MACHEREY-NAGEL
Chromatography application note

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Determination of pyrrolizidine alkaloids

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Abstract

This application note describes the determination of pyrrolizidine alkaloids using Solid Phase Extraction for analyte enrichment and sample clean-up. The eluates from SPE are finally analyzed by HPLC-MS/MS.

Introduction

A large number of plant species of the families of Boraginaceae, Asteraceae and Fabaceae produce pyrrolizidine alkaloids (PA). More than 600 PAs are currently known by science for protecting plants against herbivores. The content of PAs in plants varies widely. The amount of PAs depends on the plant species and the part of the plant, for instance the pollen of these plants could be a potential source for PAs and may lead to a contamination of honey. PAs are esters derived from a 1-hydroxymethylpyrrolizidine (necine base) and aliphatic mono-carbon or dicarbon acids (necine acids). A distinction between PAs of the retronecine, heliotridine, otonecine or platynecine type depends on the structure of the necine base. PAs have carcinogenic and genotoxic properties and their consumption in high doses can lead to acute liver damage [1,2]. Various research projects and studies conducted by the official authorities of the federal states lead to new procedures for the determination of pyrrolizidine alkaloids in different matrices. The results of these studies recommend analyzing the PA contents in herbal food and feed like tea, flour, honey etc. [3]. The German food control authority, the Federal Institute for Risk Assessment (BfR), recommends analyzing PA levels in tea and other herbal food and has published methods for analyzing PAs in different matrices [4,5].

In this application note a method for the determination of PAs has been developed. The method follows the recommendations introduced by the BfR. The identification and quantification of PAs were finally carried out by ESI mass spectrometry on modern HPLC phases like NUCLEOSHELL® RP 18plus or NUCLEODUR® C_{18} Gravity-SB.

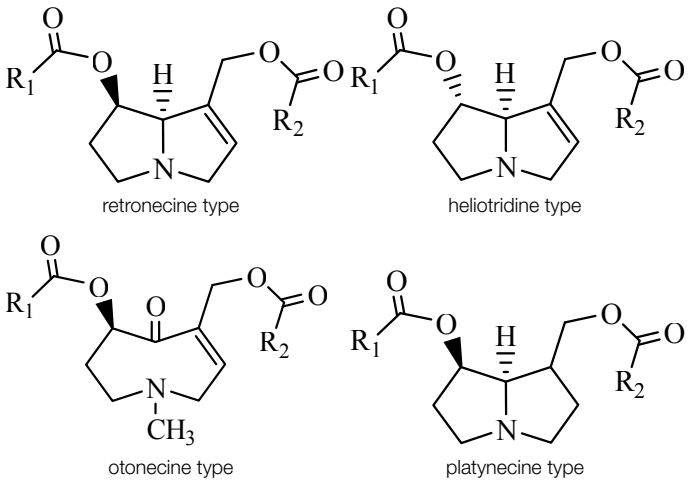


Figure 1: Necine bases of pyrrolizidine alkaloids.

Solid phase extraction using RP phases

Sample pretreatment:

The following analysis were performed with standard solutions.

Column:

CHROMABOND® HR-X, 85 µm, 3 mL, 200 mg, (REF 730931)

CHROMABOND® C_{18} ec, 6 mL, 500 mg, (REF 730014)

Conditioning:

5 mL methanol, 5 mL water

Sample application:

10 mL neutralized standard solution with a flow rate of 3 mL/min

Washing:

2 x 5 mL of water with a flow rate of 3 mL/min

Drying:

5–10 min with vacuum

Elution:

5 mL methanol

Eluent exchange:

Add 1.0 mL water as keeper. Evaporate eluate to a volume of 0.5 mL at 40 °C under a stream of nitrogen and fill up to 1.0 mL with water – methanol (95:5, v/v).

Determination of pyrrolizidine alkaloids

Solid phase extraction using very strong mixed mode cation exchanger

Column:

CHROMABOND® HR-XC, 85 µm, 3 mL, 200 mg, (REF 730952)

Conditioning:

5 mL methanol, 5 mL 0.05 M H_2SO_4

Sample application:

10 mL standard solution in aqueous or methanolic sulphuric acid (0.05 M) with a flow rate of 3 mL/min

Washing:

6 mL water with a flow rate of 3 mL/min or 6 mL methanol with a flow rate of 3 mL/min

Drying:

5 - 10 min with vacuum

Elution:

2 x 5 mL 2.5 % ammonia in methanol

Eluent exchange:

Evaporate eluate to a volume of 0.5 mL at 40 °C under a stream of nitrogen and fill up to 1.0 mL with water – methanol (95:5, v/v).

