

Simplified Analysis of Aqueous Short Chain Fatty Acids by GC/MS

Y. Nakagawa

User Benefits

- ◆ No derivatization or special consumables required
- ◆ Total run time of about 10 mins including the cooling period

Introduction

Short chain fatty acids are fatty acids with fewer than 6 carbon atoms. Such organic acids are found not only in the environment (e.g. rainwater, atmosphere), but also in the human body. They are produced by the gut microbiota and provide energy for the cells lining the colon. About 95 % of short chain fatty acids in the human body is acetic acid(C2), propionic acid(C3) and butyric acid(C4).

Derivatization is often used in an analysis for short chain fatty acids. In this article, C2-C6 fatty acids in water are analyzed without derivatization or internal standard (Fig. 1).

Materials and Methods

A 10 mM pre-mixed standard solution (MilliporeSigma: CRM46975) was diluted to 4, 10, 40, 100 μM in water. The prepared calibration solutions are aliquoted into 2 mL vials and filled to the brim in order to suppress the volatile fatty acids escaping into headspace of the 2 mL vial.

Table 1 Instrument Configurations

| | |
|-------------------|---|
| GC-MS | : GCMS-QP2020 NX |
| Auto Injector | : AOC-20i Plus |
| Auto Sampler | : AOC-20s Plus |
| Analytical Column | : SH-Rtx™-WAX (60 m × 0.25 mm I.D., df=0.5 μm) P/N: 221-75894-60 |

Table 2 Analytical Conditions

| | |
|-------------------------|---|
| GC | |
| Injection Mode | : Split |
| Injector Temp. | : 240 °C |
| Split Ratio | : 5 |
| Carrier Gas | : Helium |
| Control Mode | : Constant Linear Velocity (34.0 cm/s) |
| Column Oven Temp. | : 80 °C (2 min) → (40 °C/min) → 200 °C → (25 °C/min) → 240 °C (2 min) Total 8.60 mins |
| Purge Flow Rate | : 3.0 mL/min |
| Sample Injection Volume | : 1 μL |
| MS | |
| Ion Source Temp. | : 200 °C |
| Interface Temp. | : 240 °C |
| Measurement Mode | : SIM |
| Monitoring Ions (m/z) | : Refer to Fig. 3 |
| Loop Time | : 0.30 seconds |

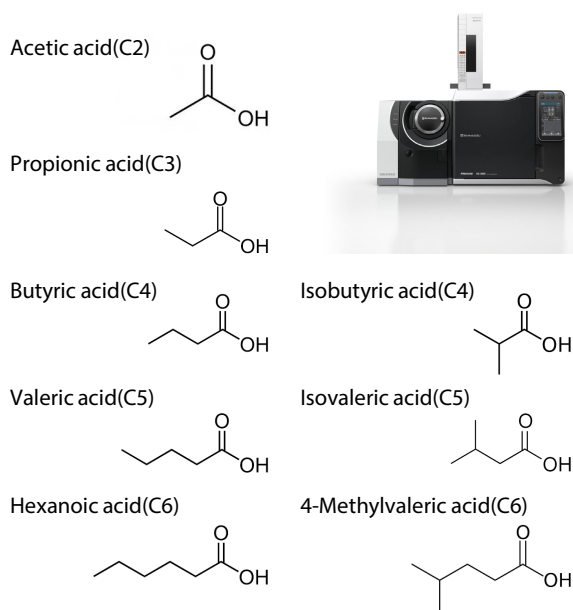


Fig. 1 Target Compounds

GC separation is crucial for a group of small compounds with the same functional group. The 10 mM pre-mixed standard was diluted several folds with DI water and the TIC chromatogram was obtained (Fig. 2).

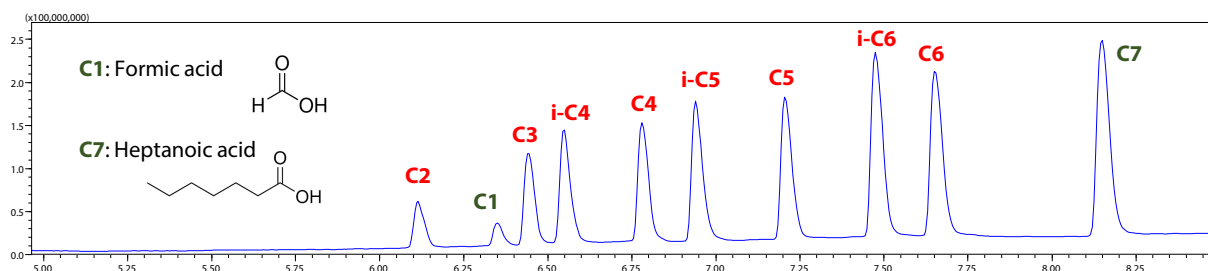


Fig. 2 Total ion chromatogram of a diluted pre-mixed standard solution

Results

Fig. 3 below shows chromatograms obtained from 10 µM solution.

