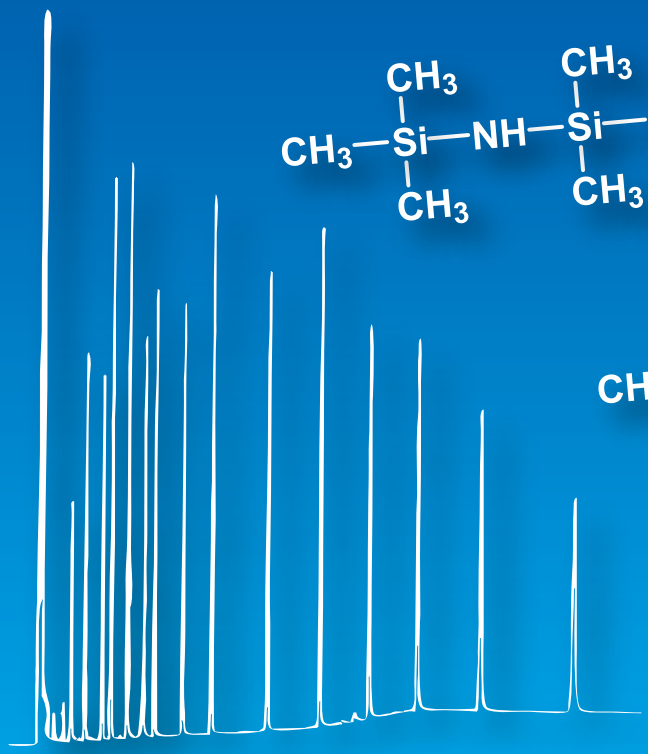
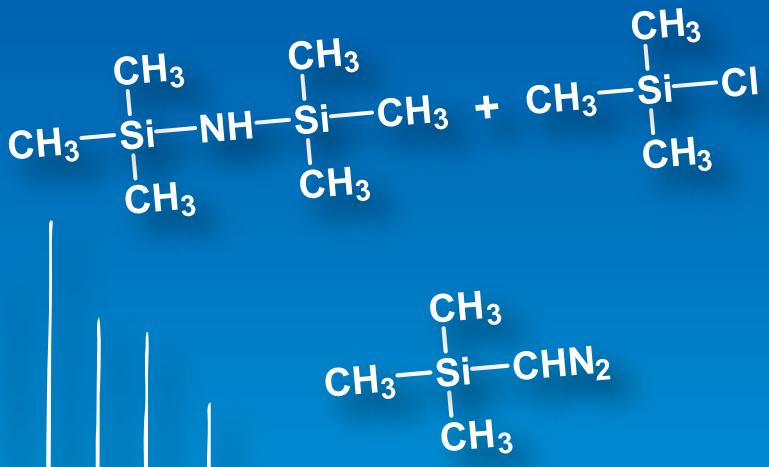
ANALYTICAL

GC Derivatization Reagents





Trimethylsilylation

Acylation

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■Other Pretreatment

Trimethylsilylation

Trimethylsilylating Reagents (A5000 series)

Gas Chromatography (GC) is widely used for analysis of various kinds of samples. The range of analytes has continued to expand to trace components in biological and environmental fields. As a result, GC derivatizing reagents for specific purposes have been under increasing demand.

Trimethylsilylating reagents (A5000 series) are GC derivatizing reagents quality-controlled for analyzing trace-level components. These reagents are highly purified so that impurities with high boiling point that would disturb the analysis (the component whose retention index is over 1500) are kept below 50 ppm per component.

1. Products

Item	Volume	Vessel	Code
BSA [=*N,O*-Bis(trimethylsilyl)acetamide]	**5 mL**	**Vial**	**A5601**
TMS-BA (BSA 25% in Acetonitrile)	**5 mL**	**Vial**	**A5602**
BSTFA [=*N,O*-Bis(trimethylsilyl)trifluoroacetamide]	**5 mL**	**Vial**	**A5603**
TMS-HT (=HMDS and TMCS in Anhydrous Pyridine)	**5 mL**	**Vial**	**A5604**
TMS-Imidazole (=SIM, *N*-Trimethylsilylimidazole)	**5 mL**	**Vial**	**A5605**

2. Precautions for Safe Handling

* Avoid moisture and keep container tightly sealed. Store in an explosion-proof refrigerator.
* Do not breathe dust/fume/gas/mist/vapors/spray.
* Avoid contact with the skin, eyes, mouth and mucous membranes.
* Use a dry syringe or micro-syringe to withdraw reagent from the vial.
* The packing of the vial is made from Teflon-coated rubber. Direct contact with rubber may cause contamination of the reagent by piercing with a needle. The reagent should be used as soon as possible after piercing.

3. Product Details

3.1 BSA [=*N,O*-Bis(trimethylsilyl)acetamide] 5 mL [A5601]

[A5601]

BSA is highly reactive towards nitrogenous compounds such as amino acids and amides, as well as compounds bearing hydroxyl or carboxyl groups. BSA cannot be used alone for the trimethylsilylation of sugars but can be used with catalytic amounts of chlorotrimethylsilane (TMCS).

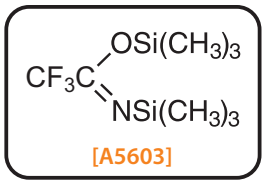
BSA is applicable to amino acids, amides, ureas, phenols, carboxylic acids, enols, sulfonic acids, steroids, uric acids, nucleic acids, and sugars.

3.2 TMS-BA (25% BSA in Acetonitrile) 5 mL [A5602]

[A5602]

TMS-BA might be separated into two layers during cool weather or by storing in a refrigerator. In that case, heat and shake to homogenize before use.

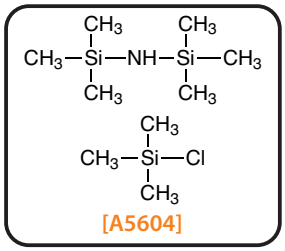
3.3 BSTFA [=*N*,*O*-Bis(trimethylsilyl)trifluoroacetamide] 5 mL [A5603]



[A5603]

Equivalent to BSA. BSTFA is effective for Flame Ionization Detector (FID) applications, and excels in activity, volatility, and solubility as compared with BSA. BSTFA by-products have high volatility and minimally disturb the analysis on GC compared to BSA. It is particularly suitable for trimethylsilylation of amino acids. (e.g. alanine and valine need to be heated at 125 °C for 15min.)

3.4 TMS-HT (=HMDS and TMCS in Anhydrous Pyridine) 5 mL [A5604]



[A5604]

TMS-HT is a pyridine solution that is mainly composed of hexamethyldisilazane and trimethylchlorosilane. Although it sometimes precipitates ammonium chloride crystals during storage, its supernatant can be used.
(HMDS=Hexamethyldisilazane, TMCS=Chlorotrimethylsilane)

Suitable for hydroxyl groups (e.g. alcohols, sugars, and steroids)

3.5 TMS-Imidazole (=SIM, *N*-Trimethylsilylimidazole) 5 mL [A5605]

[A5605]

Reacts selectively with hydroxyl groups (e.g. alcohols, sugars, steroids, and uric acids)

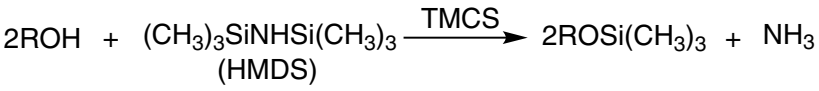
4. Overview

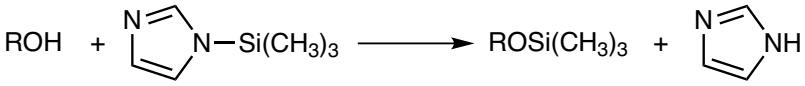
Trimethylsilylating reagents have applicability in wide range of applications such as GC analyses (e.g. separation of structurally similar materials and clinical inspection like analysis of serum amino acids, steroids, uric acids, etc.), protection of reactive groups during peptide/nucleoside synthesis, and for the separation/purification of organic compounds and inorganic acids (boronic acids, arsenic acids, and phosphoric acids, etc.).

Trimethylsilylating reagents are commonly used for GC analysis of compounds having slightly volatile polar functional groups such as hydroxyl groups, carboxyl groups, thiol groups, amino groups and imino groups. TMS reagents can convert these compounds (e.g. sugars, alcohols, phenol, steroids, amino acids, peptide and nucleic acids) into TMS ether, TMS ester, TMS thioether, and *N*-TMS which are thermally stable and volatile. Even an analyte is not stable enough to perform normal pretreatment (e.g. uronic acid) or difficult to trimethylsilylate directly (e.g. sulfonate salts), TMS reagents can be used by preparing appropriate derivatives beforehand (such as sugars, alcohols, and thiols mentioned in the example).

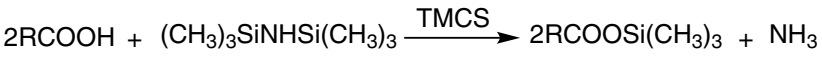
5. Reaction Formula of Trimethylsilylation

Hydroxyl compounds

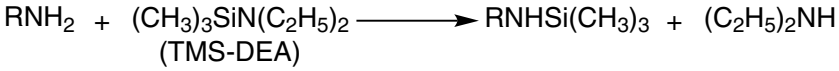




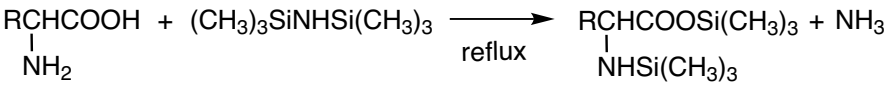
Carboxyl compounds

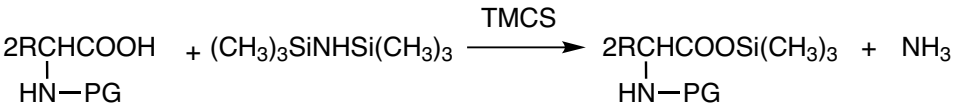


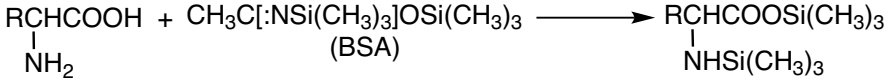
Amino compounds

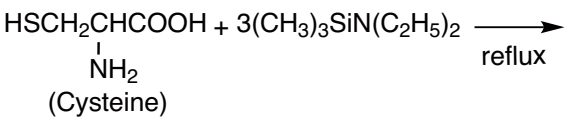


Amino Acids









6. Applications

6.1 General Procedure

[1] Sugars, Alcohols, Steroids, and others

Approx. 1 mg of a sample and either 1 mL of TMS-HT or 1 mL of SIM are placed in a dry vial, and then the vial is tightly sealed and allowed to react by shaking or heating. The supernatant can be used as a GC sample when using TMS-HT because crystals of ammonium chloride will be formed.

[2] Alcohols, Amino Acids, Amines, and others

Approx. 1 mg of a sample and 1 mL of TMS-BA are placed in a dry vial, and the vial is tightly sealed and allowed to react by shaking or heating.

6.2. Practical Application

6.2.1 Sugars

[1] Sugars in General

1 mL of TMS-HT is added to 10 mg of sugars. After shaking for 30 sec, the mixture is left for 5 min at room temperature. The supernatant can be used as a GC sample.[1)]

[2] Dissaccharides in Blood and Urine [2)]

A dry sample from 1 mL of blood/urine is added into either 50 µL of BSA :TMCS :pyridine (1:1:2) or 200 µL of BSA : TMCS : pyridine (1:1:5), and then the resulting mixture is allowed to react for 45 min at room temperature or for 20 min at 60 °C.

6.2.2 Amino acids [3)]

BSA is added to free amino acids or hydrochloride (5-10 mg), and allowed to react by heating for 1-2 h at 80 °C or for 0.5-1 h at 90 °C. Mainly Bis-TMS adduct is obtained from the free amino acids, whereas tris-TMS adduct is obtained from their hydrochlorides, respectively.

6.2.3 Catecholamines [4)]

1mg of norepinephrine is dissolved in 0.1 mL of acetonitrile, and then 0.2 mL of BSA, 0.1 mL of TMCS, and 2 µL of water are added. *N*,*N*,*O*',*O*'',*O*'''-pentakis-TMS adduct is obtained by heating for 2 h at 60 °C. However, the reaction takes 5 h to complete without adding water.

