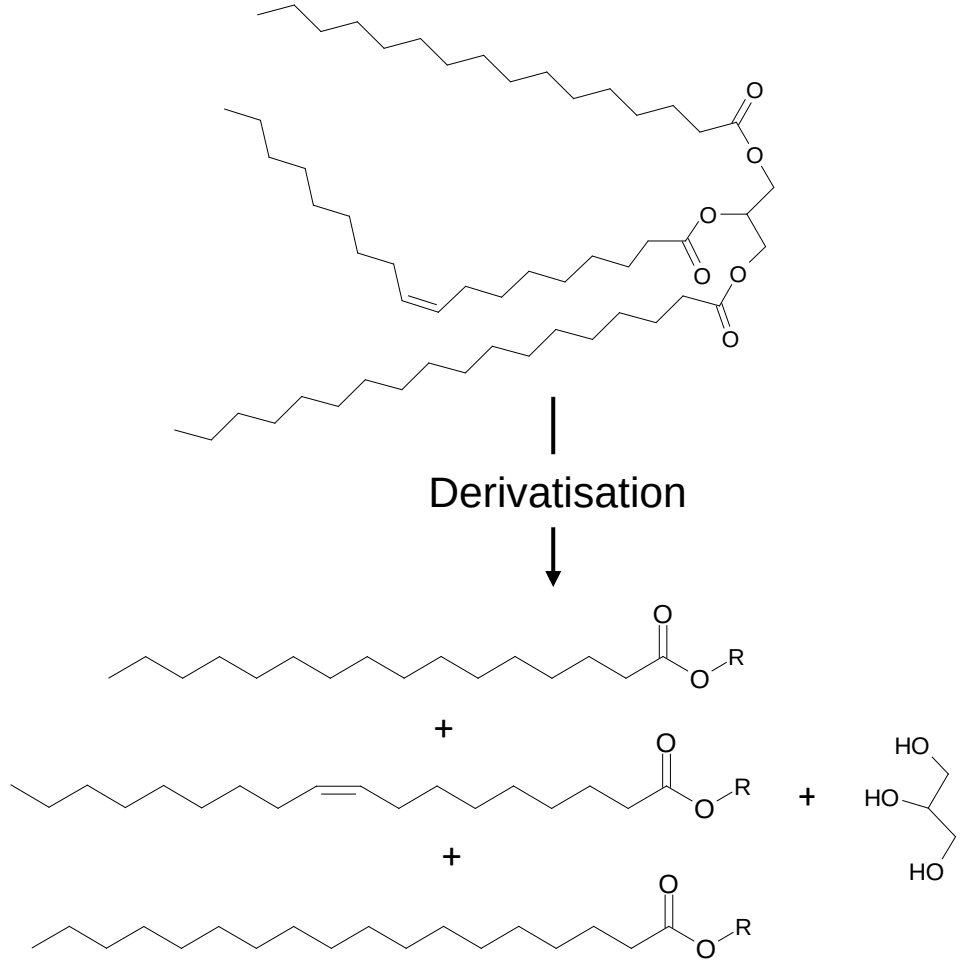
Derivatisation for GC analysis

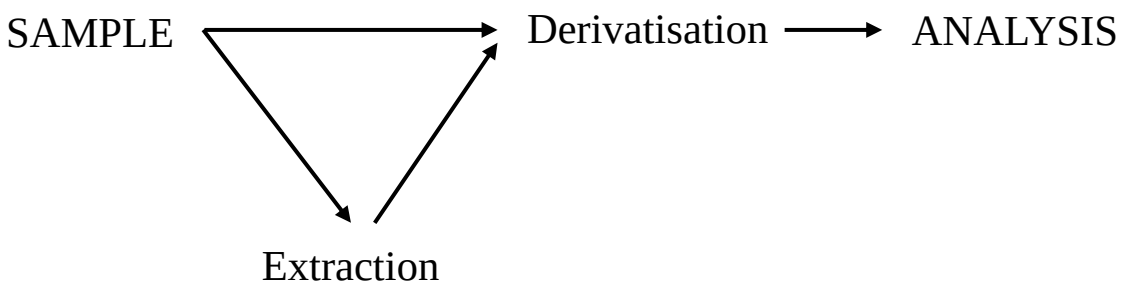
Principle of GC derivatisation

- Process, where a compound is chemically changed before the analysis to:
  - increase volatility;
  - increase thermal stability;
  - improve detection;
  - decrease adsorption in the injector;
  - improve separation of compounds.
- Mainly three types are common:
  - silylation, e.g. $–Si(CH_3)_3$;
  - acylation, e.g. $–COCH_3$;
  - alkylation, e.g. $–CH_3$; $–CH_2CH_3$.
- Many different derivatisation reagents are used
  - e.g. TMAH, BSTFA, TMTFTH, BSA, BF_3 ...

