Evaluation of matrix effects occuring during drugs of abuse analysis by LC-MS/MS

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Introduction

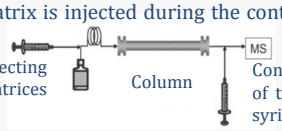
In LC-MS/MS, some co-eluting components are susceptible to increase (ion enhancement) or reduce (ion suppression) the ionization of the target analytes, producing the so-called matrix effect (ME). The aim of the present work was to assess, by two approaches, the importance of ME during the quantitative determination of drugs of abuse in three biological matrices (serum, blood and oral fluid).

Methods

The first strategy used to evaluate ME is the **post-extraction addition** [1] which involves the comparison of areas generated by the same amount of analyte, with and without the extracted matrix:

- A = neat standard in the mobile phase
- B = blank matrix extracted *then* spiked
- Absolute ME (%) = B/A x 100 (can be corrected by the internal standard (IS))
- Relative ME (%) = CV (can be corrected by the internal standard)

The second strategy used to evaluate ME is the **post-column infusion** [2] [3] which consists to monitor the detector response when an extracted matrix is injected during the continuing infusion of the target analyte:



Autosampler injecting extracted matrices (without analyte) — Column — Constant infusion of the analyte by a syringe pump

Both approaches were tested to evaluate ME of 3 biological fluids, submitted to 2 distincts sample preparations and chromatographic conditions. Blood (n=20), serum (n=20) and oral fluid (n=15) specimens were tested.

Cannabinoids: liquid-liquid extraction (hexane/ethyl acetate (9/1: v/v))

Amphetamines, cocaine, opiates, opioids and metabolites: solid phase extraction (Oasis® MCX 30mg, 1ml)

UPLC Waters® Acquity → Quattro Premier QQQ MS (ESI)

Results

Post-extraction addition

Cannabinoids

	Serum			Blood			Oral Fluid		
Compound	Mean ME without IS (%)	Mean ME with IS (%)	Relative ME with IS (%)	Mean ME without IS (%)	Mean ME with IS (%)	Relative ME with IS (%)	Mean ME without IS (%)	Mean ME without IS (%)	Relative ME with IS (%)
THC	81	100	1.6	91	101	4.0	93	102	4.1
THC-OH	125	100	1.1	109	103	4.1	N.d.	N.d.	N.d.
THC-COOH	93	100	2.6	93	104	6.9	N.d.	N.d.	N.d.

N.d. = not determined

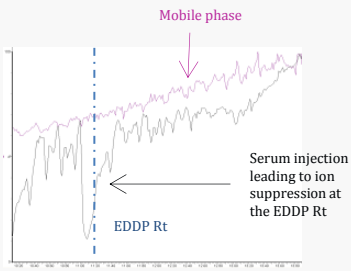
Example of post-column infusion: when ME is significant, the signal variation is substantial (for this serum: ME=22%).

Amphetamines, cocaine, opiates, opioids and metabolites

Post-extraction addition

	Serum			Blood			Oral Fluid		
Compound	Mean ME without IS (%)	Mean ME with IS (%)	Relative ME with IS (%)	Mean ME without IS (%)	Mean ME with IS (%)	Relative ME with IS (%)	Mean ME without IS (%)	Mean ME with IS (%)	Relative ME with IS (%)
Amphetamine	182	121	8.1	91	107	3.2	74	101	3.0
Metamphetamine	154	109	3.7	80	103	3.8	64	98	6.0
MDMA	166	94	5.2	93	100	3.7	51	98	2.7
MDA	179	127	3.9	92	105	3.3	50	98	4.3
MDEA	152	105	2.6	97	111	6.2	63	99	4.2
MBDB	147	108	1.9	95	102	4.9	64	102	3.2
Cocaine	117	99	1.3	93	108	3.6	88	102	1.5
Benzoylecgonine	94	102	1.8	99	104	4.9	74	102	1.1
Cocaethylene	81	100	1.4	100	106	3.5	N.d.	N.d.	N.d.
Morphine	105	99	6.6	81	104	5.8	77	102	6.5
6-MAM	129	114	4.3	134	95	3.2	83	101	3.7
Codeine	144	108	2.8	201	99	2.6	145	101	2.3
M6G	91	108	3.3	182	103	5.2	N.d.	N.d.	N.d.
M3G	79	108	4.2	85	103	6.9	N.d.	N.d.	N.d.
Hydromorphone	154	100	4.4	125	93	4.1	N.d.	N.d.	N.d.
Dihydrocodeine	141	92	5.7	171	104	3.6	N.d.	N.d.	N.d.
Hydrocodone	224	95	6.9	144	115	5.3	N.d.	N.d.	N.d.
Oxycodone	254	102	3.1	164	88	3.7	N.d.	N.d.	N.d.
Buprenorphine	42	92	5.8	58	100	7.8	N.d.	N.d.	N.d.
Methadone	73	98	0.9	97	106	2.1	N.d.	N.d.	N.d.
EDDP	76	97	1.5	89	99	5.3	N.d.	N.d.	N.d.

Example of post-column infusion: when ME is significant, the signal variation is substantial (for this serum: ME=23%).



Conclusion

The post-extraction addition approach enables a quantitative assessment of ME, whatever their extent. On the other hand, only wide ME can be detected by the post-column infusion approach. Finally, ME was compound and matrix dependent, and was corrected by the use of appropriate labeled IS, which were essential for a proper quantification of the analytes.

REF: [1] Matuszewski B.K. et al., Anal. Chem. **2003**, *75*, 3019-3030 [2] Bonfiglio R. et al., Rapid Commun. Mass Spectrom. **1999**, *13*, 1175-1185 [3] Souverain S. et al., J. Chrom. A **2004**, *1058*, 61-66