6.2.4 Steroids

[1] Hydroxysteroids

Non-sterically hindered hydroxyl group can readily be trimethylsilylated by the general procedure. The reactivity between the positions of hydroxyl groups (such as at 3-, 11-, 16-, 17-, and 20- positions) and trimethylsilylating reagents has been much discussed so far.[5-8)] For example, 10% of TMCS is added to BSA, HMDS or SIM for the trimethylsilylation of 11β-OH, whereas 20% of TMCS is added to BSA or SIM for the trimethylsilylation of 17α-OH as a catalyst, respectively. Full trimethylsilylation of cortols has also been reported.[5-7)]

[2] Methoxime-trimethylsilylation of ketosteroids

(1) 0.5 mL of pyridine and 8 mg of methoxylamine hydrochloride are added to 2 mg of steroids, and then the mixture is allowed to react for 3 h at 60 °C or overnight at room temperature. After extracting with benzene or ethyl acetate, the solvent is evaporated by N_2 flow. 0.2 mL of BSA is added to the residue and it is allowed to react for 3-5 h at room temperature. As for steroids having 11β-OH, 0.005-0.1 mL of TMCS is added as a catalyst.[9)]

(2) 50 µL of 10% pyridine solution of methoxylamine is added to 0.1 mg of steroids, and the resulting mixture is allowed to react for 15 min at 60 °C to complete the reaction. Then 50 µL of SIM is added to the mixture and is allowed to react for 2 h at 100 °C. Cortol is also thoroughly trimethylsilylated by the catalytic action of methoxylamine.

[3] Methoxime-trimethylsilylation of steroid hormones (in urine) [10)]

100 µL of pyridine solution of methoxylamine hydrochloride is added to a dried sample prepared from 5 mL of a sample of urine hydrolyzed enzymatically and undergone clean-up treatment. And then the mixture is allowed to react for 15 min at 60 °C for methoximation. After removing pyridine by N_2 flow, 100 µL of BSTFA : TMCS (5:1, v:v) is added, and the mixture is allowed to react for 2 h at 60 °C to complete the trimethylsilylation.

[4] Dexamethasone [11)]

50 µL of pyridine containing 5 mg of methoxylamine hydrochloride is added to 0.1 mg of a sample, and the mixture is allowed to react for 3 h at 60 °C to complete the reaction of a carbonyl group at the 20-position. To this 50 µL of SIM is added and the mixture is allowed to react for 5 h at 100 °C for tris-trimethylsilylation.

[5] Phytoecdysone [12)]

0.5 mg of steroids is dissolved in 20 µL of SIM, and the mixture is allowed to react for 1 h at 100 °C. All hydroxyl groups are trimethylsilylated, but the 6-positioned carbonyl groups is not affected.

7. References

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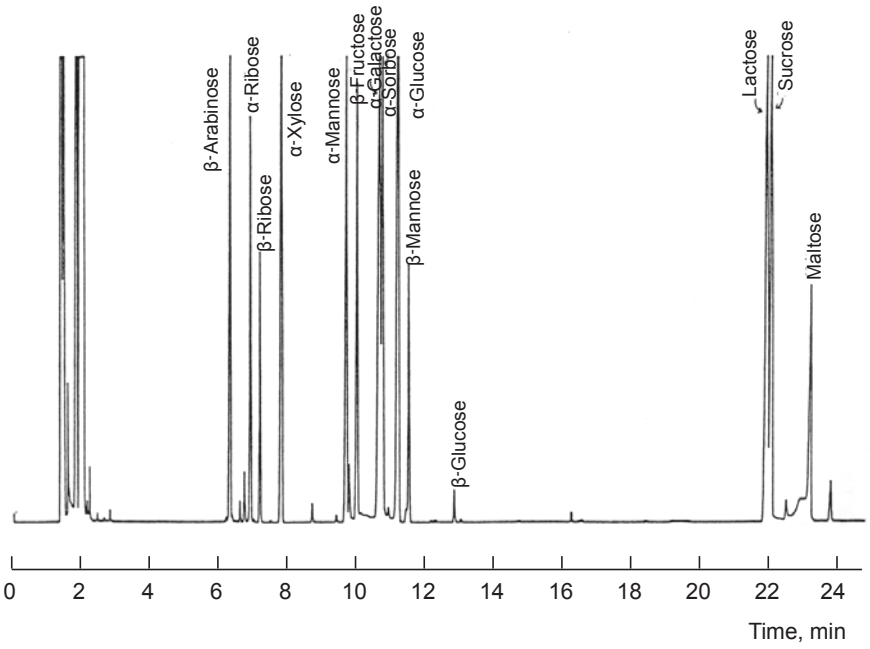
Gas Chromatogram (TMS-sugars)

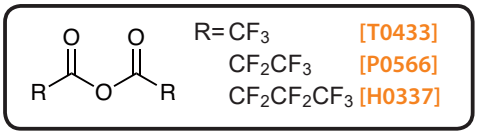
Column : 007-1
25 m × 0.25 mmI.D. × 0.25 µm
Temperature : 150 °C(5 °C / min) ～ 220 °C(10 °C / min) ～ 270 °C
Detector : FID
Inj. Mode : Split
Carrier Gas : He 30 cm/s



Acylation

Acid Anhydrides[1~3)]

Trifluoroacetic Anhydride 20 mL / 100mL / 400 mL [T0433]
Pentafluoropropionic Anhydride 5 g / 25 g [P0566]
Heptafluorobutyric Anhydride 10 g [H0337]



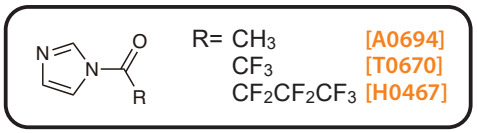
[Application Example]
Trifluoroacetylation of alcohols, amines and others[4)]

1-5 mg of sample is dissolved in 0.5 mL of solvent such as acetone or dichloromethane*, and 200 µL of trifluoroacetic anhydride is added. The mixture is allowed to react for 20-30 min at room temperature (or heated to 40 °C if necessary). After removing excess reagent and solvent by N_2-blowing, the residue is dissolved in acetone or other solvents to be used as a GC sample.

*If the sample is difficult to dissolve in such solvents, trifluoroacetic acid can be used as a solvent.

Acylated Imidazoles[5-9)]

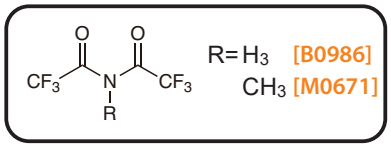
***N*-Acetylimidazole** 25 g / 500 g [A0694]
1-(Trifluoroacetyl)imidazole 5 g / 25 g [T0670]
1-(Heptafluorobutyryl)imidazole 5 g / 25 g [H0467]



Acylation reactions can proceed under mild conditions.
The resulting imidazoles are inert.

Fluorinated Acetamides[10,11)]

Bistrifluoroacetamide (=BTFA) 5 g / 25 g [B0986]
***N*-Methylbis(trifluoroacetamide) (=MBTFA)** 1 mL / 5 mL [M0671]



Trifluoroacetylation of the amino groups, hydroxyl groups and thiols can proceed under mild conditions.

[Application Example]
Trifluoroacetylation of sugars[11)]

5-10 mg sugars are placed in a 2 mL vial, then 0.5 mL of MBTFA and 0.5 mL of pyridine are added respectively. The resulting mixture is heated for about 1 h while shaking occasionally. The reaction is completed when the sample is dissolved, which can be used as a GC sample.

[References]

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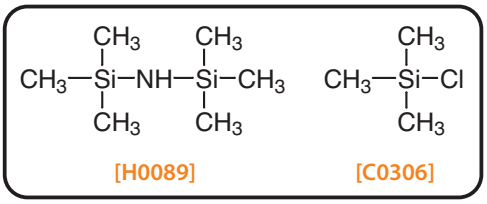
Silylation

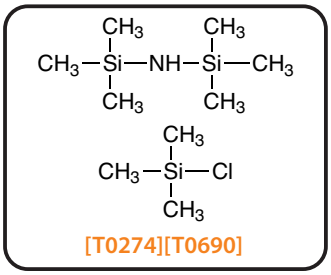
Trimethylsilylating Reagents

1,1,1,3,3,3-Hexamethyldisilazane (=HMDS) 25 mL / 100 mL / 500 mL [H0089]
Chlorotrimethylsilane (=TMCS) 25 mL / 100 mL / 500 mL [C0306]



TMCS is frequently used with HMDS. TMC is easily decomposed by moisture and generates hydrochloric acid gas in the process requiring careful handling.

TMS-HT (=HMDS and TMCS in Anhydrous Pyridine) 12 mL [T0274]
TMS-HT Kit Contents of the Kit: reagent (1mL) vial×8, 2mL empty reaction vial×8 [T0690]



TMS-HT is a pyridine solution whose principal constituents are hexamethyldisilazane (HMDS) and chlorotrimethylsilane (TMCS) and is useful for the trimethylsilylation of hydroxyl and carboxyl groups. If crystals of ammonium chlorid are appeared during storage, the supernatant can be used.

[General Procedure]

1. 1 mL of TMS-HT is added to *ca*. 1 mg of a sample in a dry vessel (preferably a vial of about 2 mL capacity), and then it is sealed and shaken (crystals of ammonium chloride are precipitated). The supernatant is injected into the GC column. In some cases, it is needed to heat to complete the reaction.
2. Apporx. 1 mg of sugar is dissolved in 0.2 mL of pyridine, and 1 mL of TMS-HT is added to the mixture. And then, white precipitate of ammonium chloride appreares immediately. After it is left at room temperature for about 5 min while intermittently shaking (if necessary, the vessel may be heated by directly immersing in a water bath: e.g. for maltose, at 80-90 °C for 2-3 min). The supernatant is used as a GC sample.

Note: If a liquid is poured into the sealed reaction vial in the kit, it is recommended to reduce the pressure of the vial in advance by using a syringe. For a solutions of sugars, use TMS-PZ.

[Application Examples]

Those that can be trimethylsilylated at room temperature

Alcohols (such as 2-methyl-2-butanol, stearyl alcohol, and oleyl alcohol),[1,2] sugars (such as xylose, cellobiose, and trehalose),[3-11,27] amino sugars, phenols (such as *o*-cresol, *m*-cresol, *p*-cresol, tricresol, and guaiacol),[12] organic acids (such as benzoic acid, salicylic acid, gentisic acid, and gallic acid),[13-17] amino acids (trimethylsilylation of DNP-methyl ester derivatives such as serine, threonine, and hydroxyproline),[18] catecholamine,[19] bile acids (trimethylsilylation of methyl ester derivatives),[20] fatty acids,[1] acids of citric acids cycle (such as α-keto glutaric acid, oxalacetic acid),[21] alkaloids (such as morphine, codeine.),[22] and steroids.[23,28]

Those that can be trimethylsilylated at about 100 °C and for 1 h

Sugar phosphate salts (such as D-erythrose-4-phosphate, D-ribose-5-phosphate, D-fructose-6-phosphate, D-glucose-6-phosphate, and D-glucose-1-phosphate) [24] and nucleoside (such as adenosine, inosine, uridine, deoxyuridine, thymidine, xanthosine, cytidine, and guanosine).[25,26]

Keto acids[21)]

In order to obtain positive results in the trimethylsilylation of α-ketoglutaric acids and oxaloacetic acids in GC, their oximes are first prepared, then converted into TMS-oxime derivatives. 10 mg of a sample and 10 mg of hydroxylamine hydrochloride are dissolved in 1 mL of dry pyridine and left for 10 min at room temperature. After that, 1 mL of TMS-HT is added, and the mixture is left for 5 min at room temperature.

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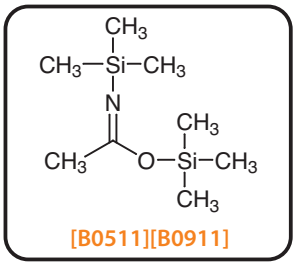
25) Y. Sasaki, T. Hashizume, *Anal. Biochem.* **1966**, *16*, 1.
26) T. Hashizume, Y. Sasaki, Protein, *Nucleic Acid and Enzyme* **1968**, *13*, 735.
27) Y. Masada, K. Hashimoto, T. Inoue, T. Sawada, *YAKUGAKU ZASSHI* **1969**, *89*, 734.
28) E. M. Chambaz, G. Maume, B. Maume, E. C. Horning, *Anal. Lett.* **1968**, *1(12)*, 749.

