Determination of ASPARTATE and GLUTAMATE by HPLC

A rapid method for analysing glutamate and aspartate using automatic OPA derivatization in the CMA/200, isocratric HPLC and fluorescence detection. An ideal method for studying neuroprotection and neurotoxicity.

APPLICATION NOTE

INSTRUMENTS and METHOD

Pump: CMA/250 LC-pump x 2
Controller: CMA/252 Gradient Controller
Degasser: CMA/260
Injector: CMA/200 Refrigerated Microsampler
Detector: CMA/280 Fluorescence Detector

Column: Nucleosil C_{18}. 5µmm, 60 x 4 mm. Knauer.
Precolumn: Nucleosil C_{18}. 5µm, 5 x 4 mm. Knauer.

Integrator or integration software.

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

Gradient:

Minutes:	0	4.0	4.1	5.0	5.1	10.0
% A:	100	100	0	0	100	100
% B:	0	0	100	100	0	0

Flowrate: 1.0 mL/minute
Detection: Exitation, 330 - 365 nm; Emission, 440 - 530 nm.
Derivatisation: OPA / 2-mercaptoethanol
Temperature Microsampler: +6 ^{0}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 conc H_3PO_4	
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, H_3BO_3	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, $C_8H_6O_2$, Mw: 134.1. Sigma P-1378	27 mg
Ethanol, 99 %	500 µL
2-mercaptoethanol, $SHCH_2CH_2OH$, Fluka	20 µL
Borate buffer (5)	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.

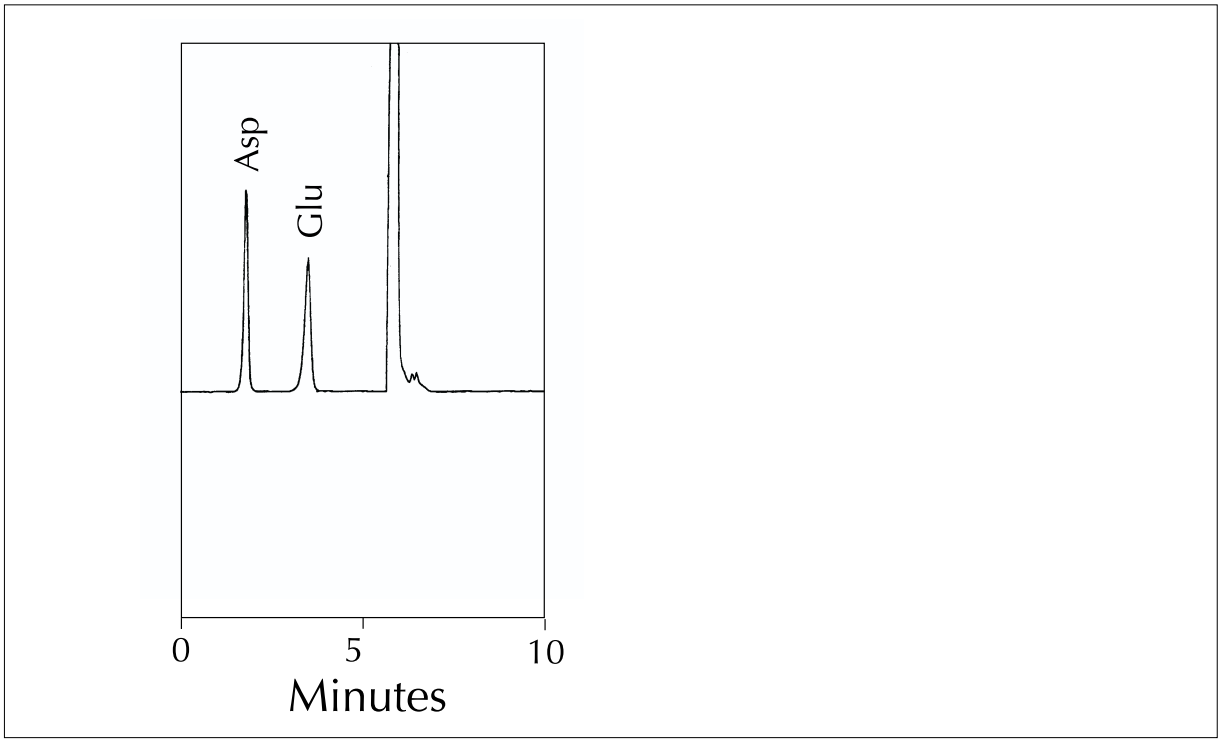
Amino acid calibrator, 1.875 µmol/L

Amino acid calibrator, 18.75 mmol/L	100 µL
Distilled water to	1000 µL

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:
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High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatisation with o-phtaldialdehyde. Analytical chemistry, Vol 51, No 11, 1979, 1667-1674.
2.Jan Kehr; CMA Applications note no 2.

CMA/Microdialysis
www.microdialysis.com

Box 2 • 171 18, Solna
Tel 08-470 10 00 • Fax 08-470 10 50

73 Princeton Street, North Chelmsford • MA 01863 • USA
Tel: +1 (978) 251-1940, (800) 440-4980, Fax: +1 (978) 251-1950

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