25455
2,6-Dibromoquinone-4-chloroimide	purum p.a., ≥98.0% (AT), for the detection of phosphatase, cyanides and phenols	537-45-1	10 g	34080
2′,7′-Dichlorofluorescein solution	Ready-to-use spray and dip reagent for chromatography, for detection of sweeteners	76-54-0	500 mL	02591
2,6-Dichlorophenolindophenol sodium salt hydrate	≥97.0% (calc. based on dry substance, AT), for detection of N-substituted barbiturates	620-45-1	1, 5, 25 g	36180
2,6-Dichloroquinone-4-chloroimide	≥99.0% (AT), for detection of vitamin B6	101-38-2	10, 50 g	35620
4-(Dimethylamino)benzaldehyde (Ehrlich's reagent)	≥99.0% (HPLC), for detection of hydroxyproline, forms colored condensation products (Schiff bases) with pyrroles and primary amines, analytical reagent for the determination of amino acids and peptides, amines, indoles, hydrazines and hydrogen peroxides; spray reagent for tryptophan; for the determination of tryptophan in proteins	100-10-7	50, 250 g	39070
4-(Dimethylamino)benzaldehyde solution (Ehrlich's reagent)	ready-to-use spray and plunge reagent for chromatography, Ehrlich's reagent, for detection of amines	100-10-7	500 mL	02560
5-(4-Dimethylaminobenzylidene) rhodanine	purum p.a., ≥98.0% (S), for detection of Ag, Au, Cu, Hg, Pd, Pt, alkaloids, antipyrin, indican, sulfonamide and urobilinogen	536-17-4	10 g	39090
Dansyl chloride	for fluorescence, ≥99.0% (HPLC), for detection of hexylamine, a fluorogenic reagent for fluorescent N-terminal derivatization of amino acids and peptides and detection by reverse phase HPLC	605-65-2	1, 5, 50 g	39220
Dimethylglyoxime	≥99% (gravimetric), for the detection of Ni, Co, Fe and Re	95-45-4	25, 100 g	33133
Diphenylcarbazone	≥95.0% (HPLC), for detection of Hg, Pb, Zn, Cd, Cr, Cu, Fe, Mo	538-62-5	5, 25 g	33153
Dragendorff reagent	ready-to-use spray and plunge reagent for chromatography, for detection of alkaloids and other nitrogen compounds in TLC	39773-75-2	100 mL	44578
Fast Blue B Salt	for microscopy (Hist.), technical grade, for detection of aflatoxins, also used for the determination of others compounds, e.g. proteins; bilirubin; enzymes	14263-94-6	10, 100 g	D9805
Fluorescence indicator green 254 nm	For thin layer chromatography	68611-47-2	50, 250 g	02554
Lead(II) acetate basic	≥33.0% as basic Pb (as PbO), ≥75.0% as total Pb (as PbO), for detection of sugar	51404-69-4	1, 5, 50 Kg, 6 x 1, 4 x 5 Kg	32306
Lead tetraacetate	purum p.a., moistened with ~15% acetic acid to stabilize, ≥95% (dried material), for detection of vicinal diols,	546-67-8	100, 500 g	15370
Manganese(II) chloride tetrahydrate	≥99%, for detection of organothio-phosphorous compounds	13446-34-9	250, 1000 g	31422
Mercury(II) chloride	eag. ISO, reag. Ph. Eur., ≥99.5% (calc. to the dried substance), for detection of barbiturates	7487-94-7	100, 500 g	31005
2-Methoxy-2,4-diphenyl-3(2H)-furanone	for fluorescence, ≥98.0% (HPLC), for detection of hexylamine, reagent for the derivatization of primary and secondary amines for HPLC; highly fluorescent derivatives are formed; Pre-column derivatization of amines; fluorescent labeling of proteins before SDS-PAGE	50632-57-0	25, 100 mg	64958
2,2-Dihydroxy-5-methoxy-1,3-indandione hydrate (5-Methoxyninhydrin)	>93% (HPLC), for detection of amines	304671-58-7	500 mg	341002
3-Methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate	≥99.0% (HPLC), for detection of aliphatic aldehydes, reagent for the determination of hexosamines in glycosamino-glycans;iIncorporated into a new peroxidase color reaction, reagent for the spectrophotometric determination of traces of selenium (IV) in environmental samples	38894-11-0	2.5, 10, 50 g	65875



