Analysis of Pyrrolizidine Alkaloids in Herbal Teas and Honey using SPE and LC-MS/MS

Albrecht Pütz[1], Dirk Hansen[2], and Jörg Baute[2]
[1]LUA Rheinland-Pfalz ILC Trier, Maximineracht 11a, 54295 Trier, Germany
[2]Phenomenex Ltd. Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany

Overview

Many plants produce pyrrolizidine alkaloids as secondary metabolites to protect them against herbivores. The occurrence of pyrrolizidine alkaloids in plants is depending on various factors including the plant species and the part of the plant. Pyrrolizidine alkaloids have shown liver toxicity in humans and animals and are therefore undesirable in food and feed.

In this application note we describe the effective analysis of pyrrolizidine alkaloids in herbal teas and honey.

Methods and Materials

Sample Preparation

The sample preparation for honey samples was done according to BfR-PA-Honig-1.0/2013[1] using 500 mg SCX sorbent and for herbal teas according to BfR-PA-Tee-2.0/2014[2] using 500 mg C18 sorbent. Compared to the above-mentioned methods, two deviations have been applied. The final dissolution of the extract was higher (F=10) and lower concentration calibration solutions, from 0.1 and 20 ng/mL in residue free matrix eluent, have been used.

LC Conditions

System: Agilent® 1260 system with binary pump
Column: Kinetex® 2.6 µm EVO C18 150 x 2.1 mm
Part No.: 00F-4725-AN
Guard: SecurityGuard™ ULTRA (AJ0-9000 + AJ0-9298)
Mobile Phase: A: 5 mmol/L Ammonium Formate and 0.1% Formic Acid in Water
B: 5 mmol/L Ammonium Formate and 0.1% Formic Acid in Methanol

Gradient:

Time (min)	%A	%B
0	95	5
0.5	95	5
7	50	50
12	5	95
15	5	95
16	95	5
24	95	5

Flow Rate: 350 µL/min
Temperature: 35 °C
Injection: 5 µL
Detector: SCIEX® QTRAP® 5500

MS Conditions

Table 1: MS Settings

Parameter	Setting
Ionization	ESI^+
Mode	MRM
Temperature	600 °C
Curtain Gas (CUR)	40 psi
Collision Gas (CAD)	Medium
IonSpray Voltage	5500 V
Gas1	60 psi
Gas2	80 psi
Q1 Resolution	Unit
Q3 Resolution	Unit
EP	10
Scheduled MRM	
MRM Detection Window	110 sec
Target Scan Time	0.7 sec

Results

Table 2: Retention Times and MRM settings of the target analytes

t_R[min]	Analyte	Q1 Mass	Q3 Mass	DP	CE	CXP
8.62	Echimidine	398.2	120.1	66	35	10
			220.1	66	25	18
8.77	Echimidine-N-Oxide	414.2	254.1	106	43	22
			352.2	106	35	16
3.65	Erucifoline	350.1	120.1	216	39	14
			138.1	216	39	12
5.34	Erucifoline-N-Oxide	366.1	94.0	191	67	12
			120.1	191	41	10
4.30	Europine	330.1	138.1	111	31	10
			254.1	111	27	20
5.43	Europine-N-Oxide	346.1	172.1	86	41	14
			111.1	86	61	10
6.75	Heliotrine	314.1	138.1	76	29	10
			156.1	76	37	14
7.30	Heliotrine-N-Oxide	330.1	172.1	136	37	14
			111.0	136	55	10
3.74	Intermedine	300.1	94.0	71	35	12
			138.1	71	27	12
5.34	Intermedine-N-Oxide	316.1	172.1	116	39	14
			94.0	116	61	12
4.39	Jacobine	352.2	120.1	151	39	10
			155.1	151	39	12
5.67	Jacobine-N-Oxide	368.1	296.1	201	35	18
			120.1	201	45	12
9.70	Lasiocarpine	412.2	120.1	136	37	10
			220.1	136	27	20
10.41	Lasiocarpine-N-Oxide	428.2	254.1	106	39	22
			94.0	106	69	12
4.21	Lycopsamine	300.1	94.0	61	35	12
			138.1	61	29	16
5.95	Lycopsamine-N-Oxide	316.1	172.1	131	39	14
			138.1	131	39	10
2.01	Monocrotaline	326.1	120.1	166	45	10
			237.1	166	33	18
4.17	Monocrotaline-N-Oxide	342.1	137.0	196	39	14
			120.1	196	45	14
6.48	Retrorsine	352.1	120.1	166	39	14
			138.1	166	39	12
6.86	Retrorsine-N-Oxide	368.1	94.1	151	71	8
			120.1	151	43	14
7.80	Senecionine	336.1	120.1	196	39	14
			138.1	196	39	12
8.16	Senecionine-N-Oxide	352.1	94.0	146	67	12
			118.0	146	41	14
6.91	Seneciphylline	334.1	120.1	141	37	10
			138.1	141	37	12
7.34	Seneciphylline-N-Oxide	350.1	94.0	166	61	12
			120.2	166	41	10
8.85	Senkirkine	366.2	168.1	171	39	14
			150.0	171	37	12
6.46	Trichodesmine	354.1	222.1	186	39	20
			120.1	186	49	10

