MICROEXTRACTION BY PACKED SORBENT CLEAN-UP FOR THE DETERMINATION OF SELECTED SYNTHETIC CATHINONES IN HAIR SAMPLES

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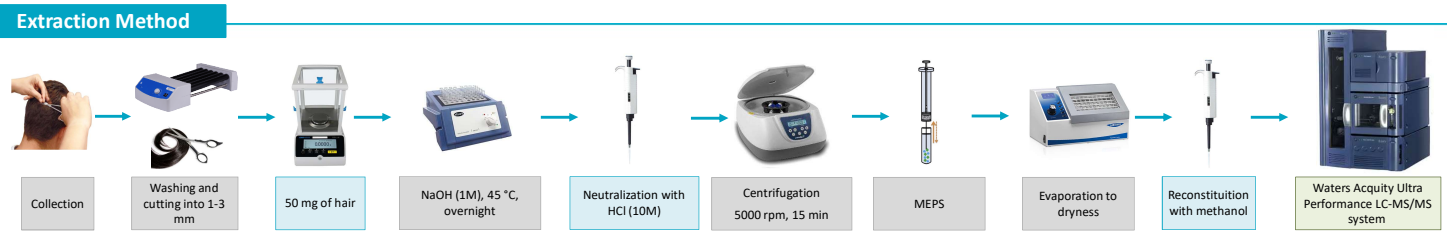
Introduction & Aims

- Synthetic cathinones are commonly known as amphetamine derivatives, not only because of their similar chemical structure to amphetamine, but also concerning their physiological and behavioral effects;
- As of today, more than 150 synthetic cathinones are monitored by the European Union Early Warning System;
- Regulatory measures have been introduced to control these substances. However, new uncontrolled derivatives keep appearing on the market to replace those that have been subjected to legislation. Thus, a need to develop new methodologies to monitor these substances.
- In this work, we develop a microextraction by packed sorbent (MEPS) clean-up methodology for the determination of 4-CEC, ethylone, pentedrone, penthylone, methylone, alpha-PVP, MDPV, and 4-Cl-alpha-PVP in hair samples;
- MEPS number of sample strokes was optimized to improve analyte response.

Extraction Method



LC-MS/MS Parameters

- Scan type: MRM
- Polarity: Positive
- Mobile phase: 2mM Ammonium formate in methanol (A) and 2mM Ammonium formate in water (B)
- Flow rate: 0.4 mL/min
- Column temperature: 45 °C
- Injection volume: 2 µL
- Run time: 14 min
- Column: Acquity UPLC® HSS T3 (100 mm × 2.1 mm; 1.8 µm)

Table 1: MRM transitions and retention times for the selected synthetic cathinones and internal standard (IS)

Analyte	Q1 (m/z)	Q3 (m/z) quantifier	Q3 (m/z) qualifier	Retention time (min)
Methylone	208	160	190	2.56
Ethylone	222	174	204	2.88
Pentedrone	192	174	91	3.80
4-CEC	212	159	144	3.82
Pentylone	236	86	188	3.92
Alpha-PVP	232	77	91	4.01
MDPV	276	175	126	4.12
4-Cl-alpha-PVP	266	195	126	4.95
Cocaine-d3 (IS)	307	185	-	3.82

Validation Parameters

Matrix effect	Selectivity and interferents	Linearity
81-109%	The method demonstrated specificity and selectivity for all analytes.	R^2 above 0.99 for all analytes; Range: 0.03 – 2.5 pg/mg for all analytes, except for pentedrone, which range was of 0.1 - 2.5 pg/mg ; and 4-CEC, which ranged from 0.05 – 2.5 pg/mg
Precision and accuracy	**Recovery**	**Carryover**
The method was precise and accurate for all analytes at all concentrations.	Recovery ranged from 15 % to 53 %. Although low for some analytes, it did not affect LOD and LLOQ values (0.03-0.1 pg/mg).	The method did not show significant carryover.

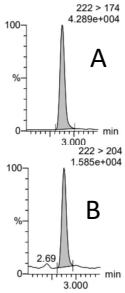
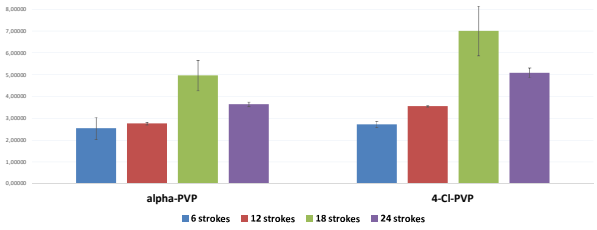


Figure 1 – Example of a chromatogram at LLOQ (0.03 pg/mg), for methylone A) quantifying transition, B) qualifying transition

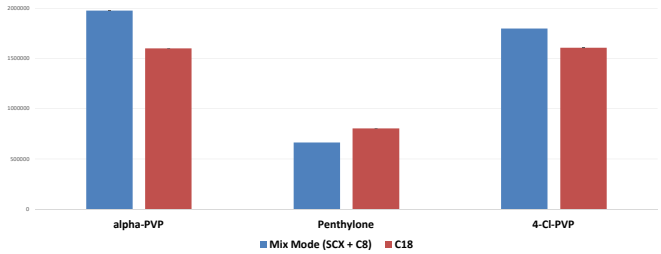
- To maximise the response obtained for each compound using MEPS as a clean-up process, both the type of extraction cartridge (sorbent) and the number of strokes were optimised. Below, some of the results obtained are presented.

Number of sample strokes - Optimisation



- At a glance, with the example for alpha-PVP and 4-Cl-PVP, it seems that 18 strokes is a better choice, but statistical analysis showed different significances between 18 and 24 strokes, which was the choice for this work.

Comparison of sorbent types



- No statistical significances were observed for any of the analytes, so M1 (80% C_8 and 20% SCX) cartridge was selected, because peak areas were better, in comparison to those obtained with extraction with a C_{18} cartridge.

Conclusions

- Successful optimization and full validation of the analytical method to determine synthetic cathinones in hair samples using MEPS as clean-up procedure and LC-MS/MS;
- The combination of this microextraction technique for clean-up with tandem mass spectrometry proved to be a simple and rapid procedure;
- Sensitive, selective, precise and accurate method, that can be applied to authentic samples.
- MEPS optimization is necessary in order to obtain better analyte resolution, in terms of peak area. In this work, 24 strokes proved to be a better approach;
- Mix mode cartridge (combination of a strong cation exchange and C_8) proves to be better for analytes extraction than only C_{18} cartridge.

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