N,O-Bis(trimethylsilyl)acetamide (=BSA) 10 mL / 100 mL [B0511]

N,O-Bis(trimethylsilyl)acetamide Kit (=BSA Kit)

Contents of the Kit: reagent (1 mL) × 8, 2 mL blank vial for reaction×8 [B0911]



[B0511][B0911]

BSA is highly reactive towards alcohols and carboxylic acids, as well as nitrogenous compounds (such as amino acids,[4,5,7] amides, ureas,[4] phenols, carboxylic acids, enol compounds,[3] sulfonic acids, steroids,[9,10,11] nucleic acids,[2] sugars[1,8]).

[General Procedure]

BSA is added to 10-50 mg of a sample placed into a dry vessel, and the vessel is sealed tightly. If necessary, it is heated at 70-80 °C for 30 min-1 h.

[Application Examples]

Steroids

0.2 mL of *N*-trimethylsilylimidazole (= SIM) is added to 1-5 mg of a sample in 0.1 mL of pyridine. After sealing the vessel, the mixture is left for 0.5-1 h at room temperature. The mixture usually can be used for a sample for GC. In the case of a sterically hindered alcohols, it is recommended to use BSA and TMCS together with SIM. For ketosteroids, after leaving for 3 h at room temperature, 0.2 mL of BSA is added and left for further 2 h at room temperature. The resulting transparent solution can be used for GC. By this method, the carbonyl group is converted into an enol TMS ether, and these derivatives can bevery useful for GC applications. Furthermore, the reaction improves by adding a trace ammounts of TMCS.

Cortol[11]

Trimethylsilylation of cortol with 3:3:2 volume mixture of SIM, BSA and TMCS affords penta-TMS derivative cortol. If BSA is used alone, hydroxyl groups at 3-, 20-, and 21-position are trimethylsilylated. When BSA and TMCS are used together, hydroxyl groups at 3-, 11-, 20-, and 21-position are trimethylsilylated.

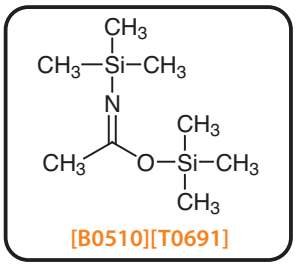
Sulfonic acids and Sulfonate salts

After the conversion to thiol derivatives, BSA is added. The mixture is trimethylsilylated by leaving for 10 min at *ca*. 80 °C.

N,O-Bis(trimethylsilyl)acetamide (25% in Acetonitrile) (=TMS-BA) 12 mL [B0510]

N,O-Bis(trimethylsilyl)acetamide Kit (25% in Acetonitrile) (=TMS-BA Kit)

Contents of the Kit: reagent (1 mL) × 8, 2 mL blank vial for reaction×8 [T0691]



[B0510][T0691]

TMS-BA is an acetonitrile solution of bis(trimethylsilyl) acetamide. It may be separated into two layers in winter or when stored in a cold place. If so, it should be homogenized by heating and shaking before use.

[Application Example]

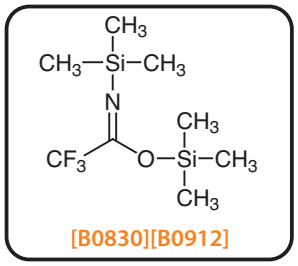
Equivalent to BSA's.

[General Procedure]

1. *ca*. 1 mg of sample and 1 mL of TMS-BA is placed into a dry vessel (about 2 mL of vial is preferable). After sealing the vessel, the reaction is proceeded by shaking or heating (e.g. for leucine, threonine, by heating for 15 min at 125 °C) to result a transparent solution. And then it is directly injected into GC.
2. *ca*. 0.5-1 mg of steroid is dissolved in 0.05-0.1 mL of an appropriate solvent (such as pyridine and acetonitrile) and the mixture is poured into 1 mL of TMS-BA. It is left at room temperature or the vial is directly heated with water bath (e.g. for estriol, for 20 min at 78-80 °C), and then it can be used as GC sample..

Note: If a liquid is injected into the sealed reaction vial in the kit, it is recommended to reduce air pressure in the vial in advance by using a syringe.

N,O-Bis(trimethylsilyl)trifluoroacetamide (=BSTFA) 5 mL / 25 mL / 100mL [B0830]
N,O-Bis(trimethylsilyl)trifluoroacetamide Kit (=BSTFA Kit)
Contents of the Kit: reagent (1mL) vial×8, 2mL empty reaction vial×8 [B0912]


[B0830][B0912]

Equivalent to BSA. BSTFA is useful in Flame Ionization Detector (FID), and excels in activity, volatility, and solubility as compared to BSA. BSTFA by-products have high volatility and minimally disturb the analysis on GC compared to BSA. It is particularly suitable for trimethylsilylation of amino acids.[4-6,13,14] (e.g. for alanine and valine, they can be trimethylsilylated by heating at 125 °C for 15 min.)

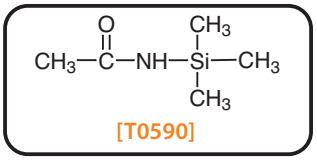
[Application Example]
Amino acids[12]

1 mg of a dry sample is placed into a vial, and then 0.24 mL each of acetonitrile and BSTFA is added. After sealing the vial, it is shaken to become a homogenized solution, and then heated with an oil bath (150 °C) for 15 min. After cooling, it can be used as a GC sample.

[References]

1) H. Mizunuma, K. Minakawa, *Igaku No Ayumi* **1968**, *65,* 679.
2) T. Hashizume, Y. Sasaki, Protein, *Nucleic Acid and Enzyme* **1968**, *13*, 735.
3) S. Ito, T. Nishina, M. Kitamura, *Rinsyoubyouri* **1968**, *16,* 599.
4) F. Shahrokhi, C. W. Gehrke, *J. Chromatogr.* **1968**, *36*, 31.
5) E. D. Smith, K. L. Shewbart, *J. Chromatogr. Sci.* **1969**, *7*, 704.
6) M. R. Guerin, W. D. Shults, *J. Chromatogr. Sci.* **1969**, *7*, 701.
7) K. A. Caldwell, A. L. Tapple, *J. Chromatogr.* **1968**, *32*, 635.
8) Y. Masada, K. Hashimoto, T. Inoue, T. Sawada, *YAKUGAKU ZASSHI* **1969**, *89,* 734.
9) E. C. Horning, M. G. Horning, N. Ikekawa, E. M. Chambaz, P. I. Jaakonmaki, C. J. W. Brooks, *J. Gas Chromatogr.* **1967**, *5*, 283.
10) E. M. Chambaz, G. Maume, B. Maume, E. C. Horning, *Anal. Lett.* **1968**, *1(12)*, 749.
11) E. M. Chambaz, E. C. Horning, *Anal. Lett.* **1967**, *1(3)*, 201.
12) C. W. Gehrke, K. Leimer, *J. Chromatogr.* **1971**, *57*, 219.
13) C. W. Gehrke, H. Nakamoto, R. W. Zumwalt, *J. Chromatogr.* **1969**, *45,* 24.
14) C. W. Gehrke, K. Leimer, *J. Chromatogr.* **1970**, *53*, 201.
15) K. Bergström, J. Gürtler, R. Blomstrnd, *Anal. Biochem.* **1970**, *34*, 74.

N-Trimethylsilylacetamide (=*N*-TMS-acetamide) 25 g [T0590]


[T0590]

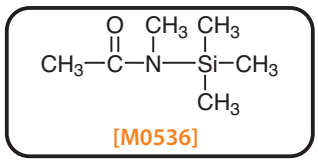
[Application Example]
Ascorbic acid (Vitamin C)

50 mg of a sample and 50 mg of octadecane (internal standard) is dissolved in 10 mL of dry pyridine. Next, 1.5 g of *N*-TMS-acetamide is added, and the mixture is left for over 4 h at room temperature.

[Reference]

M. Vecchi, K. Kaiser, *J. Chromatogr.* **1967**, *26*, 22.

N-Methyl-*N*-trimethylsilylacetamide (=*N*-Methyl-*N*-TMS-acetamide) 10 g / 25 g [M0536]


[M0536]

[Application Examples]

Amino acid

0.1 mL of *N*-Methyl-*N*-TMS-acetamide is added to a 1-2 mg samplevial and is sealed tightly and stirred for 5 min at room temperature. If the sample does not dissolve, heat to 60-100 °C. The formation of a transparent solution indicates reaction completion. The reaction is then directly injected into GC for analysis.

Others

amines, fatty acids, polyols, sugars, phenols, and alkylamines

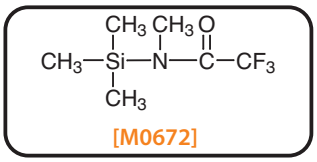
[Handling Precautions]

Avoid contact with moisture.
Store sealed under inert atmosphere in a fridge.
Do not inhale vapor.
Avoid contact with skin, eyes and clothing.

[Reference]

L. Birkofer, M. Donike, *J. Chromatogr.* **1967**, *26*, 270.

N-Methyl-*N*-trimethylsilyltrifluoroacetamide (=MSTFA) 5 mL / 25 mL [M0672]

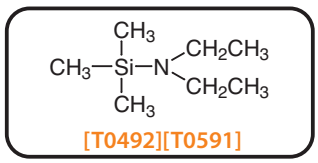

[M0672]

MSTFA is more volatile than BSTFA and BSA.[1] As its byproduct *N*-methyltrifluoroacetamide presents a further shorter retention time than MSTFA, overlapping of the peaks can be avoided. MSTFA works more effectively than BSTFA and BSA in the trimethylsilylation of steroids.[2] Amine hydrochlorides can be directly trimethylsilylated.

[References]

1) M. Donike, *J. Chromatogr.* **1969**, *42*, 103.
2) H. Gleispach, *J. Chromatogr.* **1974**, *91*, 407.

N-(Trimethylsilyl)diethylamine (=TMS-DEA) 25 mL [T0492]
N-(Trimethylsilyl)dimethylamine (=TMS-DMA) 25 mL [T0591]


[T0492][T0591]

[Application Examples]

Amino acid[1-4]

100 mol% excess of TMS-DEA or TMS-DMA (usually 1.5-2.0 mL) is added to a sample and is heated to reflux, which subsequently resulted in a transparent solution (It is preferable to remove the resulting diethylamine or dimethylamine by distillation). After cooling, it is diluted with benzene to a proper concentration to use directly as a GC sample. If a catalytic amount of TMCS or trichloroacetic acid is added, better results are acquired. This method is also applicable to samples other than amino acids.

Fatty acids in urine

0.15 mL of either TMS-DEA or TMS-DMA and 0.1 mL of TMCS is added to a trace amount of a sample in dry pyridine (0.1 mL), and then the mixture is left at room temperature.

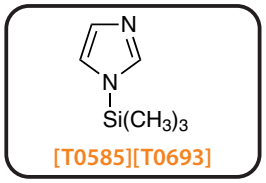
[References]

1) E. D. Smith, H. Sheppard, *Nature* **1965**, *208*, 878.
2) K. Rühlmann, W. Giesecke, *Angew. Chem.* **1961**, *73*, 113.
3) P. S. Mason, E. D. Smith, *J. Gas Chromatogr.* **1966**, *4*, 398.
4) E. D. Smith, K. L. Shewbart, *J. Chromatogr. Sci.* **1969**, *7*, 704.

TMS-Imidazole (=SIM, *N*-Trimethylsilylimidazole) 25 g / 100 g [T0585]

TMS-Imidazole Kit (=SIM Kit)

Contents of the Kit: reagent (1 mL) vial × 8, 2 mL blank vial × 8 [T0693]



[T0585][T0693]

TMS-Imidazole reacts only with hydroxyl groups, sugars, steroids, and uric acids.

