

Determination of Volatile Fatty Acids (in Rumen Fluid)

Materials:

Hewlett-Packard 6890N Gas Chromatograph
Hewlett-Packard 7683 Auto-Sampler and 7683 Injector
Supelco NUKOL fused silica capillary column, 15 m x 0.53 mm x 0.5 μ m
Centrifuge
25 ml centrifuge tubes with caps
13 x 100 polypropylene centrifuge tubes
25 mm syringe filter holders (Gelman Sciences)
25 mm glass fiber filter (0.45 μ m)
5 ml polypropylene syringes
12 x 75 mm storage tubes with caps
GC injection vials with Teflon lined caps

Reagents:

Stock standard solution for VFA analysis:

Carefully measure (with a microliter pipette) into 100 ml volumetric flasks:

1. 3.033 ml (3.182 g) Acetic Acid (MCB Cat # AX0077-1, FW 60.05, CAS # 64-19-7)
2. 1.017 ml (1.010 g) Propionic Acid (Sigma Cat # P1386, FW 74.08, CAS # 79-09-4)
3. 0.261 ml (0.253 g) Isobutyric Acid (Sigma Cat # I1754, FW 88.11, CAS # 79-31-2)
4. 1.055 ml (1.010 g) Butyric Acid (Sigma Cat # B-2503, FW 88.11, CAS # 107-92-6)
5. 0.272 ml (0.253 g) Isovaleric Acid (Sigma Cat # 129542-100ML, FW 102.13, CAS # 79-31-2)
6. 0.269 ml (0.253 g) Valeric Acid (Sigma Cat # V0125, FW 102.13, CAS # 109-52-4)

Bring to volume with 18MOhm water.

Internal standard solution for calibration standards and rumen fluid:

25% (w/v) Metaphosphoric acid solution:

250 g metaphosphoric acid (Mallinckrodt Cat # 2816-04, FW 79.98, CAS # 37267-86-0) dissolved in 900 ml H₂O, mix well and add 2 g 2-Ethylbutyric Acid (2-EB) (Aldrich Cat # 10995-9, FW 116.16, CAS # 88-09-5), and dilute to 1 liter with 18 MOhm water.

Calibration standards for VFA analysis:

The GC is calibrated using specific standards with concentrations that are close to the actual VFA concentrations in the rumen fluid. We use 4 ml, 7 ml, and 10 ml of the stock standard solution individually diluted to 50 ml with 18 MOhm water. These are our “working” standards. The calibration standards are made with 5 ml of the “working” standards and 1 ml internal standard (2-EB). Vortex well.

	Calibration Concentrations in mM		
	<u>Standard 4</u>	<u>Standard 7</u>	<u>Standard 10</u>
Acetic Acid	42.00	73.50	105.00
Propionic Acid	10.80	18.90	27.00
Isobutyric Acid	2.26	7.98	11.40
Butyric Acid	9.08	15.89	22.70
Isovaleric Acid	1.96	6.86	9.80
Valeric Acid	1.96	6.86	9.80

Procedure:

Rumen Fluid Preparation

1. If frozen, thaw rumen fluid samples at room temperature or overnight in refrigerator.
2. Mix rumen fluid well in Whirl-Pak and pour into 25 ml centrifuge tubes. Balance by weight and place in centrifuge.
3. Centrifuge at 20,000 x g for 10 minutes.
4. Pipette 5 ml supernatant into 13 x 100 ml pp centrifuge tubes. Add 1 ml internal standard (25% metaphosphoric acid 2-EB solution). Vortex and allow to stand in ice water for 30 minutes. Do not discard remaining rumen fluid. Save for ammonia and/or cobalt analysis.
5. Centrifuge tubes for 10 minutes at 10,000 x g.
6. Filter liquid through 0.45 um filter into 12 x 75 mm storage tubes. Cap and store in the refrigerator.
7. Load GC vials. The fluid is now ready to be analyzed for VFA content on the GC. Injection Volume is 1.0 µl.

References

Baumgardt, B. R., Practical Observations on the Quantitative Analysis of Free Volatile Fatty Acids (VFA) in Aqueous Solutions by Gas-Liquid Chromatography. Departmental Bulletin 1 (June 1964). Department of Dairy Science, University of Wisconsin, Madison, WI.

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Byers, F. M., Organic Acid Analysis in Rumen Fluid and Fermented Feeds. 1979, O.A.R.D.C

Fritz, J. S. and G. H. Schenk, 1979. Quantitative Analytical Chemistry. 4th Edition, Allyn and Bacon, Inc., Boston MA. Goetshen and Galyean. 1983.