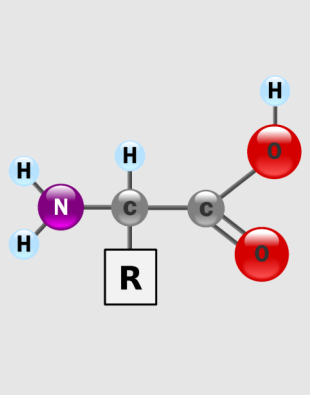
Application Note

Amino Acids



Amino acids

Introduction

Amino acids are organic compounds that contain amine ($–NH_2$) and carboxyl (–COOH) functional groups, along with a side chain (R group) specific to each amino acid [1]. Amino acids play a central role both as structural units (monomers) of peptides, proteins and as intermediates in metabolism.

Amino acid can be analyzed by anion exchange chromatography (HPAEC) in combination with integrated pulsed amperometric detection (IPAD) [2]. his method does not require derivatization. In this application note the analysis of 20 amino acids is demonstrated using the DECADE Elite electrochemical detector and SenCell, in combination with an Agilent 1260 Infinity Bio-Inert LC system. The detection is based on a 5-step PAD waveform using a gold (Au) working electrode.

Method

The LC-EC conditions are listed in table 1. The analysis was performed using an Agilent 1260 Infinity Bio-Inert LC system, consisting of an quaternary low-pressure gradient pump, autosampler and thermostatted column compartment. For detection a DECADE Elite electro-chemical detector with SenCell flow cell was used (see figure 2) in combination with an Agilent 35900E Series II Dual Channel Interface (A/D converter).

The detector signal was acquired in Agilent MassHunter software. The DECADE Elite detector was controlled via a PC using the Antec Dialogue Elite software. For the separation of the amino acids an adapted gradient profile was used from reference [3]. The details of the gradient profile are listed in table 2 on the next page.

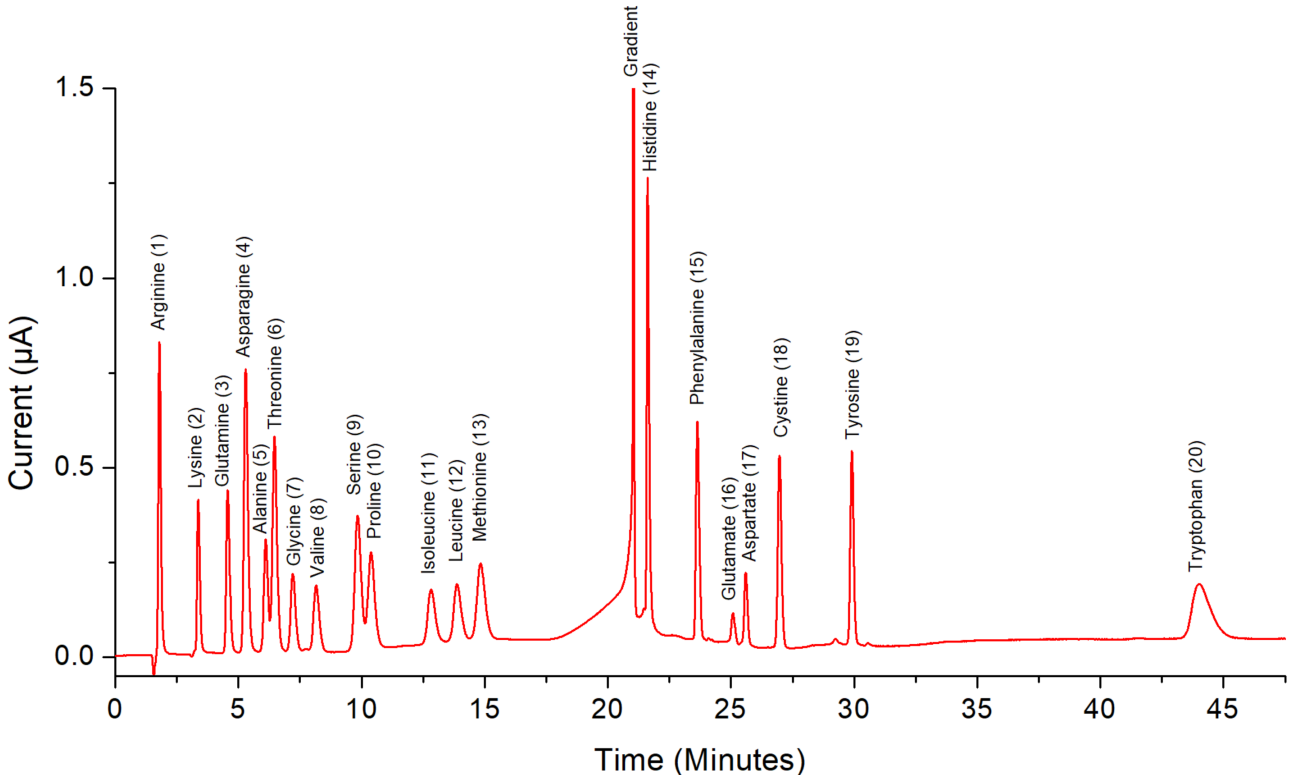


Figure 1. *Chromatograms of a 25 µL injection of an amino acid (AA) standard mix in water containing 8 µM* of (1) Arginine, (2) Lysine, (3) Glutamine, (4) Asparagine, (5) Alanine, (6) Threonine, (7) Glycine, (8) Valine, (9) Serine, (10) Proline, (11) Isoleucine, (12) Leucine, (13) Methionine, (14) Histidine, (15) Phenylalanine, (16) Glutamate, (17) Aspartate, (18) Cystine*, (19) Tyrosine and (20) Tryptophan. * Except for Cystine concentration was 4 µM.*

Table 1. LC-EC conditions

HPLC	Agilent 1260 Infinity Bio-Inert LC system with 35900E Series II Dual Channel A/D converter
LC detector	Antec Scientific DECADE Elite EC detector
Columns	BorateTrap™ Inline Trap Column, 50 x 4.0 mm ID (placed between LC pump and injector) AminoPac™ PA10 , 250 x 2 mm ID + 50 x 2 mm ID All columns: Thermo Scientific™ Dionex™
Separation	Ternary (low pressure) gradient. All mobile phases are continuously sparged with Helium 5.0.
Mobile phase (MP)	MP A: 1.0 M NaOAc MP B: MilliQ water MP C: 0.25 M NaOH
Flow rate	250 µL/min
Back pressure	about 195 bar (during separation)
$V_{injection}$	25 µL
Temperature	30 °C (separation), 35 °C (detection)
Flow cell	SenCell with Au working electrode and HyREF Palladium reference electrode, AST position 2
Potential waveform	E1, E2, E3, E4, E5: +0.22, -2.0, +0.6, -0.20 V, 0 V ts, t1, t2, t3, t4, t5: 0.2, 0.45, 0.02, 0.01, 0.07 s, 0.05s
Range	2 µA/V
I_{cell}	around 0.3 µA
ADF	0.1 Hz

For detection a 5-step PAD waveform is applied, optimized for sensitive detection of amino acids. The detection potential (E1) was + 220 mV followed by a 4-step cleaning & conditioning pulse (see table 1).

Table 2. Gradient time table

Time (min)#	*A (%)*	*B(%)*	*C(%)*
0.00	-	76.0	24.0
0.10	-	76.0	24.0
2.00	-	76.0	24.0
8.00	-	64.0	36.0
11.00	-	64.0	36.0
18.00	40.0	40.0	20.0
21.00	40.0	44.0	16.0
23.00	70.0	14.0	16.0
42.00	70.0	14.0	16.0
42.10*	-	20.0	80.0
44.10	-	20.0	80.0
44.20	-	76.0	24.0
74.00	-	76.0	24.0

#) the flow rate was constant over the complete gradient run, 250 µL/min.
**) from t=42.10 – 74.00 min: column clean-up (late eluting components),*

Results

In figure 1 on the previous page a typical chromatogram is shown obtained with a 25 µL injection of a 8 µM standard mix of 20 amino acids in water analyzed under the specified conditions. All amino acids elute within 50 minutes. The total run time is 74 minutes due to a column clean-up and equilibration step. Most of the amino acids are baseline separated, except for the couples Alanine/Threonine and Serine/Proline. The linearity, repeatability and detection limit data for all amino acids are listed in table 3. Note that in this application note only standards were used to demonstrate the performance. For the analysis of complex samples containing both sugars and amino acids it might be necessary to modify the separation conditions or apply sample preparation methods to remove the sugars prior to analysis of the amino acids. In reference [3] and [4] directions are given how to optimize the gradient profile to obtain separation of both amino acids and sugars.