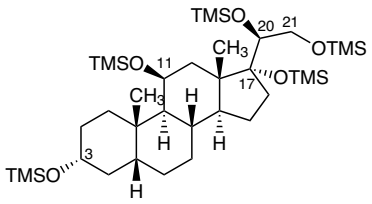
[Application Examples]

Steroids

0.2 mL of SIM (*N*-TMS-imidazole) is added to 1-5 mg of substrate in 0.1 mL of pyridine. After sealing a vial, the mixture is left for 0.5-1 h at room temperature. The mixture can usually be used as a GC sample. In the case of applying to a sterically hindered hydroxyl group, it is recommended to use BSA and TMCS together with SIM. For ketosteroids, after leaving 3 h at room temperature, 0.2 mL of BSA is added and is left for further 2 h at room temperature. The resulting transparent solution can be used for GC. By this method, carbonyl groups are converted into enol TMS ethers and these derivatives are very useful for GC. Furthermore, the reaction improves by adding a trace of TMCS.

Cortol[5)]

Trimethylsilylation of cortol with 3:3:2 volume mixture of SIM, BSA and TMCS affords penta-TMS derivative cortol. If BSA is used alone, the hydroxyl groups at 3-, 20-, and 21-position are silylated. When BSA and TMCS are used together, the hydroxyl groups at 3-, 11-, 20-, and 21-position are trimethylsilylated.



Trimethylsilylation by SIM, BSA, and TMCS

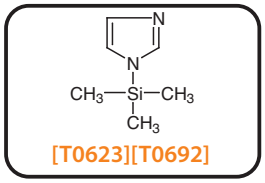
[Handling Precautions]

Avoid contact with moisture.
Store sealed under inert atmosphere in a fridge.
Do not inhale vapor.
Avoid contact with skin, eyes and clothing.

[References]

1) M. G. Horning, A. M. Moss, E. C. Horning, *Biochem. Biophys. Acta* **1967**, *148*, 597.
2) Y. Masada, K. Hashimoto, T. Inoue, T. Sawada, *YAKUGAKU ZASSHI* **1969**, *89*, 734.
3) E. C. Horning, M. G. Horning, N. Ikekawa, E. M. Chambaz, P. I. Jaakonmaki, C. J. W. Brooks, *J. Gas Chromatogr.* **1967**, *5*, 283.
4) E. M. Chambaz, G. Maume, B. Maume, E. C. Horning, *Anal. Lett.* **1968**, *1*, 749.
5) E. M. Chambaz, E. C. Horning, *Anal. Lett.* **1967**, *1 (3)*, 201.
6) M. G. Horning, A. M. Moss, E. A. Boucher, E. C. Horning, *Anal. Lett.* **1968**, *1*, 311.
7) L. T. Sennello, *J. Chromatogr.* **1971**, *56*, 121.

TMS-PZ (=N-Trimethylsilylimidazole in Anhydrous Pyridine) 12 mL [T0623]
TMS-PZ Kit Contents of the Kit: reagent (1 mL) vial × 8, 2 mL blank vial × 8 [T0692]


[T0623][T0692]

TMS-PZ is useful for the trimethylsilylation of aqueous sugar solutions. Although trimethylsilylating reagents normally need to be used under dried conditions, TMS-PZ can be used in aqueous sugar solutions.

[Application Example]

10% aqueous solution of sugar (5-10 µL) is poured into 1 mL of TMS-PZ. After the generation of slight heat, the mixture is shaken for 30 s, and is left for 5 min at room temperature or heated to 60-70 °C (e.g. for raffinose, it is heated to 60-70 °C (bath temperature) for 15 min). The resulting clear solution is directly injected into GC.

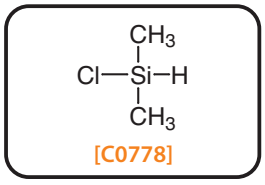
Note: If a liquid is poured into the sealed reaction vial in the kit, it is recommended to reduce air pressure of the vial in advance by using a syringe.

[Handling Precautions]

Store under inert atmosphere in a fridge.
Do not inhale vapors.
Avoid contact with skin, eyes and clothing.

Dimethylsilylating Reagent

Chlorodimethylsilane (=DMCS) 25 mL / 250 mL [C0778]


[C0778]

These reagents are for the preparation of dimethylsilyl ethers, which are more volatile than TMS ethers. TMDS and DMCS (as a catalyst) are used together.

[Handling Precautions]

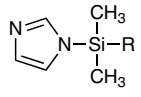
DMCS is decomposed by moisture to emit hydrogen chloride gas.

[References]

1) W. R. Supina, *et al*., *J. Am. Oil Chem. Soc.* **1967**, *44*, 74.
2) W. J. A. Vanden Heuvel, *J. Chromatogr.* **1967**, *27*, 85.
3) W. W. Wells, *et al*. in "*Biomedical Applications of Gas Chromatography.*" *H. A. Szymanski, Ed., Plenum Press, New York.* **1964**, 199.

Dimethylalkylsilylating Reagents

1-(Dimethylethylsilyl)imidazole 1 g / 5 g [D1516]
1-(Dimethylisopropylsilyl)imidazole 1 g / 5 g [D1596]
1-(*tert*-Butyldimethylsilyl)imidazole 1 g / 5 g [B1043]



R= C_2H_5 [D1516]
$CH(CH_3)_2$ [D1596]
$C(CH_3)_3$ [B1043]

Dimethylalkylsilylating reagents are used for structural analysis of hydroxysteroids by GC-MS and for analysis of prostaglandins, bile acids, and thromboxane.

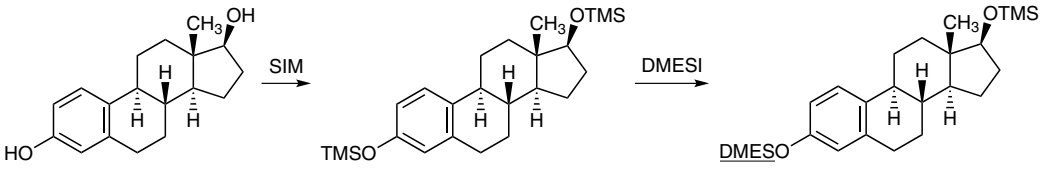
In Gas Chromatography-Mass Spectrometry (GC-MS) of hydroxysteroids, trimethylsilylating reagents such as *N*-trimethylsilylimidazole (SIM) are used in the preparation of derivatives. However, it is sometimes difficult to distinguish between alcoholic hydroxyl group and phenolic group when analyzing the structures of unknown compounds.

The dimethylalkylsilylating (DMAS) reagents have been studied and developed to improve upon the disadvantages of SIM.[1-3,8)]

The DMAS reagents are prepared by replacing one of methyl group of SIM with an alkyl group. The reaction with hydroxyl groups proceeds rapidly at room temperature similarly to TMS reagents (If the sample has a sterically hindered hydroxyl group, the reaction needs to be heated to 100 °C).

The DMAS ethers are generally more stable than the corresponding TMS ethers and also show better separation resolution in GC. The number of hydroxyl groups can be detected by comparing the methylene unit (MU) with trimethylsilylated compounds. This facilitates an accurate structural analysis of steroids by MS. It is also used for the trace analysis of biological samples such as prostaglandins[6,9,11-13)] and bile acids.[4,5,7,10)]

Trimethylsilyl ethers from the phenolic hydroxyl group have a characteristic to exchange to DMAS ethers, and vice versa in GC by "sandwich injection". This reactivity enables us to distinguish between alcoholic hydroxyl groups and phenolic groups by GC-MS.



Exchange reaction of β–estradiol from a TMS group to a DMES group

The following is an example of silylation (sandwich injection) of β–estradiol by 1-(Dimethylethylsilyl)imidazole (DMESI).

[Application Example] (Sample: β–estradiol)

Ⅰ. Procedure of the exchange reaction from TMS to DMES by "Sandwich injection"

1. Preparation of β–estradiol bis-TMS ether: 0.1 mg of β–estradiol is prepared in a sealable vial and is dissolved in 20 µL of SIM. The mixture is allowed to react for 30 min at room temperature.
2. Sandwich injection: 0.2 µL of DMESI, 0.1 µL of the mixture prepared in procedure 1 and 0.2 µL of DMESI are taken with a microsyringe successively, and then injected into the GC in one shot.

II . Gas Chromatogram

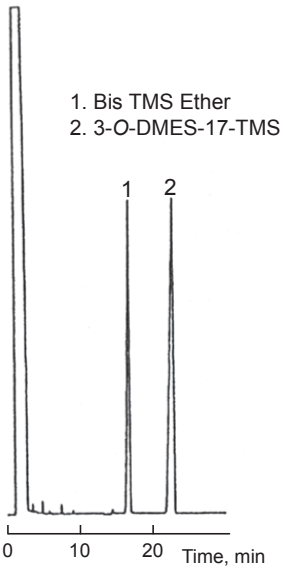


Figure 1. TMS→DMES

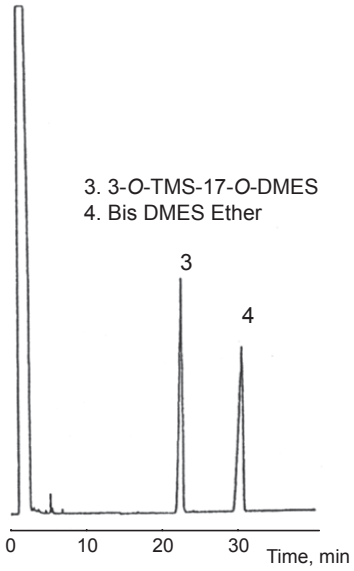


Figure 2. DMES→TMS

GC Condition of Figure 1. and Figure 2.

Column	:	007-1
		25 m × 0.53 mm I. D. × 1 μm
Temperature	:	250 °C
Detector	:	FID:23 × 27, Inj.:300 °C,
		Splitless Injection
Carrier Gas	:	He 0.3 kg/cm^2, 30 cm/s

[References]

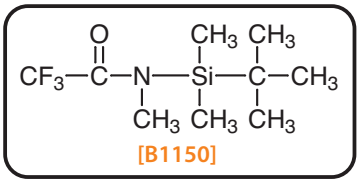
1) H. Miyazaki, M. Ishibashi, M. Itoh, T. Nambara, *Chem. Pharm. Bull.* **1975**, *23*, 3033.
2) H. Miyazaki, M. Ishibashi, M. Itoh, T. Nambara, *Biomed. Mass Spectrom.* **1977**, *4*, 23.
3) H. Miyazaki, M. Ishibashi, M. Itoh, K. Yamashita, T. Nambara, *J. Chromatogr.* **1977**, *133*, 311.
4) Y. Nishikawa, K. Yamashita, M. Ishibashi, H. Miyazaki, *Chem. Pharm. Bull.* **1978**, *26*, 2922.
5) H. Miyazaki, M. Ishibashi, K. Yamashita, *Biomed. Mass Spectrom.* **1978**, *5*, 469.
6) H. Miyazaki, M. Ishibashi, K. Yamashita, M. Katori, *J. Chromatogr.* **1978**, *153*, 83.
7) A. Fukunaga, Y. Hatta, M. Ishibashi, H. Miyazaki, *J. Chromatogr.* **1980**, *190*, 339.
8) H. Miyazaki, M. Ishibashi, K. Yamashita, *Biomed. Mass Spectrom.* **1979**, *6*, 57.
9) H. Miyazaki, M. Ishibashi, K. Yamashita, *Biomed. Mass Spectrom.* **1979**, *6*, 57.
10) H. Miyazaki, M. Ishibashi, H. Takayama, K. Yamashita, I. Suwa, M. katori, *J. Chromatogr.* **1984**, *289*, 249.
11) S.H.G. Andersson, J. Sjövall, *J. Chromatogr.* **1984**, *289*, 195.
12) H. Miyazaki, M. Ishibashi, K. Yamashita, Y. Nishikawa, M. Katori, *Biomed. Mass Spectrom.* **1981**, *8*, 521.
13) H. Miyazaki, *et al.*, *J. Chromatogr.* **1982**, *239*, 595.
14) Y. Harada, H. Miyazaki, *et al.*, *Prostaglandins* **1982**, *23*, 881.

