

Product Information

Methanolic HCl, 0.5N and 3N

Methanolic HCl (hydrochloric acid in methanol) is particularly useful for preparing methyl esters of volatile (short chain) fatty acids. Fatty acids are esterified by heating them with an anhydrous alcohol (e.g., methanol) in the presence of an acidic catalyst (e.g., HCl)* in a sealed vessel at a high temperature for a short time. In the reaction, a fatty acid molecule and an alcohol molecule are joined, with the release of a water molecule. The derivatives can be quickly and easily recovered, quantitatively, from the reaction medium.

Methanolic HCl has been used at a range of normalities, from mild to strong. The proper concentration to use is based on the conditions required to esterify the acid(s) under study. High concentrations of methanolic HCl reduce the time necessary for complete reaction, but can create extraneous byproducts that can interfere with the analysis. Also, high concentrations of the reagent can weaken rapidly, even when refrigerated, and therefore should be prepared fresh before use. Lower concentrations of methanolic HCl may be used, but longer time must be allowed for the reaction. If the concentration of the catalyst (HCl) is too high, concentration artifacts (polymers or unidentified derivatives) can form and interfere with the analysis. A good test of a derivatization procedure is to form the esters of a known fatty acid mixture, such as A-NHI-F, and analyze the derivatives by GC. Subsequently, results for the acid(s) under study can be compared against the results for the A-NHI-F mix. Methanolic HCl also can be used to prepare derivatives for HPLC and TLC applications.

Applications/Benefits

Derivatization of fatty acids, particularly volatile (short chain) fatty acids.

Clean reaction (no side reactions) with volatile byproducts.

Provides convenient, fast, quantitative derivatization.

Typical Procedure

This procedure is intended to be a guideline and may be adapted as necessary to meet the needs of a specific application. 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. Weigh 1-25mg of sample into a 5mL reaction vessel.
2. Add 3mL methanolic HCl and mix.
3. Heat at 50°C for 5-10 minutes. Allow mixture to cool to room temperature.
4. Analyze an aliquot of the organic (upper) layer.

Derivatization times vary widely, depending upon the specific compound(s) being derivatized. If derivatization is not complete, use additional reagent or reevaluate temperature/time of reaction.

Properties

Hydrogen Chloride (hydrochloric acid)

CAS Number: 7647-01-0

Molecular Formula: HCl

Formula Weight: 36.46

bp: -85°

Flash Point: na

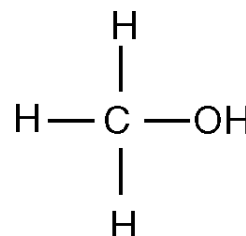
d: na

n_D: na

Appearance: colorless to slightly yellow, fuming, pungent liquid

Methanol

Structure:



CAS Number: 67-56-1

Molecular Formula: CH₃OH

Formula Weight: 32.04

bp: 64.7°C

Flash Point: 52°F (11°C)

d: 0.791

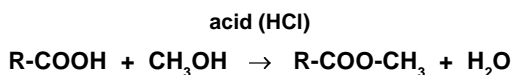
n_D: 1.3290 at 20°C

Appearance: clear colorless liquid

796-0598

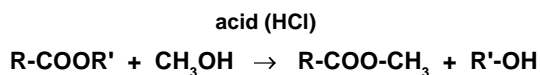
*Typical catalysts for esterification are hydrogen chloride (favored because of its acid strength and its ease of removal after the reaction), sulfuric acid (less easily removed; can contribute dehydrating and/or oxidative side reactions, charring effects), trifluoroacetic acid, dichloroacetic acid, benzene/p-toluene sulfonic acid, sulfonyl chloride, thionyl chloride, phosphorus trichloride, phosphorus oxychloride, phosphoric acid.

Esterification



Adapted from (1).

Transesterification



Adapted from (3).

Mechanism (1,2,3)

Esterification

Esterification involves heating the carboxylic acid with an acid catalyst in an alcohol solvent. The catalyst protonates an oxygen atom of the reactive COOH group, making the molecule much more reactive to nucleophiles. An alcohol molecule then combines with the protonated group, to yield the ester product (R-COO-CH₃) 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.

Transesterification

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

Methanolic HCl, 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 shelflife, even when refrigerated, and the use of old or excessively concentrated solutions (through alcohol evaporation) often produces artifacts and a significantly lower reaction yield.

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).

References not available from Supelco.

Additional Reading

R.N.M. Carballeira, F. Shalabi *The Rare Caribbean Sponge Leucosolenia canariensis: Phospholipid Fatty Acids and Sterols* Lipids, **30** (5): 467-470 (1995).
J. Miralles, G. Barnathan, R. Galonnier, T. Sall, A. Samb, E.M. Gaydou, J.M. Kornprobst *New Branched-Chain Fatty Acids from the Senegalese Gorgonian Leptogorgia piccola (white and yellow morphs)* Lipids, **30** (5): 459-466 (1995).
S. Joron, H. Robert *Simultaneous Determination of Antidepressant Drugs and Metabolites by HPLC. Design and Validation of a Simple and Reliable Analytical Procedure*. Biomed. Chromatogr., **8** (4): 158-164 (1994).

Ordering Information:

Description	Cat. No.
Methanolic HCl, 0.5N	
20 x 1mL	33354
10 x 5mL	33095
Methanolic HCl, 3N	
20 x 1mL	33355
10 x 3mL	33051
400mL	33050-U
Microreaction Vessels with Hole Caps and Septa	
pk. of 12	
1mL	33293
3mL	33297
5mL	33299
Book	
<i>Handbook of Analytical Derivatization Reactions</i> D.R Knapp	23561

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