Determination of LACTATE, PYRUVATE and ASCORBATE by HPLC.

An isocratic analysis with UV detection of lactate, pyruvate and ascorbate of particular relevance for studies of hypoxia, ischemia, stroke and free radicals. A perfect companion to the method described in application note 22. Both methods can be handled by one CMA/200 automatically injecting into two different chromatographs.

INSTRUMENTS and METHOD

Pump: CMA/250 LC-pump
Degasser: CMA/260
Injector: CMA/200 Refrigerated Microsampler
Detector: UV-detector
Column: Polypore H, 10 µm, 220 x 4.6 mm. Brownlee.
Precolumn: Polypore H, 10 µm, 30 x 4.6 mm. Brownlee.

Integrator or integration software.

Mobile phase: 2 mM sulphuric acid
Flowrate: 0.3 mL/minute
Detection: 214 nm
Temperature Microsampler:+6 °C
Column: Ambient

REAGENTS

1. Mobile phase, Sulphuric acid, 2 mmol/L
Sulphuric acid, H₂SO₄, conc. 112 µL
Distilled water to 1000 mL

CALIBRATOR

Lactate, 10 mmol/L
Lactic acid, M w: 96.08, Sigma L-2250 96.08 mg
Mobile phase 100 mL

Pyruvate, 1.0 mmol/L
Pyruvic acid, M w: 110.0, Sigma P-2256 22.0 mg
Mobile phase 200 mL

Ascorbate, 500 µmol/L
Ascorbic acid, M w: 176.13, Merck 127 17.6 mg
Metaphosphoric acid, 2.5 % 200 mL

Lactate 250 µmol/L, Pyruvate 25 µmol/L, Ascorbate 10 µmol/L
Lactate, 10 mmol/L 25 µL
Pyruvate, 1.0 mmol/L 25 µL
Ascorbate, 500 µmol/L 20 µL
Mobile phase 930 µL
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: