MACHEREY-NAGEL

Application Note 03/2022 · Chromatography

Analysis of amino acids by HPLC using 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate as derivatizing agent

MACHEREY NAGEL application department · Dr. Timo Engelsdorf *

* Philipps-Universität Marburg, FB17 – Biology, Molecular Plant Physiology, Karl-von-Frisch-Strasse 8, D-35043 Marburg

Application benefits

- Analysis of amino acids by HPLC using 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate as derivatizing agent
- Sensitive HPLC analysis on a NUCLEOSHELL® RP 18plus column

MN products

REF 763236.30
NUCLEOSHELL® RP 18plus, 2.7 µm, 150 x 3 mm

REF 728777.20
EC HPLC guard column (analytical), UNIVERSAL RP, 4 x 2 mm

REF 702107
Screw closure, N 9, PP, yellow, center hole, silicone white/PTFE red, 1.0 mm

REF 702008
Screw neck vial, N 9, 11.6 x 32.0 mm, amber, with integr. 0.2 mL insert

MN application numbers

HPLC: 129160

Keywords

Amino acids, corn, precolumn derivatization, 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, AQC

Introduction

The analysis of amino acids from feed, food and beverages is a commonly known methodology of routine control. For the determination of amino acids, the HPLC separation followed by fluorescence or UV detection is the most convenient and commonly used approach. The combination of precolumn derivatization and HPLC separation is a classic analytical methodology to generate fluorescent amino acid derivatives, including 9-fluorenyl-methyl chloroformate (FMOC-Cl), phenyl isothiocyanate (PITC), 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) and *o*-phthalaldehyde (OPA).

In this work, AQC was used as derivative reagent for the HPLC determination of 20 amino acids in standard mixtures and corn as sample matrix. The chromatographic separation was successfully developed on a NUCLEOSHELL® RP 18plus column.

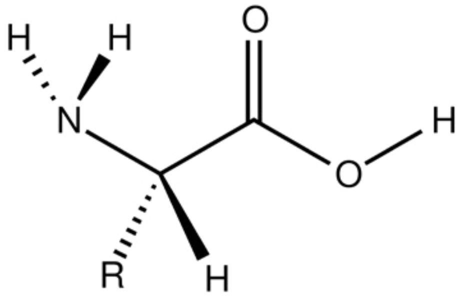

Figure 1: Structure of a generic *L*-amino acid in the „neutral“ form needed for defining a systematic name (without implying that this form actually exists in detectable amounts either in aqueous solution or in the solid state).

Sample pretreatment

Chemicals and reagents

1. 0.2 mol/L boric acid, pH = 8.8 (adjusted with sodium hydroxide)
2. AQC mixture: 3 mg/mL in acetonitrile (store in the dark at – 20°C) *

* Preparation: heat the mixture for not more than 8 minutes at 55°C, optionally place in an ultrasonic bath occasionally

Sample extraction

1. Free amino acids were extracted from leaf tissue in 80% ethanol at 80°C.
2. The extraction was repeated once.
3. Both extracts combined and concentrated on a rotary vacuum concentrator.
4. The amino acids were taken up in ultrapure water and used for the derivatization.

Precolumn derivatization

1. Use amber reaction tubes (PP) for derivatization.
2. Add 10 µL of amino acid solution.
3. Add 80 µL of boric acid.
4. Add 10 µL of AQC mixture.
5. Homogenize and heat the mixture at 55 °C for exactly! 9 min and immediatly stop reaction on ice after incubation.
6. Transfer to glass vials with insert for HPLC analysis.

Time (min)	Eluent A (percentage by volume)	Eluent B (percentage by volume)	Eluent C (percentage by volume)
0	98	2	0
17	94	6	0
20	88	12	0
29	80	20	0
32	0	60	40
34	0	60	40
38	98	2	0
40	98	2	0

Table 1: Tenary gradient elution

Analysis by HPLC-MS / MS

MN Appl. No. 129160

Chromatographic conditions

System	Agilent 1260 Infinity II Prime HPLC System
Guard column	UNIVERSAL RP, 4x2 mm (REF 728777.20)
Column	EC 150/2 NUCLEOSHELL® RP 18plus, 2.7 µm (REF 763236.30)
Eluent A	140 mM ammonium acetate + 7 mM triethanolamine in water (pH = 6.0, adjusted with acetic acid)
Eluent B	1% water in acetonitrile
Eluent C	water
Flow rate	1.1 mL/min
Temperature	37 °C
Injection volume	10 µL
Fluorescence Detection	
Excitation wavelength	300 nm
Emission wavelength	400 nm