[Related Products]

Dimethylethylchlorosilane	5 g / 25 g [D0135]
Chlorodimethylpropylsilane	5 mL / 25 mL [D1590]
Dimethylisopropylchlorosilane	5 mL / 25 mL [D1594]
tert-Butyldimethylchlorosilane	5 g / 25 g / 100 g [B0995]
N-Trimethylsilylimidazole	25 g / 100 g [T0585]
N-(*tert*-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide	1 g / 10 g [B1150]

tert-Butyldimethylsilylating Reagent

***N*-(*tert*-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide (=MTBSTFA)** 1 g / 10 g [B1150]



[B1150]

MTBSTFA is used for *tert*-butyldimethylsilylation of hydroxyl group, carboxyl group, thiol group and amino group.

tert-Butyldimethylsilylated (TBDMS or TBS) derivative is widely used for synthesis of natural products and GC-MS analysis because of its relative stability in the presence of water and highly reactive reagents (e.g. Wittig reagents, CrO_3, RMgX and RLi) and its ease of handling.

The TBDMS-Cl / Imidazole / DMF reaction conditions[1)] are generally applied when introducing *tert*-butyldimethylsilyl group. However, it is chellenging to *tert*-butyldimethylsilylate thiol groups, amino groups, and sterically hindered hydroxyl groups. Fortunately, MTBSTFA is an effective silylating agent for these functional groups. The reaction can be completed in 5-20 min at room temperature in most cases, and the reaction mixture can directly be injected into GC.

MTBSTFA is used for GC or GC-MS analysis of thiols,[2,15)] amines,[2)] polyamines,[5)] amino acids,[2,6,8,9)] dipeptides,[11)] ketone bodies,[6,7)] fatty acids,[6,10,13,16)] hydroxyeicosatetraene acids,[12,14)] leucotrienes[12)] and alkylphosphonic acids,[17)] and also is used for GC-MS analysis of prostaglandins[3)] and oxygen-containing anions.[4)]

[References]

1) E. J. Corey, *et al.*, *J. Am. Chem. Soc.* **1972**, *94*, 6190.
2) T. P. Mawhinney, *et al.*, *J. Org. Chem.* **1982**, *47*, 3336.
3) A. C. Bazan, *et al.*, *J. Chromatogr.* **1982**, *236*, 201.
4) T. P. Mawhinney, *J. Chromatogr.* **1983**, *257*, 37.
5) N. G Lay-Keow, *J. Chromatogr.* **1984**, *314*, 455.
6) W. F. Schwenk, *et al.*, *Anal. Biochem.* **1984**, *141*, 101.
7) J. M. Miles, *et al.*, *Anal. Biochem.* **1984**, *141*, 110.
8) C. J. Biermann, *et al.*, *J. Chromatogr.* **1986**, *357*, 330.
9) T. P. Mawhinney, *et al.*, *J. Chromatogr.* **1986**, *358*, 231.
10) T. P. Mawhinney, *et al.*, *J. Chromatogr.* **1986**, *361*, 117.
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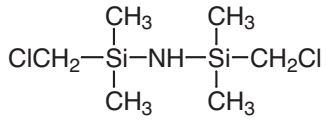
12) S. Steffenrud, *et al.*, *J. Chromatogr.* **1987**, *423*, 1.
13) K. Kim, *et al.*, *HRC&CC* **1987**, *10*, 522.
14) S. Steffenrud, *et al.*, *J. Chromatogr.* **1987**, *416*, 219.
15) D. C. Landrum, T. P. Mawhinney, *J. Chromatogr.* **1989**, *483*, 21.
16) K. R. Kim, *et al.*, *J. Chromatogr.* **1989**, *468*, 289.
17) J. G. Purdon, *et al.*, *J. Chromatogr.* **1989**, *475*, 261.

[Related Products]

1-(*tert*-Butyldimethylsilyl)imidazole	1 g / 5 g [B1043]
Allyldimethylsilyl Chloride	10 mL / 25 mL [A1275]

Halomethyldimethylsilylating Reagents [for GC-ECD]

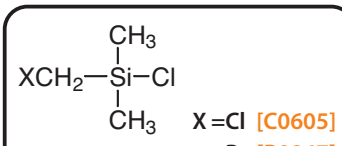
1,3-Bis(chloromethyl)tetramethyldisilazane 5 g [B0990]



[B0990]

1,3-Bis(chloromethyl)tetramethyldisilazane is used for carboxylic acid,[2] Acids,[2] phenols,[2] steroids[1,3] and sugars. Used together with CMDMCS.

(Chloromethyl)dimethylchlorosilane (=CMDMCS) 25 g / 250 g [C0605]
(Bromomethyl)dimethylchlorosilane (=BMDMCS) 5 g / 25 g [B0847]



X =Cl [C0605]
=Br [B0847]

CMDMCS and BMDMCS are used for carboxylic acid,[2] Acids,[2] phenols[2] and steroids.[1]

Halomethylsilylating reagents are highly effective when detecting trace amounts of components by an Electron Capture Detector (ECD).

[Application Example]
How to use halomethyldimethylsilyldiethylamine solution[1,2]

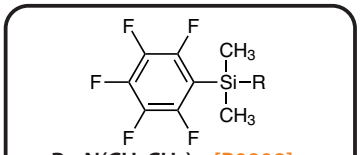
1 mL of hexane, 0.075 mL of diethylamine and 0.09 mL of (halomethyl)dimethylchlorosilane are mixed in a sealable vessel and centrifuged. 0.4 mL of the resulting supernatant is added to 100 µg of sample in 0.1 mL of ethyl acetate and refluxed for 30 min at 65 °C. The mixture is then promptly cooled to room temperature followed by adding hexane to adjust to the appropriate concentration. This solution is injected into GC.

[References]

1) C. Eaborn, D. R. M. Walton, *Chem.& Ind.* **1967**, 827.
2) C. A. Bache, L. E. St. John, D. J. Lisk, *Anal. Chem.* **1968**, *40*, 1241.
3) B. S. Thomas, D. R. M. Walton, *"The Gas Liquid Chromatography of Steroids"* ed. by J. K. Grant p199.

Pentafluorophenyldimethylsilylating Reagents [for GC-ECD]

Pentafluorophenyldimethylsilyldiethylamine (=Flophemesyldiethylamine) 100 mg [P0908]
Pentafluorophenyldimethylchlorosilane (=Flophemesyl Chloroide) 1 mL / 5 mL [P0854]



R =N(CH2CH3)2 [P0908]
=Cl [B0854]

[Application Example]
Alcohols

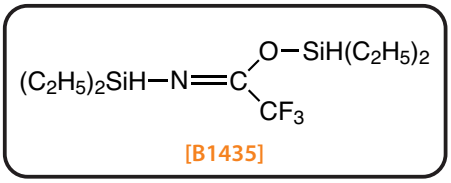
The substrate (primary alcohol) is dissolved in pyridine and 1:1 mixture of pentafl uorophenyldimethylsilyldiethylamine and pentafluorophenyldimethylchlorosilane is subsequently added. This mixture can be directly used for GC-ECD analysis. This can also be used for GC-MS analysis both in high selectivity and in high sensitivity. In the case of tertiary alcohols, the derivatization is completed by reacting for 10 min at 25 °C.

[Reference]

P. W. Burkinshaw, E. D. Morgan, *J. Chromatogr.* **1977**, *132*, 548.

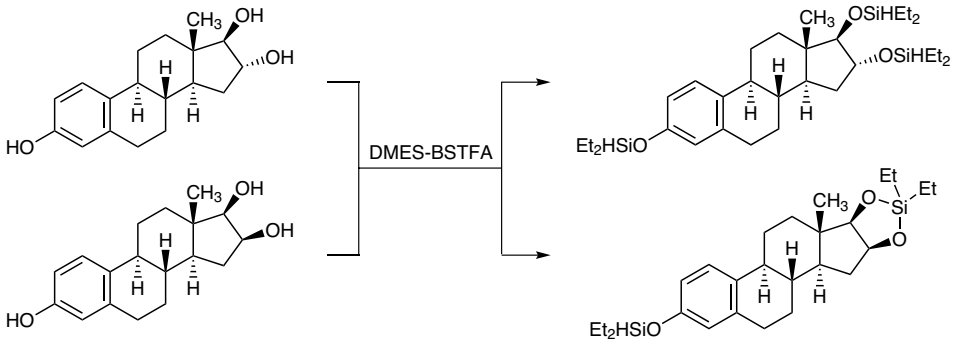
Simultaneous Cyclic Silylene and Silyl Derivatizing Reagent

N,O-Bis(diethylhydrogensilyl)trifluoroacetamide (=DEHS-BSTFA) 1 g [B1435]



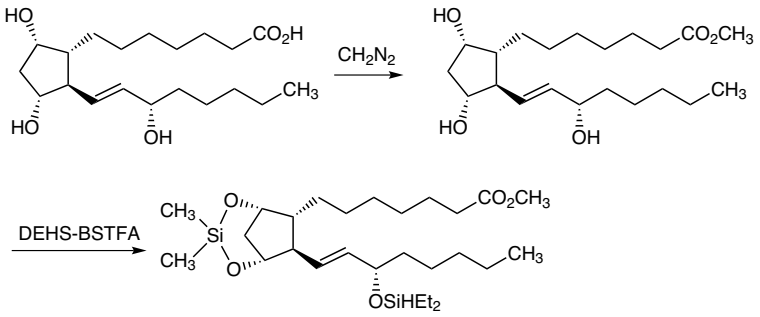
[B1435]

The analysis of 1,2- and 1,3- diols in GC's frequently involves their conversion to cyclic boronate or di-*tert*-butylsilylene derivatives. Nevertheless, for compounds with an isolated hydroxyl group, the hydroxyl group remains unreacted, and necessitates a secondary treatment such as an additional trimethylsilylation to achieve protection.



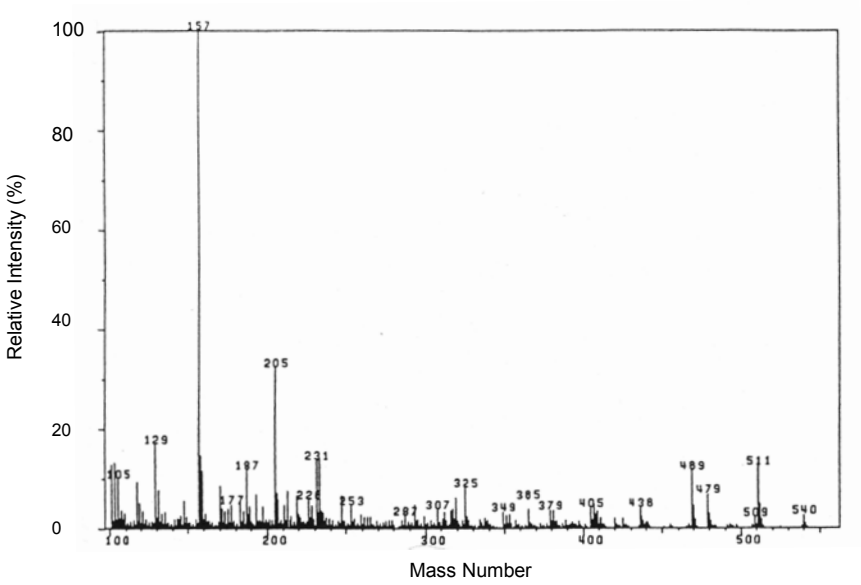
Miyazaki *et al*. have developed a single step derivatization reaction that produces both cyclic diethylsilylene (DES) from 1,2- and 1,3-diols and a diethylhydrogensilyl ether (DEHS) from a hydroxyl group by applying DEHS-BSTFA to hydroxysteroids.[1)] According to their results, as for hydroxyl groups on D rings, 1,2-*cis* diol produces cyclic DES selectively. (See above equation)

The ratio of cortisol and its metabolite 6β-hydroxycortisol in urine has been received attention as potential indicators for the function of hepatic drug-metabolizing enzymes. The MO-TMS method is generally used for ketosteroids analysis but it is not suitable for cortisol and 6β-hydroxycortisol due to difficulties encountered during separation. Ishibashi *et al*. have developed a method making it possible to simultaneously quantify the constituents in urine by converting them to MO-DEHS-DES derivatives using DEHS-BSTFA. Furthermore, Goto *et al*. have reported the use of DEHS-BSTFA as a derivatizing reagent for GC-MS analysis of abnormal bile acids containing a hydroxyl group at 4th and 6th position in fetuses and neonates.



