

## Einfache GC/MS Analyse von polaren Gallensäuren

Gallensäure spielen eine wichtige Rolle als Biomarker zur Früherkennung und therapeutischen Begleitung u.a. bei Darm- und Lebererkrankungen.

Dabei wird die übliche LC Analytik häufig durch starke Verschleppungen und niedrige Peakkapazität erschwert.

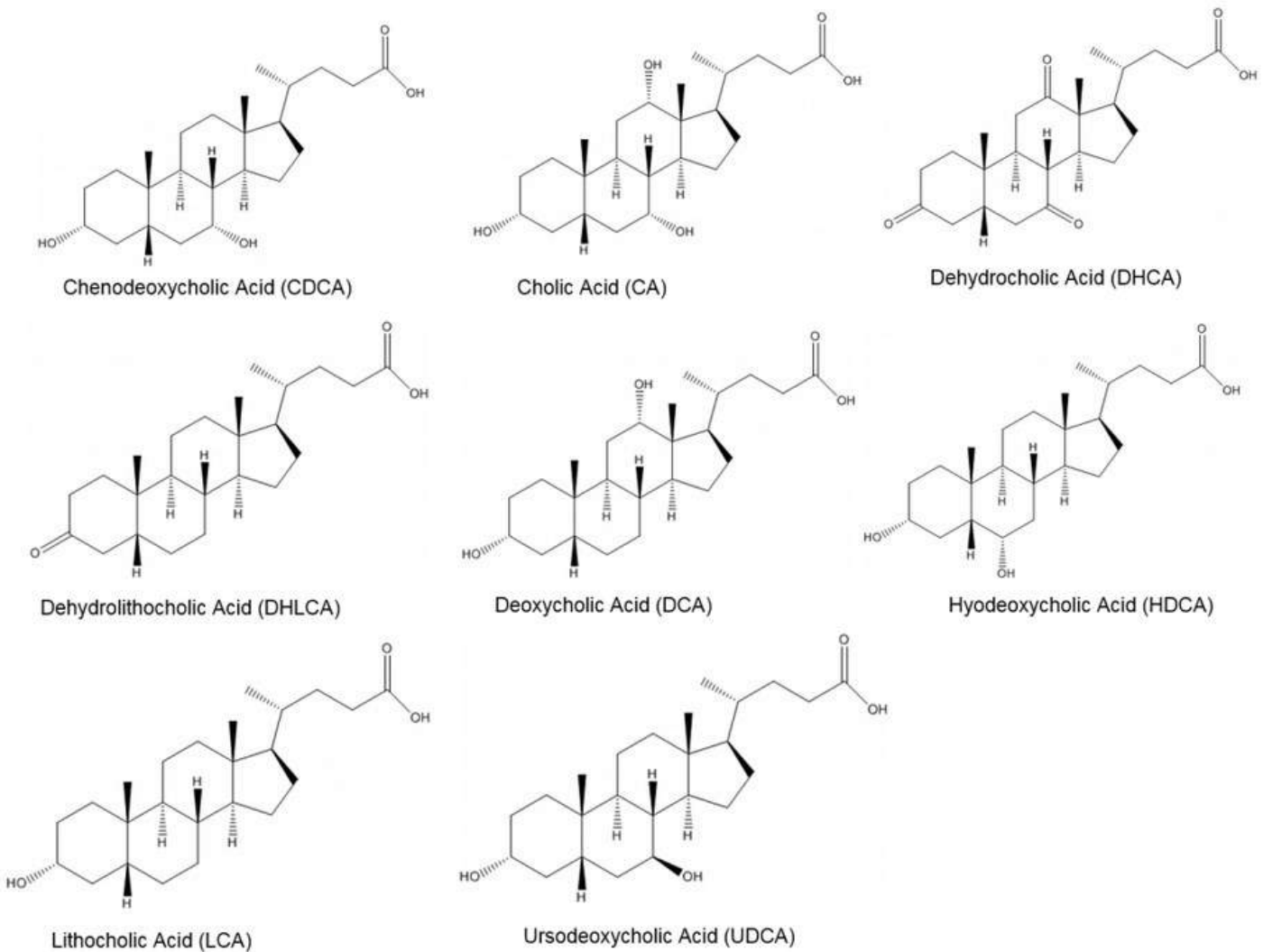
➔ Nachfolgend stellen wir Ihnen eine GC/MS Alternative nach Derivatisierung der polaren Gallensäuren mit BSTFA+1% TMCS vor. Dabei kann die Laufzeit von 30 min mit Hilfe des EzGC Calculators bis auf <7 min reduziert werden.

## Simple Analysis of Bile Acids by GC-MS

Bile acids are a group of steroidal acids with carboxyl and hydroxyl groups on the side. They are the major metabolic products of cholesterol. Bile acids play important roles as biomarkers for early diagnosis and therapeutic

monitoring of many diseases, especially liver and intestinal diseases. For instance, high levels of intestinal bile

acids, in particular, deoxycholic acid, is an indication of colon carcinogenesis.<sup>1</sup> Below are structures of some of the most common bile acids.

