

GC Derivatization Reagent Procedures

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	Reagent	Page	Procedure
Chiral Derivation			
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The following procedures are intended to be used as guidelines and may be adapted as necessary to meet the needs of specific applications. For these procedures the sample may be injected directly after dissolution is complete.

The use of derivatization grade solvents is recommended for these procedures.

As always, take proper safety precautions when using the silylating reagents. Consult the MSDS for specific handling information.

1

The work of W.C. Butts is the basis of the following procedure. These conditions have been applied to over 200 organic compounds including carboxylic acids, amines, alcohols, phenols, and nucleic acids.

Reagents

BSTFA, BSA, or MSTFA
Pyridine

Procedure

To a 1-5 mg sample, in a 1 mL Vial, add 100 μ L each of pyridine and silylating reagent. Cap and heat to 60°C for 20 minutes.

For moderately hindered or slowly reacting compounds, use BSTFA with 1% or 10% TMCS catalyst. Under extreme conditions compounds may require heating for up to 16 hours. Ketones, when derivatized using this procedure, may form 15-20% enol TMS esters. These esters can be eliminated by first forming a methoxime. Consult procedure 25 for precursor derivatization methods.

Amino acids may require reaction in a sealed tube or vial. Heat samples, cautiously, near the boiling point of the mixture until a clear solution is obtained.

Note: This method is not recommended for carbohydrates.

2

Reagents

BSTFA,
DMF

Procedure

Place 5-10 mg of sample in a 3 mL vial. Add 2000 μ l each of DMF and BSTFA. Cap and shake well. Allow solution to react overnight at room temperature. Inject directly onto the column.

This method has been developed by K. M. Brobst and C. E. Lott for the silylation of duryps and carbohydrates in concentrated aqueous solution. The reaction must be closely monitored because considerable heat and ammonia gas

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BSTFA derivatizes slightly aqueous hydroxyl compounds.

Reagents

BSTFA
Acetonitrile

Procedure

To a 25 μ L sample, in a 3 mL vial add 175 μ L acetonitrile. Seal vial and inject 800 μ L BSTFA. Agitate for 10 to 20 seconds. Let stand approximately 1 hour. Inject directly onto column

Note: Due to the reactivity of this silylating reagent with water, the amount of BSTFA should exceed that of water by 1-fold molar, and that of the analyte by 50 molar.

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This derivatization method produces stable products with easy-to-interpret mass spectra.

Reagents

MTBSTFA
Solvent of choice

Procedure

To a 1-5 mg sample, in a 1 mL Vial, add 100 μ L each of reagent and solvent. Hindered compounds should be heated in a closed vial at 60°C for 1 hour.

For alcohols and amines, use MTBSTFA with 1% *t*-BDMCS catalyst.

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Reagents

HMDS
Trifluoroacetic acid
Pyridine

Procedure

Place 30 mg of 80% soluble syrup in a 3 mL vial and dissolve in 0.5 mL of pyridine. Add 0.4 mL HMDS followed by 0.05 mL of trifluoroacetic acid. Shake for 30 seconds and then allow to



stand for 15 minutes with occasional shaking. Inject onto column.

Note: Reagents cannot be premixed.

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This method produces derivatives of dicarboxylic acids.

Reagents

MTBSTFA

Procedure

To 150 μ L of sample in a 3 mL vial, add 100 μ L of acetonitrile and 10 μ L of MTBSTFA. Seal vial and allow mixture to stand overnight. Add 100 μ L of water to hydrolyze any unreacted MTBSTFA; follow with 250 μ L of hexane. Mix vigorously and centrifuge. Decant upper hexane layer and dry to approximately 5 μ L under a nitrogen stream. Inject sample onto column.

7

Hydrox-Sil, based on the formulation developed by Sweeley, et al.; and Deriva-Sil are special formulations for the derivatization of hindered and other hard-to-silylate compounds.

Reagents

Hydrox-Sil or
Deriva-Sil

Procedure

Place 5-10 mg of sample into a 3 mL TR-Vial. Add 1 mL of reagent and mix to dissolve. (Compounds with low solubility may have to be warmed to 60°C.) Allow mixture to stand until silylation is complete, usually 5 to 10 minutes. In some cases, highly hindered compounds may require heating for several hours. Inject onto column.

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Suited for compounds where pyridine is not applicable.

Reagents

Hydrox-Sil Concentrate
Deriva-Sil Concentrate
Solvent of choice

Procedure

Working solution: Mix 1 part reagent of choice with 2 to 4 parts of the appropriate derivatization grade solvent.

Note: Working solutions of reagent and solvent are usually stable for several days if protected from moisture. The exception is DMF working solutions which should be dis-

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This method is excellent for carbohydrates, low molecular weight fatty acids, and steroids.

Reagents

Hydrox-Sil AQ

Procedure

Evaporate 5-10 mg sample to a glassy syrup in a 1 mL TR-Vial and then add 0.5 mL Hydrox-Sil AQ. Stir until dissolved; the solution may be heated to 60°C if necessary. Inject onto column.

10

This method produces TMS derivatives of unhindered, as well as highly hindered, hydroxyl compounds.