Product Name	Description	CAS Number	Pkg. Size	Cat. No.
Molybdenum Blue spray reagent	1.3% molydbenum oxide in 4.2 M sulfuric acid; For use in the detection of phospholipids and related compounds.		100 mL	M1942
Morin hydrate	Standard Fluka, for microscopy (Fl.), for the detection of Al, Be, Zn, Ga, In, Sc	654055-01-3	5, 10, 50 g	69870
N-(1-Naphthyl)ethylenediamine dihydrochloride monomethanolate	≥99.0% (AT), for detection of nitrate and nitrite	1465-25-4	5, 25, 100 g	70720
Ninhydrin	≥98%, for detection of amino acids, amines and aminosugars	485-47-2	10, 1000 g	151173
Ninhydrin reagent according to Stahl	For thin layer chromatography, 1 g ninhydrin dissolved in 475 mL 1-butanol and 25 mL acetic acid		100 mL	17975
Orcinol monohydrate	from lichens, for detection of glycolipids	6153-39-5	5, 10, 100 g	O1875
Phosphomolybdic acid solution	ready-to-use spray and plunge reagent for chromatography, for detection of alkaloids	12026-57-2	100, 500 mL	02553
Phthaldialdehyde	for fluorescence, ≥99.0% (HPLC), for detection of amino acids, for precolumn derivatization of amino acids for HPLC separation. For flow cytometric measurements of protein thiol groupsfor HPLC separation. For flow cytometric measurements of protein thiol groups	643-79-8	1, 5, 50 g	79760
Potassium dichromate	ACS reagent, reag. ISO, reag. Ph. Eur., ≥99.8%, for detection of bile acids and lipids	7778-50-9	250 g, 1, 50 Kg	31255
Potassium hexachloroplatinate(IV)	Pt, 38-41% , for detection of alkaloids	16921-30-5	1, 5, 25 g	520861
Rhodamine B Solution	ready-to-use spray and dip reagent for chromatography, for detection of lipids	81-88-9	100 mL	02558
Sodium 1,2-naphthoquinone-4-sulfonate (Folin's reagent)	≥97.0% (T), Folin's reagent, for detection of Isonicotic hydrazide, amines and amino acids	521-24-4	10 g	70382
Sulfanilic acid	≥99.0% (T), for detection of nitrites 99%, for detection of phenols, reagent for the dehydrogenation of hydroaromatic compounds, undergoes photoinduced cyclo-addition reactions with stilbene derivatives and, β-unsaturated carbonyl compounds, fluorescence quencher	121-57-3	5, 100, 500 g	86090
Tetrachloro-1,4-benzoquinone		118-75-2	25, 100 g	232017
Tetracyanoethylene	98%, for detection of dienes	670-54-2	1, 5, 10, 25 g	T8809
N,N,N′,N′-Tetramethyl-p-phenylenediamine dihydrochloride	≥97.0% (AT) , for detection of osazones	637-01-4	5, 25 g	87890
8-Quinolinethiol hydrochloride	97%, for determination of metal ions	34006-16-1	1 g	359785
p-Toluene-4-sulfonic acid monohydrate	ReagentPlus®, 98.5%, for acetyl detection	6192-52-5	5, 100, 500 g	T35920
2,3,5-Triphenyltetrazolium chloride	≥99.0% (AT), reducing sugars	298-96-4	10, 50g	93140
Zinc chloride	≥98.0% (KT)	7646-85-7	500 g, 1, 5 Kg	276839
Aluminum chloride - ethanol solution	for thin layer chromatography, ~11% in ethanol/water	7371-55-3	100 mL	28672
Aniline phthalate solution p.a.	p.a., for thin layer chromatography	50930-79-5	100 mL	08545
Berberine chloride dihydrate	technical, ≥90% (AT)	141433-60-5	10, 50 g	14050
Brilliant Green ≥ 95.0%	Dye content ~90 %	633-03-4	25, 100, 500 g	B6756
Boron trifluoride	~10% in ethanol (~1.3 M)		100 mL	05576
N-Bromosuccinimide	≥95.0% (RT)	128-08-5	100, 500 g, 1 Kg, 6 x 100 g, 6 x 1 Kg	B81255
Chloramine T trihydrate	for the detection of halogens and bromate, ≥98.0% (RT	7080-50-4	50, 250 g, 1 Kg	23270
Copper(II) acetate monohydrate	≥99.0% (RT)	6046-93-1	100, 500 g	61148
Copper(II) nitrate trihydrate	99-104%	10031-43-3	100, 500 g	61194
Copper(II) sulfate pentahydrate	ACS reagent, crystallized, ≥99.0% (RT)	7758-99-8	250 g, 1Kg, 6 x 1 Kg	31293
Creatinine	≥99.0% (NT)	60-27-5	10, 50 g	C4255
1-Dansylpiperazine	BioChemika, for fluorescence, ≥99.0% (HPLC)	86516-36-1	100, 500 mg	39367
4-(Dimethylamino)cinnamaldehyde	≥98.0% (HPLC)	6203-18-5	5, 25 g	39421
Dimethylglyoxime	ACS reagent, for the detection of Ni, ≥99.0% (TLC)	95-45-4	25, 100, 500 g	40390
2,5-Dimethoxytetrahydrofuran, mixture of cis and trans	cis+trans, ≥97.0% (GC)	696-59-3	50, 250 mL, 1 L	D137103
3,5-Dinitrobenzoic acid	≥98.0% (HPLC)	99-34-3	100, 500 g	121258
2,4-Dinitrophenylhydrazine-hydrochloric acid solution	~0.005 M in ethanol, for thin layer chromatography	119-26-6	100 mL	18189
Fluorescamine spray reagent 0.05% fluorescamine in acetone	0.05% fluorescamine in acetone		100 mL	F5928
1-Fluoro-2,4-dinitrobenzene	≥98.0% (GC)	70-34-8	50, 250 g	42085
Iodine	ACS reagent, ≥99.8% solid	7553-56-2	5,100, 500 g, 6 x 500 g, 1, 2.5 Kg	207772
Iron(III) chloride	anhydrous, ≥97.0% (RT)	7705-08-0	50, 250 g, 1 Kg	157740
Lead(II) acetate basic	for sugar analysis (acc. to Horne), anhydrous, ≥72% Pb basis (KT)	51404-69-4	100, 500 g, 1 Kg	32306
Michler's ketone	≥99.0% (HPLC)	90-94-8	10, 25 g	147834

Derivatization Reagents for TLC (contd.)

Product Name	Description	CAS Number	Pkg. Size	Cat. No.
4-Nitrobenzenediazonium tetrafluoroborate	≥97.0% (T)	456-27-9	10, 50 g	294438
Palladium(II) chloride 60% as Pd, purum, anhydrous	anhydrous, 60% Pd basis	7647-10-1	1, 5, 25 g	76050
Picrylsulfonic acid solution ~1 M in H2O, purum.	~1 M in H_2O	2508-19-2	10, 50 mL	92823
Pinacryptol yellow	for photographic purposes	25910-85-4	1 g	80540
Rhodamine B for fluorescence, BioChemika	for fluorescence	81-88-9	1 g	83689
Rhodamine 6G	suitable for fluorescence, BioReagent	989-38-8	250 mg, 1 g	83697
Sodium phosphate monobasic dihydrate	≥99.0% (T)	13472-35-0	250 g, 1 Kg	71505
Sodium bicarbonate	≥99.0% (T)	144-55-8	1, 5 Kg	71630
Sodium hydroxide	≥98.0% (T), pellets	1310-73-2	500 g, 6 x 500 g, 1, 5 Kg, 6 x 1 Kg	S8045
Sodium hydroxide	puriss. p.a., ACS reagent, K ≤0.02%, ≥98.0% (T), pellets	1310-73-2	500 g, 1, 5 Kg	71690
Sodium hydrosulfite	≥82% (RT)	7775-14-6	50, 250 g, 1 Kg	71699
Sodium hydrosulfite	≥80% (RT)	7775-14-6	500 g, 1 Kg, 6 x 1 Kg	157953
5-Sulfosalicylic acid dihydrate	≥99.0% (T)	5965-83-3	25, 100, 500 g	S7422
3,3′,5,5′-Tetramethylbenzidine	≥98.0% (NT)	54827-17-7	1, 5, 25 g	87748
Thymol	≥99.0% (GC), purum	89-83-8	100, 500 g	T0501
Tin(II) chloride dihydrate	ACS reagent, ≥98.0% (RT)	10025-69-1	100, 500 g	208035
p-Toluenesulfonic acid monohydrate	≥98.5% (calc. on dry substance, T), puriss.	6192-52-5	100, 500 g, 6 x 100 g, 6 x 500 g	T35920
1,3,3- Trimethyl-2-methyl-eneindoline liquid	≥96.0%	118-12-7	100 mL	92550
2,4,6-Trimethylpyridine	≥99.0% (GC)	108-75-8	100, 500 mL	27690
2,3,5-Triphenyltetrazolium chloride	≥99.0% (AT)	298-96-4	10, 50 g	93140
Uranyl acetate dihydrate	ACS reagent, ≥98.0% (T)	6159-44-0	5, 25, 100 g	73943
8-Quinolinethiol hydrochloride	≥95.0% (CHN), yellow	34006-16-1	1, 5 g	359785
Vanillin	1.0 M in diethyl ether	121-33-5	100, 800 mL	V1104
Zinc chloride	≥98.0% (KT)	7646-85-7	500 g, 6 x 500 g, 1, 5 Kg, 6 x 1 Kg	31650
Zirconium(IV) oxychloride octahydrate	≥99.0% (AT)	13520-92-8	25, 100, 500 g	31670
Aerosol® 22			100, 500 mL	A9753
Bispyrazolone	for the detection of cyanide, ≥98.0% (TLC)	7477-67-0	5 g	15156
Fluorescein mercuric acetate	for the determination of disulfide groups, ~90% (UV)	32382-27-7	5 g	46980
4-(4-Nitrobenzyl)pyridine	≥98.0% (NT)	1083-48-3	5, 25, 250 g	73210
Phospray			200 mL	33047-U
Fluorescamine			100, 200 mL	34653
Bromothymol Blue		76-59-5	200 mL	34656
Manganese(II) chloride tetrahydrate	≥99%	13446-34-9	250 g, 1 Kg	31422
Mercury(II) chloride	≥99.5% (calc. to the dried substance)	7487-94-7	100, 500 g	31005



