High-Throughput Analysis of Pyrrolizidine Alkaloids in Tea by Liquid Chromatography Vacuum Differential Mobility Spectrometry-Mass Spectrometry

FP421

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Overview

- Separation of diastereomeric pyrrolizidine alkaloids using differential ion mobility spectrometry-mass spectrometry (DMS-MS) at low pressure (33 mbar).
- Combination of vacuum DMS-MS with a short column in trap/elute setup to increase throughput in quantitative analysis.
- Comparison of analytical performance of the short LC-vDMS-MS (5 min) versus a reported LC-MS assay (12 min) [2].

Introduction

In recent years, the monitoring of phytotoxic compounds such as, pyrrolizidine alkaloids (PAs) in food matrices has gained interest. PAs are a complex mixture of diastereomers and more than 600 and their N-oxides forms were identified in over 6000 plants. Many LC-MS and GC-MS assays have been developed, but these methods require extensive sample preparation and long analyses times. Differential ion mobility is powerful for the separation of isomers and for filtering chemical noise. In the present work, the potential of vacuum DMS (vDMS) for enhanced selectivity and short LC columns are investigated for the analysis of diastereomeric PAs in tea samples for improved sample throughput.

Four set of PA diastereomers were investigated. Intermedine, echinatine, lycopsamine, indicine (*m/z* 300) and their N-oxide forms; intermedine-N-oxide, echinatine-N-oxide, indicine-N-oxide, lycopsamine-N-oxide (*m/z* 316) have no selective fragments under CID and coeluted in LC. Senecivernine and senecionine (*m/z* 336) cannot be distinguished by MS/MS but they partially coeluted in LC. Jacobine is the only diastereomer of *m/z* 352 that has a selective fragment (*m/z* 352>155) and can be distinguished from retrorsine, senecivernine-N-oxide and senecionine-N-oxide (*m/z* 352); which have different retention times under isocratic conditions at 15% MeOH. The orthogonality of vDMS as second dimension to LC-MS allowed the separation of co-eluting analytes without compromising analysis time. Several parameters in vDMS were optimized to achieved separation of diastereomers such as nature of solvent, amplitude of the waveform, compensation voltage, temperature and pressure. Sample preparation was simplified due to the combination of short column and trap/elute setup to vDMS-MS detection. Finally, PA diastereomers were analyzed in tea samples using the new developed short LC-DMS-MS method and compared to a LC-MS reference method.

Analytes

	PAs	LC-MS-MS (MRM mode) Q1 (m/z)	Q3 (m/z)	CE (V)	IS	RT (min)	Short LC-DMS-MS (SIM mode) RT (min)	CV (V)	DV (V)
1	Intermedine (InT)	300.1	94.1 138.1	27 21	Atp	4.79	0.93	-18.16	800
2	Echinatine (Ech)	300.1	94.1 138.1	20 31	Atp	4.86	0.91	-19.46	800
3	Lycopsamine (Lyc)	300.1	94.1 138.1	28 22	Atp	4.89	0.93	-18.06	800
4	Indicine (InD)	300.1	94.1 138.1	25 20	Atp	4.87	0.92	-16.85	800
5	Intermedine-N-oxide (InT-N)	316.1	172.1 94.1	30 44	Atp	5.34	1.30	-18.36	800
6	Echinatine-N-oxide (Ech-N)	316.1	172.1 111.1	30 41	Atp	5.25	1.31	-22.47	800
7	Lycopsamine-N-oxide (Lyc-N)	316.1	172.1 138.1	25 30	Atp	5.47	1.39	-21.07	800
8	Indicine-N-Oxide (InD-N)	316.1	172.1 94.1	30 43	Atp	5.36	1.30	-18.36	800
9	Jacobine (Jac)	352.1	155.1 120.1	25 20	Epi	4.76	0.93	-13.34	800
10	Retrorsine (Ret)	352.1	120.1 138.1	30 30	Epi	5.88	1.49	-12.54	800
11	Senecivernine-N-oxide (SeV-N)	352.1	94.1 118.1	30 30	Epi	7.15	2.49	-14.45	800
12	Senecionine-N-oxide (SeC-N)	352.1	94.1 118.1	30 30	Epi	7.28	2.82	-14.85	800
13	Senecivernine (SeV)	336.1	120.1 94.1	25 25	Epi	6.88	2.23	-13.24	800
14	Senecionine (SeC)	336.1	120.1 94.1	25 25	Epi	6.97	2.39	-13.34	800
15	Atropine (Atp)	290.3	93.1 124.2	35 30	N.A.	6.53	1.95	-16.22	800
16	Epi-jacobine (Epi)	352.1	155.1 120.1	25 20	N.A.	6.35	1.71	-14.15	800