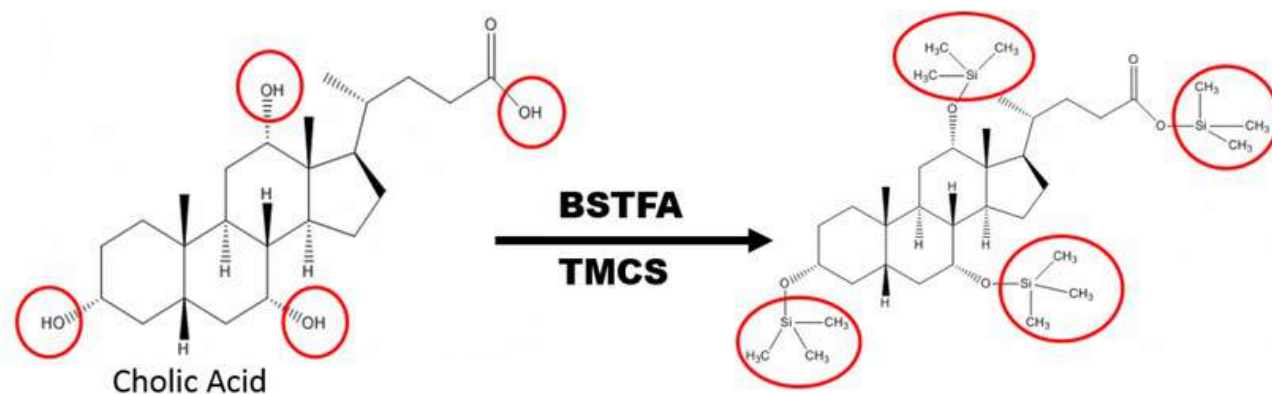


LC-MS and LC-MS/MS are commonly used for bile acid analysis in biological fluids without derivatization. Disadvantages of LC-based methods include relatively high carry-over on LC columns and low peak capacity.

GC is a robust, simple and inexpensive lab tool for volatile or semi-volatile compound analysis. The only drawback of GC versus HPLC for bile acid analysis is polar functional groups (carboxyl and hydroxyl) need to be derivatized into GC-detectable forms. Trimethylsilylating reagents, such as N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyl-N(trimethylsilyl)trifluoroacetamide (MSTFA), are commonly used for derivatization of both hydroxyl and carboxyl groups. Choosing the right reagents can be tricky. Kumar et al.<sup>2</sup> compared four types of derivatization mixtures: MSTFA:NH<sub>4</sub>I:DTE (500:4:2, 40 IL, 60 C, 30 min), MSTFA:TMSI:TMCS (100:2:5, 40 IL, 60 C, 30 min), MSTFA:TMCS (100:1, 40 IL, 60 C, 30 min), and BSTFA:TMCS (100:1, 40 IL, 60 C, 30 min). Test results showed that MSTFA:NH<sub>4</sub>I:DTE (500:4:2, v/w/w) has the highest efficiency for bile acid derivatization. However, the mixture must be freshly prepared and thus is not convenient for fast analysis. Fortunately, my colleague Amanda Rigdon developed a one-step method for [cannabinoid acids derivatization](#) using [BSTFA+1% TMCS](#), and the reaction is only 30 min.

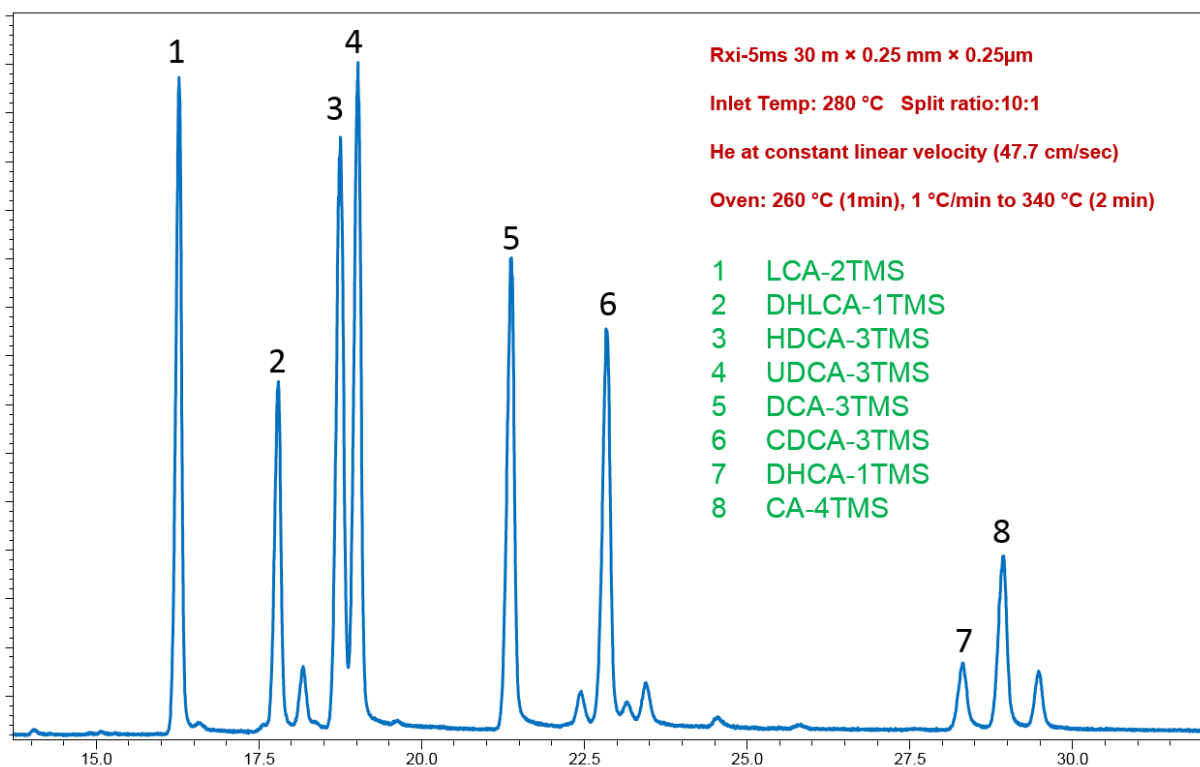
Here, the same derivatization method was applied to bile acids, and the preliminary results are very promising. Basically, this reagent simply replaces the hydrogen in the –OH or –COOH groups with a trimethylsilyl group (see the picture below). The procedure is simple and straightforward.

