Determination of volatile fatty acids (VFA) in water-based solutions using ethyl acetate extraction

Protocol # A – 10 **Revised 02/07/2024**

Objective

To determine the concentration of volatile fatty acids in water-based solutions using ethyl acetate extraction.

Materials

Polypropylene tubes (Fisher; cat no. 02-681-200)	GC vials (ThermoScientific; cat no. 6ASV9-1P)	Red caps
Caps for polypropylene tubes (Fisher; cat no. 02-681-207)	GC caps (ThermoScientific; cat no. 6ASC9ST1)	1 mL pipette & tips
12 × 75 mm glass tubes (Fisher; cat no. 14-961-26)	Transfer pipettes (Fisher; cat no. 13-711-9AM)	Sulfuric acid
5 mL pipette & tips	Ethyl acetate	DI water
10 mL syringe	Needles	

Reagents

Stock VFA standards

25% (wt/vol) meta-phosphoric acid solution containing 2 g/L of crotonic acid

Table 1. Preparation of stock VFA standards.

VFA	MW	RT	Volume to add, µL	mg/mL (approx. in rumen)	m*M*
Acetic	60.05	5.0	410	4.3	71.61
Propionic	74.08	5.6	220	2.19	29.56
Isobutyric	88.1	5.8	10	0.08	0.91
Butyric	88.1	6.4	140	1.32	14.98
Isovaleric	102.13	6.7	10	0.05	0.49
2 MB	102.13	7.5	10	0.05	0.49
Valeric	102.13	8.1	45	0.41	4.01
Caproic	116.16	8.9	10	0.025	0.22

Note: Stock VFA standards and meta-phosphoric and crotonic acid solutions are available in the refrigerator in bldg. 8004 or made upon request by the Lab Manager. If standards are >6 months old, prepare new standards.

Stock VFA standard preparation

1. Two independent stock VFA standards are prepared in 100 mL volumetric flasks
2. Each acid from Table 1 is added to each volumetric flask and filled to volume with DI water
3. These reagents are transferred to 125 mL bottles, fitted with a blue stopper, and crimp sealed

Table 2. Dilution of stock VFA standards to create working standards.

Standards	VFA, %	VFA standard to add, mL	DI Water, %	DI water to add, mL
0	0	0	100	4
1	25	1	75	3
2	50	2	50	2
3	75	3	25	1
4	100	4	0	0

Table 3. Meta-phosphoric and crotonic acid solution preparation.

Total reagent, mL	Meta-phosphoric acid, g	Crotonic acid, g	
100	25	0.2	Fill to volume with DI water
1000	250	2	
2000	500	4	

Working VFA standard preparation

1. Using a 10 mL syringe and needle, transfer the appropriate amount of stock VFA solution and DI water to labeled polypropylene tubes (according to Table 2)
    a. Use a separate syringe and needle for the stock standards and DI water (3 of each)

2. Acidify working standards with the appropriate amount and concentration of sulfuric acid
    a. Standards must be prepared and acidified according to the preparation of samples
    b. Example: If samples received 1 mL of 20% sulfuric acid per 100 mL of rumen fluid, then standards should receive 0.04 mL of 20% sulfuric acid per 4 mL of working standard

3. From this point, working VFA standards should be prepared as samples below

Sample preparation

1. Assuming samples have been stored in the freezer:
    a. Defrost samples using a fan
    b. Vortex samples to homogenize
2. Centrifuge strained ruminal fluid, in duplicated polypropylene tubes, at 10,000 × g for 15 min
    a. If using 15 mL conical tubes to store samples, transfer 3.5 mL of fluid into each of two separate polypropylene tubes – these are now the duplicated tubes, a & b
    b. Centrifuge cups **must be balanced by weight**
    c. Caps should be removed before centrifuging

3. Pipette 2.0 mL of the supernatant from the original tubes (just centrifuged) into another set of prelabeled polypropylene tubes
4. Add 0.4 mL of meta-phosphoric and crotonic acid solution to supernatant
    a. If there is not enough sample remaining to retrieve 2.0 mL of supernatant, adjust the amount of meta-phosphoric and crotonic acid solution so that a 5:1 ratio of supernatant to meta-phosphoric and crotonic acid solution is maintained

5. Freeze the sample with meta-phosphoric and crotonic acid solution overnight in the -20°C freezer
6. **Next day:** Defrost, vortex, and centrifuge samples at 10,000 × g for 15 min
    a. Centrifuge cups **must be balanced by weight**
    b. Caps should be removed before centrifuging

7. Pipette 1 mL of the sample into labeled glass tubes (12 × 75 mm)
    a. Add 2 mL of ethyl acetate to samples and standards for extraction
    b. Cap the glass tubes with the red caps
    c. Vortex the glass tubes
        i. Allow the layers to separate before moving to the next step
8. Using a transfer pipette, carefully aspirate the top layer and transfer to labeled GC vials
    a. If you disturb the layers so that they are again mixed, just recap the tube, vortex, and allow the layers to separate again
9. Add GC vials to carousel for VFA analysis (Fig. 1)

VFA data collection and calculations

When all of your samples have been analyzed, please let the Lab Manager know and she will retrieve the raw values for you. Each sample will create a report – the information you need can be found in the report. Please see the Lab Manager for training on how to transfer the data from the report to the VFA excel spreadsheet.

References

Ruiz-Moreno, M., E. Binversie, S. W. Fessended, and M. D. Stern. 2015. Mitigation of in vitro hydrogen sulfide production using bismuth subsalicylate with and without monensin in beef feedlot diets. J. Anim. Sci. 93:5346-5354. doi:10.2527/jas2015-9392