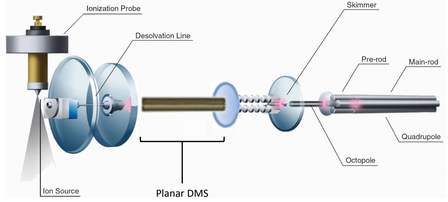


Figure 1. Schematic representation of the vacuum Differential Ion Mobility- Mass Spectrometry (vDMS-MS).[1]

Materials and methods

Sample preparation

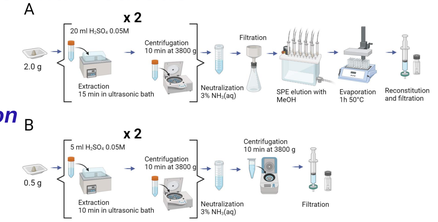
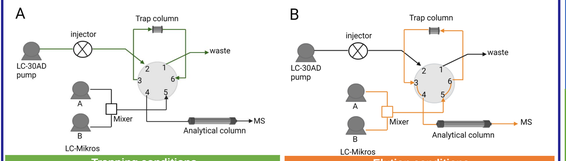


Figure 2. Tea sample preparation workflow: A) follow the BFR protocol [2] that includes extraction with 0.05 M sulfuric acid by sonication, centrifugation, neutralization, filtration, solid phase extraction, evaporation and reconstitution. B) a simplified protocol that excluded the solid phase extraction, evaporation and reconstitution steps for LC-DMS-MS analysis in trap/elute mode.

Short LC-DMS-MS method



Trapping conditions	
Flow rate	100 µL/min
Time	0.25 min
Mobile phase	15% MeOH:H2O,0.1% FA (isocratic)

Elution conditions	
Flow rate	100 µL/min
Time	4.75 min
Mobile phase	15% MeOH:H2O,0.1% FA (isocratic)

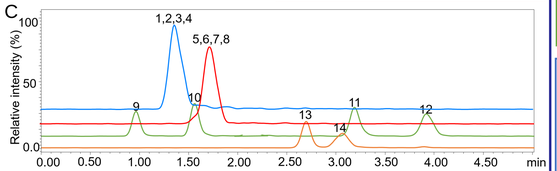


Figure 3. Short LC-DMS-MS configuration. A trap column Reprosil –Pur C18-AQ (10x 0.5 mm, 5 µm), and a short column Kinetex XB-C18 (50 x 1.0 mm, 5 µm) was used to analyzed the samples in 2 steps: front-flush injection **(a)** and back-flush elution of analytes **(b)** acquired in SIM mode **(c)** XIC of PAs (500 ng/ml) in SIM mode.

LC-MS method [2]

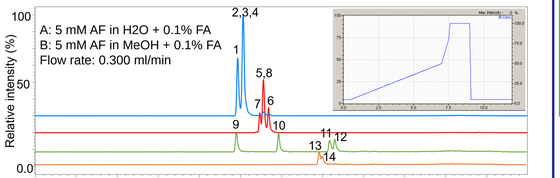


Figure 4. LC-MS method follow BFR protocol, using a Kinetex XB-C18 column (150 x 2.1 mm, 1.7 µm). Extracted ion chromatogram of PAs (500 ng/ml) acquired in MRM mode by LCMS-8060 with ballistic gradient of 12 minutes.

Results

CV scans at DV=800 v

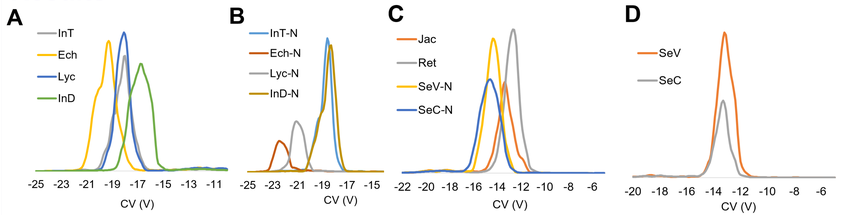


Figure 5. Measured correction voltage (CV) at DV=800 v by infusion of diastereomeric PAs (500 ng/ml) in SIM mode. Vacuum DMS was used in scan mode (CV stepped by 0.1 volts) for optimized separation using 15% MeOH as modifier at flow rate 100 µL/min. CV values in volts for diastereomers **(A)** *m/z* 300 InT (-18.16), Ech (-19.46), Lyc (-18.06), InD (-16.85). **(B)** *m/z* 316 InT-N (-18.66), Ech-N (-22.47), Lyc-N (-21.07), InD-N (-18.36). **(C)** *m/z* 352 Jac (-13.34), Ret (-12.54), SeV-N (-14.44), SeC-N (-14.85). **(D)** *m/z* 336 SeV (-13.24) and SeC (-13.34)

Method performance of LC-DMS-MS (SIM) and LC-MS (MRM)