The chromatography application database

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- Free access to the MN application database: *https://chromaappdb.mn-net.com*

Chromatogramms

Figure 2: a

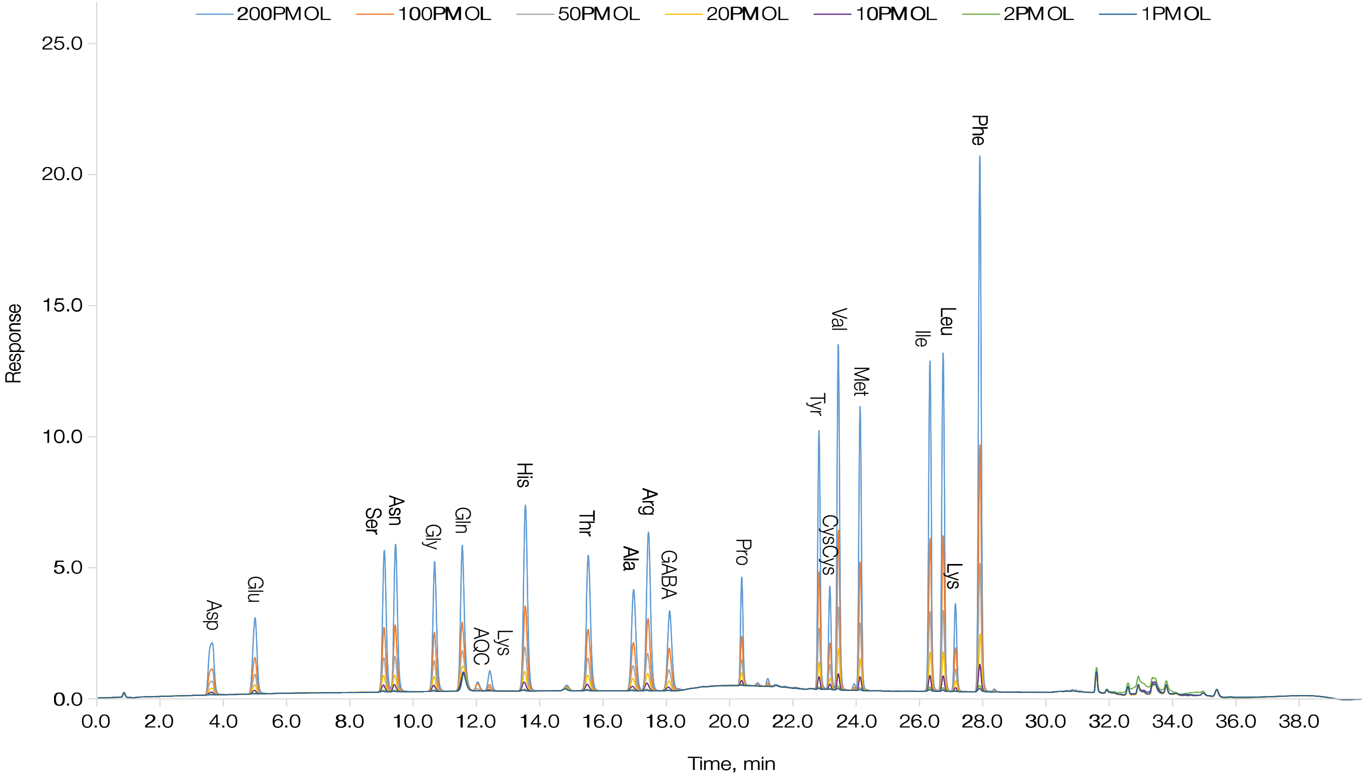

Figure 2: b

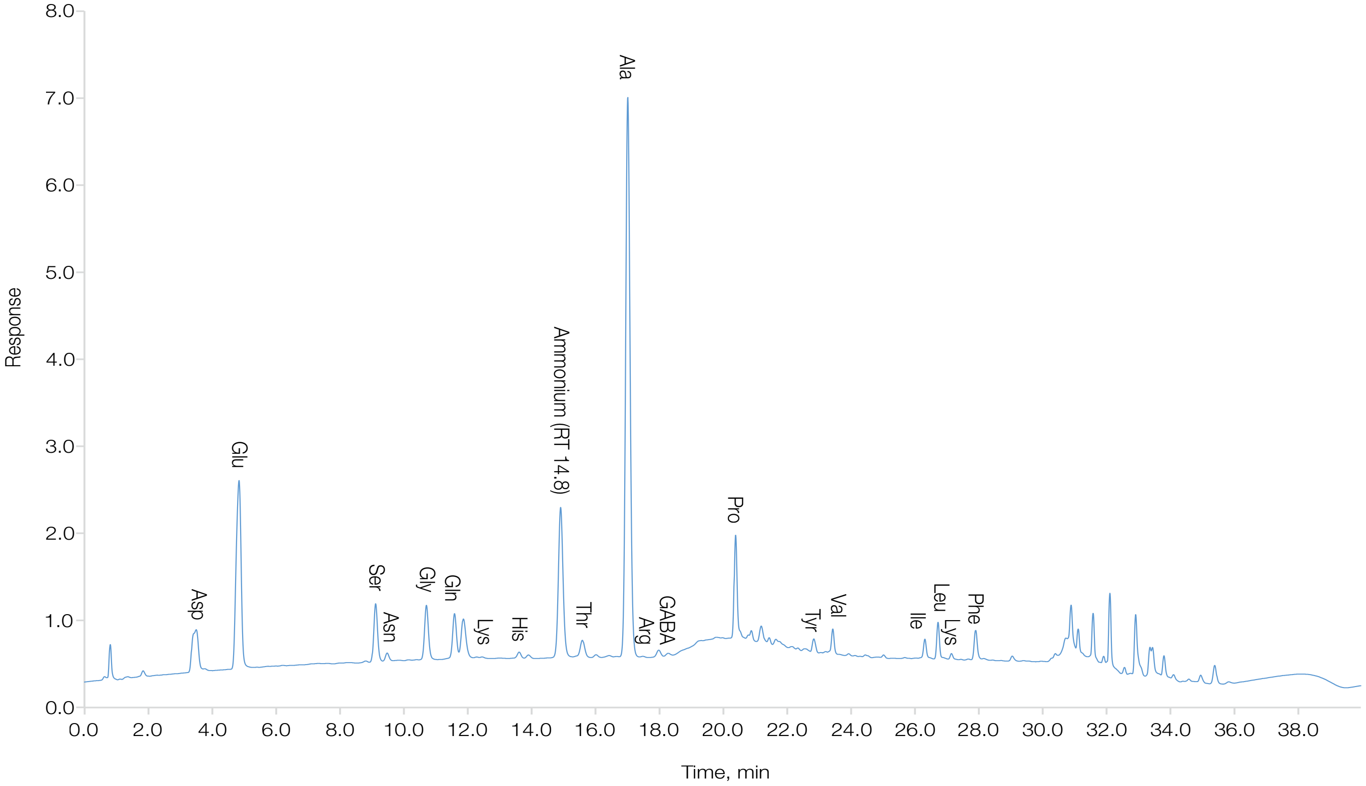

Figure 2: Chromatogram of samples: a = overlay of standard mixtures, b = sample extract of corn

Retention times and lower limit of detection (LOD)

Peak number	Amino acid	Abbreviation	Retention time (min)	LOD (pmol)
1	Aspartic acid	Asp	3.60	2
2	Glutamate	Glu	4.95	2
3	Serine	Ser	9.01	1
4	Asparagine	Asn	9.36	1
5	Glycine	Gly	10.10	1
6	Glutamine	Gln	11.56	1
19a	Lysine	Lys_1	12.35	10
7	Histidine	His	13.46	1
8	Threonine	Thr	15.48	2
9	Alanine	Ala	16.91	2
10	Agrinine	Arg	17.37	1
11	γ-aminobutyric acid	GABA	18.04	2
12	Proline	Pro	20.36	1
13	Tyrosine	Tyr	22.81	1
14	Cystine (Cysteine dimer)	CysCys	23.15	1
15	Valine	Val	23.42	1
16	Methionine	Met	24.11	1
17	Isoleucine	Ile	26.32	1
18	Leucine	Leu	26.74	1
19	Lysine	Lys_2	27.14	2
20	Phenylalanine	Phe	27.90	1

Table 2: Retention times and LOD of 20 precolumn derivatized amino acids.

Conclusion

This application note presents the reliable and successful determination of 20 amino acids using the combination of precolumn derivatization and HPLC separation that leads to excellent LOD´s given in table 2.

The chromatographic separation of derivative amino acids was performed by using core-shell particles that are well known for fast and high-efficient separations combined with a reasonably low back pressure. In this work, a subsequent analysis was developed on a NUCLEOSHELL® RP 18plus column as shown in figure 1.

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MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com