High Throughput Analysis of Antidepressant Drugs in Human Plasma by Liquid Chromatography Vacuum Differential Mobility Spectrometry-Mass Spectrometry

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Overview

- Analysis of isobaric antidepressants drugs 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 quantitative analysis.
- Comparison in method performance of short LC-vDMS-MS (2 min) and a classical LC-MS assay (8 min).

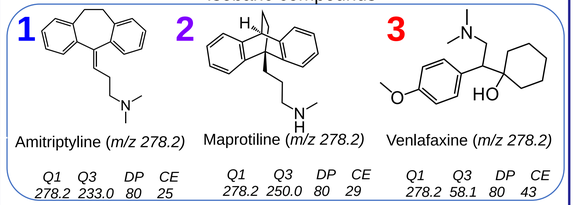
Introduction

In recent years Differential Mobility Spectrometry (DMS) devices have been applied as additional filter dimension in liquid chromatography - mass spectrometry analysis (LC-MS), for the elimination of chemical noise or the improvement of selectivity. Fast LC-MS analysis of drugs and metabolites in biological samples often suffers in limited chromatographic selectivity, which jeopardize the quantification of isobaric/isomeric compounds. With most current setup the DMS is operated at atmospheric pressure. In the present work, the potential of vacuum DMS is investigated for the high throughput analysis of isobaric antidepressants in human plasma as model compounds using a short LC column operated in trap/elute mode.

The simplest way to reduce the analysis time of an LC-SRM/MS assay is to minimize chromatographic separation. This becomes problematic when isobaric or isomeric drugs are co-eluting with the analytes in complex biological matrices such as plasma. Nortriptyline (*m/z* 264), Desipramine (*m/z* 267) and imipramine (*m/z* 281) have different molecular weight and can be distinguished by their *m/z*. Amitriptyline, maprotiline and venlafaxine are isobars (*m/z* 278) that can be distinguished by some MS/MS mass fragments but on cost of assay performance. The introduction of DMS as second orthogonal dimension to LC-MS allows separation of co-eluting analytes in LC without compromising analysis time. Several parameters can be considered to optimize the vDMS separation including amplitude of the waveform, compensation voltage and pressure. The combination of short LC and vDMS for analyte baseline separation enabled rapid quantitative analysis (2 min) of the isobaric antidepressant drugs with improved S/N ratio and selectivity. The short LC/vDMS/MS method showed similar quantitative performance compared to the classical LC-MS method.

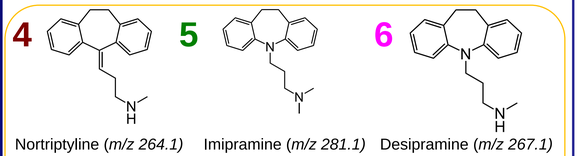
Analytes

Isobaric compounds



	Amitriptyline (*m/z 278.2*)				Maprotiline (*m/z 278.2*)				Venlafaxine (*m/z 278.2*)			
	Q1	Q3	DP	CE	Q1	Q3	DP	CE	Q1	Q3	DP	CE
	278.2	233.0	80	25	278.2	250.0	80	29	278.2	58.1	80	43

Structural related compounds



	Nortriptyline (*m/z 264.1*)				Imipramine (*m/z 281.1*)				Desipramine (*m/z 267.1*)			
	Q1	Q3	DP	CE	Q1	Q3	DP	CE	Q1	Q3	DP	CE
	264.1	233.0	80	21	281.1	86.1	80	25	267.1	72.0	80	29

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. Kirchherr, H. and W. N. Kühn-Velten (2006). "Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: A multi-level, single-sample approach." Journal of Chromatography B **843**(1): 100-113.
3. Bravo-Veyrat S, Hopfgartner G (2018). "High-throughput liquid chromatography differential mobility spectrometry mass spectrometry for bioanalysis: determination of reduced and oxidized form of glutathione in human blood". Journal of Chromatography B 410(27):7153-61.

Methods

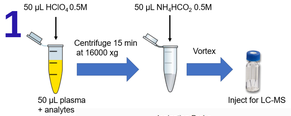


Figure 1. Plasma sample preparation workflow: i) perchloric acid 0.5 M for protein precipitation, ii) centrifugation and iii) sample pH is reduced using ammonium formate 0.5 M.

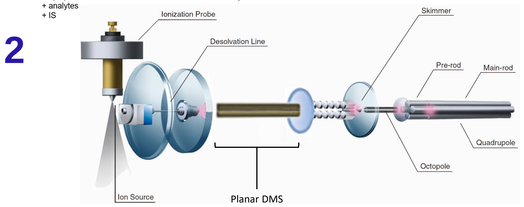
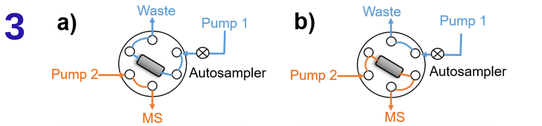


Figure 2. Schematic representation of the vacuum Differential Ion Mobility- Mass Spectrometry (vDMS-MS).



Trapping conditions	
Flow rate	100 µL/min (LC-30AD pump)
Time	0.25 min
Mobile phase	5% CH_3CN in H_2O + 0.1% FA in isocratic

Elution conditions	
Flow rate	50 µL/min (Nexera Mikros LC)
Time	1.75 min
Mobile phase	CH_3CN + 0.1% FA in isocratic

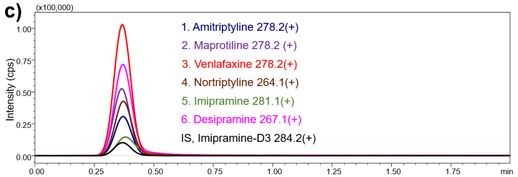


Figure 3. Short LC-DMS-MS configuration. A short column Luna Omega C18 (20 x 0.5 mm, 5 µm 100A°) was used to analyzed the samples in 2 steps: front-flush injection **(a)** and back-flush elution of analytes **(b)** acquired in SIM mode. **(c)** XIC of antidepressants drugs (400 ng/ml).

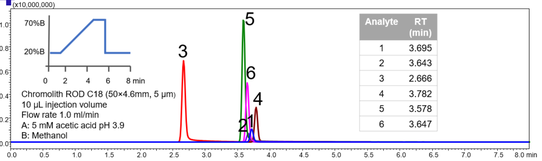


Figure 4. Classical LC-MS method. Extracted ion chromatogram of antidepressants drugs (400 ng/ml) using Nexera LC system with LC-30AD pump acquired in MRM mode by LCMS-8050 with ballistic gradient of 8 minutes.

Results

CV/DV scans by infusion in vDMS

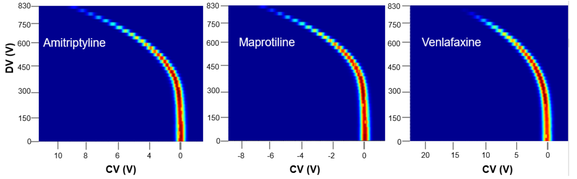


Figure 5. Measured correction voltage (CV) and dispersion voltage (DV) by infusion of 500 ng/ml for isobaric analytes in SIM mode. Vacuum DMS was used in scan mode (DV ramp from 0 to 830 V and CV stepped by 0.2 volts)

µLC-vDMS-MS

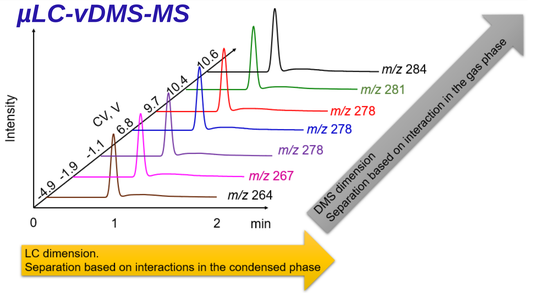


Figure 6. Representative XIC of antidepressants drugs by short LC-vDMS-MS acquired in SIM mode illustrated the separation by 2 dimensions (LC x DMS) based on different interactions. Isobaric compounds are separated on the DMS dimension at DV=760 V and the corresponding CV= -1.1 (Map), 6.8 (Ami) and 9.7 (Ven) volts.