TLC Applications by Compound Class

Application Group	Description	CAS Number	Cat. No.
Aflatoxins	Fast Blue B Salt	14263-94-6	D9805
Aliphatic Aldehydes	3-Methyl-2-benzothiazolinone hydrazone hydrochloride	38894-11-0	65875
Alkaloids	Dragendorff's reagent	39773-75-2	44578
	Phosphomolybdic acid solution	12026-57-2	'02553
	Potassium hexachloroplatinate(IV)	16921-30-5	520861
	Zinc chloride	7646-85-7	31650
Amines	Potassium hexachloroplatinate(IV)	16921-30-5	520861
	4-(Dimethylamino)benzaldehyde (Ehrlich's reagent)	100-10-7	39070
	Ninhydrin	485-47-2	151173
	2,2-Dihydroxy-5-methoxy-1,3-indandione hydrate (5-Methoxyninhydrin)	304671-58-7	341002
Antibiotics	4-(Dimethylamino)benzaldehyde (Ehrlich's reagent)	100-10-7	39070
	Ninhydrin	485-47-2	151173
Amino Acids, Related Compounds	Ninhydrin	485-47-2	151173
	Sodium 1,2-naphthoquinone-4-sulfonate (Folin's reagent)	521-24-4	70382
Antioxidants	Phosphomolybdic acid solution	12026-57-2	02553
Barbiturates	2,6-Dichlorophenolindophenol sodium salt hydrate	620-45-1	36180
	Mercury(II) chloride	7487-94-7	31005
Base Metals (Pb, Zn, Cd, Cr, Cu, Fe, Mo)	Diphenylcarbazone	538-62-5	33153
Bile Acids	Potassium dichromate	7778-50-9	31255
Cyanides	2,6-Dibromoquinone-4-chloroimide	537-45-1	34080
Dienes	Tetracyanoethylene	670-54-2	T8809
Flavanoids and Foodstuffs	2-Aminoethyl diphenylborinate	524-95-8	42810
	Bromocresol Green solution	76-60-8	02559
	Antimony(III) chloride reagent (free of CHC)	–	21623
Glycolipids	Orcinol monohydrate	6153-39-5	O1875
Glycosids	Lead tetraacetate	546-67-8	15370
Glycine	4-Chloro-7-nitrobenzofurazan	10199-89-0	25455
Halogens	Chloramine T trihydrate	7080-50-4	23270
Hexylamine	Dansyl chloride	605-65-2	39220
	2-Methoxy-2,4-diphenyl-3(2H)-furanone	50632-57-0	64958
Indole Derivatives	4-(Dimethylamino)benzaldehyde (Ehrlich's reagent)	100-10-7	39070
Iron	2,2'-Bipyridyl	366-18-7	14454
Lipids	Antimony(III) chloride reagent (free of CHC)	–	21623
	Bromothymol Blue	76-59-5	114413
	Dragendorff's reagent	39773-75-2	44578
	Molybdenum Blue spray reagent	–	M1942
	Ninhydrin	485-47-2	151173
	Orcinol monohydrate	6153-39-5	O1875
	Rhodamine B solution	81-88-9	02558
Nickel	Dimethylglyoxime	95-45-4	33133
Nitrate and Nitrite	N-(1-Naphthyl)ethylenediamine dihydrochloride monomethanolate	1465-25-4	70720
Organic Acids	Bromocresol Green solution	76-60-8	02559
Osazones	N,N,N',N'-Tetramethyl-1,4-phenylenediame dihydrochloride	637-01-4	87890
Phenols	Tetrachloro-1,4-benzoquinone	118-75-2	232017
	2,6-Dibromoquinone-4-chloroimide	537-45-1	34080
	Phosphomolybdic acid solution	12026-57-2	02553
Precious metals (Cu, Ag, Au, Hg, Pt)	5-(4-Dimethylaminobenzylidene)rhodanine	536-17-4	39090
Steroids	Antimony(III) chloride reagent (free of CHC)	–	21623
	4-(Dimethylamino)benzaldehyde (Ehrlich's reagent)	100-10-7	39070
	Phosphomolybdic acid solution	12026-57-2	02553
Sugars and Derivatives	Ninhydrin	485-47-2	151173
	2',7'-Dichlorofluorescein solution	76-54-0	02591
	Lead tetraacetate	546-67-8	15370
Terpenes	Phosphomolybdic acid solution	12026-57-2	02553
Urobilinogene	5-(4-Dimethylaminobenzylidene)rhodanine	536-17-4	39090
Vitamins	Antimony(III) chloride reagent (free of CHC)	–	21623
	Potassium hexachloroplatinate(IV)	16921-30-5	520861

Derivatization Accessories

Micro Reaction Vessels

Micro reaction vessels are cone shaped inside for easy removal of small samples by syringe or micropipette. These vials are made of heavy-wall borosilicate glass and are supplied with PTFE-faced red rubber septum and cap. This glassware can be autoclaved or centrifuged. The maximum temperature of the septa is less than 121 °C and cannot be autoclaved.

Capacity (mL)	OD/HT/Depth (mm)	Cap Diameter (mm)	Qty.	Cat. No.
Clear glass (graduated) with open top cap				
0.3	16.5 x 34 x 23	15	12	33291
1	16.5 x 40 x 33	15	12	33293
2	16.5 x 58 x 48	15	12	33295
3	20.5 x 42 x 42	20	12	33299
5	20.5 x 61 x 58	20	12	33299
10	26 x 72 x 69	24	12	27479
Clear glass (graduated) with solid top cap				
0.3	16.5 x 34 x 23	15	12	27035
1	16.5 x 40 x 33	15	12	27036
2	16.5 x 58 x 48	15	12	27037
3	20.5 x 42 x 42	20	12	27038-U
5	20.5 x 61 x 58	20	12	27039

Mini-Vap Concentrator Evaporator

The 6-Port mini-vap concentrator/evaporator processes six miniature vials or containers at one time. It comes with six stainless steel needles, a fine control needle valve, and three feet (0.9 m) of plastic tubing. The adjustable Mini-Vap is a versatile concentrator/evaporator that can be used with a single container. The unique design allows for any size container, from the smallest vial to a 250 mL beaker. The Mini-Vap includes a needle valve for fine metering of air or nitrogen drying gas. This is ideal for a variety of needs in evaporation or concentration, and is a useful addition to the analytical laboratory.

