Determination of PURINES by HPLC

Analysis of purines with UV detection, e.g. hypoxanthine which is of particular relevance for hypoxia, ischemia and stroke studies especially in the light of free radical formation.

INSTRUMENTS and METHOD

Pump: CMA/250 LC-pump

Degasser: CMA/260

Injector: CMA/200 Refrigerated Microsampler

Detector: UV-detector

Column: Nucleosil C_{18}. 3µm, 100 x 4 mm. Knauer.

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

Integrator or integration software.

Mobile phase: 0.01 M ammonium phosphate, pH 6.0 : Methanol (95 : 5)

Flowrate: 0.8 mL/minute

Detection: 254 nm

Temperature Microsampler: +6 ^{0}C
Column: Ambient

REAGENTS

1. Ammonium phosphate, 2 mol/L

Ammonium phosphate, $(NH_4)H_2PO_4$, Mw: 115.03	23 g
Distilled water to	100 mL

2. Ammonium phosphate buffer, 0.01 mol/L, pH 6.0

Ammonium phosphate, 2 mol/L	(1)	5.0 mL
Distilled water to		900 mL
Adjust pH to 6.0 with H_3PO_4		
Distilled water to		1000 mL

3. Mobile phase

Ammonium phosphate buffer (2)	950 mL
Methanol	50 mL

APPLICATION NOTE

CALIBRATOR

Hypoxanthine, 1 mmol/L

Hypoxanthine, Mw: 136.1. Sigma H-9377.	13.61 mg
Sodium hydroxide, 1 mol/L	10 -100 µL (to solve)
Distilled water to	100 mL

Xanthine, 1 mmol/L

Xanthine, Mw: 152.1. Sigma X-0626.	15.21 mg
Sodium hydroxide,1 mol/L	10 - 100 µL (to solve)
Distilled water to	100 mL

Inosine, 1 mmol/L

Inosine, Mw: 268.2. Sigma I-4125.	26.82 mg
Distilled water to	100 mL

Heat to solve.

Guanosine, 1mmol/L

Guanosine, Mw: 283.2. Sigma G-6752.	28.32 mg
Distilled water to	100 mL

Heat to solve.

Adenosine, 1 mmol/L

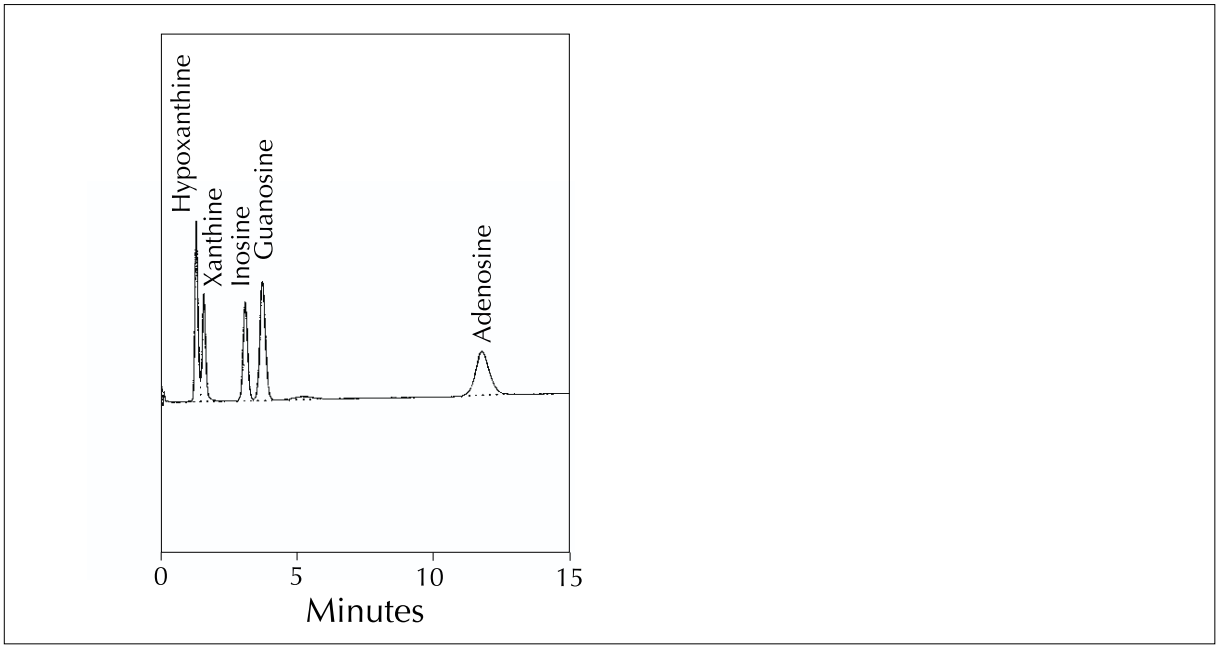
Adenosine, Mw: 316.3. Sigma A-7636.	31.63 mg
Distilled water to	100 mL

Hypoxanthine, Xanthine, Inosine, Guanosine, Adenosine, 10 mmol/L

Hypoxanthine,1 mmol/L	1000 µL
Xanthine, 1 mmol/L	1000 µL
Inosine, 1 mmol/L	1000 µL
Guanosine, 1 mmol/L	1000 µL
Adenosine, 1 mmol/L	1000 µL
Distilled water to	100 mL

ANALYSIS

1. Let pump run with eluent for at least 30 minutes to equilibrate the column.
2. Inject 10 µL of distilled water to check that there are no extra peaks.
3. Pipette samples to be analyzed (calibrators and unknowns) into sample vials and place in Microsampler.



References:

1. Zetterström T, Vernet L, Ungerstedt U, Tossman U, Jonzon B and Fredholm BB; Purine levels in the intact rat brain, studies with an implanted perfused hollow fibre. Neurosci. Lett 29 (1982)

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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