

Determination of AMINO ACIDS by HPLC

An extensive range of amino acids using automatic OPA derivatization in the CMA/200, gradient HPLC and fluorescence detection with the CMA /280 Fluorescence Detector.

INSTRUMENTS and METHOD

Pump: Gradient pump; Low pressure mixing.
Degasser: CMA/260
Injector: CMA/200 Refrigerated Microsampler
Detector: CMA/280 Fluorescence Detector
Column: Nucleosil C₁₈. 5µm, 60 x 4 mm. Knauer.
Precolumn: Nucleosil C₁₈. 5µm, 5 x 4 mm. Knauer.

Integrator or integration software.

Mobile phase:

A: 0.1 M sodium acetate, pH 6.95 : Methanol : Tetrahydrofuran (92.5 : 5 : 2.5)
B: Methanol : Tetrahydrofuran (97.5 : 2.5)

Gradient:

Minutes:	0	6	8	12	15	18	20	24	24.5	26	35
% A:	100	100	90	75	60	60	40	0	0	100	100
% B:	0	0	10	25	40	40	60	100	100	0	0

Flowrate: 1.2 mL/minute

Detection: Excitation, 330 - 365 nm; Emission, 440 - 530 nm.

Derivatisation: OPA / 2-mercaptoethanol

Temperature Microsampler: +6 °C
Column: Ambient

REAGENTS

1. Acetate buffer, 100 mmol/L, pH 6.95

Sodium acetate, Mw: 82.03 8.2 g
Distilled water to 900 mL
Adjust pH to 6.95 with acetic acid
Distilled water to 1000 mL
Filter through a 0.45 µm filter.

2. Mobile phase A

Acetate buffer (1) 925 mL
Methanol 50 mL
Tetrahydrofuran 25 mL

3. Mobile phase B

Methanol 975 mL
Tetrahydrofuran 25 mL

4. Borate buffer, 0.4 mol/L, pH 9.3

Boric acid, H₃BO₃ 2.47 g
Distilled water to 90 mL
Adjust pH to 9.3 with sodium hydroxide
Distilled water to 100 mL

5. Derivatisation reagent, OPA 40 mmol/L

o-phthaldialdehyde, C₈H₆O₂, Mw: 134.1 Sigma P-1378 27 mg
Ethanol, 99 % 500 µL
2-mercaptoethanol, SHCH₂CH₂OH, Fluka 20 µL
Borate buffer (4) 4.5 mL

Store in refrigerator. Let "age" for 24 hours before use.
Add 5 µL 2-mercaptoethanol once a week.
Can be used for two weeks.

CALIBRATOR

Amino acid calibrator, 18.75 µmol/L

Sigma, AA-S-18*, 2.5 mmol/L

75 µL

Distilled water to

10.0 mL

Freeze in portions of 1000 µL at -20°C.

Amino acid calibrator, 1.875 µmol/L

Amino acid calibrator, 18.75 µmol/L

100 µL

Distilled water to

1000 µL

* The components in AA-S-18 are:

Alanine, ammonium chloride, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine.

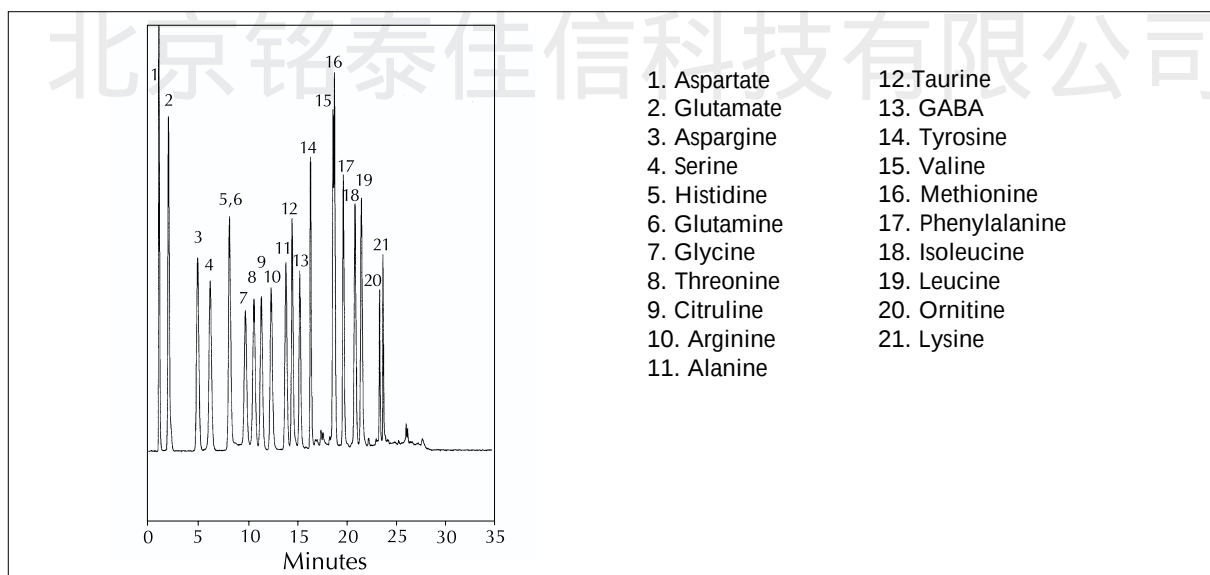
If additional amino acids are to be analysed, they can be added to the calibration solution.

ANALYSIS

Let the pumps run with eluents for at least 30 minutes to equilibrate the column.

Inject 10 µL of distilled water to check that there are no extra peaks.

1. Pipette 10 µL of samples to be analyzed (calibrators and unknowns) into sample vials and place in Microsampler.
2. Add derivatization reagent (5) to vial in reagent position (63) in Microsampler.
3. Program Microsampler to add 10 µL derivatisation reagent to sample to be injected. Let react for 60 seconds before injection. Inject 10 µL - 18 µL.



References:

1. Lindroth P and Mopper K;

High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatisation with o-phthalaldehyde.

Analytical chemistry, Vol 51, No 11, 1979, 1667-1674.

2. Jan Kehr; CMA Application note no 2.

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