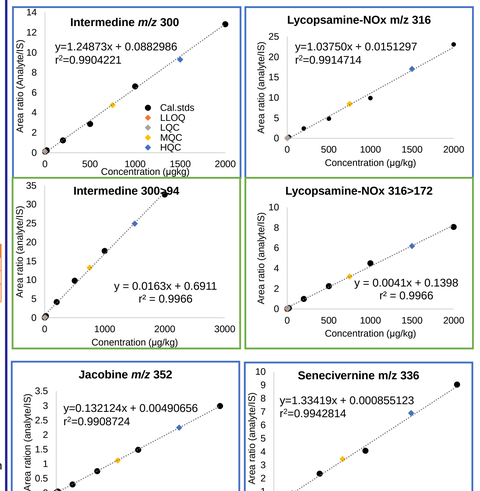


Figure 6. Selected calibration curves of pyrrolizidine alkaloids spiked in green tea matrix (2 to 2000 µg/kg). Triangles represents the accuracy QC levels (LLOQ, LQC, MQC, HQC). Samples were analyzed by the short LC-DMS-MS (acquired in SIM mode) highlighted in blue and LC-MS/MS (acquired in MRM mode) in green trace. Epi-jacobine and atropine-D_3 was used as internal standards to correct matrix effect.

QC level	Method		InT	Lyc-N	Ret	SeC
LLOQ	LC-vDMS-MS (SIM)	Accuracy%	108	107	102	101
		Precision%	1.3	7.50	2.90	4.50
(2 µg/kg)		S/N	205	17.7	17	29.5
	LC-MS (MRM)	Accuracy%	92.2	105	102	102
		Precision%	2.50	4.00	5.40	3.50
		S/N	114	151	41.6	22.7
LQC	LC-vDMS-MS (SIM)	Accuracy%	113	105	111	104
		Precision%	1.30	3.10	2.40	1.90
(6 µg/kg)		S/N	511	21.2	97.7	40.6
	LC-MS (MRM)	Accuracy%	101	104	104	108
		Precision%	3.90	3.50	3.40	4.90
		S/N	414	427	161.3	110
MQC	LC-vDMS-MS (SIM)	Accuracy%	93.4	104	93.4	101.5
		Precision%	4.80	8.20	9.00	4.60
(750 µg/kg)		S/N	2770	1374	7310	2738
	LC-MS (MRM)	Accuracy%	97.4	99.8	101	105
		Precision%	9.80	0.40	4.40	2.70
		S/N	5430	15651	5317	2576
HQC	LC-vDMS-MS (SIM)	Accuracy%	92.8	111	98.5	95.2
		Precision%	8.90	4.20	2.90	5.70
(1500 µg/kg)		S/N	6688	4361	13025	6367
	LC-MS (MRM)	Accuracy%	96.5	99	104	106
		Precision%	6.10	6.60	2.50	2.70
		S/N	9556	26153	8841	6418

Figure 7. Table summarized the accuracy and precision values of PAs spiked in green tea matrix obtained at different QC levels using short LC-DMS-MS (SIM mode) and LC-MS/MS (MRM mode).

Application to quantification of PAs in tea samples

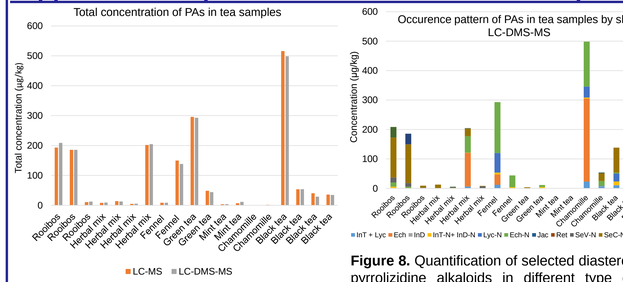


Figure 8. Quantification of selected diasteromeric pyrrolizidine alkaloids in different type of tea samples purchase in swiss supermarkets.

Conclusions

- The combined short C18 column with trap/elute LC setup hyphenated to vDMS-MS detection enables to simplify the sample preparation, higher throughput analysis (5 min) and improved selectivity compare to a classical LC-MS method.
- LC-DMS-MS enables accurate quantitation of 10 out of 14 diastereomeric PAs with higher throughout (<1h), compare to the classical LC-MS method in which 9 out of 14 diastereomeric could be separated.
- LC-vDMS-MS method in SIM mode has similar performance (linearity, accuracy and precision) compared to a reported LC-MS method in MRM.

References

1. Shvartsburg, A. A., A. Haris, R. Andrzejewski, A. Entwistle and R. Giles (2018). "Differential Ion Mobility Separations in the Low-Pressure Regime." Analytical Chemistry **90**(1): 936-943.

2. Method description BfR-PA-Tee-2.0/2014; Determination of pyrrolizidine alkaloids (PA) in plant material using SPE-LC-MS/MS