11.4 Amino acid analysis by hydrolysis method (Non Oxidised)

This method is used for the quantitative determination of amino acids in materials such as grain, feedstuff, milk, etc., which do not just consist of pure protein.

Reagents

- 6 N Hydrochloric acid
- Lithium citrate buffer: pH 2.20

Equipment

- Serial heating mantle set up for 250 ml round bottom flasks with water-cooled condensers.
- Rotary evaporator
- Amino acid analyser (Fig. 11.2)

Procedure

1. Accurately weigh 200 mg of sample into a 250 ml round bottom flask. Add 2 glass beads.
2. Add 160 ml of 6 N HCl. Reflux at 110ºC for 23 hours.
3. Filter solution into a 250 ml measuring cylinder, make it up 200 ml with DI water.
4. Take a 50ml aliquot, place it into a 250 ml round bottom flask, and remove the acid using a rotary evaporator connected to a vacuum pump (water bath temperature 60ºC). Rinse the residue twice with DI water and evaporate to dryness. Store 50 ml in a specimen jar in case you need to repeat the procedure discard once you are happy with the results.

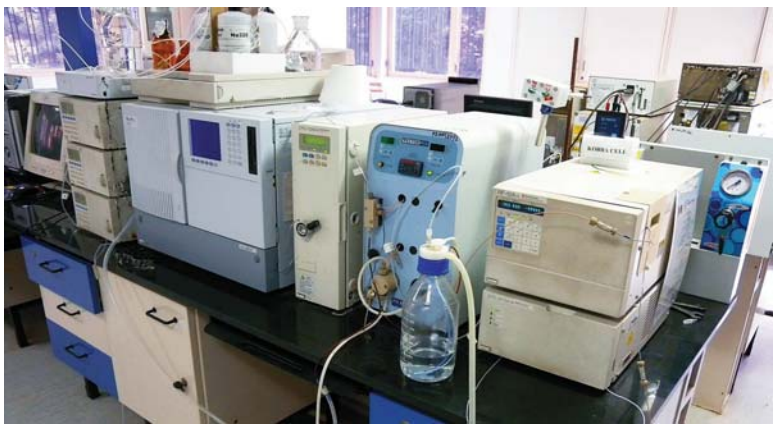

Fig. 11.2 Amino acid analyzer

5. Pipette 5 ml of diluting buffer into the residue, and transfer to a plastic scintillation vial.
6. Filter about 0.5 ml through a 2 um filter, into a vial, so that it is ready to be used, with the appropriate buffer dilution. The samples are run under the “Hydrolysis program” of the Biochrom 20.

- The dilution is worked out according to the Nitrogen% of the sample, i.e. the higher the Nitrogen content the higher the dilution.

Hydrolysis method (Oxidised)

This method is used for the quantitative determination of cystine and methionine, (both acids must be converted into their stable oxidized forms using performic acid) to convert them to cysteic acid and methionine sulphone. During the oxidation tyrosine and histidine will be almost completely destroyed.

Reagents

- Performic acid/hydrogen peroxide oxidation mixture:

To make	**10 ml**	**20 ml**	**30 ml**
Hydrogen peroxide 30%	1 ml	2 ml	3 ml
Perfomic acid 88%	9 ml	18 ml	27 ml
Liquid phenol	50 mg	100 mg	150 mg

Weigh the liquid phenol into a 50 or 100 ml conical flask, then add the other two reagents and mix, incubate for 1 hour at 30ºC., using a water bath.

- Sodium disulphite.
- 6 N Hydrochloric acid + phenol: (50 ml 6 N HCl, containing 50 mg phenol).
- Lithium citrate buffer: pH 2.20.

Equipment

- Serial heating mantle set up for 250 ml round bottom flasks with water-cooled condensers.
- Rotary evaporator and amino acid analyser

Procedure

1. Accurately weigh 100 mg of sample into a 100 ml round bottom flask, and place them with enough ice to cover a third of the flask.
2. Prepare the oxidation mixture, cool it together with the samples in ice.
3. Add 5 ml of solution to each sample, swirl the flask to mix in the solution.
4. Cover each flask with parafilm, and leave in the fridge for 16 hours. This is where the oxidation occurs.
5. To stop the reaction, add 840 mg of Sodium bisulphate.
6. The hydrolysis is performed with 60 ml of 6 N HCl containing 60 mg phenol, add boiling chips, and reflux at @ 110ºC for 23 hours.
7. Filter solution into a 100 ml measuring cylinder, make it up to 100 ml with DI water.
8. Take a 50 ml aliquot, place it into a 250 ml round bottom flask, and remove the acid using a rotary evaporator connected to a vacuum pump (water bath temperature 60ºC). Rinse the residue twice with DI water and evaporate. Store 50 ml into a specimen jar in case you need to repeat the procedure, discard once you are happy with the results.
9. Pipette 5 ml of diluting buffer into the residue, and transfer to a plastic scintillation vial.
10. Filter about 0.5 ml through a 2 um filter, into a vial, so that it is ready to be used, with the appropriate buffer dilution.*

    *The dilution is worked out according to the Nitrogen % of the sample, i.e. the higher the Nitrogen content the higher the dilution.

References: Biotronic amino acid analyser LC 5001 Manual.