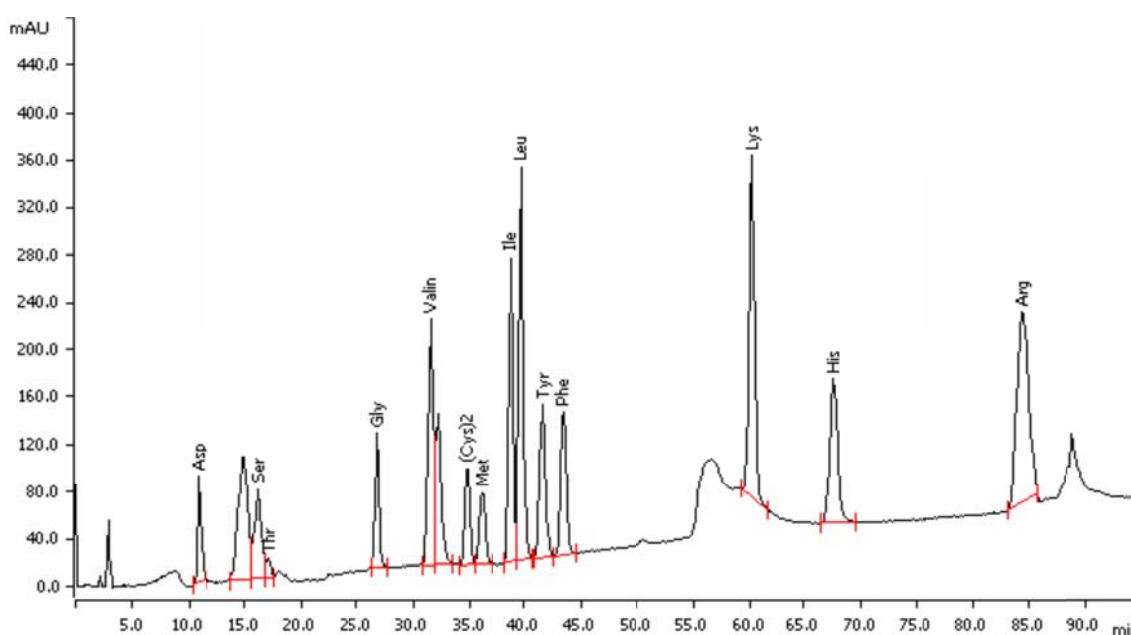


Amino acids in cell culture growth medium applying UV/VIS detection after post-column reaction with ninhydrin at 120 °C



Cell culture growth media contain all required components to keep cells alive. Here the amino acid composition is analyzed. A binary gradient separates in this example amino acids. The post-column reaction with ninhydrin requires a temperature of 120 °C, while the samples need to be cooled for stability.

Results

| Amino acid | µmol/L | Amino acid | µmol/L | Amino acid | µmol/L |
|------------|--------|--------------------|--------|------------|--------|
| Asp | 2.5 | (Cys) ₂ | 2.5 | Phe | 2.5 |
| Ser | 2.5 | Met | 2.5 | Lys | 2.5 |
| Thr | 2.5 | Ile | 2.5 | His | 2.5 |
| Gly | 1.25 | Leu | 2.5 | Arg | 2.5 |
| Val | 2.5 | Tyr | 2.5 | | |

Method description

Sample

Cell culture growth solution

Sample preparation

Filtration through 0.2 µm, partial loop injection

Column

| | |
|----------------------------------|------------|
| Metrosep Amino Acids 1 - 100/4.0 | 6.4001.410 |
|----------------------------------|------------|

| | |
|-------------------------|------------|
| Metrosep BP 1 Guard/2.0 | 6.1015.000 |
|-------------------------|------------|

Solutions

| | |
|-----------|---|
| Eluent A: | 42.6 mmol/L lithium citrate, 10.6 mmol/L phenol, pH 2.8 (HCl) |
|-----------|---|

| | |
|----|--|
| B: | 42.6 mmol/L lithium citrate, 1.0 mol/L lithium chloride, 10.6 mmol/L phenol, pH 4.2 (HCl) |
|----|--|

| | |
|---------------------|---|
| 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) |
|---------------------|---|

Analysis

| | |
|--------------|--------|
| UV detection | 570 nm |
|--------------|--------|

| | |
|-----------|--------|
| Reference | 700 nm |
|-----------|--------|

Parameters

| | |
|------------------|------------|
| Flow rate column | 0.4 mL/min |
|------------------|------------|

| | |
|---------------|------------|
| Flow rate PCR | 0.2 mL/min |
|---------------|------------|

| | |
|------------------|-------|
| Injection volume | 20 µL |
|------------------|-------|

| | |
|------------------|----------|
| P _{max} | 10.0 MPa |
|------------------|----------|

| | |
|----------------|--------|
| Recording time | 95 min |
|----------------|--------|

| | |
|--------------------|-------|
| Column temperature | 50 °C |
|--------------------|-------|

| | |
|-----------------|--------|
| PCR temperature | 120 °C |
|-----------------|--------|

| | |
|--------------------|--------|
| Measuring duration | 300 ms |
|--------------------|--------|



Instrumentation

| | |
|--|------------|
| 850 Professional IC Cation – HP Gradient | 2.850.1220 |
|--|------------|

| | |
|------------------------------|------------|
| 872 Extension Module IC Pump | 2.872.0010 |
|------------------------------|------------|

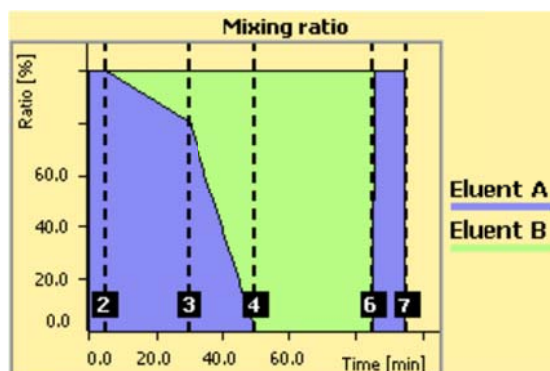
| | |
|----------------------------------|------------|
| 887 Professional UV/VIS Detector | 2.887.0010 |
|----------------------------------|------------|

| | |
|--------------------------|------------|
| 886 Professional Reactor | 2.886.0110 |
|--------------------------|------------|

| | |
|-----------------------------|------------|
| 889 IC Sample Center - cool | 2.889.0020 |
|-----------------------------|------------|

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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