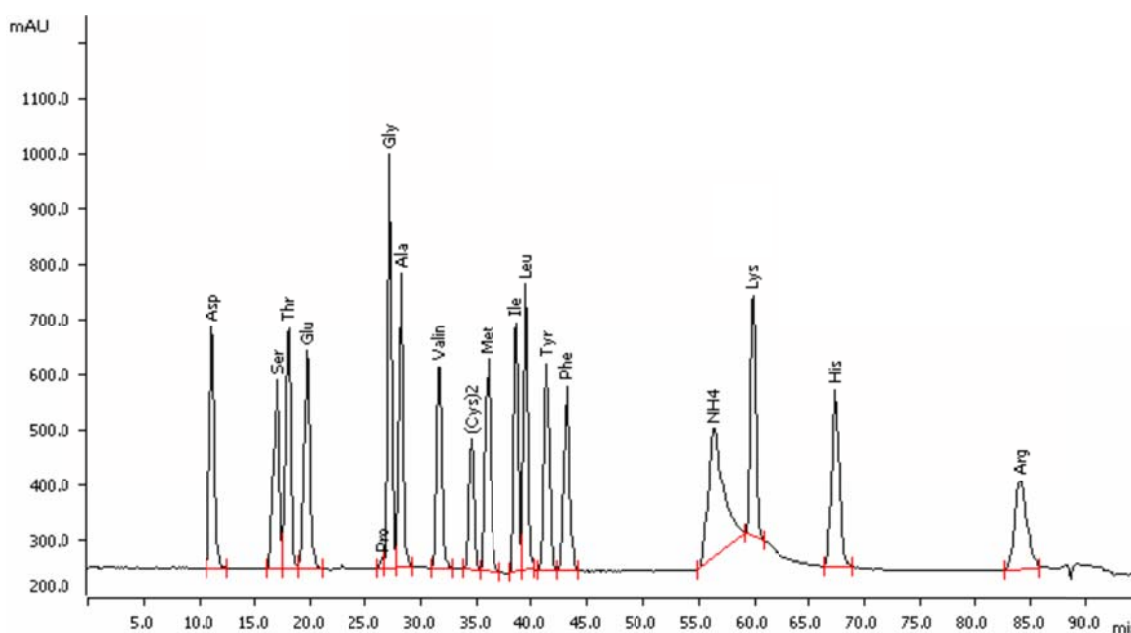


Amino acids applying UV/VIS detection after post-column reaction with ninhydrin at 120 °C



The determination of amino acid is an important task in pharmaceutical and biochemical applications. A binary gradient separates in this example 17 amino acids of a commercially available standard solution. The post-column reaction with ninhydrin requires a temperature of 120 °C, while the samples need to be cooled for stability.

Results

Amino acid	mmol/L	Amino acid	mmol/L	Amino acid	mmol/L
Ala	2.5	His	2.5	Pro*	2.5
Arg	2.5	Ile	2.5	Ser	2.5
Asp	2.5	Leu	2.5	Thr	2.5
(Cys) ₂	1.25	Lys	2.5	Tyr	2.5
Glu	2.5	Met	2.5	Val	2.5
Gly	2.5	Phe	2.5		

* Pro at 440 nm (not shown)

Method description

Sample

Standard solution

Sample preparation

Direct partial loop injection

Column

Metrosep Amino Acids 1 - 100/4.0	6.4001.410
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Metrosep BP 1 Guard/2.0	6.1015.000
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Solutions

Eluent A:	42.6 mmol/L lithium citrate, 10.6 mmol/L phenol, pH 2.8 (HCl)
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B:	42.6 mmol/L lithium citrate, 1.0 mol/L lithium chloride, 10.6 mmol/L phenol, pH 4.2 (HCl)
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Post-column reagent	0.11 mol/L ninhydrin + 2.5 mmol/L Hydrindantin in (1:1) = (DMSO:2 mol/L lithium acetate at pH 5.2)
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Analysis

UV detection	570 nm / 440 nm
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Reference	700 nm
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Parameters

Flow rate column	0.4 mL/min
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Flow rate PCR	0.2 mL/min
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Injection volume	4 µL
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P _{max}	10.0 MPa
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Recording time	95 min
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Column temperature	50 °C
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PCR temperature	120 °C
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Measuring duration	300 ms
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Instrumentation

850 Professional IC Cation – HP Gradient	2.850.1220
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872 Extension Module IC Pump	2.872.0010
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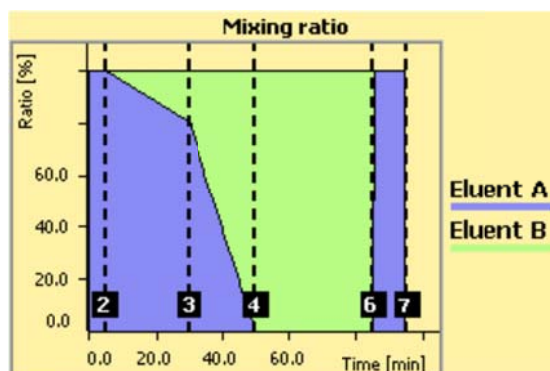
887 Professional UV/VIS Detector	2.887.0010
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886 Professional Reactor	2.886.0110
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889 IC Sample Center - cool	2.889.0020
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Gradient

Time [min]	Eluent A [%]	Eluent B [%]	Curve	Flow [mL/min]
Start	100	0		0.4
5.0	100	0	Linear	0.4
30.0	80	20	Linear	0.4
50.0	0	100	Linear	0.4
85.0	0	100	Linear	0.4
85.1	100	0	Linear	0.4
95.0	100	0	Linear	0.4



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