Ishibashi *et al.* have used DEHS-BSTFA to induce F_{α}PG (e.g. prostaglandin (PG) $F_{1\alpha}$, $F_{2\alpha}$, and 6-keto $PGF_{1\alpha}$, and 13,14-dihydro-15-keto $PGF_{2\alpha}$), thromboxane (TX) B_2 and 11-dehydro TXB_2 to cyclic DES derivatives. Detailed analysis by GC/MS have indicated that the resulting cyclic DES derivatives show a characteristic mass spectrum.[3,4)]

In this way, DEHS-BSTFA is used as an effective derivatization reagent for GC-MS analysis of hydroxysteroids, bile acids, and prostaglandins.



Mass spectrum of DEHS-DES derivatives of $PGF_{1\alpha}$ Methyl Ester

[References]

1) H. Miyazaki, M. Ishibashi, M. Itoh, K. Yamashita, *Biomed. Mass Spectrom.* **1984**, *11*, 377.
2) M. Ishibashi, H. Takayama, Y. Nakagawa, N. Harima, *Chem. Pharm. Bull.* **1988**, *36*, 845.
4) M. Ishibashi, K. Watanabe, K. Yamashita, *J. Chromatogr.* **1987**, *391*, 183.
5) K. Watanabe, M. Ishibashi, N. Harima, S Krolik, *Chem. Pharm. Bull.* **1989**, *37*, 140.

Esterification

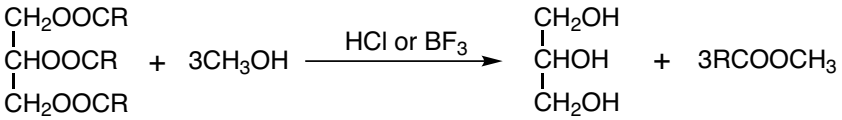
Acid Catalyst in Anhydrous Alcohols

BF_3 - Butanol Reagent (10-20%)	1 mL×10 [X0034]
BF_3 - Isopropanol Reagent (10-20%)	1 mL×10 [X0035]
BF_3 - Propanol Reagent (10-20%)	1 mL×10 [X0037]
BF_3 - Methanol Reagent (10-20%)	1 mL×10 [X0036]
HBr - Ethanol Reagent (10-20%)	25 mL / 100mL / 500 mL [H0959]
HBr - Methanol Reagent (5-10%)	25 mL / 500 mL [X0043]
HCl - Butanol Reagent (5-10%)	1 mL×10 [X0039]
HCl - Methanol Reagent (5-10%)	1 mL×10 [X0038]
HCl - Methanol Reagent (5-10%)	25 mL / 100mL / 500 mL [X0041]

Experimental procedures differ from types of esterification reagents or purposes. Typical applications are shown below. Please refer to the references for details.

[General Procedures]

- 500 mg of substrate (e.g. stearic acid or linolenic acid) is placed into a test tube, and 1 mL of HCl-MeOH or BF_3-MeOH is added. After attaching a reflux condenser, the mixture is heated to reflux for about 0.5 - 1 h. Then cooled to room temperature, 1 mL of distilled water is added and followed by extraction with 1 mL of hexane. The hexane solution is directly injected into GC as a sample.
- After the esterification of trace fatty acids extracted from a biological sample, only esters will be obtained from the sample containing unsaponificated components by microsublimation.[1)]
- Free fatty acids from oil can be adsorbed onto a resin (Amberlite IRA-400) and can be directly esterified on the resin and subsequently extracted.[5)]
- When analyzing the composition of fatty acids in glycerides, esterification of free fatty acids (obtained by saponification) can be applicable. However, it is more convenient to obtain esters directly by transesterification since the reaction occurs in one step.



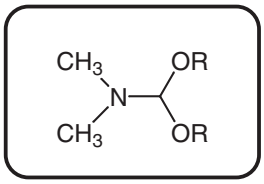
CAUTION: Wear appropriate PPE and open reaction vessles with extreme care after cooling, as it irritates the eyes, skin and bronchitis, and is also corrosive and may still be under pressure. Store in a cool place to avoid an increase in internal pressure of the container.

[References]

1) Esterification with HCl-alkanol W. Stoffel, *Anal. Chem.* **1959**, *31*, 307.
2) Esterification with BF_3-alkanol L. D. Metcalfe, *Anal. Chem.* **1961**, *33*, 363.
3) Ester interchange with HCl-alkanol M. E. Mason, *Anal. Chem.* **1964**, *36*, 583.
4) Ester interchange with BF_3-alkanol F. E. Luddy, *J. Am. Oil Chem. Soc.* **1968**, *45*, 549.
5) Esterification of absorbed fatty acid on resin Hornstein, *Anal. Chem.* **1960**, *32*, 540.
6) Esterification with BCl_3-2-Chloroethanol D. D. Woodhem, *J. Agr. Food Chem.* **1971**, *19*, 186.

N,*N*-Dimethylformamide Dialkylacetals

***N*,*N*-Dimethylformamide Dimethyl Acetal**	25 mL [D2071]
***N*,*N*-Dimethylformamide Dimethyl Acetal**	0.5 mL×10 [D1332]
***N*,*N*-Dimethylformamide Diethyl Acetal**	5 mL / 25 mL [D1294]
***N*,*N*-Dimethylformamide Dipropyl Acetal**	5 mL / 25 mL [D1301]
***N*,*N*-Dimethylformamide Dibutyl Acetal**	5 mL / 25 mL [D1302]
***N*,*N*-Dimethylformamide Di-*tert*-butyl Acetal**	5 mL / 25 mL [D1303]
***N*,*N*-Dimethylformamide Dineopentyl Acetal**	5 mL / 25 mL [D1595]



The listed compounds (except *N*,*N*-Dimethylformamide Dineopentyl Acetal) act as esterification reagents for fatty acids and can readily provide the corresponding alkyl esters. In addition, these reagents can be used to convert amino acids into the corresponding *N*-dimethylaminomethylene-*O*-alkyl esters in one step. These dimethylformamide acetals are liquid at room temperature, are easy to handle, and are stable at room temperature as long as stored away from moisture.

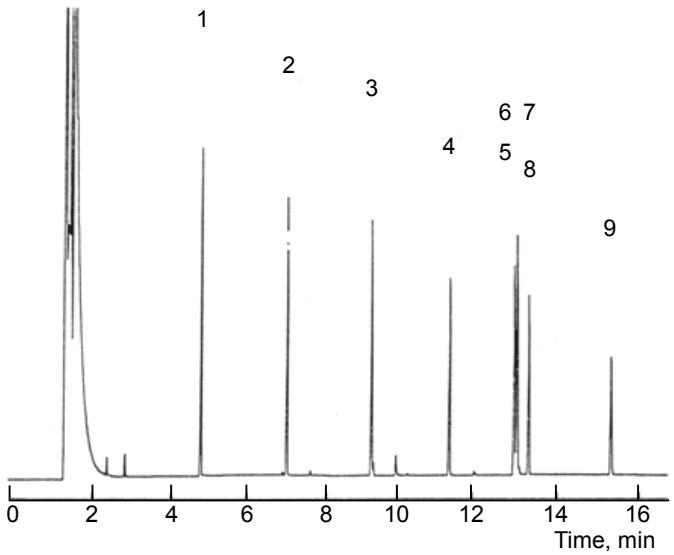
[Application Examples]

1. Esterification of fatty acids[1)]

$$R'COOH + (CH_3)_2NCH(OR)_2 \longrightarrow R'COOR + ROH + HCON(CH_3)_2$$

5 mg of fatty acid is placed into a vial and then 100 µL of an esterification reagent is added. The reaction is completed upon dissolution. The reaction mixture can be injected directly into GC.

Using this method, after washing with water, extraction and condensation procedures is generally not required. In addition, water is not produced as a byproduct during the reaction. If the sample is a solid with long carbon chains, a solvent can be added and heated slightly. The reaction time can be shortened for the completion if some samples are dissolved in a variety of solvents (e.g. pyridine, benzene, methanol, chloroform, dichloromethane, THF, DMF, etc.) because these reagents cannot be used as proper solvents.



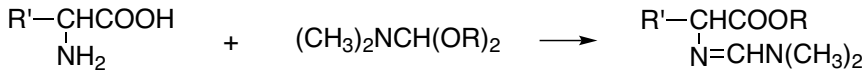
Chromatogram of fatty acids methylesterificated by DMF-DMA

GC Condition

1. C_{10}
2. C_{12}
3. C_{14}
4. C_{16}
5. $C_{18:2}$
6. $C_{18:1}$
7. $C_{18:3}$
8. C_{18}
9. C_{20}

Column	:	007-1, 25 m × 0.25 mm I. D. × 0.25 µm
Temperature	:	100 °C~(10 °C/min)~240 °C
Detector	:	FID: $2^3 \times 2^5$
Injection	:	300 °C
Carrier Gas	:	He: 0.9 kg/cm^2, 30 cm/s

2. Reaction with amino acids[2)]

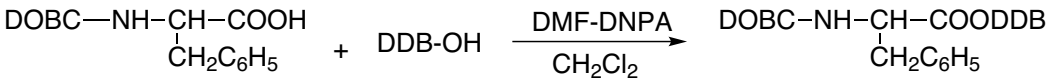


The reaction is completed when the reaction mixture becomes a solution. Although various reaction solvents can be used, acetonitrile is the most recommended for this reaction. Most amino acids react in acetonitrile solution (1:1) and the reaction is completed at 100 °C for 20 min, while aspartic acid requires longer reaction time.

An *N*-dimethylaminomethylene alkyl ester can be obtained from an amino acid by this reaction.

3. *N*,*N*-Dimethylformamide Dineopentyl Acetal (=DMF-DNPA)[3, 4)]

DMF-DNPA itself does not act as an esterification reagent but mediates esterification.



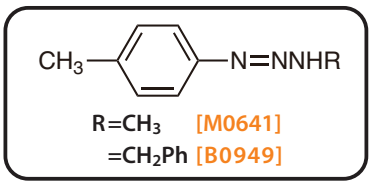
DOBC=n-$C_{10}H_{21}O$-$C_6H_4CH_2O$-CO-
DDB=n-$C_{12}H_{25}$-C_6H_4-CH_2-

[References]

1) J. P. Thenot, E. C. Horning, M. Stafford, M. G. Horning, *Anal. Lett.* **1972**, *5*, 217.
2) J. P. Thenot, E. C. Horning, *Anal. Lett.* **1972**, *5*, 519.
3) A. Kirrmann, J. J. Delpuech, *Compt. Rend.* **1965**, *260*, 6600.
4) J. J. Delpuech, *Bull. Soc. Chim. France* **1966**, 1624.

1-Alkyl-3-*p*-triazenes

1-Methyl-3-*p*-tolyltriazene 1 g / 25 g [M0641]
1-Benzyl-3-*p*-tolyltriazene 1 g / 25 g [B0949]



1-Alkyl-3-*p*-tolyltriazenes react with carboxylic acids rapidly under mild conditions to give the corresponding esters in high yields.[1)]

These reagents can also be used for alkylation of phenols,[2)] imides and enolized ketones.[3)] Furthermore, it has been reported that these reagents can be used for the alkylation of alcohols[3)] and thiols[4)] in the presence of a catalyst such as trimethoxyaluminium.

[Application Examples]

1. Methylesterification of 3,5-dinitrobenzoic acid[1b)]

25 mL of ether solution of a sample (1.50 g, 7.1 mmol) is slowly added to 10 mL of ether solution of 1-Methyl-3-*p*-tolyltriazene (1.05 g, 7.0 mmol) with occasional stirring. During solution addition, the reaction mixture turns red with the evolution of N_2. After

$$CH_3C_6H_4N{=}NNHCH_3 + (NO_2)_2C_6H_3COOH \longrightarrow (NO_2)_2C_6H_3COOCH_3 + N_2 + CH_3C_6H_4NH_2$$

the evolution of N_2 is completed (about 1 h), the ether solution is washed with 5M-HCl to remove the by-product toluidine. The mixture is washed with 5% sodium carbonate solution and dried over anhydrous Na_2SO_4. The ether is removed by concentration to obtain a methyl ester (1.11-1.42 g, 70-90%, light yellow-brown crystal, mp 106-107.5 °C). The residue is recrystallized from ether to give small plate crystal. (mp 107-107.5 °C). A variety of esters can be prepared from the other corresponding triazenes using this procedure.

