

STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Volatile fatty acids (VFA)
BIOVIT-nr.: Arb1132

1. Introduction:

Volatile fatty acids (VFA), are separated by gas chromatography. Column, gas flow and temperature program are taken from the application GC_FF00653. (www.restek.com/chromatogram/view/GC_FF00653).

2. Reagents:

"Homemade" external standard with content of acids corresponding to average values for rumen fluid. This is made from pure concentrated acids and diluted with distilled H₂O (MilliQ) added 5% formic acid. Content of acids should be: Acetic acid: 60, propionic acid 20, iso-butyric acid 1.5, butyric acid 20, iso-valerian acid 1.5 and valerian acid 1.5; all in millimol per liter (mM).

Internal standard: 30 mM solution of 2-methyl valeric acid diluted with distilled H₂O added 5 % formic acid.

3. Risk assessment

4. Equipment

- GC: Trace 1300 with autosampler (Thermo Scientific).
- Software: Chromeleon (Thermo Scientific).
- Column: Stabilwax – DA; 30m, 0.52 mm ID, 0.25 µm (Restek, cat. # 11023).

5. Special remarks

It is very important to have a clean system. The formic acid makes all parts of the system dirty, such as injection needle and liner. Check that all parts are clean before starting and check for carry over and that the injection needle is working properly during the analysis.

The operator must be familiar with the use of GC and software for the withdrawal of areas. Separate Excel file is used for calculating concentrations in mmol/L and molar percentage for the individual acids. Sum of acetic acid, propionic acid and butyric acid, as a rule, shows 95% of the amount of acid. If internal standard is used, all areas (both in standards and samples) must be divided by the area of the internal standard. This will correct for variations in injected volume as well as changes in instruments sensitivity during the analysis.

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6. Sample material:

Rumen fluid added concentrated formic acid (normally 5% FA) or frozen rumen fluid. The samples are centrifuged in 20min. at 3000 rpm. 1 mL is pipetted into Eppendorf tubes and centrifuged one more time for 10min. at 13000rpm.

7. Work procedure

Dilute the internal standard solution 1:29 (finale concentration: 1 mM).

Add 400 µL of the rumen fluid sample and 800 µL internal standard solution (1mM) into a 2mL GC-vial.

Do the same for the external standard. (400 µL standard + 800 µL internal standard).

For analysis: Set up the following sequence:

- 3 x water (**only 0.1 µL**) for cleaning the system
- 1 standard
- 8 samples
- 1 standard
- 3 x water (**only 0.1 µL**) for cleaning the system
- 1 standard
- 8 samples
- 1 standard
- 3 x water

Etc. (always end the sequence with 3 x water)

Column: Stabilwax®-DA, 30 m, 0.52 mm ID, 0.25 µm

Injector temperature: 250 °C

Injection method: Split 3:200

Carrier gas: Helium

Gas flow: 3 mL/min (constant flow)

Start temperature: 90 °C (2 min)

Temperature program: 10 °C/min to 150 °C, 50 °C/min to 250°C (1min)

Detector temperature: 275 °C

Injection volume: 0.3 µL

8. Calculations

$$\frac{\text{Area Fatty acid in sample}}{\text{Area Fatty acid in standard}} \times \text{mmol/L Fatty acid in standard} = \text{mmol/L Fatty acid in sample}$$

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Remember to correct for any dilutions.

If internal standard is used, all areas should be divided by the area of internal standard first.

Excel sheet for calculating is to be found under:

Labmal -> VFA
«VFA beregningsark»

For preparation of external standard (or alternatively standard 1-3):

Labmal -> VFA
«std utregning»

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