Dimensions (L/W/H)	Qty.	Cat. No.
1.5 x 8.5" (adjustable)	1	22970
7.5 x 1.5 x 0.75" (19 x 4 x 2 cm)	1	22971

Block Heater (block sold separately)

- Multi-purpose use
- Heats from ambient to 150 °C
- Holds modular heating blocks
- Analog controls

This block heater is ideal for incubation and activation of cultures, enzyme reactions, immunoassays, and a variety of laboratory applications. There are two separate temperature control knobs. The low range knob adjusts from ambient to 100 °C and the high range knob adjusts from 75 °C to 150 °C. The dimensions are 12.4 x 8 x 3.5" (31.5 x 20.3 x 8.9 cm). The electrical specifications are 120V, 50/60 Hz 0.9 amps/100 watts and the weight of the heater is 5.8 lbs (2.6 kg).

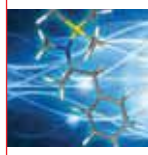
Description	Qty.	Cat. No.
Block Heater		
110 V	1	33315
240 V	1	33318-U
Block		
Block #1, 8 holes for 3 & 5 mL reaction vessels	1	33316
Block #2, 12 holes for 16 mm tubes and 0.1-2 mL reaction vessels	1	33317-U

Manual Injection Syringes

The most popular syringes are available in multi-unit packages, at considerable cost savings. These syringes have a temperature limit of 50 °C for cemented needles and 115 °C for removable needles.

- Eliminate downtime by having syringes on hand
- Reduce lab expenses by ordering in multipacks
- Save time by ordering less often

Volume (µL)	Description	Bevel tip Needle	Qty.	Cat. No.
10	701N (fixed needle)	26s gauge, 51 mm	6/pk	20779
10	701RN (removable needle) SGE syringe	26s gauge, 51 mm	6/pk	20793
10	10F (fixed needle)	26 gauge, 50 mm	6/pk	21934-U



HPLC Accessories

ASI Pump Replacement Parts

Analytical Scientific Instruments (ASI) cartridge check valve design offers self-priming convenience, rugged, crush proof construction, rapid response time (ball seats more quickly, for less pulsation and a more stable flow), and replaceable outlet filters (to protect system from particles).
If your pump model is not listed, please call us. UHMW PE - ultra-high molecular weight polyethylene.

Description	Pkg.	Cat. No.
Agilent®/HP Pump Replacement Parts (ASI)		
For use with 1090		
Inlet Cartridge	1	504734
Bio-Rad® Pump Replacement Parts (ASI)		
For use with 1330, 1350 Bio-Rad For use with 590, 600E, 6000 Waters		
Inlet cartridge	1	501204
For use with 1330, 1350 Bio-Rad		
UHMW PE Pump Seal	1	501921
SSI Pump Replacement Parts (ASI)		
For use with 1330, 1350 Bio-Rad For use with 200, 220, 222, 300 SSI For use with Extended Flow 510EF, 600EF, 6KEF, 6KAEF Waters For use with M-45, 501, 590, 600E, 6000 Waters		
Outlet Cartridge	1	501905
Gilson® Pump Replacement Parts (ASI)		
For use with 5S, 10S, 5SC, 10SC Elder For use with A, B, E Gilson		
ASI HPLC Pump Part for Gilson	1	502006
For use with 5S, 10S, 5SC, 10SC Gilson For use with Gilson 5S, 5SC		
UHMW PE Pump Seal	1	504653

Postcolumn Reactors

Postcolumn Reactor Module

Increase detection sensitivity for amino acids, proteins, carbohydrates, pesticides, inorganic ions, other samples.

The heated reactor cartridge in the ASI Model 310 Postcolumn Reactor Module mixes reagent with column effluent efficiently and with minimum peak dispersion. Unlike conventional PTFE tube coil reactors, the rugged reactor cartridge can be used at pressures up to 3000 psi, at 150 °C, without rupturing. We recommend using a low volume static mixer, such as the binary input housing/mixer cartridges listed on this page, with the Model 310 module. Install the mixer in line, prior to the reactor cartridge, to combine the reagent with the column effluent. A pump is required for delivering reagent to the system.

Description	Pkg.	Cat. No.
Postcolumn Reactor Module		
120 V, 0.50 mL (Reaktor)	1	54976
120 V, 1.0 mL (Reaktor)	1	54973
220 V, module only	1	54971
Postcolumn Reactor Cartridge (fits all heater modules) Replacement cartridges for all ASI Model 310 postcolumn Reactor Modules.		
0.15 mL	1	54978
0.50 mL	1	54979
1.0 mL	1	54980-U

ASI Static Mixers

- Reduces baseline noise
- Increases sensitivity
- Increases reaction efficiency in postcolumn derivatization
- Improves accuracy in gradient mixing for microbore analyses

A highly efficient cross-flow shearing mechanism in the ASI static mixer produces vortex mixing over a wide range of flow rates. Use the binary input housing to combine two flowpaths into one, such as in postcolumn or gradient mixing applications. Use the in-line housing when additional mixing is needed in a single flowpath. Within each product series (micro, low and high volume) the mixer cartridges are interchangeable. We recommend the 250 µL cartridge for large peak volumes, and the 50 µL or 150 µL sizes for smaller volumes. Use the Micro-Mixer Cartridges only with Micro-Mixer Housings and the Low Volume Mixer Cartridges only with the Low Volume Mixer Housings.

Component Assemblies

Choose a housing and a cartridge within each volume group. In-line, binary or ternary refers to the number of lines going into the mixer housing.

Description	Pkg.	Cat. No.
Micro-Mixer Static Mixers (2 - 25 µL)		
Stainless Steel		
Housing, In-Line	1	56665-U
Housing, Binary	1	56666-U
2 µL cartridge	1	56661-U
5 µL cartridge	1	56662-U
10 µL cartridge	1	56663-U
25 µL cartridge	1	56664-U
Low Volume Static Mixer		
Stainless Steel		
Housing, In-Line	1	57548
Housing, Binary	1	57549
Housing, Ternary	1	500488
50 µL cartridge	1	57545
150 µL cartridge	1	57546
250 µL cartridge	1	57547
PEEK		
Housing, In-Line	1	500496
Housing, Binary	1	500518
50 µL cartridge	1	500445
150 µL cartridge	1	500453
250 µL cartridge	1	500461
High Volume Static Mixer		
Stainless Steel		
350 µL In-Line	1	500534
500 µL In-Line	1	500550
500 µL Binary	1	500569

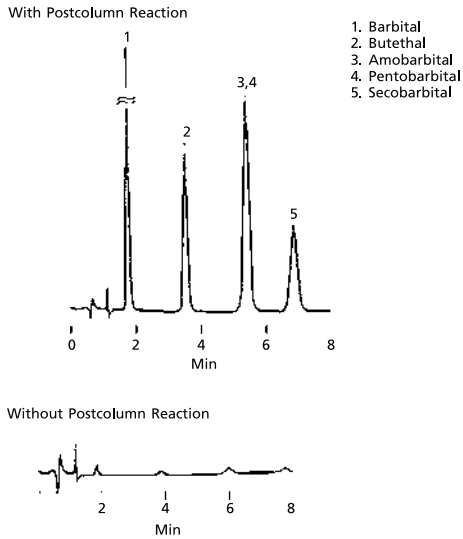
Postcolumn Reactor

Assemble Your Own System and Save!

