# Determination and quantification of fatty acids in speleothems and cave drip water using HPLC-ESI-IT/MS

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## Introduction

Cave drip water, speleothems and the proxies preserved within them have significant potential to record palaeoenvironmental changes in the regional vegetation[1]. The use of stalagmites provides valuable information because they form a chemically closed system which does not change much after lithification, they grow continously[2] and are amenable to precise $^{230}$Th/U-dating[3]. The most common proxies measured in speleothems are inorganic proxies, in particular carbon and oxygen isotopes, but more recently the importance of organic matter analyses in this field is examined.

This study focuses on the research of lipid biomarkers. The lipids contained in stalagmites originate from the overlying soil and different plants, bacteria and fungi. Therefore different compositions of lipids may provide records of environmental changes[4].

In the following a new method for the extraction of fatty acids (saturated) from stalagmites and cave drip water and their measurement by HPLC-ESI-IT/MS is presented.

Table 1: Used fatty acid standards.

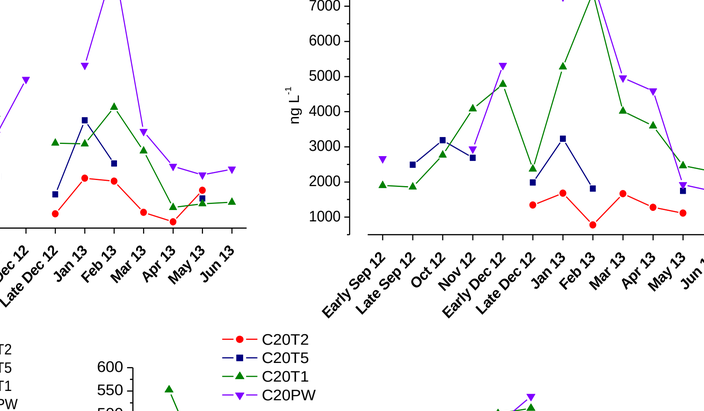
| Common name | Structure | Abbr. |
|---|---|---|
| Lauric acid | $CH_3(CH_2)_{10}COOH$ | C12 |
| Myristic acid | $CH_3(CH_2)_{12}COOH$ | C14 |
| Palmitic acid | $CH_3(CH_2)_{14}COOH$ | C16 |
| Stearic acid | $CH_3(CH_2)_{16}COOH$ | C18 |
| Arachidic acid | $CH_3(CH_2)_{18}COOH$ | C20 |

Table 2: Parameters used during measurement.

| HPLC | Agilent 1100 Series |
|---|---|
| Column | Varian Pursuit XRs 3 C8<br>50x2 mm<br>temperature: 50 °C |
| Gradient | 0 min ACN/$H_2$0 80% / 20%<br>5 min ACN/$H_2$0 99% / 1%<br>25 min ACN/$H_2$0 99% / 1%<br>26 min ACN/$H_2$0 80% / 20% |
| Flow rate | 200 µL min$^{-1}$ |
| Detector | Bruker HCT+ (ESI-IT/MS) |
| Polarity | negativ |
| Mode | MRM (multiple reactions monitoring)<br>m/z: 199, 227, 255, 283, 311 |

## Experimental

Stalagmite samples → precleaning with DCM/MeOH (9:1) → dissolving in 4M HCl → ACN was added as a modifier → solid phase extraction (SPE):




Drip water samples → ACN + 0,4M HCl was added → SPE

## Results

Table 3: Calibration data including SPE procedure.

| | C12 | C14 | C16 | C18 | C20 |
|---|---|---|---|---|---|
| Slope | 4.37E+04 | 1.40E+05 | 5.65E+05 | 1.36E+05 | 1.10E+05 |
| Intercept | 6.36E+04 | 1.36E+06 | 1.47E+08 | 2.81E+07 | 6.63E+05 |
| $R^2$ | 0.9979 | 0.9873 | 0.9946 | 0.9136 | 0.9948 |
| RSD/% | 0.9-1.4 | 0.1-6 | 1.1-2 | 3-8.6 | 0.1-3.4 |
| LOD/ng | 1.23 | 0.77 | 3.81 | 55.97 | 2.54 |
| LOQ/ng | 4.08 | 2.57 | 12.69 | 186.56 | 8.48 |