Figure 1a: XiC of Standards with concentration of 0.5 ng/mL of each pyrrolizidine alkaloid

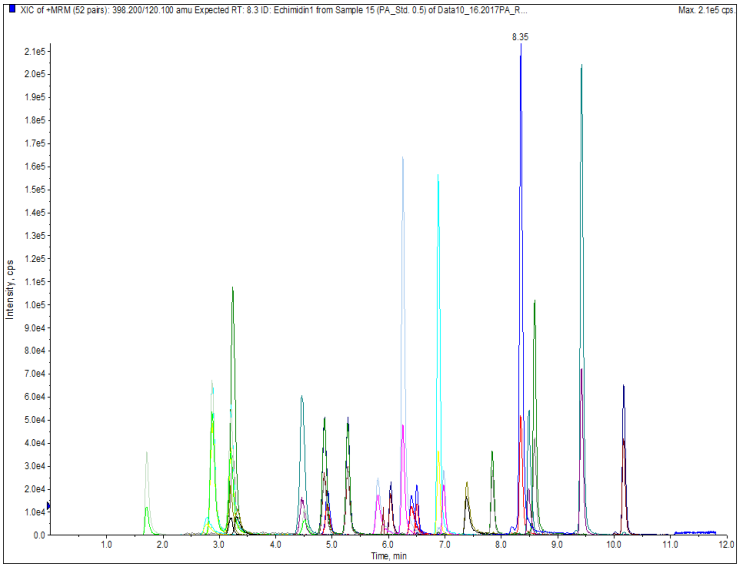

Figure 1b: XiC of blank injection

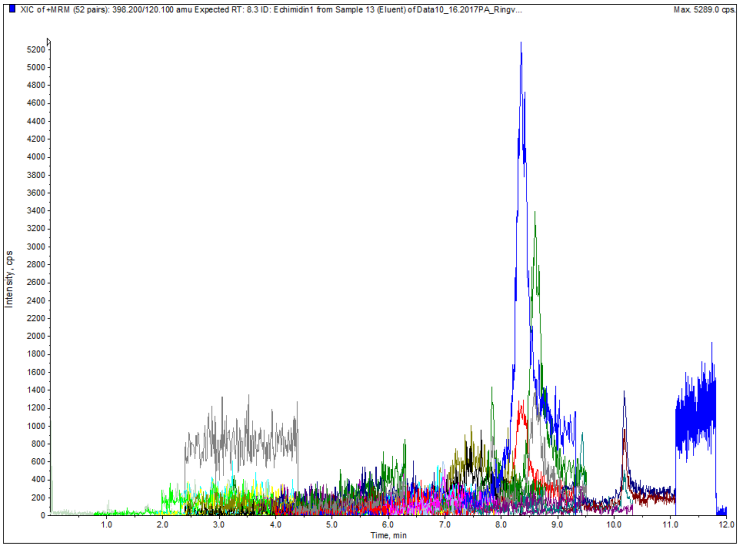

Figure 2a: Calibration curve first transition for Echimidine, Europine, Heliotrine, Lasiocarpine and Lycopsamine in honey

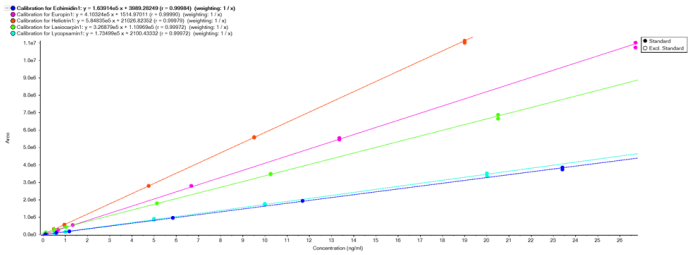

Figure 2b: Calibration curve first transition for Monocrotaline, Retrorsine, Senecionine, Senecipylline, and Senkirkine in honey

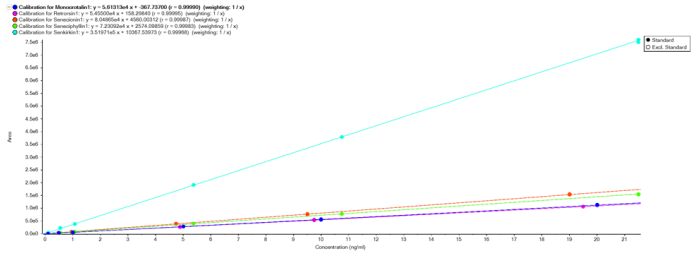

Figure 2c: Calibration curve first transition for Trichodesmine, Europine-N-Oxide, Heliotropine-N-Oxide, Intermedine, and Lasiocarpine-N-Oxide in honey

