

DETERMINATION OF METHIONINE, THREONINE, LYSINE, AND CYSTINE IN FODDERS AND RAW MATERIALS BY CAPILLARY ELECTROPHORESIS

INTRODUCTION

The method enables fast quantitative determination of amino acids methionine, threonine, lysine, and cystine in feeds, mixed fodders, and raw materials.

MEASURING METHOD

Capillary electrophoresis method for the determination of amino acids is based on the differential migration of ionic forms of amino acids in a narrow quartz capillary under the influence of the applied electric field. Identification of amino acids is performed basing on their own absorbance in the UV range at 190–200 nm. Capillary electrophoresis is performed twice: first for the determination of lysine, threonine, and cystine in borate buffer at 20 °C and then for methionine in borate buffer with β -cyclodextrin at 40 °C.



CONCENTRATION RANGES

Ranges of percentage for analyzed amino acids in samples are presented in the table.

Amino acids	Percentage of amino acids, % (w/w)
Lysine	0.25–10.0
Threonine	0.25–3.0
Methionine	0.2–2.0
Cystine	0.3–3.0

ADVANTAGES OF CAPILLARY ELECTROPHORESIS

Capillary electrophoresis has several advantages in comparison with the method of amino acid determination in the mixed fodders by amino acid analyser:

- Absence of derivatization
- Low analysis cost
- Absence of an expensive chromatographic column
- Short analysis time

EQUIPMENT AND REAGENTS

The following equipment and reagents are used in measurements:

- CAPEL[®]-105 capillary electrophoresis system with a special capillary cassette for the amino acid analysis
- Distilled deionized water
- Sodium hydroxide
- Hydrochloric acid
- Sodium tetraborate
- *L*- or *D,L*-Amino acids
- β -Cyclodextrin
- Benzoic acid

All reagents must be of analytical grade or better.

Data acquisition and integration is accomplished with PC with Windows[®] 95/98/NT/ME/2000/XP, using "Chrom&Spec[®]" chromatographic software.

PREOPERATIONAL PROCEDURES

Preparation should include the following steps:

- sampling and sample pretreatment
- preparation of solutions
- capillary conditioning

MEASUREMENT PROCEDURE

Sampling is accomplished in accordance with the standard certified protocol.

Capillary conditioning is done by rinsing the capillary consequently by 1M hydrochloric acid, water, 1M sodium hydroxide, water and background electrolyte.

Hydrolysis of fodders is done according to the standard protocol in a close vessel with 6 M hydrochloric acid within 14–16 hours at 110 °C.

Upon the removal of the excess of the acid, the sample solution is analyzed by capillary electrophoresis in accordance with the program that is preset in CAPEL®-105 system.

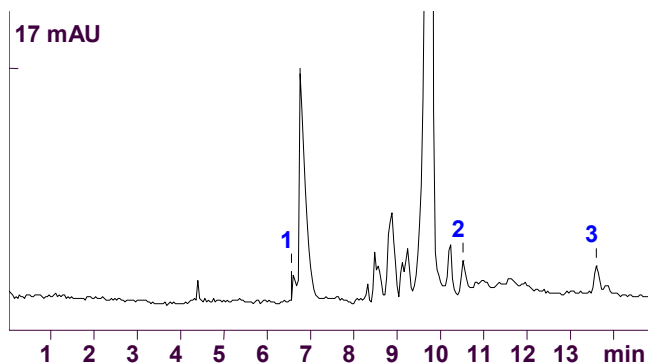
DATA PROCESSING

Data analysis, integration and calculation of concentrations is done by the “Chrom&Spec® for Windows®” software.

EXAMPLES OF REAL ANALYSIS

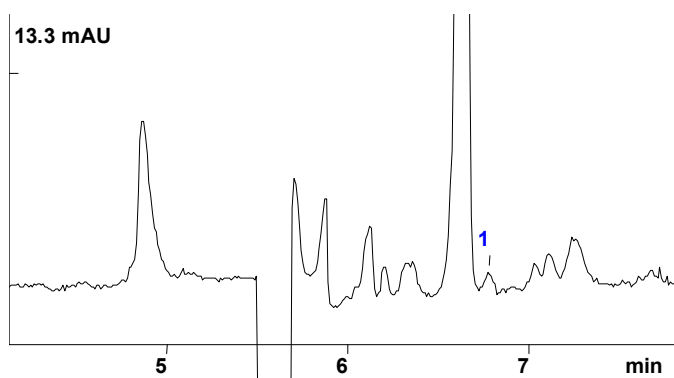
Sample: fish flour (100 mg)

- 1 – lysine (3.12%)
2 – threonine (2.24%)
3 – cystine (0.56%)



Sample: fish flour (100 mg)

- 1 – methionine (1.35%)



LITERATURE

Komarova N. V., Kamentsev J. S., Solomonova A. P., and Anufrieva R. M. Determination of amino acids in fodders and raw materials using capillary zone electrophoresis // Journal of Chromatography B. – 2004. – V. 800. – No. 1–2. – P. 135–143.