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions for NUCLEODUR® C_{18} Gravity-SB

Column:

EC 50/2 NUCLEODUR® C_{18} Gravity-SB, 1.8 µm (REF 760593.20)

Eluent A:

315 mg ammonium formate are dissolved in 5 mL of water, 1 mL of formic acid is added and filled up to 1000 mL with water

Eluent B:

315 mg ammonium formate are dissolved in 5 mL of water, 1 mL of formic acid is added and filled up to 1000 mL with methanol

Gradient:

5–40 % B in 6 min, 40–80 % B in 1.5 min, 80–100 % B in 0.1 min, 100 % B for 1.4 min, 100–5 % B in 0.1 min, 5 % B for 5.9 min

Flow rate:

0.3 mL/min

Temperature:

30 °C

Injection volume:

5 µL

Chromatographic conditions for NUCLEOSHELL® RP 18plus

Column:

EC 150/2 NUCLEOSHELL® RP 18plus, 2.7 µm (REF 763236.20)

Eluent A:

315 mg ammonium formate are dissolved in 5 mL of water, 1 mL of formic acid is added and filled up to 1000 mL with water.

Eluent B:

315 mg ammonium formate are dissolved in 5 mL of water, 1 mL of formic acid is added and filled up to 1000 mL with methanol

Gradient:

5–40 % B in 6 min, 40–80 % B in 1.5 min, 80–100 % B in 0.1 min, 100 % B for 1.4 min, 100–5 % B in 0.1 min, 5 % B for 5.9 min

Flow rate:

0.3 mL/min

Temperature:

40 °C

Injection volume:

5 µL

MS conditions:

API 3200, ion source ESI, positive ionization mode, curtain gas 15 psi, ion spray voltage 4500 V, temperature 650 °C, nebulizer gas 40 psi, turbo gas 50 psi, CAD 3.0 psi

MRM transitions

Analyte	RT [min] on NUCLEODUR® C_{18} Gravity-SB	RT [min] on NUCLEOSHELL® RP 18plus	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Monocrotaline (Mc)	1.84	4.23	326.2	120.2	291.2
Erucifoline (Er)	3.36	5.09	350.3	120.3	94.1
Intermedine (Im)	3.60	5.73	300.3	94.2	138.1
Lycopsamine (La)	3.84	5.89	300.3	94.2	138.1
Jacobine (Jb)	3.89	5.71	352.3	94.1	120.2
Europine (Eu)	3.93	5.85	330.3	138.2	172.3
Monocrotaline-*N*-oxide (McN)	4.03	5.60	342.3	137.0	94.2
Erucifoline-*N*-oxide (ErN)	4.35	5.78	366.3	94.2	168.3
Europine-*N*-oxide (EuN)	4.45	6.24	346.3	172.2	328.3
Intermedine-*N*-oxide (ImN)	4.60	6.45	316.3	172.3	138.0
Jacobine-*N*-oxide (JbN)	4.60	6.15	368.2	120.2	94.2
Lycopsamine-*N*-oxide (LaN)	4.80	6.60	316.3	172.3	138.0
Trichodesmine (Td)	5.00	7.00	354.3	120.2	94.1
Retrorsine (Re)	5.09	6.88	352.3	94.1	120.2
Heliotrine (Hn)	5.32	7.38	314.3	138.2	156.3
Seneciphylline (Sp)	5.33	7.23	334.3	120.1	94.1
Retrorsine-*N*-oxide (ReN)	5.48	7.08	368.2	94.2	120.2

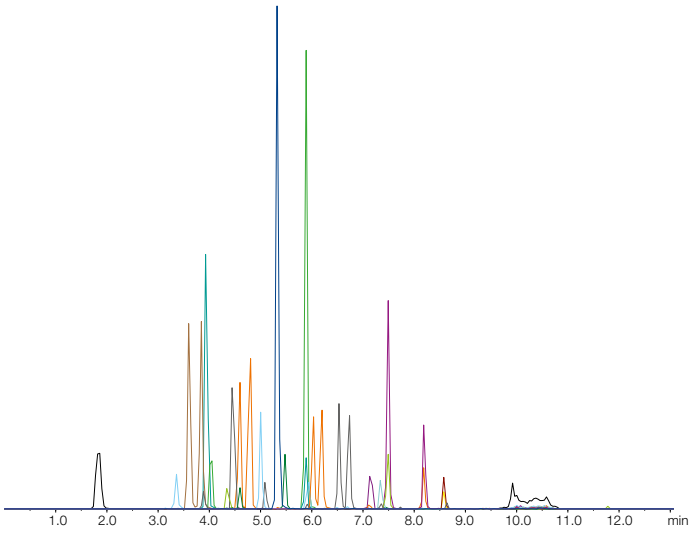
Determination of pyrrolizidine alkaloids

