Simultaneous extraction of alkaline addictive drugs from post-mortem human adipose tissues and GC/MS detection

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AIMS: The Authors present a modified method [1] for the efficient extraction of alkaline addictive drugs and their major metabolites from human adipose tissue. The procedure combines acidic purification, extraction on Extrelut column and GC/MS analysis, with deuterated internal standards.

METHODS: Adipose tissues were collected during autopsy of three decedents, all drug abusers. Homogenized, adipose tissue (10 g), are placed in 10 ml of chloroform and 1 mcg /g of appropriate deuterated internal standards are added with agitation (morphine-d3, 6-monoacetylmorphine-d3, cocaine-d3, benzoylecgonine-d3, MDMA-d5). The mixture is then alkalinized with concentrated ammonium hydroxide, extracted for 10 min and centrifuged. The aqueous layer is discarded, and the chloroform layer is filtered through paper.
The chloroform layer is extracted with 10 ml of 1N sulphuric and centrifuged. The aqueous layer is recovered, made alkaline, introduced in a Extrelut-20 column (Merck) and extracted with 15 ml of chloroform. After drying the eluate is reconstituted in methanol (50 mcl), and injected in GC/MS-EI operating in full scan mode. The remained sample is dried, silylated by BSTFA/TMCS 1% 50 mcl (30 min, 70°C) and injected in GC/MS-EI operating in full scan mode.
The same addictive drugs were determined in blood samples of the three decedents with routine Forensic toxicology laboratory methods.
To evaluate recovery and accuracy of extraction, to 10 g adipose tissue drug free homogenized was spiked 1 mcg/g of a mix of drugs (heroine, 6-monoacetylmorphine, morphine, cocaine, benzoylecgonine, MDMA, ethaverine, codeine). Vortexed for 15 seconds and extracted with the described method. Eluate is reconstituted in methanol, spiked with 1 mcg /g deuterated standard (morphine-d3, 6-monoacetylmorphine-d3, cocaine-d3, benzoylecgonine-d3, MDMA-d5), silylated and injected in GC/MS-EI operating in full scan mode.
This extraction method was useful for both parent drugs and their metabolites. In analyzed adipose tissues cocaine and MDMA were identified and quantified in unsilylated samples; while benzoylecgonine, morphine, 6-MAM were determined in BSTFA silylated specimens.

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RESULTS: In real forensic samples, addictive drugs (cocaine, 6-monoacetylmorphine, morphine and MDMA) were quantified; adulterants (lidocaine) and other drugs (nicotine, caffeine) were identified too.
Case 1- Blood: cocaine 60 ng/ml; benzoylecgonine 450 ng/ml; morphine 360 ng/ml, also caffeine and lidocaine. Adipose tissue: cocaine 110 ng/g; benzoylecgonine 65 ng/g; morphine 121 ng/g also lidocaine, nicotine and caffeine.
Case 2- Blood: benzoylecgonine 1040 ng/ml; 6-monoacetylmorphine 50 ng/ml; morphine 1120 ng/ml. Adipose tissue: cocaine 37 ng/g; benzoylecgonine 187 ng/g; 6-monoacetylmorphine 15 ng/ml; morphine 260 ng/g.
Case 3- Blood: benzoylecgonine 910 ng/ml; morphine 76 ng/ml.
Adipose tissue: cocaine 16 ng/ml; benzoylecgonine 65 ng/ml; morphine 12 ng/ml. The extraction allowed high cocaine and morphine recoveries > 70. The accuracy was different for each of the compounds, varying from 65% (morphine) to 84% (cocaine).

CONCLUSIONS: The developed extraction procedure of alkaline drugs and their major metabolites from human adipose tissue was evaluated for accuracy and recovery. The method is easy and fast and produces clean extracts suitable for gaschromatography/mass spectrometry determination, while the acid purification followed by solid-phase extraction achieved the best compromise between recovery and chromatographic profile.

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