2. Methylesterification of fatty acids and its application for GC

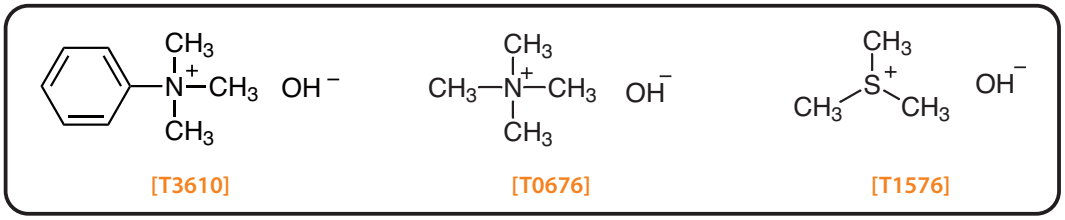
1 mL of 10% ether solution of 1-methyl-3-*p*-tolyltriazene is added to *ca*. 50 mg of mixture of fatty acid in a flask. The mixture is refluxed in a water bath for 30 min. After cooling, 1 mL each of hexane and HCl (1:10) are added and the mixture is shaken with periodic venting. After being left for some minutes , 1 µL of the hexane layer is injected into GC.

[References]

1) a) E. H. White, H. Scherrer, *Tetrahedron Lett*. **1961**, *21*, 758.
b) E. H. White, A. A. Baum, D. E. Eitel, *Org. Synth*. **1968**, *48*, 102.
c) *Ukrain. Khim. Zhur*. **1952**, *18*, 631.
2) *Ukrain. Khim. Zhur*. **1954**, *20*, 284.
3) *Ukrain. Khim. Zhur*. **1955**, *21*, 496.
4) *Ukrain. Khim. Zhur*. **1955**, *21*, 628.

On-Column Methyl Esterification Reagents

Phenyltrimethylammonium Hydroxide (=PTAH) (8.5% in Methanol) 25 mL / 100 mL [T3610]
Tetramethylammonium Hydroxide (=TMAH) (10% in Methanol) 25 mL / 100 mL / 500 mL [T0676]
Trimethylsulfonium Hydroxide (0.2mol/L in Methanol) 5 mL / 25 mL [T1576]



The following applications are for PTAH (8.5% in Methanol) and TMAH (10% in Methanol) as on-column methylation reagents in the sample vaporization chamber of GC's.

[Application Examples]

Methylesterification of fatty acids

Esterification by diazomethane is often carried out for GC analysis of heat-labile and relatively highly polar fatty acids. However, the reagent is difficult to handle due to its extreme toxicity and explosiveness, and the reaction often does not proceed quantitatively.

PTAH is very effective for "on-column methylation" and the reaction readily and rapidly proceeds quantitatively. Furthermore, it is safe and easy to handle. For example, Middleditch *et al*. showed efficient esterification and analysis in the separation of esterificated fatty acids.[9] Namely, 1 mg of fatty acid mixture is dissolved in 0.5 mL of 0.2 M methanol solution of PTAH at room temperature and 1 mL of the above mixture is directly injected into the sample vaporization chamber. In this reaction, it is hypothesized that phenyltrimethylammonium salts generated from the acids at room temperature, produce esters and dimethylaniline as a byproduct by thermolysis in vaporization chamber.

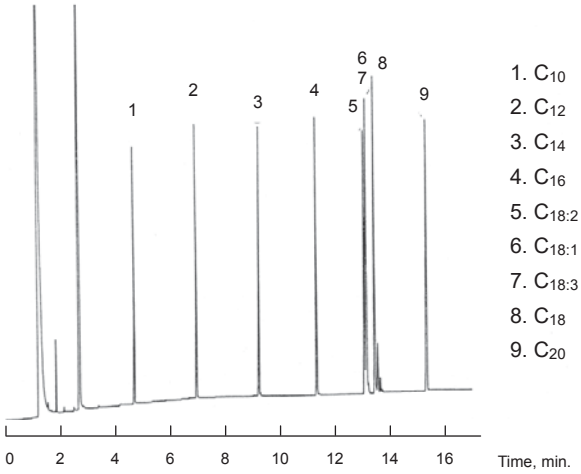
The use of TMAH includes the esterification of the carboxylic acids by Robb *et al*.[4] and the methylation of purine and pyrimidine bases.[5]

Methylation of barbituric acids

Martin *et al*.[3] have found they obtained better separation ability with sharp spectra peaks by injecting methylated barbituric acids into GC compared to injecting free acids directly.[2] However, it takes time and labor for methylation. Stevenson[1] has applied "on-column methylation" by TMAH to the analysis of barbituric acids. Namely, 1 mL of 0.1 M methanol solution of TMAH was added to each 1 mg of the acids and then the resulting mixture was partially injected into GC. They have found that the reaction proceeded quantitatively in the molar ratio 1:4, sample-reagent.

PTAH is also used as an "on-column methylation" reagent for barbituric acids,[6-8] sedatives,[6,8] xanthines,[5] phenolalkaloids,[7] diphenylhydantoin sodium salt,[8] etc. and gives good results for GC analysis.

500 μL of 0.2 M methanol solution of PTAH is added to 1 mg of fatty acid mixture and the resulting mixture is injected into the GC column.



1. C_{10}
2. C_{12}
3. C_{14}
4. C_{16}
5. $C_{18:2}$
6. $C_{18:1}$
7. $C_{18:3}$
8. C_{18}
9. C_{20}

Chromatogram of fatty acid mixture methylated by PTAH

GC Condition

Column	:	007-1,
		25 m × 0.25 mm I. D. × 0.25 μm
Temperature	:	100 °C~(10 °C/min)~240 °C
Detector	:	FID: $2^3 \times 2^5$
Injection	:	300 °C

[References]

1) G. W. Stevenson, *Anal. Chem.* **1966**, *38*, 1948.
2) A. B. Svendsen, *J. Pharm. Sci.* **1962**, *51*, 318.
3) H. F. Martin, J. L. Driscoll, *Anal. Chem.* **1966**, *38*, 345.
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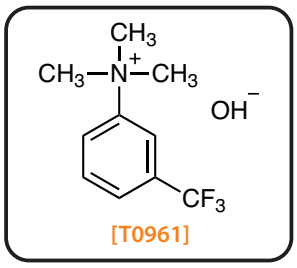
5) J. MacGee, *Anal. Biochem.* **1966**, *14*, 305.
6) *Chemical&Engineering News* **1971**, April 12, p.13.
7) E. Brochmann-Hanssen, T. O. Oke, *J. Pharm. Sci.* **1969**, *58*, 370.
8) M. J. Barrett, The Clinical Chemistry Newsletter p.3, No.1, Spring (1971). (published by the Perkin-Elmer Corp.)
9) B. S. Middleditch, D. M. Desiderio, *Anal. Letters* **1972**, *5*, 605.

3-(Trifluoromethyl)phenyltrimethylammonium Hydroxide (=*m*-TFPTAH) (5% in Methanol)

25 mL [T0961]



[T0961]

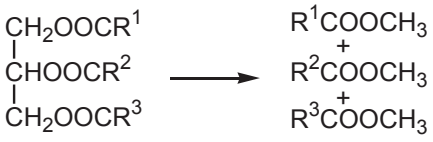
3-(Trifluoromethyl)phenyltrimethylammonium Hydroxide is used as an ester exchange reagent for triglycerides and others. It can used for the detection of triglyceride-constituent fatty acids by GC.

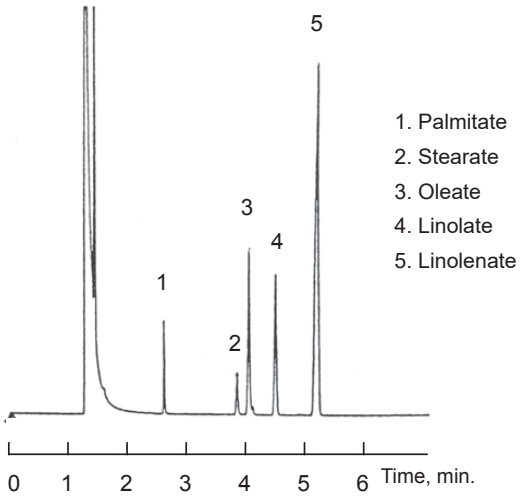
By injecting the mixture of *m*-TFPTAH and triglyceride into GC, chromatogram of methyl esters of triglyceride-constituent fatty acids can be obtained quantitatively. GC analysis of triglyceride-constituent fatty acids becomes substantially easier compared with a conventional methyl esterification method such as using sodium methoxide. *m*-TFPTAH reagent is easy to use and reacts with fatty acids without affecting double bonds in them. It also can be used as an on-column methylation reagent for fatty acids.[1,2)]

[Application Example]

Transesterification of linseed oil

10 mg of linseed oil in a vial is dissolved with 0.5 mL of toluene. Next, a 200 µL of 5% methanol solution of *m*-TFPTAH is added. The vial is tightly closed and left for 15 min at room temperature. 1 µL of the reaction mixture is directly injected into GC.





1. Palmitate
2. Stearate
3. Oleate
4. Linolate
5. Linolenate

Column : 007-23
0.25 mmI.D. × 25 m × 0.25 µm
Temperature : 180 °C

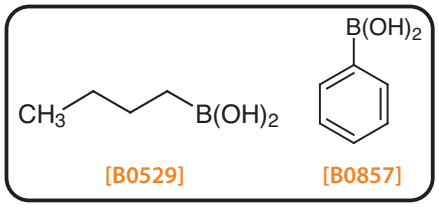
Capillary gas chromatogram of methyl esters of fatty acids from linseed oil

[References]

1) W. C. Kossa *et al.*, *J. Chromatogr. Sci.* **1979**, *17*, 177.
2) J. MacGee, K. G. Allen, *J. Chromatogr.* **1974**, *100*, 35.

Cyclic Boronate Esterification Reagents

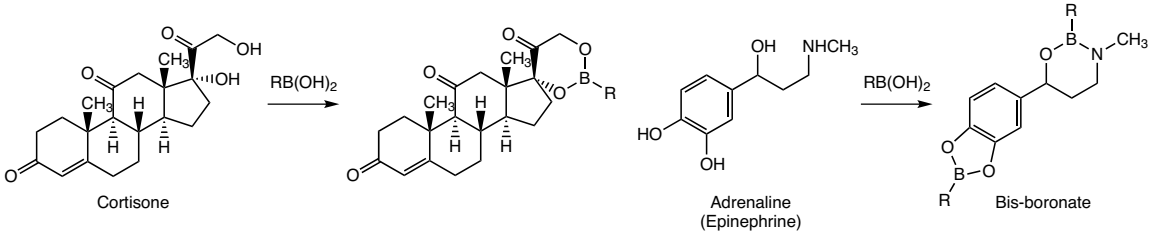
Butylboronic Acid (contains varying amounts of Anhydride) 1 g / 5 g / 25 g [B0529]
Phenylboronic Acid (contains varying amounts of Anhydride) 5 g / 25 g / 250 g [B0857]



These reagents readily react with diols, hydroxy acids and hydroxy amines at room temperature or by slight warming to generate cyclic boronates. They can be used for GC or GC-MS analysis of hydroxy acids (e.g. tartaric acid, lactic acid, salicylic acid), catecholamines, corticosteroids, and brassinolide.

[Application Example]
Corticosteroid[1,2)]

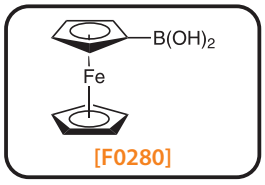
10 µmol each of steroids and butyl boronic acid are dissolved in 1 mL of ethyl acetate and the mixture is allowed to react for 5 min at room temperature.



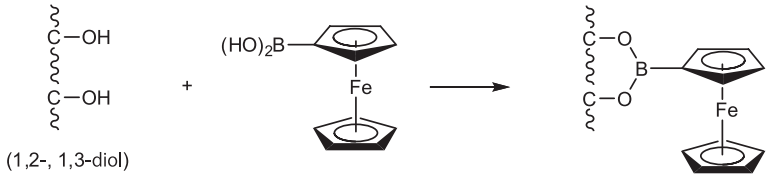
[References]

1) C. J. W. Brooks, *et al.*, *J. Chromatogr.* **1971**, *54*, 193.
2) C. J. W. Brooks, *et al.*, *J. Chromatogr. Sci.* **1971**, *9*, 18.