Analyte	RT [min] on NUCLEODUR® C_{18} Gravity-SB	RT [min] on NUCLEOSHELL® RP 18plus	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Heliotrine-*N*-oxide (HnN)	5.89	7.80	330.3	172.3	138.2
Seneciphylline-*N*-oxide (SpN)	5.93	7.60	350.3	94.1	120.3
Senecivernine (Sv)	6.04	8.08	336.3	120.2	94.1
Senecionine (Sc)	6.20	8.00	336.3	120.2	94.1
Senecivernine-*N*-oxide (SvN)	6.53	8.38	352.3	94.1	120.2
Senecionine-*N*-oxide (ScN)	6.74	8.25	352.3	94.1	120.2
Echimidine (Em)	7.15	8.76	398.3	120.2	55.2
Echimidine-*N*-oxide (EmN)	7.34	8.80	414.3	254.3	55.1
Senkirkine (Sk)	7.49	8.90	366.3	168.3	94.2
Lasiocarpine (Lc)	8.19	9.31	412.3	120.2	220.2
Lasiocarpine-*N*-oxide (LcN)	8.58	9.49	428.3	94.1	254.3

Table 1: MRM transitions for the analysis of pyrrolizidine alkaloids.

Chromatograms

a)



b)

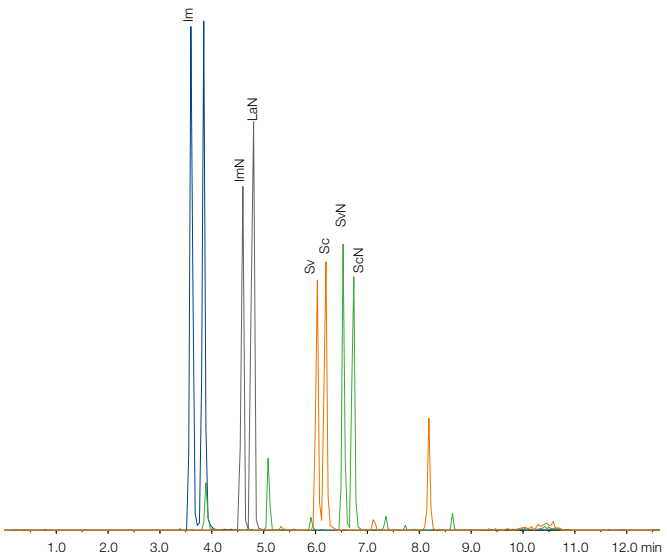
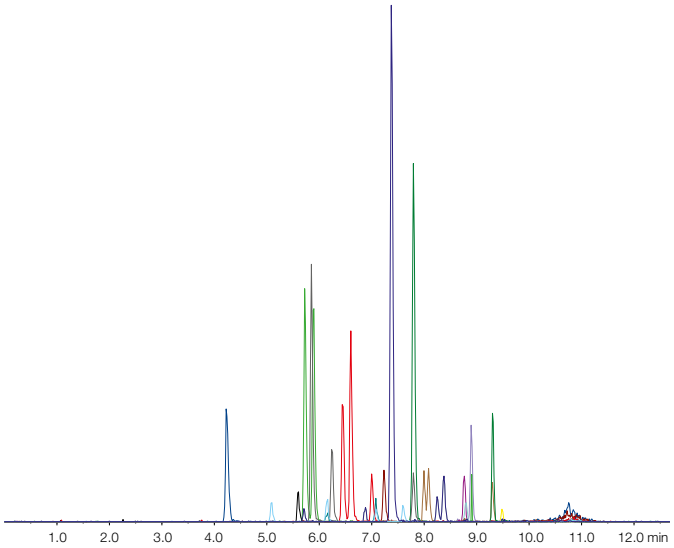


Figure 2: Chromatograms of solid phase eluate (c = 50 ng/mL) on EC 50/2 NUCLEODUR® C_{18} Gravity-SB, 1.8 µm column.

a)



b)