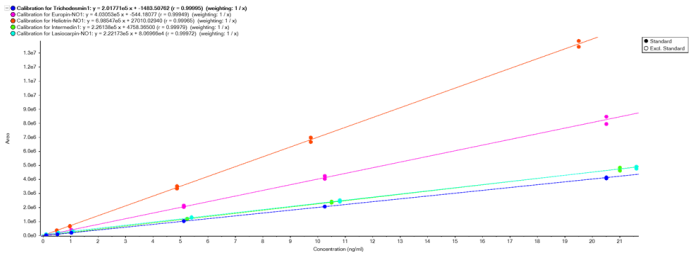

Figure 2d: Calibration curve first transition for Monocrotaline-N-Oxide, Retrorsine-N-Oxide, Senecionine-N-Oxide, Seneciphylline-N-Oxide, and Echimidine-N-Oxide in honey

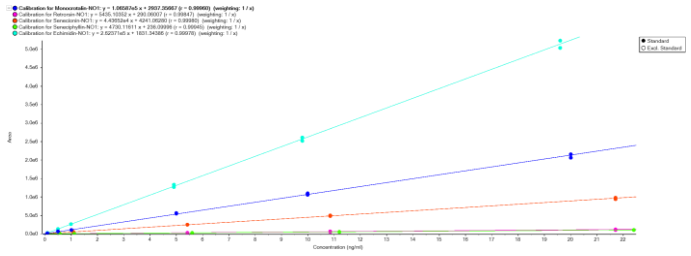

Figure 2e: Calibration curve first transition for Erucifoline, Erucifoline-N-Oxide, Intermedine-N-Oxide, Jacobidine, Jacobidine-N-Oxide, and Lycopsamine-N-Oxide in honey

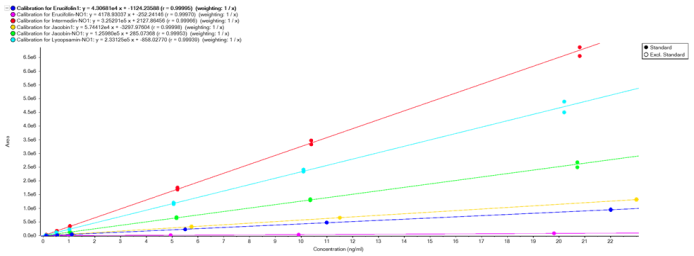

Figure 3a: Calibration curve first transition for Echimidine, Europine, Heliotrine, Lasiocarpine and Lycopsamine in herbal tea

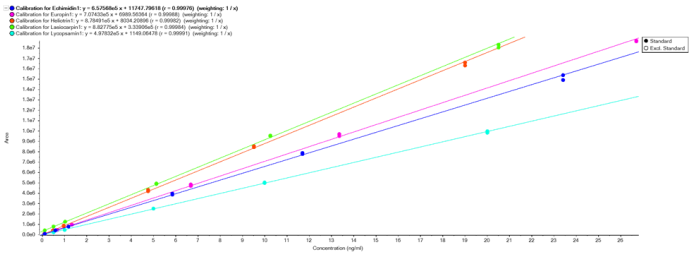

Figure 3b: Calibration curve first transition for Monocrotaline, Retrorsine, Senecionine, Senecipylline, and Senkirkine in herbal tea

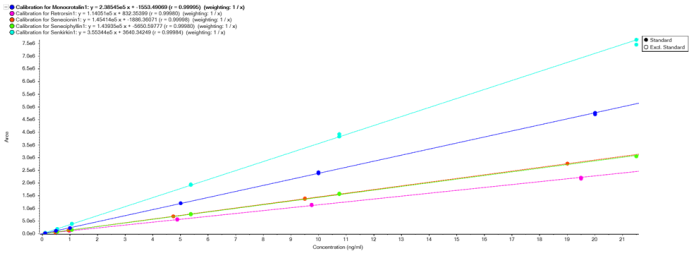

Figure 3c: Calibration curve first transition for Trichodesmine, Europine-N-Oxide, Heliotropine-N-Oxide, Intermedine, and Lasiocarpine-N-Oxide in herbal tea