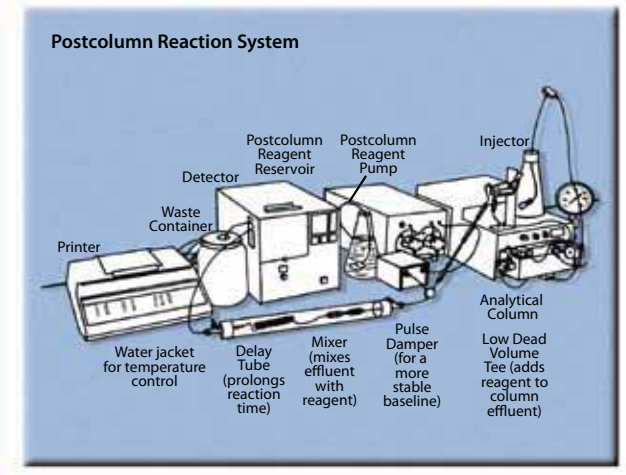
The equipment needed to perform postcolumn reactions can be relatively simple. These components enable you to easily and economically construct your own system. We recommend using a 5 cm × 4.6 mm column filled with 250 mm beads when peak volumes are large. Our Mixing Column Hardware Kit (Cat. No. 58319), contains a 5 cm × 4.6 mm I.D. column blank, two fittings, two frits, and 2 in./5 cm of 1/16 in. tubing. For small peak volumes, use a column filled with 75 mm beads, or a single bead string reactor (30 cm of 0.5 mm I.D. PTFE tubing filled with 250 mm beads).

Use our ready-to-use single bead string reactors, or prepare your own from our PTFE tubing, 1/16 in. internal unions, and silane treated glass wool (for terminating the reactor). The delay tubes (Cat. Nos. 59206 and 59207) are knitted PTFE tubing.

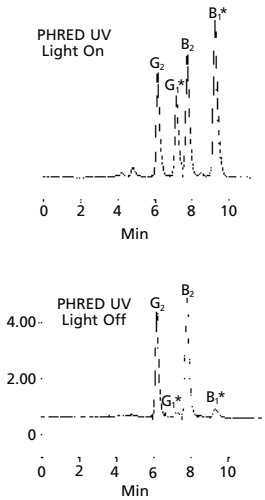
Improve sensitivity for amino acids, proteins, carbohydrates, inorganic ions, pesticides, and other samples. In postcolumn reactions, column effluent is mixed with a reagent before it enters the detector. The reaction can increase detection sensitivity or enable you to use more selective conditions (e.g., a different UV wavelength). The reaction can be as simple as changing the pH of the effluent, but the results often are significant. A postcolumn reaction system can be used to perform derivatizations or other reactions. It can be used with fluorescence, electrochemical, conductivity, and UV/visible detectors.



Deprotonization of barbiturates, an instantaneous reaction, gives a twenty-fold increase in sensitivity. The reaction also improves selectivity by shifting the UV absorption maximum from 220 nm to 240 nm.



PHRED: Photochemical Reactor Enhanced Detection



*Requires derivatization for fluorescence detection. By replacing chemical derivatization, photochemical derivatization simplifies this procedure.

References

1. C.Wolf, R. W. Schmid, J. Liq. Chromatogr. 13: 2207 (1990).
2. L. Dou, I. S. Krull, Anal. Chem. 62: 2599 (1990).
3. W. J. Bachman, J. Stewart, LC/GC 7: 38 (1989).
4. I. S. Krull, C. M. Selavka, M. Lookabaugh, W. R. Childress, LC/GC 7: 758 (1989).
5. TheReporter XII, #4, pp. 6-7.
6. H. Joshua, American Laboratory April 1995, p. 361.
7. TheReporter Vol. 16, no. 3, p. 9.

Description	Pkg.	Cat. No.
Postcolumn Reaction Single Bead String Reactors		
Acid Washed	1	59204
Acid Washed/Silanized	1	59205
Postcolumn Reaction Glass Beads		
75 µm Acid Washed	25 g	59200-U
250 µm Acid Washed	25 g	59202
75 µm Acid Washed/Silanized	25 g	59201
250 µm Acid Washed/Silanized	25 g	59203
Postcolumn Reaction Delay Tubes		
10 ft. (3 m) x 0.5 mm I.D.	1	59206
10 ft. (3 m) x 0.8 mm I.D.	1	59207
Postcolumn Reaction TFE PTFE Tubing		
10 ft L x 1/16 in O.D. x 0.031 in I.D.	1	58700-U
10 ft L x 1/16 in O.D. x 0.023 in I.D.	1	58701
10 ft L x 1/16 in O.D. x 0.012 in I.D.	1	58702
Postcolumn Reaction System Accessories		
Column Water Jacket	1	58450-U
Glass Wool	50 g	20411
	250 g	20410
SSI LO-Pulse Damper	1	58455
Union	1	22997-U
Guard Column Hardware Kit	1	58319
Tee	1	58283

Description	Pkg.	Cat. No.
PHRED: Photochemical Reactor and Accessories		
PHRED Photochemical Reactor, 100V	1	57400-U
Knitted Reactor Coils		
5 m x 0.25 mm, 0.25 mL	1	57402
15 m x 0.25 mm, 0.75 mL	1	57404
5 m x 0.50 mm, 1.0 mL	1	57405
10 m x 0.50 mm, 2.0 mL	1	57406
20 m x 0.50 mm, 4.0 mL	1	57411
20 m x 0.25 mm, 1.0 mL	1	57410-U
10 m x 0.25 mm, 0.5 mL	1	57403
Replacement Support Plate, 15 m x 0.50 mm, 0.5 mL	1	57401
Reflective Support Plate, 15 m x 0.50 mm, 3.0 mL	1	57407
Reflective Support Plate, stainless steel, for use with PHRED Reactor	1	57408

Solvents and Reagents - HPLC Buffers

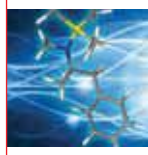
Key to Abbreviations

AT – Argentometric (Silver) Titration*; GC – Gas Chromatography; HPLC – High Performance Liquid Chromatography; KT – Complexometric Titration*; NT – Nonaqueous Titration*; RT – Redox-Titration*; T – Acidimetric Titration*

* Assay indicated in mass % (weight/weight)