- Method developement and validation was performed using liquid-liquid extraction (LLE) and SPE.
- LLE showed matrix interferences and was not suitable for actual samples.
- Calibration data and linear regression plots of the optimized method were obtained performing spiking experiments. Results are listed in Table 3.
- Recovery of the extraction procedure was determined and ranged from 30% (C20) to 103% (C14).
- First applications to stalagmite samples (Figure 1) and cave drip water (Figure 2) were performed and correlated to $\delta^{13}$C measurements (Table 4).




Figure 2: Fatty acid concentrations in cave drip water samples of dripping sites T1, T2, T5 and pool water, Herbstlabyrinth- Adventhöhle cave system.

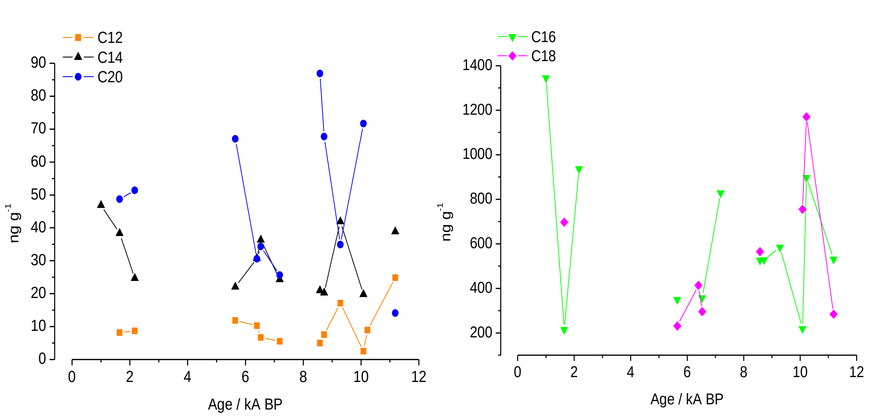



Figure 1: Fatty acid record of NG01, Herbstlabyrinth- Adventhöhle cave system.

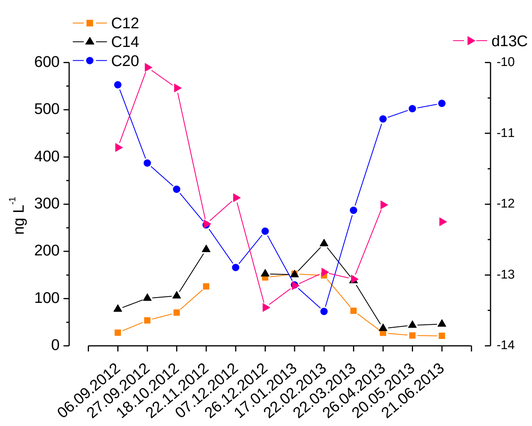



Figure 3: C12, C14 and C20 concentrations of dripping site T1 plotted against corresponding $\delta^{13}$C values.

Table 4: Correlation coefficients of drip water measurements.

| | $\delta^{13}$C | | | |
|---|---|---|---|---|
| | T1 | T5 | T2 | PW |
| C12 | -0.60 | -0.90 | NA | NA |
| C14 | -0.45 | -0.82 | NA | NA |
| C16 | -0.52 | NA | NA | NA |
| C18 | -0.46 | -0.67 | NA | NA |
| C20 | 0.50 | 0.79 | NA | NA |

## Outlook

- Application to further cave drip water and stalagmite samples and correlation with measurements of isotopes and inorganic trace elements.
- Identification of other organic compounds in speleothems using high-resolution MS (Orbitrap) and online measurements using FAPA-MS.

**Literatur:**

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