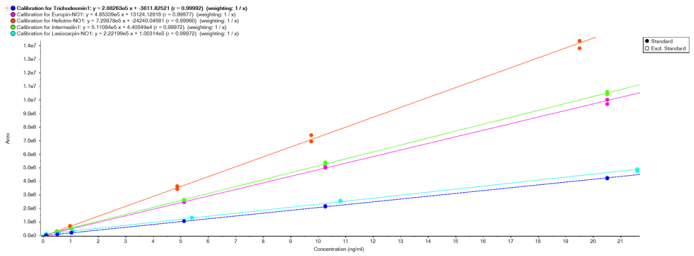

Figure 3d: Calibration curve first transition for Monocrotaline-N-Oxide, Retrorsine-N-Oxide, Senecionine-N-Oxide, Seneciphylline-N-Oxide, and Echimidine-N-Oxide in herbal tea

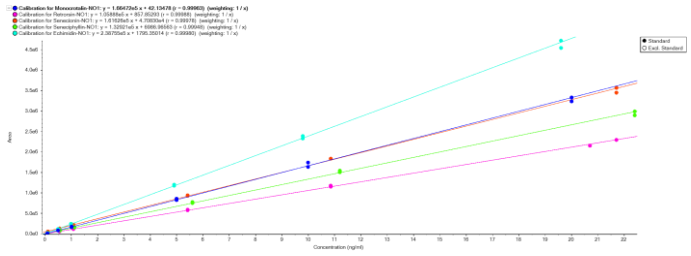

Figure 3e: Calibration curve first transition for Erucifoline, Erucifoline-N-Oxide, Intermedine-N-Oxide, Jacobidine, Jacobidine-N-Oxide, and Lycopsamine-N-Oxide in herbal tea

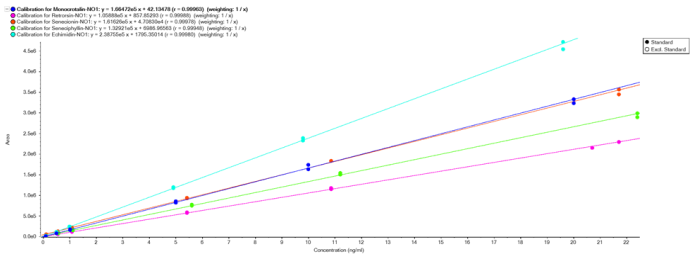

Table 3: Limits of detection (LOD) and quantification (LOQ) for the target analytes

Analyte	Herbal Teas		Honey	
	LOD [µg/kg]	LOQ [µg/kg]	LOD [µg/kg]	LOQ [µg/kg
Echimidine	1.0	4.0	0.1	0.3
Echimidine-N-Oxide	1.0	4.0	0.1	0.3
Erucifoline	1.0	4.0	0.1	0.3
Erucifoline-N-Oxide	1.0	4.0	0.1	0.3
Europine	1.0	4.0	0.1	0.3
Europine-N-Oxide	1.0	4.0	0.1	0.3
Heliotrine	1.0	4.0	0.1	0.3
Heliotrine-N-Oxide	1.0	4.0	0.1	0.3
Intermedine	1.0	4.0	0.1	0.3
Intermedine-N-Oxide	1.0	4.0	0.1	0.3
Jacobine	1.0	4.0	0.1	0.3
Jacobine-N-Oxide	1.0	4.0	0.1	0.3
Lasiocarpine	4.0	10.0	0.1	0.3
Lasiocarpine-N-Oxide	4.0	10.0	0.1	0.3
Lycopsamine	1.0	4.0	0.1	0.3
Lycopsamine-N-Oxide	1.0	4.0	0.1	0.3
Monocrotaline	1.0	4.0	0.1	0.3
Monocrotaline-N-Oxide	1.0	4.0	0.1	0.3
Retrorsine	1.0	4.0	0.1	0.3
Retrorsine-N-Oxide	1.0	4.0	0.1	0.3
Senecionine	1.0	4.0	0.1	0.3
Senecionine-N-Oxide	1.0	4.0	0.1	0.3
Seneciphylline	1.0	4.0	0.1	0.3
Seneciphylline-N-Oxide	1.0	4.0	0.1	0.3
Senkirkine	1.0	4.0	0.1	0.3
Trichodesmine	1.0	4.0	0.1	0.3

Conclusion

The presented method allows a reliable and robust quantification of pyrrolizidine alkaloids in herbal teas and honey. In herbal teas the average recovery for the pyrrolizidine alkaloids was in the range between 75 and 100% with the exception of Lasiocarpine(66%) and Seneciphylline (71%). **Table 3** shows the limits of detection (LOD) and limits of quantification (LOQ) for all pyrrozilidine alkaloids in herbal teas and honey.

References

1 *BfR Testing Method: BfR-PA-Honig-1.0/2013 (German), German Federal Institute for Risk Assessment*

2 *BfR Testing Method: BfR-PA-Tee-2.0/2014 (German), German Federal Institute for Risk Assessment*

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Canada
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China
t: +86 400-606-8099
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Czech Republic
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Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
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New Zealand
t: +64 (0)9-4780951
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Norway
t: +47 810 02 005
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Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
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