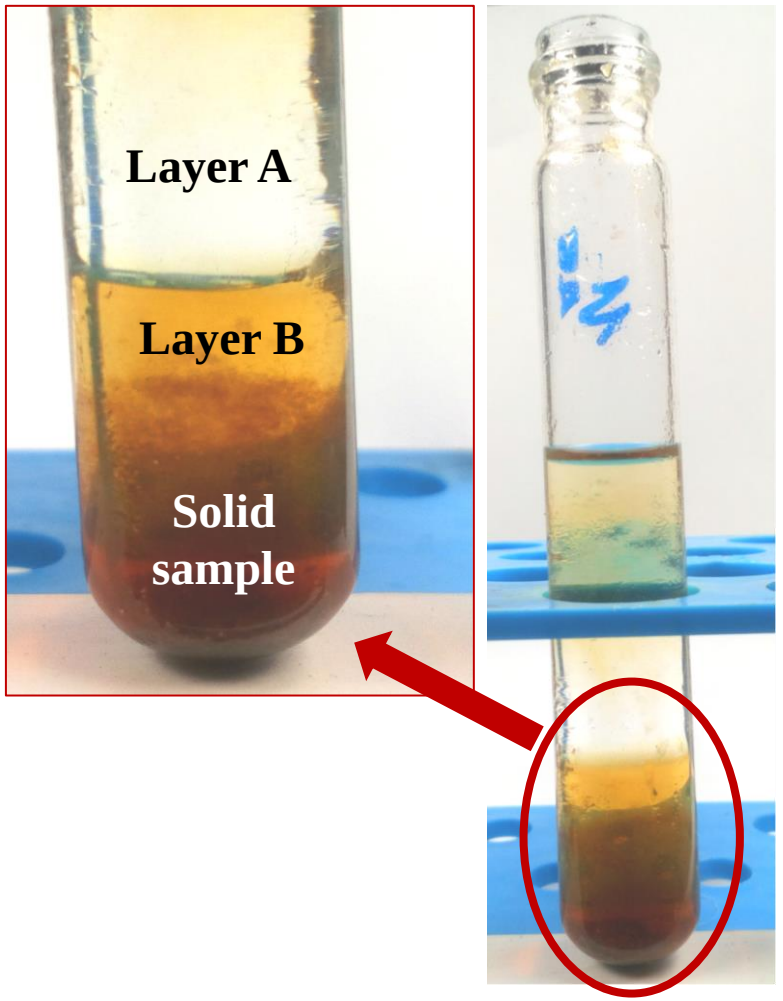


Example. Derivatisation of a triglyceride molecule (oil component) to produce fatty acid derivatives

Sample preparation

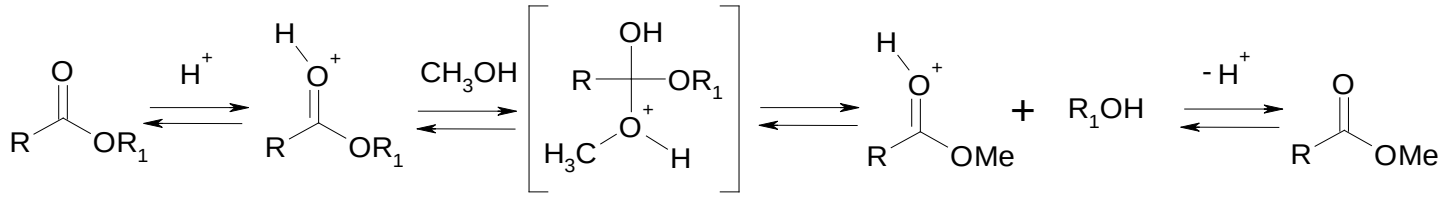


- Extraction – separation process, where an analyte of interest is separated from the mixture/other components usually with solvent(s).
- Necessity of extraction depends on the sample and analysis
  - Mostly complex samples with different components
    - E.g. lipids from ceramic matrix, adhesives from textiles
  - Used to separate compounds before derivatisation
- Derivatisation is necessary for making molecules volatile and separable in GC analysis