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

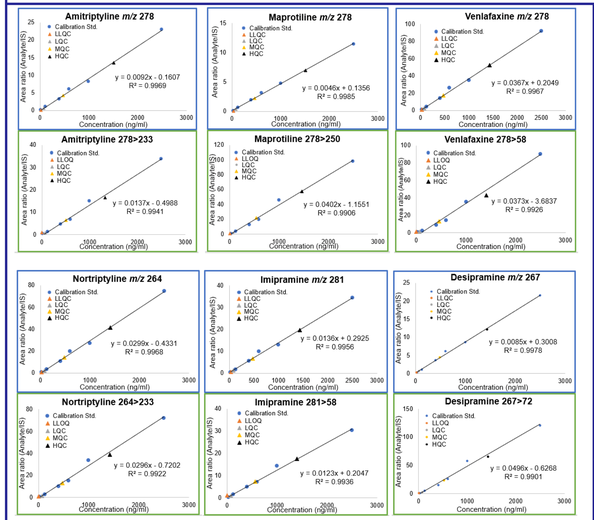


Figure 7. Quantitative analysis of plasma spiked with antidepressants drugs (25 to 2500 ng/mL). Triangles represents the accuracy QC levels (LLOQ, LQC, MQC, HQC). Samples were analyzed by the short LC-DMS-MS (acquired in SIM mode) highlighted in blue and LC-MS/MS (acquired in MRM mode) in green trace. Imipramine-D_3 was used as internal standard to correct matrix effect in short LC-DMS-MS in contrast to LC-MS was used the corresponding analog deuterium labelled compound. Amitriptyline-D_3 (for isobaric compounds), nortriptyline-D_3, desipramine-D_3 and imipramine-D_3.

Compound	Method		Amitriptyline	Maprotiline	Venlafaxine	Nortriptyline	Imipramine	Desipramine
LLOQ (25 ng/ml)	LC-vDMS-MS (SIM)	Accuracy (%)	102.3	91.75	104.4	100	106.7	97.33
		Precision (%)	10.4	8.75	12.9	8.91	10.2	9.94
		S/N	52	41	350	253	243	117
	LC-MS (MRM)	Accuracy (%)	120	100	114	101	110	108
		Precision (%)	6.12	8.59	6.58	2.85	1.97	4.68
		S/N	327	702	50	384	436	1637
LQC (80 ng/ml)	LC-vDMS-MS (SIM)	Accuracy (%)	100.1	95.43	93.44	99.3	106.1	86.13
		Precision (%)	8.12	6.71	7.23	12.5	11.3	5.63
		S/N	424	178	1492	694	622	367
	LC-MS (MRM)	Accuracy (%)	93.3	89.3	87.4	87.0	89.8	92.0
		Precision (%)	5.94	4.84	4.28	9.45	7.13	3.67
		S/N	594	1296	120	1096	2157	2343
MQC (500 ng/ml)	LC-vDMS-MS (SIM)	Accuracy (%)	98.53	93.99	94.82	93.3	93.60	98.43
		Precision (%)	7.44	10.8	9.59	8.46	9.12	8.15
		S/N	1964	448	6551	2135	2868	901
	LC-MS (MRM)	Accuracy (%)	99.5	108	92.3	96.6	111	104
		Precision (%)	7.80	6.43	4.29	5.66	5.85	10.9
		S/N	8018	12012	402	8805	24475	17128
HQC (1500 ng/ml)	LC-vDMS-MS (SIM)	Accuracy (%)	100.4	94.07	93.70	96.5	95.28	92.03
		Precision (%)	11.4	9.57	7.02	6.35	9.17	9.21
		S/N	2797	898	6702	3585	3946	1499
	LC-MS (MRM)	Accuracy (%)	91.5	97.3	93.8	94.9	93.4	95.0
		Precision (%)	9.29	6.15	9.29	7.33	4.94	6.39
		S/N	17696	18303	860	17219	41603	34722

Figure 8. Table summarized the accuracy and precision values of plasma samples spiked with antidepressants drugs obtained at different QC levels (LLOQ, LQC, MQC, HQC) and signal to noise ratio (S/N) using short LC-DMS-MS (1 µL injection, SIM mode acquisition) and LC-MS/MS (10 µL injection, MRM mode acquisition).

Conclusions

- LC-vDMS-MS method in SIM mode has similar performance (linearity, accuracy and precision) compared to a classical LC-MS method in MRM.
- The combined short C18 column with trap/elute LC setup hyphenated to vDMS-MS detection offer higher throughput analysis (2 min) for large cohort of samples in clinical applications compare to published LC-MS (8 min).
- The use of vDMS bring the advantage to tune the assay selectivity without compromising analysis time, while the background noise is reduced improving sensitivity.