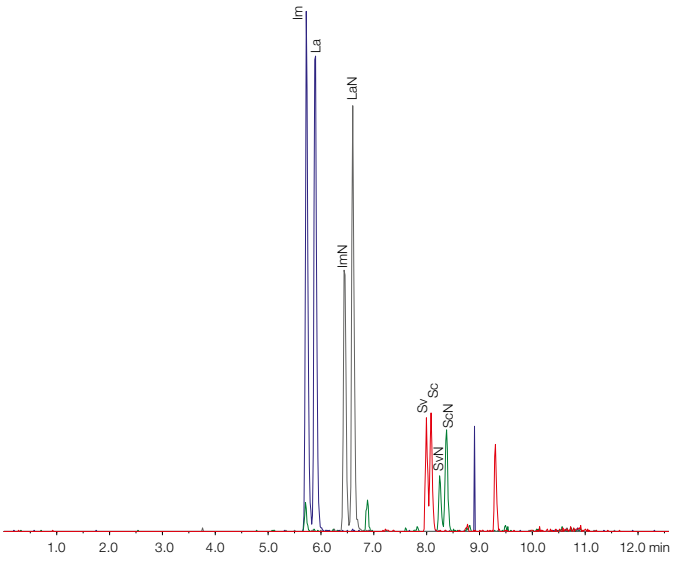


Figure 3: Chromatograms of solid phase eluate (c = 50 ng/mL) on EC 150/2 NUCLEOSHELL® RP 18plus, 2.7 µm column.

Determination of pyrrolizidine alkaloids

Recovery rates

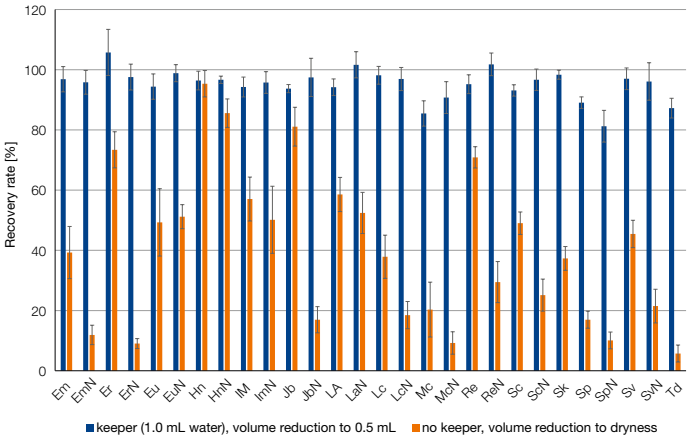


Figure 4: Effects on eluent exchange by using water as keeper for volume reduction.

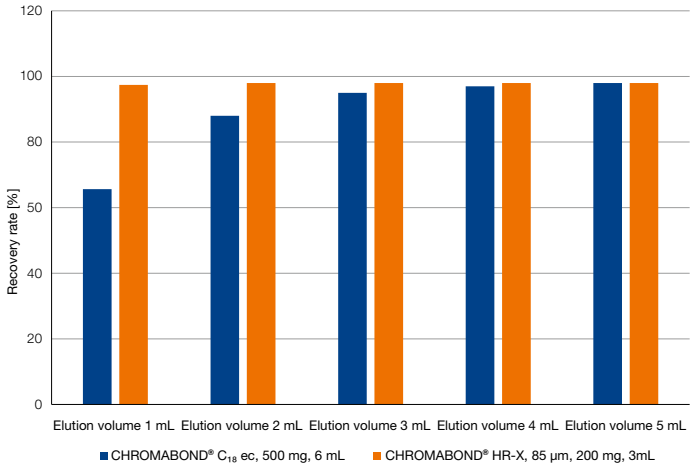


Figure 5: Elution behavior for pyrrolizidine alkaloids using RP phases.

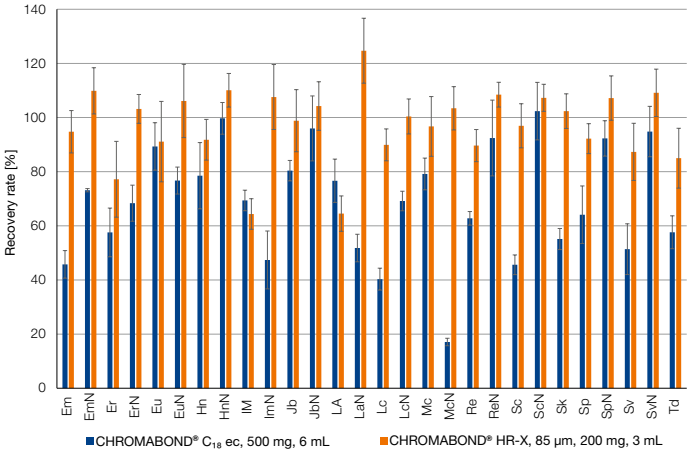


Figure 6: Recovery rate of solid phase eluates for pyrrolizidine alkaloids using RP phases.