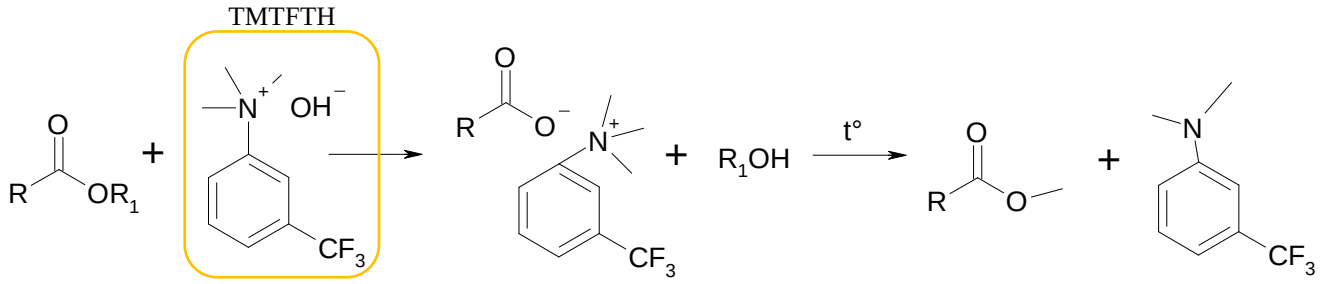
Methylation examples

- **Acid-catalysed methylation**
  - H_2SO_4 + MeOH for 4 h at 70 °C
  - Extraction with hexane
  - Produces methylated analytes



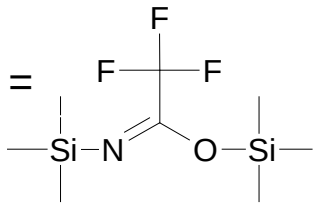
Method from: 1) Craig, O. E.; Saul, H.; Lucquin, A.; Nishida, Y.; Taché, K.; Clarke, L. et al. *Nature*, **2013**, 496, 351-354.
2) Correa-Ascencio, A., Evershed, R.P., *Anal. Methods*, **2014**, 6, 1330–1340.
3) Papakosta, V., Smittenberg, R., Gibbs, K., Jordan, P., Isaksson, S., *Microchem. J.*, **2015**, 123, 196-200.

- **TMTFTH (aka Meth-Prep II) derivatisation**
  - Solvent (toluene, benzene) and TMTFTH 5% solution in MeOH are added directly on the sample
  - The sample is sonicated for 30 min
  - After 24h the solution is analysed
  - Produces methylated analytes

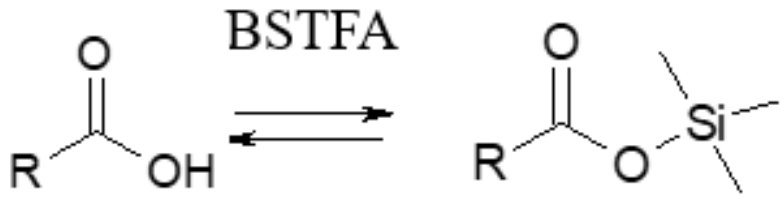


Method from: 1) Manzano, E.; Rodriquez-Simón, L.R.; Navas, N.; Checa-Moreno, R.; Romero-Gámez, M.; Capitan-Vallvey, L.F. *Talanta*, **2011**, 84, 1148-1154.
2) Sutherland, K. *J. Chromatogr. A.* **2007**, 1149, 30-37.

Trimethylsilylation examples

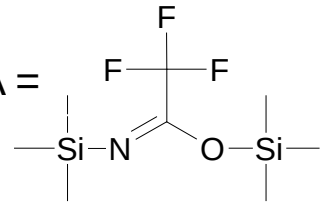
BSTFA = 

- **Extraction + BSTFA derivatisation**
  - Solvent extraction using DCM (or $CHCl_3$)/MeOH mixture
  - Derivatisation of –OH and –COOH groups with BSTFA to produce silylated derivatives
  - Produces trimethylsilyl derivatives

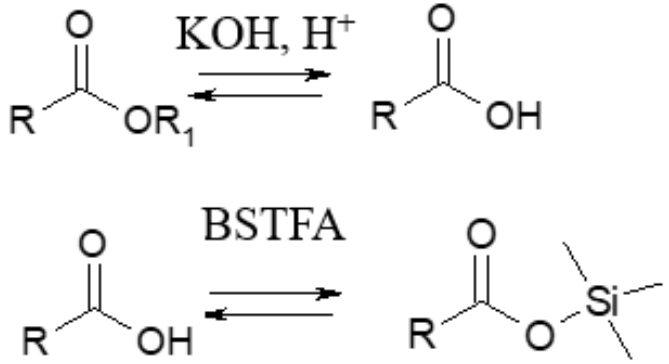


Method from: 1) R. P. Evershed, C. Heron and L. J. Goad, *Analyst*, **1990**, 115,1339–1342.
2) Correa-Ascencio, A. & Evershed, R.P. 2014. *Anal. Methods*, **2014**, 6, 1330–1340