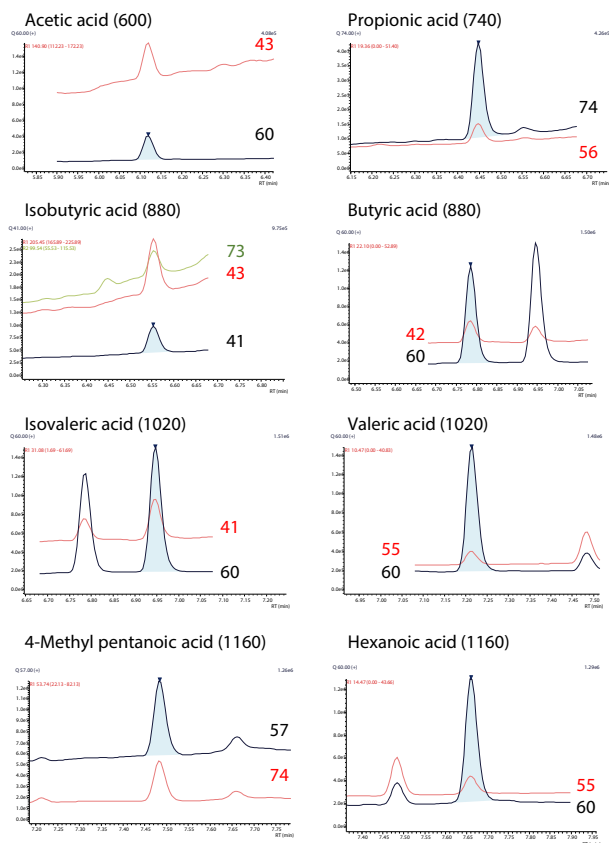


Fig. 3 10 µM spike with ng/mL conversion in parentheses

Calibration curves (quadratic) were drawn from 4 to 100 µM/mL. The concentrations in µg/mL at the smallest and largest calibrator points are tabulated below.

Table 3 Smallest and largest calibrator points in calibration curves

| Compound Name | µg/mL conversion | |
|-------------------------|------------------|--------|
| | 4 µM | 100 µM |
| Acetic acid | 0.24 | 6.0 |
| Propionic acid | 0.30 | 7.4 |
| Isobutyric acid | 0.35 | 8.8 |
| Butyric acid | 0.35 | 8.8 |
| Isovaleric acid | 0.41 | 10.2 |
| Valeric acid | 0.41 | 10.2 |
| 4-Methyl pentanoic acid | 0.46 | 11.6 |
| Hexanoic acid | 0.46 | 11.6 |

GCMS-QP and GCMS-TQ are trademarks of Shimadzu Corporation in Japan and/or other countries. Rtx is either a trademark or a registered trademark of Restek Corporation in the United States and/or other countries.

Discussions

Repeatability was measured by 7 consecutive injections of aqueous sample at 10 µM (Table 4).

Table 4 % RSD at 10 µM (n=7)

| Compound Name | % RSD |
|-------------------------|-------|
| Acetic acid | 6.0 |
| Propionic acid | 4.4 |
| Isobutyric acid | 3.9 |
| Butyric acid | 5.3 |
| Isovaleric acid | 5.1 |
| Valeric acid | 6.9 |
| 4-Methyl pentanoic acid | 8.5 |
| Hexanoic acid | 9.2 |

Table 5 10 µM (n=7) concentrations (bank subtraction used for acetic and propionic acids)

| | Acetic Acid | Propionic Acid | Isobutyric acid |
|---|-------------|----------------|-----------------|
| 1 | 9.57 | 11.38 | 10.29 |
| 2 | 9.71 | 11.37 | 10.17 |
| 3 | 10.56 | 11.91 | 9.91 |
| 4 | 10.17 | 11.71 | 10.10 |
| 5 | 10.82 | 12.16 | 10.90 |
| 6 | 10.61 | 11.49 | 9.48 |
| 7 | 13.33 | 13.20 | 9.81 |

It is recommended to use an internal standard such as deuterated acetic acid if lower %RSDs are required. Quantitation results will be biased high at a low concentration region of the calibration curve and may require an internal standard to be injected at a low level. If the concentration of the internal standard is too high, it will not properly reflect the target compounds' behavior at low concentrations.

Conclusion

Analysis of aqueous short chain fatty acids is conducted with GCMS-QP2020 NX.

No derivatization or internal standard was used in this experiment and the measured concentration range was 4 µM to 100 µM. Concentration by steam distillation or dilution with water can be possibly explored to bring a target concentration into this measurement range(1, 2).

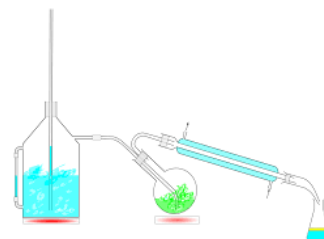


Fig. 4 Steam distillation

- J.B. Zijlstra, J.Beukema, B.G.Wolthers, B.M.Byrne, A.Groen and J.Dankert, Pretreatment methods prior to gas chromatographic analysis of volatile fatty acids from faecal samples, Volume 78, Issue 2, January 14th 1977, Pages 243-250, ISSN 0009-8981
- D.C.Dyer, A new method of steam distillation for the determination of the volatile fatty acids, including a series of colorimetric qualitative reactions for their identification, J.Biol. Chem. 1917, 28: 445-473