TMAH
Product Specification

TMAH (trimethylanilinium hydroxide),* 0.2M in methanol, is an esterification reagent used to form methyl derivatives, especially of molecules having replaceable protons attached to nitrogen (N-methyl derivatives). It is particularly useful for methylating barbiturates, sedatives, xanthine bases, phenolic alkaloids, dilantin, anticonvulsants, and fatty acids. TMAH is a preferred reagent for derivatizing barbiturates, because most are nitrogen-bearing molecules. TMAH can be used in flash alkylation, in which the analyte is derivatized in the injection port of the gas chromatograph, typically at an injection port temperature of 200-300°C. Barbiturates, biological fluids, and thermally stable fatty acids are suited to this type of derivatization. Flash methylation with TMAH eliminates degradation of barbiturates and their N-methyl derivatives, especially in the derivatization of phenobarbital.

*Note: The acronym TMAH is used for trimethylanilinium hydroxide, but TMAH also is used for tetramethylammonium hydroxide, $(CH_3)_4NOH$. Always verify the reagent by the full name.

Features/Benefits

Provides convenient, fast, quantitative derivatization of nitrogen-bearing molecules.

Preferred reagent for derivatizing barbiturates (except meprobamate, which is analyzed as the free base).

Typical Procedure

This procedure is intended to be a guideline and may be adapted as necessary to meet the needs of a specific application. Moisture can hinder the reaction. Always take proper safety precautions when using an esterification reagent – consult MSDS for specific handling information.

Prepare a reagent blank (all components, solvents, etc., *except sample*), following the same procedure as used for the sample.

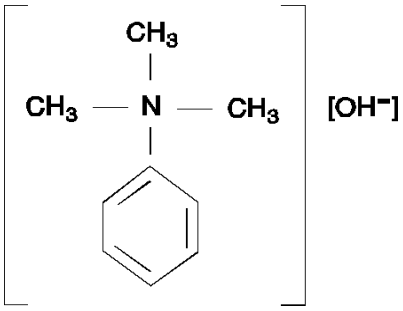
1. Extract sample with appropriate solvent, or weigh 1-10 mg of sample into a reaction vessel. If appropriate dilute neat sample with solvent.
2. Add TMAH reagent (begin with equal amounts of sample and TMAH; up to 1000-fold molar excess of reagent has been used).
3. Analyze a 1µL aliquot of the material by GC (direct injection).

Flash Alkylation

Draw 1µL TMAH, then 1µL sample, then 1µL TMAH into a 10µL syringe. Inject into the heated GC injection port. Alternatively, either the first or the second aliquot of TMAH can be omitted.

Properties

TMAH
Structure:



Elemental Formula:
mixture of trimethylphenylammonium iodine, silver oxide, methanol

bp: 65°C at 760mm Hg

d: 1.10

n_D^{20}: 0.790 at 20°C

Appearance:
clear, colorless liquid

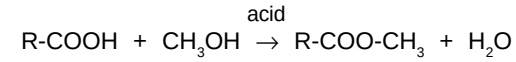
796-0717

Derivatization times will vary widely, depending upon the specific compound(s) being analyzed. If derivatization is not complete (chromatogram peak is smaller than expected), add additional reagent or reevaluate the injection port temperature. If unexpected peaks are present in the chromatogram, reevaluate the amount of TMAH added (excess reagent could cause sample decomposition), the time allowed for the sample and reagent to mix, and the injection port temperature. A low injection port temperature can produce poor responses (incomplete reaction) and/or peak tailing. A high injection port temperature can break down analytes, or produce ghost peaks, poor peak symmetry, baseline drift, or poor response.

Note: Use of TMAH can cause ring opening in ring-containing pharmaceuticals. The extent of this occurrence is affected by the concentration of the reagent and the length of the reaction time. A buffering method has been reported to minimize this problem – see Skinner, *et al., Anal. Chem.,* **45:** 574 (1973) and Osiewicz, *et al., J. Chromatogr.* **88:** 157 (1974).

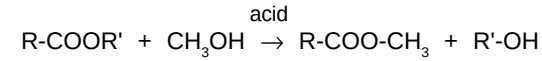
Esterification

General Mechanism



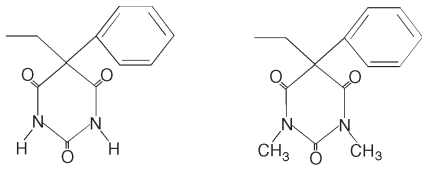
Adapted from (1).

Transesterification



Adapted from (3).

Action of TMAH



Adapted from (1).

796-0716

Mechanism (1,2,3)

Esterification involves heating the molecule bearing the amino, hydroxyl, carboxyl, or other reactive group with an acid catalyst in an alcohol solvent. The catalyst protonates an oxygen atom or the nitrogen atom of the reactive group, making the molecule much more reactive to nucleophiles. An alcohol molecule then combines with the protonated group, to yield the ester product (e.g., R-COO-CH_3, R-N-CH_3) with loss of water. 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. 2,2 dimethoxypropane can be introduced into the reaction mixture to react with the water, yielding acetone. Other water scavengers are anhydrous sulfuric acid and graphite bisulfate.

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 also is 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 conversion rate also is influenced by the reaction temperature – the reaction generally is conducted near the boiling point of the alcohol.

Toxicity - Hazards - Storage - Stability

TMAH is a flammable, toxic liquid. It may irritate eyes, skin, and/or the respiratory system. Recommended storage conditions for the unopened product are stated on the label. Store opened reagent in a sealed bottle or ampul. **If you store an opened container or transfer the contents to another container for later reuse validate that your storage conditions adequately protected the reagent**.

Use only in a well ventilated area and keep away from ignition sources. Moisture can hinder the reaction – it may be necessary to dry the solvents before conducting the reaction.

The reagent has a limited shelf-life, even when refrigerated, and the use of old or excessively concentrated solutions (through solvent evaporation) often produces artifacts and a significantly lower reaction yield, particularly for polyunsaturated fatty acids.

References

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.
3. *Bailey's Industrial Oil and Fat Products*, fifth edition, Vol. 5, John Wiley & Sons, New York (1995).

Additional Reading

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).

Ordering Information:

Description	Cat. No.
TMAH (0.2M in methanol)	
10 x 1mL	**33358-U**
10mL	**33097-U**
Microreaction Vessels, Caps, and Septa	
1mL, pk. of 12	**33293**
3mL, pk. of 12	**33297**
5mL, pk. of 12	**33299**
Books	
Handbook of Derivatives for Chromatography K. Blau and J. Halket	**26566-U**
Handbook of Analytical Derivatization Reactions D.R Knapp	**23561**

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 800-359-3044 or 814-359-5468) for expert answers to your questions.