Reagents

Deriva-Sil Concentrate

Procedure

To 5-10 mg of sample in a 3 mL vial, add 0.5 mL Deriva-Sil Concentrate followed by approximately 1 mL of solvent. Additional solvent may be added as necessary to aid dissolution. In some cases, the sample may be warmed to 60°C for 10-15 minutes. Inject onto column.

Resultant TMS ethers may be eliminated by first making the methoxime derivatives. Consult procedure 3 for precursor derivatization.

11

Particularly useful for the derivatization of wet sugar samples and hindered hydroxyl groups, TMSI may be used either neat or with solvent.⁴⁷

Reagents

TMSI
Solvent of choice

Procedure

Mix 5-10 mg sample and 0.5 mL TMSI in a 3 mL TR-Vial. If necessary, add derivatization grade solvent to dissolve the sample. Stir occasionally until dissolved. The sample may be heated to 60°C for 10-15 minutes as needed.



This method results in the prevention of dual perfluoroacetyl and TMS derivatives of amino acids, catecholamines, alkaloids, and other compounds containing both amino and hydroxyl functions. TMSI protects the hydroxyl group while leaving the amines free for derivatization by perfluoroacetyl reagent.

12

This method results in the preparation of dual perfluoroacetyl and TMS derivatives of amino acids, catecholamines, alkaloids, and other compounds containing both amino and hydroxyl functions. TMSI protects the hydroxyl group while leaving the amines free for derivatization by the perfluoroacetyl reagent.

Reagents

TMSI

HFBI, PFPI, or TFAI

Acetonitrile

Procedure

Place 1-3 mg of sample in a 1 mL vial and dissolve in 0.1 mL acetonitrile. Add 0.2 mL TMSI, cap and heat to 60°C for 3 hours. Add 0.1 mL of the desired perfluoroacetylimidazole and heat again to 60°C for 30 minutes. Inject sample onto column.



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In conjunction with TMA, the following perfluoroacyl reagents derivatize amines, alcohols, and phenols for ECD or FID. Unlike other base catalysts, TMA does not cause interfering peaks at high EC sensitivities.

Reagents

HBA, PFPA, or TFAA

0.05 M Triethylamine (TMA) in benzene

Benzene

Aqueous Ammonia

Procedure

In a 5 mL vial, dissolve 50 µg (250 µg for FID) of sample in 0.5 mL benzene. Add 0.1 mL of 0.05 M TMA in benzene followed by 10 µL of desired anhydride. Cap the vial and heat to 50°C for 15 minutes. Cool and add 1 mL of a 5% aqueous ammonia solution. Shake for 5 minutes, separate the benzene layer, and inject it directly onto the column.

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For the derivatization of alcohols, amines, amides, and phenols with imidazoles.

Reagents

HFBI
Toluene

Mg SO₄

Procedure

To 1-2 mg of sample, in a 3 mL TR-Vial, add 2 mL toluene followed by 0.2 mL of imidazole. Cap and heat to 60°C for 15-30 minutes. Cool and wash with 3 x 2 mL H₂O. Dry over MgSO₄ and inject onto the chromatograph.

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This procedure, developed by M. Donike, allows for the rapid derivatization of amines.

Reagents

MBTFA
Solvent of choice

Procedure

To 10 mg sample, in a 1 mL TR-Vial, add 0.2 mL MBTFA and 0.5 mL solvent. Cap and heat to 60-100°C for 15 to 30 minutes. Hindered compounds may require additional heating.

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Based on the procedure developed by J. E. Sullivan and L. R. Schewe, this method uses MBTFA for the derivatization of sugars for FID.

Reagents

MBTFA
Pyridine

Procedure

To 5-10 mg of sample, in a 3 mL TR-Vial, add 0.5 mL MBTFA and 0.5 mL pyridine. Cap and heat to 65°C for 1 hour with occasional shaking. Cool prior to injection.

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This procedure uses a pentafluorobenzoyl chloride (PFBCl) to provide rapid formation of the derivatives of amines and phenols.

Reagents

PFBCl
2.5 N NaOH
Mg SO₄
Methylene chloride

Procedure

In a 5 mL vial dissolve 25-50 mg of sample in 2.5 mL of 2.5 N NaOH. Add 0.1 g of PFBCl, cap, and shake vigorously for 5 minutes. Extract the derivative into methyl chloride; dry over MgSO₄. Inject directly onto column.

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This two-step sequential derivatization procedure, developed by S. Wilk and co-workers, comes closest to being a general technique.

Reagents

PFPOH
PFPA

Procedure

To 1 mg of sample, in a 3 mL TR-Vial, add 50 µL PFPOH and 200 µL of PFPA. Heat to 75°C for 15 minutes and then evaporate to dryness under N₂. Add an additional 100 µL PFPA and heat to 75°C for 5 minutes. Evaporate to dryness and dissolve in ethyl acetate prior to injection.