- Place 100 µL of bile acid standards (~100 ppm) into an [interlock vial](#)
- Evaporate the solvent under nitrogen
- Add 50 µL of ethyl acetate and 50 µL of [BSTFA+1% TMCS](#)
- Incubate for 30 min at 70 °C
- Cool down and dilute with ethyl acetate if necessary



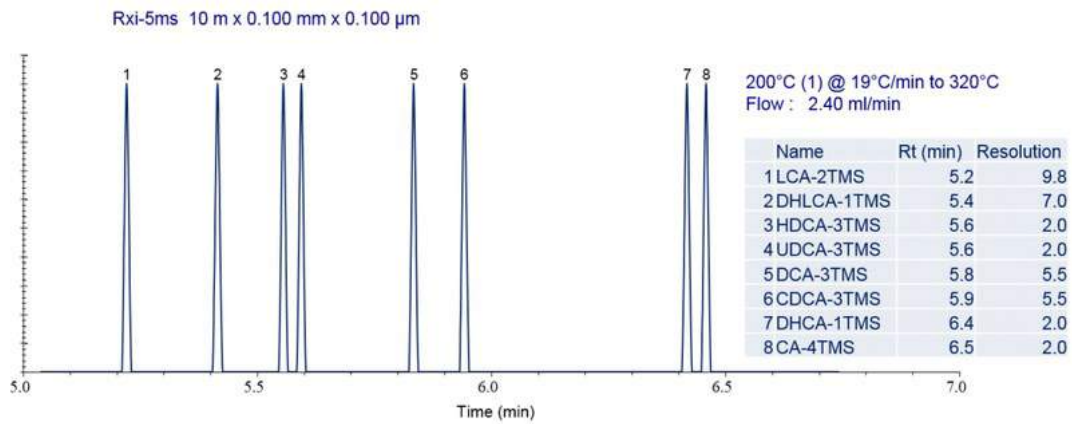
The derivatized bile acid standards were analyzed by GC-MS under full scan mode using an [Rxi-5ms](#) 30 m × 0.25 mm × 0.25 µm column. The chromatogram below shows good separation of closely related bile acids (isomers and enantiomers), such as HDCA and UDCA.

### Mixed bile acids standards (derivatized with BSTFA+1% TMCS)



The reaction and GC conditions (reagent/bile acid amount ratio, reaction temperature, time, column dimensions, etc.) can be further optimized. Hopefully, this preliminary work could open the door for the fast GC analysis of bile acids. Keep in mind there are some limitations on conjugated bile acids.

GC analysis time too long? EZGC can help! Here is one of the modulation solutions using shorter column. The total analysis time is less than 10 min.



## References

- [1] (a) Jensen, R. G.; Clark, R. M.; deJong, F. A.; Hamosh, M.; Liao, T. H.; Mehta, N. R., The lipolytic triad: human lingual, breast milk, and pancreatic lipases: physiological implications of their characteristics in digestion of dietary fats. *Journal of pediatric gastroenterology and nutrition* **1982**, *1* (2), 243-55;  
(b) Reddy, B. S., Diet and excretion of bile acids. *Cancer research* **1981**, *41* (9 Pt 2), 3766-8.
- [2] Kumar, B. S.; Chung, B. C.; Lee, Y. J.; Yi, H. J.; Lee, B. H.; Jung, B. H., Gas chromatography-mass spectrometry-based simultaneous quantitative analytical method for urinary oxysterols and bile acids in rats. *Anal. Biochem.* **2011**, *408* (2), 242-52.

**Haben Sie Fragen zu dieser Problematik oder benötigen Sie weitere Informationen dazu?  
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