High Throughput Analysis of Amphetamines in Urine with On-line-Solid Phase Extraction-Liquid-Chromatography-Tandem Mass Spectrometry

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AIMS: A completely integrated on-line SPE-LC-MS/MS automated method was developed and fully validated for the direct analysis of 7 amphetamines in urine. The combination of high throughput on-line SPE with the well-known sensitivity and specificity given by the MS/MS resulted in the elimination of the bottleneck associated with the sample preparation requirements and with the higher sensitivity, accuracy and precision.

METHODS: The method involves a fully automated SPE system (Spark Symbiosis Pharma), which allows the simultaneous extraction of the second sample in one clamp and the elution of the first sample in a second clamp, as such, achieving an optimal use of the extraction time without manual transfers and pre-concentration steps. This system offers the entire process of conditioning (methanol, water and ammonium formate buffer 10 mM pH 4), sample application (to cation exchange mode cartridges), washing (ammonium formate buffer and methanol: water (50:50 (v,v))) and elution (5% ammonia in methanol), taking place at constant flow rates, yielding better precision in comparison with off-line driven extraction procedures.

Chromatographic separation was achieved using an Atlantis dC18 column, eluted with a mixture of ammonium bicarbonate buffer 10 mM pH 8 and methanol. The applied LC gradient ensured the elution of all the drugs within 16 min and produced chromatographic peaks of acceptable symmetry. Selectivity of the method was achieved by a combination of retention time, and two precursor-product ion transitions for the non-deuterated analogues.

RESULTS: The method was fully validated using only 50 µL of urine, including linearity (25 - 1000 ng/mL), intra-assay and inter-assay precision (RSD < 15%, except for MDMA which was < 19%), LOQ (25 ng/mL), LOD (ranged from 0.5 ng/mL to 2.5 ng/mL), accuracy (> 93%), matrix effects and stability studies. External quality controls (at two concentration levels) containing most of the compounds used during method validation also demonstrated the accuracy of the method.

The method was subsequently applied to authentic urine samples, previously screened with an immunoassay technique, obtained from roadside controls, suicide attempts and from forensic and toxicology cases. The measured concentrations varied considerably.

CONCLUSIONS: The validation and actual sample analysis results show that this method is rugged, precise, accurate, and well suitable for routine analysis where more than 100 samples may be non stop analyzed in 48 hours, with a minimum sample handling.

Keywords: On-line SPE-LC-MS/MS, Urine, Amphetamines