Ferroceneboronic Acid (contains varying amounts of Anhydride) 100 mg / 1 g [F0280]



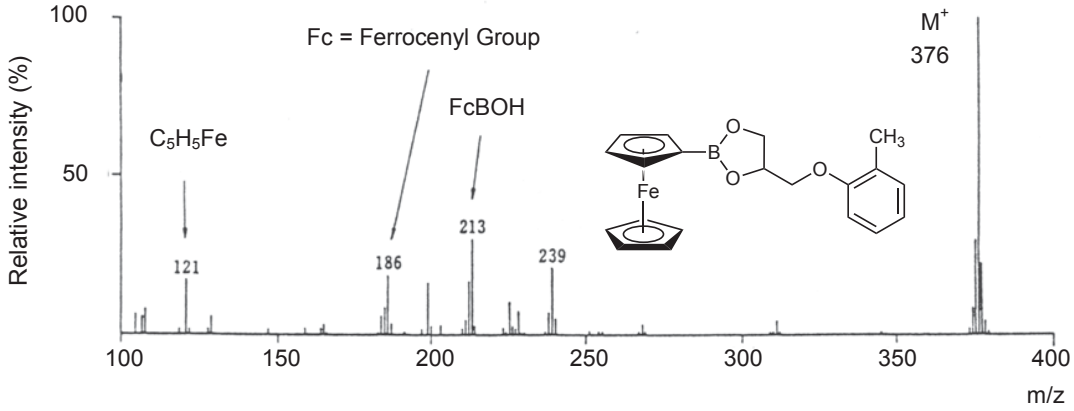
Alkyl boronic acids react with diols to form cyclic boronates, and are used for both GC and GC-MS analysis. The ferroceneboronic acid is a useful derivatizing reagent especially for GC-MS analysis.



Brooks *et al.* have reported that cyclic boronate derivatives give a characteristic spectrum in the Electron Impact (EI) MS (an example is shown in the figure below). The derivatives show strong molecular ion peaks and isotope peaks derived from the isotope atoms such as ^{10}B, ^{54}Fe and ^{57}Fe, which consequently facilitate the identification of diols. Moreover, major fragment ions are derived from reagent molecules, not from sample molecules (in the figure, m/z 239, 213, 186 and 121). Therefore, it is suitable for mass chromatography by SIM.

[General method for cyclic boronate derivatives]

100 µL of substrate is dissolved in dry pyridine. 1.1 equiv. of ferroceneboronic acid is dissolved in dry pyridine and added to the sample solution. The reaction mixture is allowed to react at 70 °C for 30 min and then pyridine is removed by nitrogen gas flow. The resulted mixture is dissolved in 100 µL of ethyl acetate and used as a sample for GC or GC-MS.



Mass spectrum of mephenesin cyclic boronate derivative

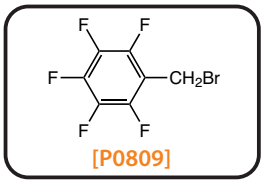
[Reference]

C. J. W. Brooks, W. J. Cole, *J. Chromatogr.* **1986**, *362*, 113.

Pentafluorobenzyl Esterificatoin Regent [for GC-ECD]

Pentafluorobenzyl Bromide

1 g / 5 g / 25 g [P0809]



[P0809]

[Application]

For carboxylic acids, phenols,[1] sulfonamides,[2] thiols and organic acids.[3-6]

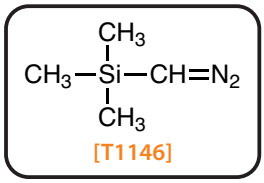
[References]

1) H. Ehrsson, *Acta Pharmaceutica Suecica* **1971**, *8*, 113.
2) O. Gylledhaal, H. Ehrsson, *J. Chromatog.* **1975**, *107*, 327.
3) F. K. Kawahara, *Anal. Chem.* **1968**, *40 (6)*, 1009.
4) F. K. Kawahara, *Anal. Chem.* **1968**, *40 (13)*, 2073.
5) F. K. Kawahara, *Environ Sci. & Tech.* **1971**, *5 (3)*, 235.
6) F. K. Kawahara, *Environ Sci. & Tech.* **1976**, *10 (8)*, 761.

Safe Methyl Esterification Reagent

Trimethylsilyldiazomethane (=TMS-Diazomethane) (*ca.* 10% in Hexane, *ca.* 0.6 mol/L)

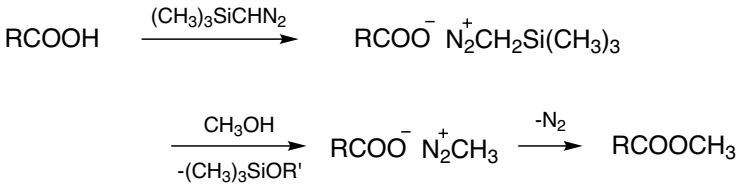
10 mL / 25 mL / 100 mL [T1146]



[T1146]

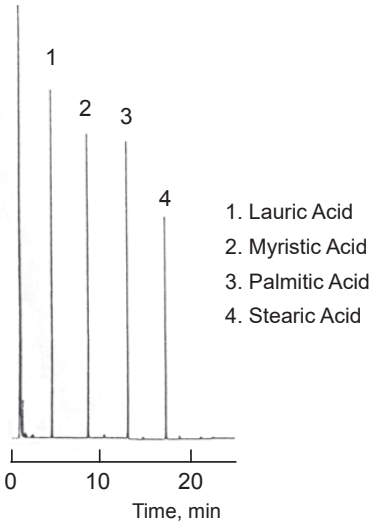
Diazomethane has long been used as a standard reagent for methyl esterification. However, it has many disadvantages including its high toxicity (e.g. acute and carcinogenic) and explosion hazard, and it also requires a detailed preparation before use.

On the contrary, TMS–diazomethane has low toxicity. Moreover, it can form methyl esters from various kinds of carboxylic acids quickly and quantitatively in the presence of methanol.



[Application Example]

0.1 mmol of fatty acids is dissolved in 1 mL of benzene containing 20% methanol and then 0.5 mL of this reagent is added. The mixture is stirred vigorously and left at room temperature for 30 min and used as a GC sample.



1. Lauric Acid
2. Myristic Acid
3. Palmitic Acid
4. Stearic Acid

GC Condition

Column : 007-5
25 m × 0.25 mm I. D. × 0.25 µm
Temperature : 150 °C → 250 °C (5 °C/min)
Carrier Gas : He 30 cm/s

[Reference]

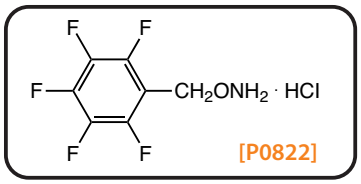
N. Hashimoto, T. Aoyama, T. Shioiri, *Chem. Pharm. Bull.* **1981**, *29*, 1475.

Other Pretreatment

Reagent for Preparation of Ketosteroid Oxime [for GC-ECD]

O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine Hydrochloride (=*O*-PFBHA·HCl)

1 g / 5 g [P0822]



O-(2,3,4,5,6-Pentafluorobenzyl)hydroxyamine hydrochloride (*O*-PFBHA·HCl) is an oxime derivatizing reagent used to detect trace amount of ketosteroids such as testosterone and progesterone by GC analysis with an electron capture detector (ECD). [1,2)]

GC analysis with ECD has been extensively carried out for the analysis of steroids in biological tissue. However, only a few steroids have sufficient electron captivity and thus a variety of derivatizing reagents with electron capture groups have been studied and developed in order to increase the detection sensitivity. Although perfluorocarboxylic chlorides or anhydrides[3)] are commonly used as esterification reagents for this purpose, they produce strong acids as a byproduct, which also reacts with steroids. In addition, it is known that incorrect recognition in analysis can occur since one steroid can often form a number of isomeric derivatives, resulting in multiple peaks. Pentafluorophenylhydradine[4,5)] has a disadvantage with the thermostability of its derivatives formed on steroids are not sufficient.

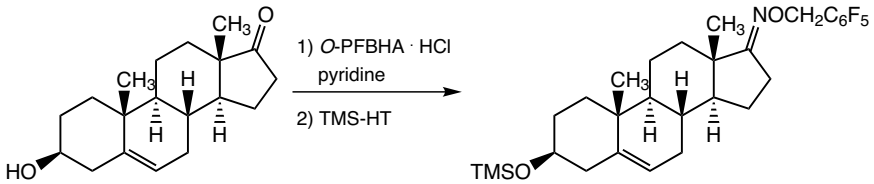
O-PFBHA·HCl is a novel derivatizing reagent for ketosteroids that solves the above-mentioned disadvantages. It reacts with traceketosteroids (1-5 ng) under a mild conditions and affords pentafluorobenzyloxime (*O*-PFBO) derivatives with few by-products. The resulting oximes have high heat stability and also excellent sensitivity to the ECD. For example, the sensitivity is 5 pg (5 x 10^{-12} g) for testosterone and 1 - 0.1 ng for other steroids.

The excess reagent can be easily removed by washing with acid and the unreacted hydroxyl groups in steroids become ready for GC analysis by trimethylsilylation.

Below application is the analysis of dehydroepiandrosterone extracted from human serum.

[Application Example][1)]

An extract from serum containing epiandrosterone acetate (approx. 1 µg, as an internal standard) is dissolved in 2 drops of pyridine. *O*-PFBHA·HCl (0.2 mg) is added to the mixture and is allowed to react for 1h at 60 °C. After diluting with 3 mL of hexane, the mixture is washed with water (1 mL), 0.1 mol/L HCl (1 mL), 0.1 mol/L aqueous solution of sodium hydroxide (1 mL), and water (1 mL), followed by centrifugation. And then hexane is evaporated to obtain the residue (*O*-PFBOs). After that, the hydroxyl group is trimethylsilylated with TMS-HT. It is evaporated and dried, and then the residue is dissolved in 1 mL of hexane and 2 µL of the mixture is injected into GC.[1)]



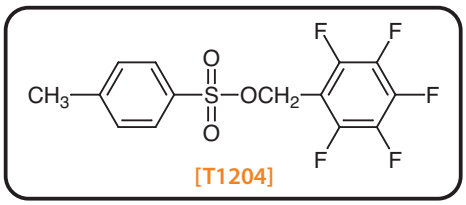
Dehydroepiandrosterone

[References]

1) T. Nambara, K. Kigasawa, T .Iwata, M. Ibuki, *J. Chromatogr.* **1975**, *114*, 81.
2) K. T. Koshy *et al.*, *J. Chromatogr. Sci.* **1975**, *13*, 97.
3) P. G. Devaux, E.C. Horning, *Anal. Lett.* **1969**, *2*, 637.
4) J. Attal *et al.*, *Anal. Biochem.* **1967**, *20*, 394.
5) R. A. Mead *et al.*, *J. Chromatogr. Sci.* **1969**, *7*, 554.

Derivatizing Reagent of Inorganic Anions

Pentafluorobenzyl *p*-Toluenesulfonate (=PFB-Tosylate) 5 g [T1204]



[T1204]

Pentafluorobenzylation using PFB-Tosylate allows for the analysis of inorganic anions (Br^-, I^-, CN^-, S_2^-, NO_2^-, NO_3^-, SCN^-) by GC. Moreover, using ECD as a detector allows for highly sensitive analyses of trace amount of inorganic anions. This reagent can be used for GC analysis of pentafluorobenzylation of carboxylic acids, phenols and others.

[Application Example]

1 mL of a sample, 0.2 mL of 0.1 mol/L aqueous solution of tetra-*n*-amylammonium chloride (TAAC), and 1 mL of 0.1 mol/L dichloromethane solution of this reagent are placed in a screw capped 10 mL brown bottle, and it is tightly sealed. After stirring for 30 min, the lower layer of the mixture is injected into GC.

Measuring Range

Anions	Derivatives	Measuring Range (FID)
Bromide	PFB-Bromide	30～300 ppm
Cyanide	PFB-Cyanide	10～100 ppm
Iodide	PFB-Iodide	50～500 ppm
Nitrite	PFB-Nitrite	45～450 ppm
Nitrate	PFB-Nitrate	25～250 ppm
Sulfide	PFB-Sulfide	6.5～65 ppm
Thiocyanate	PFB-Thiocyanate	20～200 ppm

[Reference]

K. Funazo, *et al.*, *J. Chromatogr.* **1985**, *346*, 215.

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