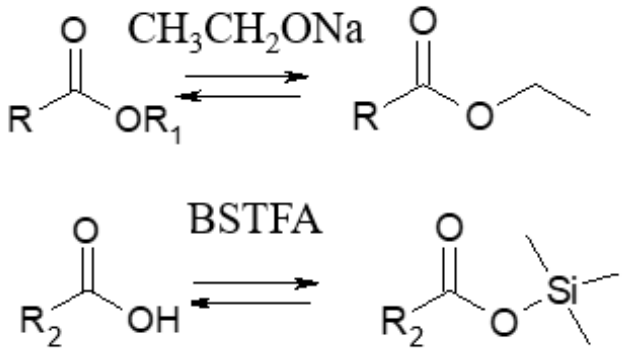
Trimethylsilylation examples

BSTFA = 

- **KOH + BSTFA derivatisation**
  - Adding KOH in ethanol, 2h at 60 °C
  - Neutralisation with acid (trifluoroacetic acid)
  - Extractions
  - Solvent + BSTFA
  - Produces mainly trimethylsilyl derivatives



- **NaOEt + BSTFA derivatisation**
  - Adding NaOEt in ethanol, 1.5h at 70 °C
  - Removal of excess NaOEt with NH_4Cl
  - Solvent + BSTFA
  - Ethylated derivatives of compounds containing ester group, trimethylsilylated derivatives of compounds containing –OH, -COOH group



Method from: Lluveras, A.; Bonaduce, I.; Andreotti, A.; Colombini, M.P. *Anal. Chem*., **2010**, 82, 376-386.

Method from: van den Berg, J.D.J.; van den Berg, K.J.; Boon, J.J. *Prog. Org. Coat*., **2001**, 41, 143-155.

Archaeology

Conventional techniques for lipid analysis

Cultural Heritage

Traditional analytical procedures with corresponding samples

Solvent extraction (followed by)

1) + BSTFA
- „Gentle“ procedure
- More variable molecular profile (incl. TAGs, DAGs)
- More traditional, widely used

2) + saponification with NaOH or KOH; followed by BSTFA or BF_3 (esp. if GC-C-IRMS analysis needed)

Meth-Prep II (TMTFTH)

- Used more recently
- Stable results
- Little labor-intensive
- Also analysis of absolute quantities
- Small sample sizes (no sample transfers)

Acid-catalysed methylation

- Faster, esp. if GC-C-IRMS needed
- Higher lipid yield
- Extraction and derivatisation combined
- More „destructive“ (results in FAs, no TAGs, DAGs)
- More used for old (poorly preserved) & small samples
- No-need for rapid runs (extractions stable in freezer)
- The most labor-intesive and time-consuming

KOH/NaOEt + BSTFA

1) KOH+BSTFA
- Extensively used
- Analysis of absolute quantities
- Unstable quantitative results

2) NaOEt+BSTFA
- Analysis of free fatty acids
- Multible derivatives

Comparison of derivatisation methods: Tammekivi, E.; Vahur, S.; Kekišev, O.; van den Werf, I. D.; Toom, L.; Herodes, K. Leito, I., *Anal. Methods*, **2019**, 11, 3514-3522.

Other examples of derivatisation methods

- On the last slide there were only some examples how lipids can be derivatised
- For other materials (waxes, resins, proteins, saccharides, polymers) there are also numerous derivatisation methods
- Here, we present only some possibilities, that all have their advantages and disadvantages:

Material	Example of derivatisation methods for specific material
Natural waxes, resinous materials, lipids	TMTFTH, Acid-catalyzed methylation, KOH + BSTFA, diazomethane (DA), methyl or ethyl chloroformate, MTBSTFA, BF_3
Synthetic polymers	TMAH; HMDS; BSTFA with pyrolysis GC
Proteins	Hydrolysis + TMBSTFA derivatisation, methyl or ethyl chloroformate derivatisation, alcohol + trifluoroacylation
Saccharides	Hydrolysis + trimethylsilylation, acetic anhydride, methoxiamine hydroxide

Further reading: Doménech-Carbó, M.T. *Anal. Chim. Acta*, **2008**, 621, 109-139.

Summary

Derivatisation – process, where a compound is chemically changed

Derivatisation is used to **improve** the chromatographic analysis

The most common derivatisations are **methylation**, **silylation** and **alkylation**

Extraction can be used before or during derivatisation to ease the analysis of complex samples