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The following method uses common reagents for the esterification of organic acids.¹⁰⁰

Reagents

BF₃/methanol or BF₃/*n*-butanol

Procedure

To 100 mg organic acid in a 5 mL TR-Vial, add 3 mL BF₃/methanol or BF₃/*n*-butanol. Heat to 60°C for 5 to 10 minutes. Cool and transfer to a separatory funnel along with 25 mL hexane. Wash 2 times with saturated NaCl solution, dry over anhydrous Na₂SO₄, and evaporate solvent to concentrate the sample. Inject sample onto column.

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Rapid procedure for the derivatization of both carboxylic and amino acids.

Reagents

DMF-Dialkylacetal of choice:

- DMF-Dimethylacetal,
- DMF-Diethylacetal,
- DMF-Di-*n*-butylacetal, or
- DMF-Di-*n*-propylacetal

Solvent of choice

Procedure

In a 1 mL TR-Vial dissolve the sample in 0.5 mL of a 1:1 solvent/reagent mixture by heating to 100°C.

Note: Use pyridine for fatty acids, acetonitrile for amino acids.

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Flash Alkylation is suited for biological fluids and thermally stable fatty acids.

Reagents

TMAH or
TBH
Toluene

Procedure

Extract tissue or biological fluid with toluene. Transfer 4 mL extract into a nipple tube and evaporate under N₂ at 60°C. Add 25 µL TMAH or TBH solution to dissolve residue and inject 4 µL directly onto the chromatograph. Set injection port temperature at 260°C or above.

22

This procedure is for an extractive alkylation. Pentazocine, certain barbiturates, sulfonamides, and drugs based on 1,4-benzodiazepine, are examples of drugs alkylated through the ion-extraction process. These reactions are generally mild, fast, and simple.

Reagents

PFBBr
TBA-H-SO₄
0.2 M NaOH
Methylene chloride

Procedure

In a 3 mL TR-Vial, add 0.2 mg of sample in 1 mL of methylene chloride. Then add 1 mL each of 0.1 M TBA-H-SO₄ and 0.2 M NaOH. To this solution, add 25 µL of PFBBr and cap. Shake for 20-30 minutes at 25°C. The solution may be injected directly for FID. For ECD, evaporate methylene chloride to dryness and dissolve the residue in benzene.



Care should be taken when handling TPC. This reagent should be kept refrigerated. Exposure to moist air can lead to decomposition, and acid exposure may cause racemization.

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For reactions with amines, the TPC coupling is carried out in a dry inert solvent, such as chloroform or ethyl acetate.

Reagents

TPC

Triethylamine (TEA)

Chloroform

6 N HCl

Mg SO₄**Procedure**

Place 5-10 mg of the sample in a pressure acylation tube and cover with 1 mL of chloroform. To this add 5 drops of triethylamine and 1 mL TPC. Cap the tube and heat at 40°C for 15 minutes. Cool to room temperature and add 1 mL 6 N HCl. Shake tube to dissolve solids. Allow tube to stand until the two layers separate. Remove lower organic layer and dry with 0.1 g anhydrous Mg SO₄. Inject a 5 µL sample into the GC.

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Derivatization with TPC has become a common method for the determination of amphetamines.

Reagents

TPC

Triethylamine (TEA)

Chloroform

6 N HCl

Mg SO₄**Procedure**

Dissolve 50 mg of sample in 2 mL of dry chloroform. Add 4 mL of TPC reagent. Stir or shake the mixture and then add 41 mg of dry triethylamine. Continue to stir for 15 minutes. Wash with 3 mL of 6 N HCl and then with water. Add Mg SO₄ and allow mixture to stand for 15 minutes. Inject aliquot onto GC.

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Prior to the coupling of an amino acid with TPC, the acid portion is converted to its methyl ester.

Reagents

TPC

Triethylamine (TEA)

Chloroform

6 N HCl

Thionyl chloride

Methanol

Mg SO₄**Procedure**

Place 5-10 mg of sample in an acylation tube. Add 1 mL of thionyl chloride-methanol solution (1:10, prepared by adding thionyl chloride, dropwise, to the methanol that is being cooled in a dry ice/ acetone bath). Place the open acylation tube in an oil bath at 90°C and allow the contents to boil off until a solid residue remains. To the residue, add 1 mL of TPC reagent and 5 drops of TEA. Cap mixture and heat again to 90°C for 5 minutes. Allow to cool and add 1 mL 6 N HCl. Shake tube to dissolve solids. Allow the two layers to separate. Remove organic layer and dry with Mg SO₄. Inject onto column.

Note: For these procedures, it is critical that the TEA be added to the mixture last. However, excess TEA will not hinder the reaction. After TEA addition, verify that the pH of the mixture is basic; if not, add more TEA until a basic pH is attained.

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Like TPC, MCF requires refrigeration. It should not be exposed to moist air or acid conditions.

Reagents

MCF

Pyridine, dry

6N HCL aq, MgSO₄**Procedure**

Place 15 mg of sample into a 3 mL vial. Add 1.5 mL of MCF and 10 µL dry pyridine. Sonicate or shake solution the mixture for 10 minutes. Wash the mixture with 1ml of 6N HCL aq. Remove the organic layer, dry it with MgSO₄ and inject onto the column.

