

Simultaneous determination of 6 amphetamines and analogues in hair and urine by LC-ESI-MS/MS: Application to the determination of MDMA after the absorption of a single dose of “Ecstasy”

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AIMS: We present an application of Liquid Chromatography- triple stage quadrupole-tandem Mass Spectrometry with electrospray probe (LC-ESI-MS/MS) for the determination of traces of amphetamine (A), metamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”), 3,4-methylenedioxyethamphetamine (MDEA), and N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) in biological specimens. This technique is one of the most powerful analytical methods to be used for analysis of urine and hair to solve drug facilitated crimes in forensic expertise. In fact, hair analysis is a very useful sample matrix when the victim lodges a complaint several days after the suspected intake of the drug and/or when the drug involved has a short half-life, providing only short-term information when analyzing biological fluids. Moreover, hair analysis enables to show the victim’s drug abstinence outside the period of the offence.

MATERIALS AND METHODS: To 20 mg of decontaminated and cut hair, 100 pg/mg of deuterated amphetamines and analogs (Cerilliant provided by Promochem), were added as internal standards. Hair specimens were digested with NaOH 1M for 15 min at 80°C. Extraction was performed with hexane/ethyl acetate (2/1). After centrifugation, the organic layer was filtered with PTFE 0.2 µm then evaporated to dryness at ambient temperature. Urine was extracted with Toxi-tube A® (Varian) and with 1 ng/mL of deuterated analogs. The residues were reconstituted by 200 µl of MeOH and transferred in glass vials. Ten microliters were injected into the LC-ESI-MS/MS triple quadrupole TSQ Quantum Ultra (ThermoElectron). Separation was achieved on a C₁₈-column (Uptisphere ODB 150 x 2 mm – 5 µm) at 30°C. Mobile phase (formate buffer 2mM pH 3 / ACN) was delivered in gradient mode for a total run time of 20 min. The detection was performed in positive and SRM mode and allowed the simultaneous detection of A, MA, MDA, MDMA, MDEA, and MBDB. To each pseudomolecular ion 4 product ions were acquired at a scan time of 0.1 s with a width of 1.0 a.m.u. Detection limits (LoDs) ranged from 0.1 - 0.5 ng/mL in urine, and cut off from 5 - 20 pg/mg in hair depending on the molecule.

RESULTS: We applied this method to the analysis of urine and hair of a 16-year-old girl for the determination of amphetamines and analogs after the suspected intake of “ecstasy” in a soft drink without her knowledge. Twelve hours later she had the first symptoms of an abnormal behavior. The victim went in a first laboratory where urine #1 was collected and analyzed by immunoassay (amphetamines positive). After her complaint 8 days post ingestion, urine #2 and blood were collected for forensic purposes. All biological fluids were kept frozen. Hair was collected two months after the offence. Analysis of urine #1 showed the presence of MDMA (37 µg/mL) and MDA (4 µg/mL) by LC-DAD. Urine #2, blood and hair were analyzed by LC-ESI-MS/MS: blood was tested negative for all amphetamines and analogs, but urine #2 was tested positive for MDMA at 0.42 ng/mL, and hair at 22 pg/mg (LoD ~5 pg/mg). MDA was not detected in urine #2. A detection limit for MDMA of 0.1 ng/mL in urine allowed the detection of a single dose of “ecstasy” up to 8 days.

CONCLUSIONS: These results demonstrate the power of LC-MS/MS as well as hair analysis for the elucidation of drug facilitated offences or crimes. To our knowledge, it is the first description of the determination of MDMA in hair after the absorption of a single dose of “ecstasy” by a non drug user.

KEYWORDS: *MDMA, Amphetamines, Hair, LC-MS/MS*

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