13.3 Estimation of total volatile fatty acids by gas chromatography

Chromatography is a physical method for separating components in a mixture. The basis of separation lies within the separating column, which is small-diameter tubing packed with a medium of large surface area. A mobile phase percolates through the stationary medium. The name gas chromatography denotes that the mobile phase is a gas.

The gas liquid chromatography (GLC) has a stationary phase, a liquid spread over the surface of a solid support. In GLC, a stream of carrier gas flows through the column. A sample is injected into the carrier gas near the head of packed chromatographic column. Separation of various components of the sample occurs as a result of multiple forces by which the column material tends to retain these components. In the gas phase the components move towards the outlet, but they are selectively retarded by the stationary phase. Consequently, all components pass through the column at varying speeds and emerge in the inverse order of their retention by the column material. Upon emerging from column the gaseous phase immediately enters a detector attached to the column. Here the individual components register a series of signals which appear as peaks on the chromatogram.

The chromatographic peaks are used for qualitative and quantitative detection of the components. A typical chromatogram consists of a baseline and the peaks. The distance between peaks is influenced by the rate of flow of gas, the column conditions and column temperature. However, peak area is related to the concentration of component. Therefore, under constant conditions, a comparison between a standard reference with that of sample will tell about the concentration of a component.

Preparation of sample extract

Draw rumen liquor from a cannulated animal and filter it through 4 layers of muslin cloth.

Reagents

- VFA (C_2-C_5 standard solution (25 mg/ml) – Transfer 2.5 g of individual volatile fatty acid (acetic, propionic, butyric, valeric and iso-valeric), in separate 100 ml volumetric flask and make up the volume with distilled water.
- Lactic acid standard solution (100 mg/ml) – Transfer 2.5 g lactic acid in a 25 ml volumetric flask and make up the volume.
- Pivalic acid internal standard (2.5 mg/ml) – Dissolve 0.5 g pivalic acid in distilled water and dilute to 200 ml.
- Oxalic acid (0.12 M) – Dissolve 3.782 g oxalic acid dehydrate in water and dilute to 250 ml.
- Composite working standard – Pipette 1 ml of C_2-C_5 standard solution (reagent 1), 2.5 ml lactic acid standard solution (reagent 2), 10 ml pivalic acid (reagent 3) and 25 ml oxalic acid solution (reagent 4) into a 100 ml volumetric flask and dilute to volume.

Procedure

1. Set the GLC according to manufacturers' instructions to the following operating conditions:

Gas flows	Nitrogen	24 ml/min
	Hydrogen	50 ml/min
	Air	500 ml/min
Temperature		
	Injection	220ºC
	Detector	240ºC
	Oven	175ºC

2. Make repeat 1.0 µl injections of the composite working standard until a stable response is obtained. The total chromatographic run time is approximately 30 minutes.

Examination of the sample extract

Pipette 5 ml of strained rumen liquor into a 10 ml volumetric flask, add 1 ml of the pivalic acid solution (reagent 3) and 2.5 ml of oxalic acid solution (reagent 4), then dilute to volume. Centrifuge the resulting extract at 2600 g for 5 minutes. Inject 1.0 µl of the supernatant and integrate the peaks eluted.

Calculation

Calculate mg/ml of each acid in the strained rumen liquor from the expression:

Conc. of unknown = (RF_1/RF_2 x standard conc. x 2)

Where,

$$RF_1 = \frac{\text{Area of sample component peak}}{\text{Area of internal standard}}$$

$$RF_2 = \frac{\text{Area of known peak}}{\text{Area of internal standard}}$$

Reference: Applied Animal Nutrition Research Techniques by Raman Malik and SK Sirohi, NDRI, Karnal – 132 001, Haryana.