

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 °C Column: Ambient

REAGENTS

1. Ammonium phosphate, 2 mol/L

Ammonium phosphate, (NH ₄)H ₂ PO 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 ₃ PO ₄		
Distilled water to		1000 mL

3. Mobile phase

Ammonium phosphate buffer (2)	950 mL
Methanol	50 mL

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, 1 mmol/L

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

Adenosine, 1 mmol/L

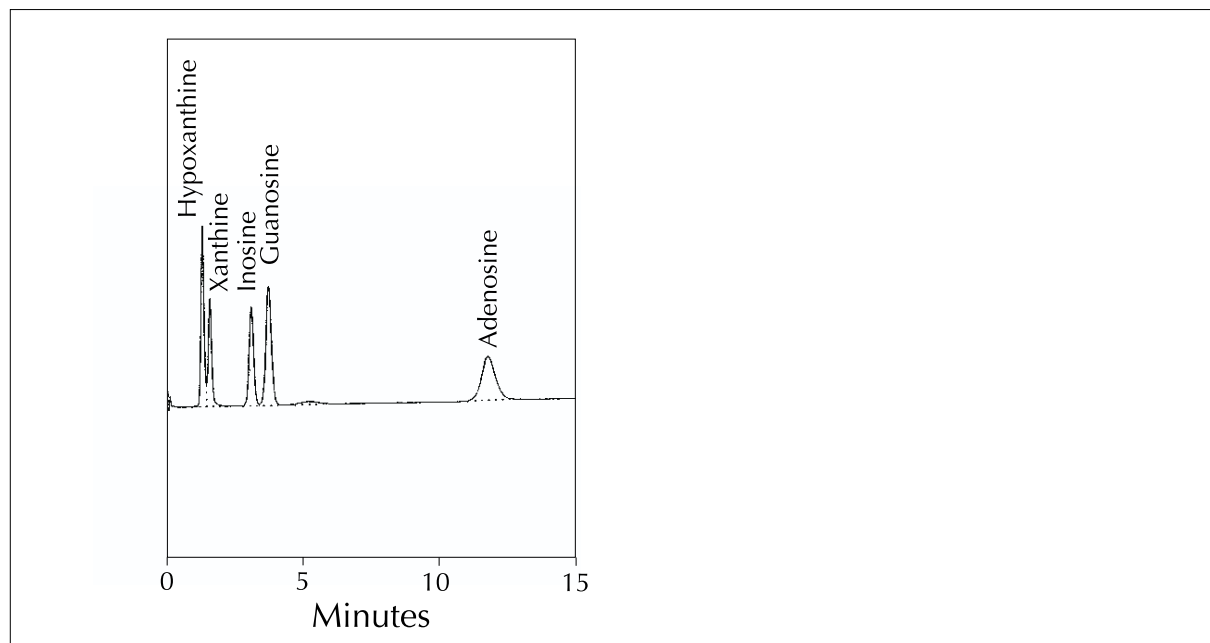
Adenosine, Mw: 316.3. Sigma A-7636. 31.63 mg
Distilled water to 100 mL

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)

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