

## 11.7 Fatty acid analysis of milk

### Reagents

- Ethanol (absolute)
- 5 N sodium hydroxide (NaOH) (200 g of NaOH pellets / 1 litre of distilled water).
- 5 N hydrochloric acid (HCL) (500 ml of concentrated acid added to 500 ml of distilled water)
- Petroleum ether (PE) (Boiling range 40-60°C)
- 1% sulphuric acid in Methanol.
- 5% Salt solution (NaCl)

### Procedures

Mix milk samples thoroughly by shaking.

### Saponify

- Pipette 2 ml of a milk sample into a 25 ml stoppered test tube
- Add 1 ml of ethanol
- Add 1 ml of 5 N sodium hydroxide (NaOH)
- Shake well and cover with foil
- Place in oven @ 80°C for 1.5-2 h.
- Remove from oven
- Allow to cool.

### Acidify

- Add 5 N hydrochloric acid (HCL) approx 2 ml.
- Invert test tube with care.
- pH must be checked for each sample using pH paper till acid (must be pink)
- When sample has cooled sufficiently
- Extract fatty acids – add 4 ml of petroleum ether, shake well, pipette the supernatant into a labelled 15 ml test tube.
- Repeat above step – pooling the extracts
- Evaporate pooled PE extracts to dryness in a warm water bath under a stream of nitrogen.

### **Methylate**

- To the dried sample add 3 ml of 1% sulphuric acid in methanol
- Reflux on a heating block at 50-60°C for 1.5 h
- Add 3 ml of 5% NaCl (salt solution)
- Add 3 ml of PE
- Cool, shake well
- Centrifuge
- Decant supernatant into GLC vial and cap
- Make sure samples are clearly labelled with number and date
- Analyze the fatty acid composition using gas chromatograph (Fig. 11.3).



*Fig. 11.3 Gas chromatograph*

**Reference:** Christie, W.C. 1993, Gas chromatography and lipids. The Oily Press Ltd., Dundie, Scotland, UK.; Gulati S.K. (1976). Protected triacylglycerol and sterol supplements for ruminants. MSc thesis, Macquarie University, North Ryde, NSW, Australia.