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.

APPLICATION NOTE

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 ^{0}C
	Column: Ambient

REAGENTS

1. Mobile phase, Sulphuric acid, 2 mmol/L

Sulphuric acid, H_2SO_4, conc.	112 µL
Distilled water to	1000 mL

CALIBRATOR

Lactate, 10 mmol/L

Lactic acid, Mw: 96.08, Sigma L-2250	96.08 mg
Mobile phase	100 mL

Pyruvate, 1,0 mmol/L

Pyruvic acid, Mw: 110.0, Sigma P-2256	22.0 mg
Mobile phase	200 mL

Ascorbate, 500 µmol/L

Ascorbic acid, Mw: 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

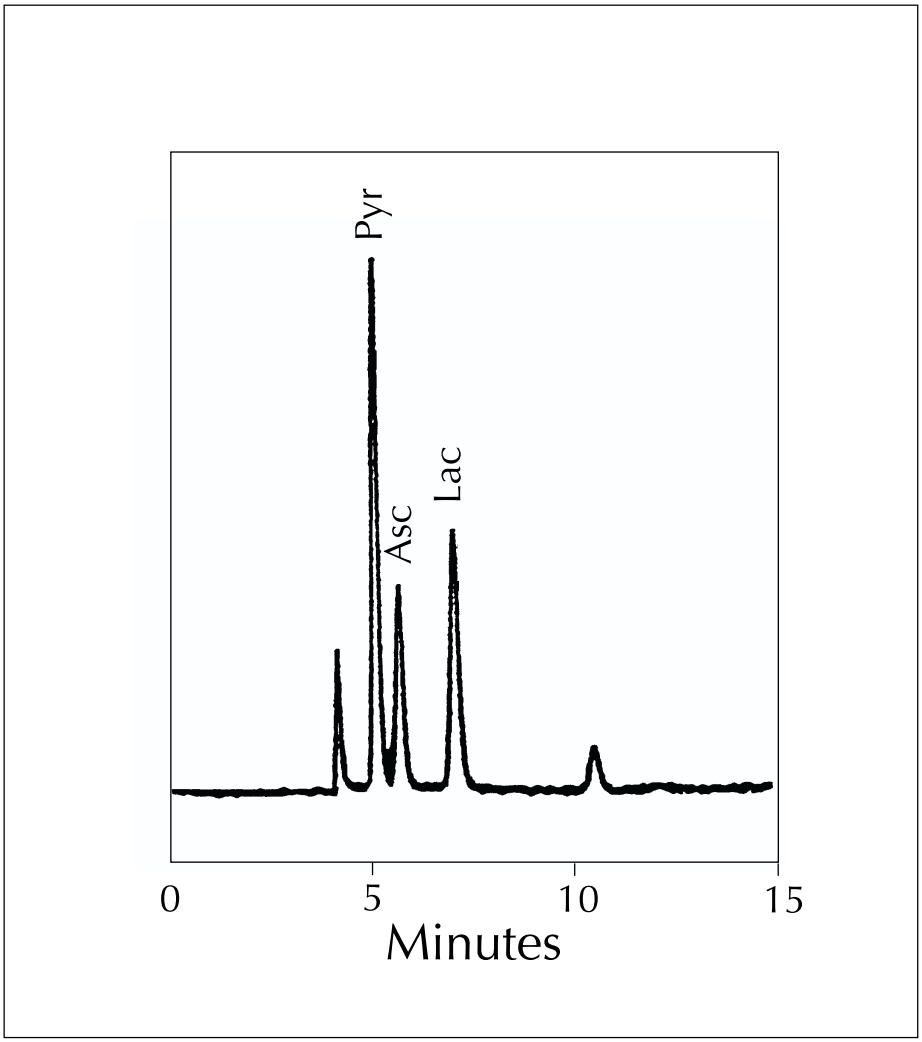
Lactate, 10 mmol/L	25 µL
Pyruvate, 1.0 mmol/L	25 µL
Ascorbate, 500 µmol/L	20 µL
Mobile phase	930 µL

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:
1.Hallström Å, Carlsson A, Hillered L and Ungerstedt U. Simultaneous determination of lactate, puruvate and ascorbate in microdialysis samples from rat brain, blood, fat and muscle using high performance liquid chromatography. Journal of Pharmacological methods, 22 113-124. 1989.

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

June 1997