HPLC Buffers - Solution

CAS No.	Compound	Pkg.	Cat. No.
64-19-7	Acetic acid solution, for HPLC, 49-51% (T)	100, 500 mL	45754
1336-21-6	Ammonium hydroxide solution, for HPLC, ~10% NH_3 in H_2O (T)	100 mL, 1 L	17837
366793-17-1	Dihexylamine acetate solution, for ion chromatography	6 x 25 mL	92467
211676-91-4	Dipentylamine acetate solution, for ion pair chromatography, ion pair reagent for HPLC/MS	6 x 25 mL	85318
114389-69-4	Dipropylamine acetate solution, for ion pair chromatography, ion pair reagent for HPLC/MS	6 x 25 mL	89789
64-18-6	Formic acid solution, for HPLC, 50% in water, 49-51% (T)	100, 500 mL	09676
75-75-2	Methanesulfonic acid solution, for ion chromatography	10, 100 mL, 1 L	17834
7664-38-2	Phosphoric acid, for HPLC, 85-90% (T)	100, 500 mL	79606
7664-38-2	Phosphoric acid solution, for HPLC, 49-51%	500 mL	79607
–	Potassium hydroxide solution, for HPLC, ~45% (T)	100, 500 mL	03564
1310-73-2	Sodium hydroxide solution, 50-52% in H_2O, eluent for ion chromatography	500 mL	72064
7664-93-9	Sulfuric acid solution, for HPLC, 49-51% (T)	100, 500 mL	84733
121-44-8	Triethylamine, for HPLC, ≥99.5% (GC)	10 x 2 mL	17924
554-68-7	Triethylamine hydrochloride, for HPLC, ≥99.0% (AT)	50, 250 g	96249
76-05-1	Trifluoroacetic acid, for HPLC, ampule, ≥99.0% (GC)	10 x 1 mL	91707
58828-90-3	Trimethylammonium bicarbonate buffer, for HPLC, volatile buffer, 1 M pH 8.5	100 mL	17899
77-86-1	Trizma® base, ≥99.7% (T)	100, 500 g, 1 kg	93350



TLC Accessories

TLC Sprayers & Reagents

Sequential Spraying Makes Drug and Lipid Detection Simpler

Drugs: Amphetamines, phenothiazines, and methaqualone can be sequentially detected on one plate, at the sensitivities shown in the table (narcotics, alkaloids). Remove the plate from the tank, dry it (5 min, 110 °C), cool it, and spray it with fluorescamine (Cat. No. 34653). After 5-10 minutes, amphetamines will exhibit bright green fluorescence under long-wave UV.

Next, warm the plate to 110 °C for 1-2 min. Phenothiazines will develop color and tranquilizers will exhibit fluorescence under long-wave UV. Finally, spray the plate with neutral iodoplatinate (Cat. No. 34651) to detect methaqualone and hydrolyzed methaqualone.

Similarly, amphetamines and alkaloids can be sequentially detected on one plate. Remove the plate from the tank, dry it (5 min, 110 °C), cool it, and examine it under long-wave UV for quinine, quinine metabolites, and demerol.

Next, spray the plate with ninhydrin (Cat. No. N0757), heat it at 90 °C for 10 min, then spray it heavily with diphenyl-carbazone, for an intense response from amphetamines and methamphetamines. Spray the plate with acidic iodoplatinate (Cat. No. I0256). Alkaloids will appear as spots of various colors. To aid in identifying specific compounds, change or intensify certain colors by over-spraying the plate with Dragendorff's reagent (Cat. No. D7518). Finally, spray the plate with ammoniacal silver nitrate and heat it (10 min, 110 °C), to detect morphine.

Lipids: Ninhydrin and Phospray can be used to visualize separated lipids. Dry the plate, spray it with ninhydrin, and gently warm it. Amino containing compounds (phosphatidyl ethanolamine, etc.) will appear as pink spots. Mark these spots to avoid confusion in the next step. Cool the plate to room temperature and spray it with Phospray to reveal phosphorus-containing compounds (phosphatidyl choline, phosphatidyl ethanolamine etc.).

Description	Pkg.	Cat. No.

Phosphomolybdic Acid Spray Reagent

- 10% Phosphomolybdic acid in ethanol
- For the detection of lipids, steroids, lactones, keto acids, hydroxy acids, unsaturated fatty acids, and phenolic compounds.
- 10% Phosphomolybdic acid in ethanol.
- Store at: 2-8 °C.

Phosphomolybdic Acid Reagent	100 mL	P4869

Ninhydrin Spray Reagent

- Detects amino acids, amines and amino sugars
- Contains 0.2% ninhydrin in ethanol
- Store at: 2-8 °C

Ninhydrin Reagent	100 mL	N1286

Iodoplatinate Spray Reagent

- For use in the detection of alkaloids, amines and organic nitrogen compounds
- 0.15% Potassium chloroplatinate and 3% potassium iodide in dilute hydrohloric acid

Iodoplatinate Reagent	100 mL	I9157

Molybdenum Blue Spray Reagent

- For use in the detection of phospholipids and related compounds
- 1.3% molydbenum oxide in 4.2 M sulfuric acid
- Molybdenum Blue reagent

Molybdenum Blue Reagent	100 mL	M1942

Ninhydrin Fixer Spray Reagent

- Acidified ethanol containing 1% (v/v) saturated cupric nitrate
- Fixative for ninhydrin chromatograms
- Store at: 2-8 °C

Ninhydrin Fixer Spray Reagent	100 mL	N1411

Other Spray Reagents

Dragendorff Reagent, Ready-to-use	100 mL	44578
Phospray	200 mL	33047-U
Flourescamine	100 mL	34653
Bromothymol Blue	200 mL	34656

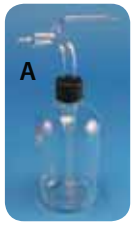
Sprayers

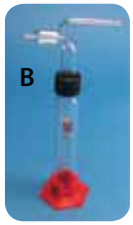
Chromatography Sprayer

Provide a fine, uniform spray that is optimized for the development of TLC plates. Also suited for use in electrophoresis.

- Adjustable spray pattern using thumb on vent hole
- Greaseless, screw-threaded joint will not seize; a simple turn of the threaded cap pulls joint apart safely
- Threaded SafetyBarbs™ prevent accidental breakage during tubing installation or removal
- Uses low-pressure gas or air

Description	Pkg.	Cat. No.
Chromatography Sprayer		
10 mL	1	Z529710
50 mL	1	Z529729
125 mL	1	Z529737
250 mL	1	Z529745
Flask-type Sprayer		
Erlenmeyer flask with #15 Ace-Thred with sprayer head.		
75 mL	1	Z190373
250 mL	1	Z129178
Replacement Flask, 75 mL	1	Z190381
Replacement Flask, 250 mL	1	Z129186
Replacement Head only	1	Z190403
Bottle-type Sprayer (A)		
240 mL	1	Z126306
Reagent Sprayer		
250 mL	1	58005
Tube-type Sprayer (B)		
Tube has hexfoot stand and sprayer head.		
50 mL	1	Z126292
Replacement Sprayer Head for Bottle- and Tube-type Sprayers		
Erlenmeyer flask with #15 Ace-Thred with sprayer head	1	Z407267





Aerosol Products

Sigma TLC Spray Box

- Completely disposable.
- Contains knock out vents for better ventilation.
- Easy to assemble.
- Compatible with most Sigma sprays.

Description	Pkg.	Cat. No.
Sigma TLC Spray Box		
~14 in. x 14 in. x 14 in.	5	S1509

Nalgene® Aerosol Spray Bottle

Reusable HDPE bottle with PP cap uses air pressure to propel contents from container. Bottle is filled and pressurized manually. Just pump the cap to charge the system. Two nozzles are included with each bottle to adjust spray from a fine mist to a heavy stream. Net fill 100 mL bottle.

180 mL	1	Z279250

Preval Spray Unit

Compact, well-designed spray canister delivers fine mist with CFC-free propellant. Permits control of reagent delivery, for even coverage and minimal waste. Gas canister serves as handle; cap fits standard 38-400 thread reagent bottles. Kit includes one propellant canister and one 6-ounce glass bottle.