Table 3. Linearity, Repeatability and LOD

Compound	*tr (min)*	*R #*	*RSD, Area (%)**	*LOD (nmol/L)ł*
Arginine	1.80	1.0000	2.10	65
Lysine	3.38	0.9998	0.74	24
Glutamine	4.58	0.9997	1.91	57
Asparagine	5.30	0.9996	1.42	40
Alanine	6.12	0.9999	0.87	27
Threonine	6.47	1.0000	0.63	21
Glycine	7.22	0.9999	1.45	45
Valine	8.17	0.9999	1.42	46
Serine	9.85	1.0000	1.48	48
Proline	10.40	0.9999	1.06	33
Isoleucine	12.85	0.9999	1.39	44
Leucine	13.90	0.9998	1.49	42
Methionine	14.87	0.9998	1.97	60
Histidine	21.63	0.9995	1.06	32
Phenylalanine	23.65	0.9998	1.32	40
Glutamate	25.08	0.9995	1.51	44
Aspartate	25.60	0.9995	1.98	56
Cystine	26.97	0.9998	1.63	49
Tyrosine	29.91	0.9999	1.35	44
Tryptophan	44.02	0.9999	2.01	64

#) The linearity was determined using a 4-point calibration curve based on the responses of the amino acids (area) of a 1, 2, 4 and 8 µM standard.
*) The RSD of the peak areas were determined using the responses of a 1 µM standard. Population n=10.
ł) the LOD's were calculated based on the response (area) of the lowest calibration standard of 1 µM (n=10), where the LOD = 3.3 x std deviation of the response / slope.

However, if amino acids are to be analyzed in samples containing much higher concentrations of carbohydrates (vegetables, plants etc.), optimization of the separation only will not be sufficient. In this case a sample preparation step is required to eliminate the sugars from the sample. In reference [5] an inline sample preparation method is described were the amino acids are trapped on a short polymeric cation exchange column under acidic conditions, prior to analysis. Under these conditions the sugars are neutral and will be washed from the trapping column during this step. The amino acids are subsequently released under alkaline conditions, by reversed flushing of the trapping column with mobile phase (initial gradient conditions).

References

1. Wikipedia, Amino Acids,https://en.wikipedia.org/wiki/Amino_acid
2. A. P. Clarke et al., An Integrated Amperometric Waveform for the Direct, Sensitive detection of Amino Acids and Amino Sugars Following Anion-Exchange Chromatography, Anal. Chem., 71 (1999), 2774–2781.
3. P. Jandik et al., Analyzing mixtures of amino acids and carbohydrates using bi-modal integrated amperometric detection, J. of Chrom. B, 732 (1999) 193–201
4. H. Yu et al., Simultaneous determination of amino acids and carbohydrates HPAE chromatography with IPAD, J. of Chrom. A, 966 (2002), 89-97
5. P. Jandik et al., Simplified in-line sample preparation for amino acid analysis in carbohydrate containing samples, Journal of Chromatography B,758 (2001), 189–196
6. Netherlands Institute of Ecology (NIOO-KNAW), https://nioo.knaw.nl/en/about-nioo

Acknowledgement

The method development and all data presented in this application note are courtesy of Mrs. Ciska E. Raaijmakers, Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands. NIOO-KNAW is a research institute focused on fundamental and strategic research in the field of animal -, plant - and microbial ecology in terrestrial and freshwater environments. The scientists of the department of Terrestrial Ecology are experts in the ecology, physiology and chemistry of plants, and in soil ecology, entomology and behavioral biology. They investigate the interactions between plants, the animals and diseases that damage plants above and below the ground, and the natural enemies of these attackers [6].

Ordering information

ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrates Analyzer - gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Software#	
195.0035	Clarity CDS single instr. incl LC, AS module

#) Antec ECD drivers are available for Chromeleon CDS, OpenLAB CDS and Empower CDS. The ALEXYS Carbohydrates Analyzer (full system) can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS. Please contact Antec for more details.

Fig. 2. *ALEXYS Carbohydrate Analyzer consisting of the ET210 eluent tray (for N_2 blanketing), a P6.1L quaternary LPG pump, AS6.1L autosampler, CT2.1 column thermostat, and the DECADE Elite electrochemical detector.*

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