Analysis of amphetamines in urine by CE-ESI-MS

Application Note

Drug Testing

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Abstract

Amphetamines and derivatives are powerful stimulants of the central nervous system, often misused by recreational users. Chronic abuse of amphetamines can lead to hallucinations and psychosis; dysphoria and depression might occur upon withdrawal. Immunoassays have been widely applied to analyze amphetamines and related drugs in biological matrices. However, these methods are not specific enough and have to be confirmed by a second more precise technique. A sensitive and quick method using capillary electrophoresis (CE) with UV detection is routinely applied for the analysis of amphetamines in tablets or biological matrices[1]. For a more precise identification which also reveals the nature of unknowns, mass spectrometric (MS) detection is suitable. An optimized method was developed and is presented here[2].

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Experimental

CE-ESI-MS analysis was performed using the Agilent Capillary Electrophoresis system with CE-MS capillary cassette coupled to the Agilent LC system which was equipped with electrospray source and orthogonal sprayer for CE-MS. The Agilent ChemStation software was used for instrument control. Standard amphetamine solutions (1 mg/mL) in methanol were purchased from Alltech (Deerfield, IL, USA). All other chemicals were of analytical grade or better. The urine samples were subjected to liquid-liquid extraction (aqueous borate vs. 1-chlorbutane)[2] prior to injection.

Results

A validated assay for the analysis of amphetamines by CE with UV-detection uses bare fused silica capillary and a Tris-phosphate buffer as background electrolyte[1]. This buffer is not suitable for MS detection since non-volatiles decrease the ionization efficiency during the electrospray process. The CE separation parameters therefore had to be optimized to allow high sensitivity MS detection. Formic acid was chosen as background electrolyte as it fulfills the volatility requirement. Instead of bare fused silica, a capillary coated with poly(vinylalcohol) (PVA) was used. This resulted in better resolution due to the suppressed electroosmotic flow. The MS acquisition parameters were also optimized. The sheath liquid composition (nature and concentration of organic and acidic modifier) was investigated using the peak height of a standard analyte as response factor (data not shown). Furthermore, the electrospray parameters were investigated using a 2^{6-1} fractional factorial design (table 1).

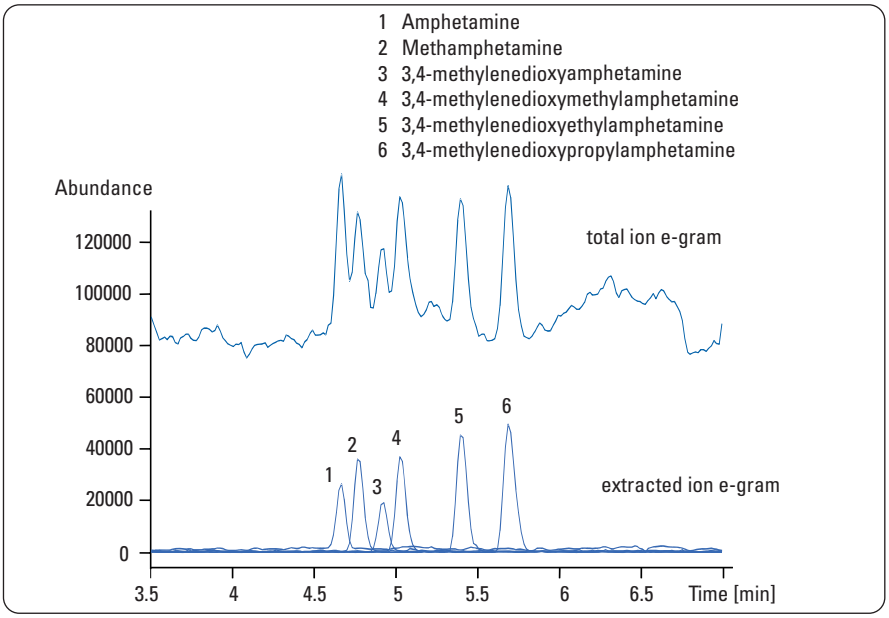

Figure 1
CE-ESI-MS of spiked urine after liquid-liquid extraction.

Chromatographic conditions

Sample:	urine spiked with amphetamines, (1 µg/mL each)
Injection:	5 sec @ 50 mbar sample
Capillary:	Poly(vinylalcohol)-coated capillary, total length 53 cm, internal diameter 50 µm
Buffer:	100 mM formic acid, pH 2.4
Voltage:	30 kV
Temperature:	15 °C
Preconditioning:	2 min flush with buffer, at 1 bar
Sheath liquid:	0.5 % formic acid in 50 % aqueous, isopropanol (4 µL/min)
Nebulizing gas:	nitrogen, 11 psi
Drying gas:	nitrogen, 6 L/min, 200 °C
Acquisition:	positive mode, Vcap -3 kV, fragmentor 50 V
Scan range:	120–400 m/z

Level	Nebulization pressure (psi)	Electrospray voltage (V)	Drying gas flow rate (L/min)	Temperature (°C)	Fragmentor voltage (V)	Sheath liquid flow rate (µL/min)
-1	6	3'000	6	200	50	4
0	11	4'000	8	250	80	6
+1	16	5'000	10	300	110	8

Table 1
Coded values of experimental factors.

Peak area and fragmentation as well as peak width were examined in order to estimate the sensitivity and the influence of the sheath liquid. The optimized method (figure 1) was then used for the analysis of urine samples, spiked with amphetamines.

References

1.
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