Preval Spray	1	Z365556

Aerosol® 22

Aerosol 22	100 mL	A9753
Aerosol 22	500 mL	A9753

TLC Developing Tanks

Rectangular TLC Developing Tanks, Complete with Lid

Description	Pkg.	Cat. No.
Tanks (L x W x H, cm)		
7.5 x 15.5 x 8.0	1	Z204226
12.1 x 10.8 x 8.3	1	Z146226
17.5 x 6.2 x 6.8	1	Z204196
17.5 x 11.0 x 6.2	1	Z204188
17.5 x 16.0 x 6.2	1	Z204161
17.5 x 16.0 x 8.2	1	Z204153
27.5 x 26.5 x 7.0	1	Z126195
Lids only		
for Z204226	1	Z412090
for Z146226	1	Z146234
for Z204196	1	Z412082
for Z204188	1	Z412074
for Z204153	1	Z412066
for Z204161	1	Z412058
for Z126195	1	Z146218

Reference

1. Dip Reagents for Visualization in TLC. J. Chem. Educ. 73, 77 (1996)

TLC Development Chamber (L x OD, mm)

Single slot chamber

95.9 x 25.4	1	Z278629

Reference

1. Levine, S. G., J. Chem. Educ. 73, 77 (1996)

TLC Desiccation Cabinet

Acrylic cabinet provides complete visability of stored TLC plates under desiccating conditions. Stores up to 24 (20 × 20 cm) plates. Plates should be oven dried before storage for optimal results.

Desiccation Cabinet	1	Z266086

Pyrex® Rectangular Chromatography Jar

Corning® 6944

Ground to the close tolerances needed for tight cover fit. Jar edges are ground flat within 0.25 mm. Covers are not supplied. Do not use heat, pressure or vacuum applications

Description	Pkg.	Cat. No.
Pyrex® Rectangular Chromatography Jar		
137 mm L x 162 mm W x 267 mm H	6	CLS69444L
181 x 238 x 324	6	CLS694411L

Cylindrical Tanks

Can be used as a staining chamber. Made of glass with lid. Compact size offers good plate visibility and minimal solvent use.

Glass Tank, 6.5 cm O.D. x 10.5 cm H	1	Z243906
	6 x 1	Z243906
Glass Tank, 6.5 x 21.0	1	Z243914
	3	Z243914
Tank Lid	1	Z407259

Developing Chamber

Single slot chamber, 95.9 mm L x 25.4 mm O.D.	1	Z278629
For use with 10 cm x 10 cm Plates	1	Z266019
For use with 20 cm x 20 cm Plates	1	Z266000

Latch-Lid™ TLC Developing Chambers

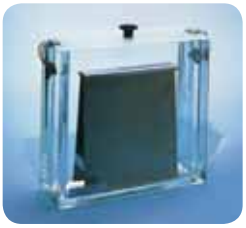
Latch-Lid units have a unique stainless steel latching device that holds the lid and tank firmly in place. This permits complete saturation of the chamber with solvent vapor for uniform development of chromatograms. Glass tank features flat ground tops, inner and outer rims beveled for safety, and flat ground bottoms for stability. Accessorize with multi-plate TLC racks for simultaneous development of up to six TLC plates.

For use with 10 cm x 10 cm Plates	1	Z266019
For use with 20 cm x 20 cm Plates	1	Z266000

TLC Plate Racks

Description	Pkg.	Cat. No.

TLC Plate Rack



Holds two 20 × 20 cm, 10 × 20 cm, or 5 × 20 cm TLC plates in a standard glass developing chamber.

Stainless Steel Rack	1	Z266027

Aluminum Multi-Plate Racks

For use with Latch-Lid™ developing chambers.

For use with 10 cm x 10 cm plates	1	Z266043
For use with 20 cm x 20 cm plates	1	Z266035

PTFE Multi-Plate Racks

For use with Latch-Lid developing chamber. Can accommodate up to six TLC plates.

For use with 10 cm x 10 cm plates	1	Z266078
For use with 20 cm x 20 cm plates	1	Z266051

TLC Plate Storage Racks

Used to carry and store TLC plates without damage to media. Racked plates may be air or oven dried. Holds up to 10 standard plates in either a vertical or horizontal position. Racks are stainless steel with a carrying handle.

For use with 10 cm x 10 cm plates	1	Z266108
For use with 20 cm x 20 cm plates	1	Z266094

TLC Plate Holder

Horizontal and vertical grooves hold 25 TLC plates of any size in an upright position. Lightweight body resistant to usual TLC solvents and chemicals. Great for desiccator storage of plates.

Analtech 5002	1	Z265284

Adsorbent Scrapers

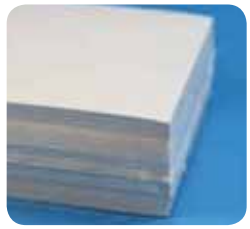
Description	Pkg.	Cat. No.

Scraper and Blades

Ideal flat 13 mm steel blade tapered to a sharp edge for rapid removal of adsorbent from TLC plates. Aluminum handle; replaceable blade.

Analtech 0500	1	Z265268
Replacement Blades	5	Z265276

TLC Saturation Pads



Placement of pads with solvent in the development chamber helps to assure rapid and uniform saturation of the atmosphere with solvent vapor. Pads improve reproducibility and reduce "edge effects".

10 cm x 10 cm	100	Z265241
10 cm x 20 cm	100	Z265233
20 cm x 20 cm	100	Z265225

Cutting Tools

Description	Pkg.	Cat. No.

Diamond Glass Cutter

Scores/breaks single or double-strength glass. Nickel-plated brass cutter with wooden handle.

Diamond Glass Cutter	1	Z169064

Scoring Tool

Good for cutting glass plates. Tungsten carbide tip.

Scoring Tool	1	23746

Hamilton® TLC Syringes

Description	Pkg.	Cat. No.

Diamond Glass Cutter

PTFE coats the final 3/4 in. of this 2 in. (51 mm) long cemented needle. This allows for more reproducible sample spotting on TLC plates.

- needle length: 51 mm (2 in.)
- coated tip

1701, volume 10 µL, size 26s ga	1	Z264385
1702, volume 25 µL, size 22s ga	1	Z264393
1705, volume 50 µL, size 22 ga	1	Z264407
1710, volume 100 µL, size 22 ga	1	Z264415

Troubleshooting in Derivatization

The first step in the development of any derivatization method should be to employ techniques and procedures that will prevent problems. Following are some points to consider when developing a method:

1. Glassware for Derivatization

Vials with 0.1-10.0 mL capacity accommodate sample plus solvent and reagent in quantities typically used in chromatography. Vials must be suitable for temperature extremes. Vials supplied with open-center screw caps can be sealed with rubber septum stoppers or Teflon®-lined discs. The heavy walls and excellent sealing properties of Supelco micro-reaction vials allow samples to be heated safely to moderately high temperatures. Ground bottoms give these vials added stability on a flat surface, and are convenient for pencil markings. Thermostatically controlled heating units with aluminum blocks drilled to fit the vials precisely are available from Supelco/Sigma-Aldrich and other manufacturers. Although a Teflon lining generally is quite inert, it can be dissolved by some samples and reagents.

