Subject: Research Methods in Entomology

Topic: Amino Acid Analyzer (AAA)

Class: MS (Replica)

Department of Zoology

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Course Code: Zoo-E-Ento-501

Content

▶ Introduction to AAA

▶ Features

▶ Principle

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Introduction

▶ AAA is specifically optimised for analysis of free amino acids.

▶ PURPOSE:

Detection of presence of AA in variety of solutions, such as

▶ ☐ extracellular and intracellular fluids.

▶ ☐ plant and animal tissues.

▶ ☐ broths, and fruits.

▶ ☐ beverage juices.

▶ ☐ Detection of presence of hydrolyzed AA, such as found in protein, collagen, peptides, and processes foods.

AMINO ACID ANALYZER

Key Features

- • AAA is fully automated.

- • Enables to analyze AA automatically without sample preparation.

- • Reproducible results while minimizing errors between operators.

- •Easily analyze 40 kinds of primary and secondary AA with pre-established analysis methods.

AMINO ACID ANALYZER

AMINO ACID ANALYZER
Principle

▶ The system utilizes <u>ion-exchange chromatography</u> incorporating post column reaction with ninhydrin and subsequent detection in the <u>visible region spectrum</u>.

Types of AA Analysis

▶ The following 2 groups of tests are important –

▶ Screening tests - Quantitative tests to monitor treatment or confirm an initial diagnosis.

▶ Specific tests - that identify an unknown amino acid or metabolite

AMINO ACID ANALYZER WORKING

▶ Sample preparation:

▶ Peptides, proteins or other high molecular compounds have to be removed and this could be done by

▶ ☐ Acid precipitation (by using Sulfosalisilic acid)

▶ ☐ Ultra filtration

▶ ☐ Ultracentrifugation

AMINO ACID ANALYZER SAMPLE PREPARATION

▶ pH Adjustment:

▶ Each AA has isoelectric pH (PI) and its charge will be natural at this pH.

▶ ☐ By increasing H ions it will be positively charged.

▶ ☐ By increasing OH ions it will be negatively charged.

AMINO ACID ANALYZER
Steps for sample analysis

▶ The sample go through 4 steps:

▶ 1. Autosampler.

▶ 2. Separation column reaction.

▶ 3. Coil reaction.

▶ 4. Photometer.

Autosampler

- Before sample analysis commercially available standard solution are analyzed to calibrate instrument.

- □Glutamine is not present in standard as it decomposes quickly. Therefore - Fresh glutamine standard solution is prepared, - Then the freshly prepared sample is injected, - And glutamine peak is identified from retention time.

- □Sample is inserted in the specified place then the autosampler inject 130 µl of the sample and pass it to separation column.

Separation Column Reaction

▶ Separation column is the stationary phase.

▶ • Buffers are the mobile phase. •

▶ Special pump pumps the buffer to the separation column.

▶ • As AA are eluted at different pH medium different pH values buffers are used.

▶ • Ion exchange reaction take place and positively charge AA are bound to negatively charged sites in the separation column.

▶

Separation Column Reaction

▶ They are eluted by the continuous flow of cation, or by increasing cation concentration developed by gradient formation.

▶ Once the final amino acid is eluted regeneration solution is used to regenerate the separation column by removal of amino acids remnant on the column.

Coil Reaction

▶ After separation instrument add the ninhydrin solution that react with the products at 130° C.

▶ All products give purple color and are estimated at 570 nm except proline and hydroxyproline they give yellow color and are estimated at 440 nm.

Photometer

After the ninhydrin reaction, colored species then are detected with a spectrophotometer at two wavelength 570 and 440 nm.

Quantity of colored complex produced is directly proportional to concentration of particular AA present in sample.

Recorder

- Photometer is linked to a two channel recorder where a series of peaks representing the AA are recorded.

- AA are identified by comparison of retention times of components in specimen to those of reference compounds.

- Information is transferred to a specific computer program where Quantitation of each AA could be done.

AMINO ACID ANALYZER Applications

► • Estimate amount of peptide/protein.

► • Estimate degree of purity.

► • Determine amino acid composition.

► • Estimate amount of unusual amino acids.

► • Identifying proteins in databases.