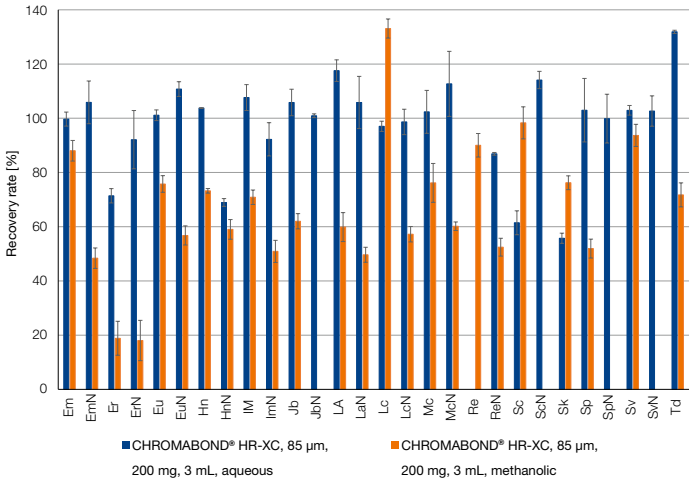


Figure 7: Recovery rate of solid phase eluates for pyrrolizidine alkaloids using very strong mixed mode cation exchanger.

Conclusion

The results show that the determination of PAs could be carried out successfully with all the tested products for solid phase extraction and HPLC. By using RP SPE phases it was possible to achieve good recovery rates for the many PAs. The hydrophobic polystyrene-divinylbenzene copolymer, CHROMABOND® HR-X was more suitable for PA enrichment because of the higher recovery rates and the better elution behavior than the octadecyl modified silica phase. Eluent exchange is another important method part for the determination of PAs. The results show that the recovery rates get better by adding water as keeper to the solid phase eluates before volume reduction.

Depending on sample matrix, solid phase extraction should be done with organic sample extracts, for instance for the matrices honey or flour. For this kind of sample preparation/extraction another solid phase material type with ionic interaction mechanism should be used. CHROMABOND® HR-X leads also to good recovery rates.

The chromatographic results are presented in figure 2 and 3. The identification and quantification of PAs were carried out successful by ESI mass spectrometry on NUCLEODUR® C_{18} Gravity-SB or NUCLEOSHELL® RP 18plus column in less than 15 minutes. The four critical peak pairs were well separated on both column types.

In summary the presented application describes a quick and convenient method for the determination of pyrrolizidine alkaloids in different sample matrices.

References

[1] Wiedenfeld, H.; Roeder, E.; Bouaul, T.; Edgar, J., Pyrrolizidine Alkaloids – Structure and Toxcity. V&R Uni Press, Bonn University Press 2008.

[2] Roeder, E., pyrrolizidinalkaloidhaltige Arzneipflanzen, Deutsche Apotheker Zeitung 1992, 45 (132), 2427–2435.

[3] EFSA Panel on Contaminants in the Food Chain (CONTAM) 2011.

[4] Determination of pyrrolizidine alkaloids in honey by SPE-LC-MS/MS, Method Protocol BfR-PA-Honey-1.0/2013.

[5] Determination of pyrrolizidine alkaloids in plant material by SPE-LC-MS/MS, Method Protocol BfR-PA-Tea-2.0/2014.

Determination of pyrrolizidine alkaloids

Additional information

The following applications regarding “Determination of pyrrolizidine alkaloids” and further applications can be found on our online application database at *www.mn-net.com/apps*

SPE:	MN Appl. No. 306620 (RP phases)
	MN Appl. No. 306630 (cation exchanger)
HPLC:	MN Appl. No. 127490 (NUCLEODUR C_{18} Gravity-SB)
	MN Appl. No. 127480 (NUCLEOSHELL RP 18plus)

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 763236.20, EC 150/2 NUCLEOSHELL® RP 18plus, 2.7 µm

REF 760593.20, EC 50/2 NUCLEODUR® C_{18} Gravity-SB, 1.8 µm

REF 730931, CHROMABOND® HR-X, 85 µm, 200 mg, 3 mL

REF 730952, CHROMABOND® HR-XC, 85 µm, 200 mg, 3 mL

REF 730014, CHROMABOND® C_{18} ec, 500 mg, 6 mL

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red