2. Deactivation of Glassware

Because the surface of laboratory glassware is slightly acidic, it can adsorb some analytes — particularly amines and certain pesticides. In low-level analyses, such losses can be significant. To prevent sample loss through adsorption, glassware used in low level analyses usually is silanized. Silanization masks the polar Si-OH groups on the glass surface by chemically binding a non-adsorptive silicone layer to the surface, in effect "derivatizing" the glass. In the most common silanization procedure, the glassware is treated with a solution of 5-10% dimethyldichlorosilane (DMDCS) in toluene for 30 minutes. The deactivated glassware is rinsed with toluene, then immediately thereafter with methanol.

Adding a compound that competes for the adsorptive sites on the glass surface also can reduce adsorption. A small amount (often less than 1%) of an alcohol, such as butanol, added to the solvent significantly reduces adsorption losses.

3. Sample Handling

Most lab personnel transfer samples and reagents with pipettes. For sensitive reagents, we recommend using a microliter syringe, which reduces exposure to atmospheric moisture. Syringes with Teflon-tipped plungers are more convenient than conventional syringes with all-metal plungers, particularly for transferring volatile reagents. The Teflon plunger tip forms a better seal and facilitates withdrawal of the reagent from a sealed vial. Any syringe will retain some reagent in the barrel. A syringe with an all-metal plunger, if not properly cleaned, is prone to corrosion and seizing. The best cleaning procedure is to remove and wash the plunger, and use a vacuum to pull solvent through the syringe. A seized plunger sometimes can be freed by soaking the syringe in a container filled with methanol.

4. Reaction Time

Reaction time varies greatly among compounds. Many materials can be derivatized by the reagents described here in a matter of seconds or minutes at room temperature, while others require extended periods at elevated temperatures. For a compound with unknown reactivity, the progress of the derivatization can be monitored by periodic chromatographic analysis of aliquots of the reaction mixture. The appearance and subsequent response of product peaks can be used to determine the reaction progress. Heating often increases the yield of derivative and/or shortens the reaction time. Before using heat, consider the thermal stability of the analytes and reagents involved.

5. Water

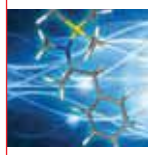
Water in the reaction mixture often can hinder the reaction and/or hydrolyze the derivative, reducing the yield of derivative for analysis. Tightly seal opened reagents during storage. If necessary, add sodium sulfate to the reaction mixture to trap water present in the sample. Samples can also be dried using gentle heating or under a stream of dry nitrogen. On humid days, keep glassware, syringes, etc. in a dry box.

6. Derivatization Reagents

Use only the highest purity reagents available to minimize interferences. Purchase the smallest quantity possible for your application. Allow refrigerated reagents to come to room temperature and gently mix prior to use (including those in sealed ampules). Once opened, reagents should be stored in tightly closed containers in a dry environment. In general, silyl reagents can withstand small amounts of moisture. The water will react with the reagent and thus be removed chemically. When used in excess (as they usually are) there should still be enough active reagent present. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) will darken when exposed to moisture.

7. Method Blanks

When doing a derivatization reaction, a blank should always be run along side the sample. The blank should contain the reagent and any solvents used. Analysis of a blank can help ensure that no artifact peaks are miss-identified as analyte derivatives in the final sample mixture.



Troubleshooting and Analysis

With few exceptions, possible causes and remedies listed here specifically address the derivatization process. It is assumed that an appropriate column and analytical conditions, and other general considerations, are used.

The following chart lists some common problems experienced when doing derivatization, along with suggested causes and remedies.

Symptom	Possible Cause	Remedy
Missing peaks or solvent peak only	1. Impurities in solvent, starting material, catalysts, or extract may interfere with derivatization (e.g. plasticizers from vial, inorganics used in sample synthesis, preservatives or antioxidants in solvents).	1. Use only highest purity materials at all steps in sample preparation process.
	2. Reagent deteriorated.	2. Use fresh reagent. Store reagent properly to prevent oxygen/water contamination, temperature damage (see product specification sheet).
	3. Reagent : sample ratio too low.	3. Use more reagent for same amount of sample.
	4. Rate of reaction too slow.	4. Reevaluate reagent concentration, time, temperature. Consider heating the reaction mix (consider thermal stability of the analytes and reagents).
	5. Water in reaction mix.	5. Remove water by adding sodium sulfate to sample.
	6. Wrong reagent.	6. Reevaluate reagent selection.
	7. Sample adsorbed to glassware.	7. Deactivate glassware and inlet sleeve (GC).
Extra peak(s)	1. Impurities from sample, solvent, reagents, sample vial, other labware.	1. Inject solvent and reagent blanks, solvent rinse from unused vial, etc. to isolate source of impurities.
	2. Derivative undergoing hydrolysis.	2. Remove water by adding sodium sulfate to sample. Store reagent properly to prevent oxygen/water contamination.
	3. Sample with >1 active hydrogen not fully derivatized.	3. Increase reaction time and/or temperature
	4. Side products from derivatization reaction.	4. Decrease reaction temperature and/or time.
Low detector response	1. Low yield of derivative – reaction did not go to completion.	1. Add more reagent, increase temperature or heating time, or add catalyst.
	2. Detector (FID) dirty; occurs with silylated derivatives.	2. Clean FID.
	3. Old sample; derivative may not be stable.	3. Prepare fresh sample.
No phase separation after adding reagent and heating (some alkylation reagents)	1. Septum in reaction vial not sealed; volatile portion of reagent evaporated off.	1. Prepare new sample – be sure vial is sealed tightly and septum is in good condition.
Low yield	1. Wrong reagent.	1. Reevaluate reagent selection.
	2. Solvent interfering with reaction	2. Choose solvent that does not have an active hydrogen, alcohol, or enolizable ketone group (e.g., hexane, toluene, etc.).
	3. Impurities in solvent, starting material, catalysts, or reagent that interfere with derivatization reaction.	3. Use only highest purity materials, including carrier solvent (if used).
	4. Reagent deteriorated.	4. Use freshly opened reagent; increase lifetime of reagents by storing to prevent oxygen/water contamination.
	5. Reagent: Sample ratio too low	5. Increase amount of reagent used.
	6. Rate of reaction too slow.	6. Increase reaction temperature and/or add catalyst/proton scavenger.
	7. Water in reaction mixture.	7. Make sure sample is dry; remove water from sample by adding sodium sulfate, gentle heating,or under a stream of dry nitrogen
	8. Sample adsorption.	8. Use silanized vials; check instrumentation (GC inlet, etc) for active sites.

References

1. Supelco Bulletin 909A, "Guide to Derivatization Reagents for GC". Sigma-Aldrich Publication T196909A.
2. The Reporter Volume15, No. 4. Sigma-Aldrich Publication T296024.
3. "Derivatization in Gas Chromatographic Analysis". Sigma-Aldrich publication T408164.
4. K. Blau; J. Halket, Handbook of Derivatives for Chromatography, Second Edition, John Wiley & Sons, New York,1993.

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