

Development of a method for analysis of opiates and cocaine by GC/MS-MS

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Introduction

The determination of drug abuse is critical in forensic science. The toxicological lab of medico-legal institute of Strasbourg wants to switch the analysis of opiates and cocaine from a GC-MS method mode Full Scan to a GC/MS-MS method mode multiple reactions monitoring (MRM). The aim is to develop a method for the simultaneous identification and quantification of opiates: 6-monoacétylmorphine (6-MAM), morphine (MOR), codéine (COD), pholcodine, codéthylène (COD) and dihydrocodéine (DHC) along with cocaine (COC) and its metabolites: benzoylecgonine (BZE), cocaéthylène (CE), ecgoninéméthylester (EME) and anhydroecgoninéméthylester AEME. Two methods of extraction in whole blood were tested for choosing which one gives better extraction efficiency. Liquid-liquid-extraction (LLE) and solid-phase-extraction (SPE) were employed.

Material and methods

The method was developed with the aid of a pure solution containing 200ng of standard analytes and their deuterated internal standard which are used for the quantification. These compounds cannot be directly analyzed by GC because they are not volatile enough. So a derivation with BSTFA 1%TMCS is necessary. GC separation was performed by using a 30m Varian Factor Four Capillary Column VF-5ms (0.25mm ID and 0.25 µm film thickness). Samples were analyzed with a Varian 450 Gas chromatograph equipped with a Varian 320-MS Triple Quad Mass-Spectrometer. The detection is performed by using MRM, so a parent ion, a quantifier ion and a qualifier ion must be determined for each analyte. The extraction efficiency of two different extraction procedures was estimated. The LLE extraction consists of several purification steps at pH = 8.4, 1.0 and 8.4 respectively. On the other hand the SPE extraction method required C18 cartridge, on which several steps of washing and rinsing, with bicarbonate buffer and solvent, were necessary.

Results and discussion

The gradient obtained allows the separation of all compounds except BZE, CE, COD and MOR which were co-eluted two by two. Thanks to the different parent ions of this compound, the mass spectrometer allows to separate them. The initial temperature of the oven was 80°C for 1 min, it was then increased 10°/min until 190°C and was already increased 30°/min until 290°C, where it was held for 3 min. The total run time was 23.33 min. Helium at constant flow rate of 1 mL/min was used as a carrier gas. Injection was pulsed in split mode with a rate of 20 and the injector temperature was 270°C. Table 1 recaps all parent ions, daughter ions and retention times, obtained for each compound. SPE and LLE were calculated with relative standard deviation (RSD) values below 20%. The estimation shows that SPE gives better extraction efficiency than LLE, except for the DHC which is extracted below 50%. Although SPE costs more than LLE, it provided better extraction efficiency.

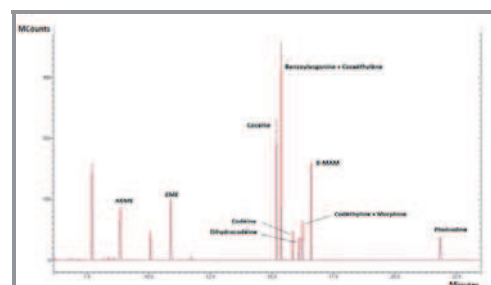


Figure 1

Compound	Retention time [min]	Parent ions (m/z)	Quantifier ions (m/z)	Qualifier ions (m/z)
Pholcodine	10.48	114	70	58
6-MAM	11.32	195	287	160
6-MAM (IS)	11.32	402	290	160
Morphine	11.07	429	287	220
Morphine (IS)	11.07	442	290	220
Codéthylène	11.02	385	132	234
Codéine	10.63	375	234	163
Codéine (IS)	10.63	374	237	163
DHC	10.48	373	282	148
EME (IS)	9.79	376	288	122
BZE	9.87	340	82	122
BZE (IS)	9.87	243	85	122
CE	9.88	196	82	122
CE (IS)	9.88	199	85	122
COC	9.33	182	82	90
COC (IS)	9.33	185	85	90
EME	8.55	96	96	82
EME (IS)	8.55	99	99	82
AEME	5.39	135	132	122
AEME (IS)	5.39	134	135	122

Table 1

Compound	% SPE efficiency (n=2)	% LLE efficiency (n=2)
Pholcodine	87	70
6-MAM	80	48
Morphine	95	24
Codéthylène	94	52
Codéine	93	53
Dihydrocodéine	41	53
CE	69	24
BZE	65	16
Cocaine	69	27
EME	88	0
AEME	114	2

Table 2

Figure 1: Final chromatogram
Table 1: Substances analyzed, internal standards and MRM parameters
Table 2: Extraction efficiency for SPE and LLE

Conclusion

The initial results showed very promising performance. However, the optimization of the photografting needs more experiments in order to enable the incorporation of a broad range of surface chemistry at specific defined locations on the monolith. Indeed, the obtained retention factors are in line with those of a classic thermal grafting.



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