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Rapid determination of methadone in postmortem whole blood and cerebellum

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AIM: Quantitative results on methadone obtained with internal standards in brain are sparsely published within the last three decades [1]. In post-mortem cases the amount of the drug at its site of action is significant for interpretation. A screening method for systematic toxicological analysis (STA) using SPE is suitable to detect methadone but problematic for quantitative results since it gives poor recoveries and is time-consuming in case of re-quantification.

Our aim was to develop a rapid method for the determination of methadone in postmortem whole blood and brain tissue, in particular in decomposed material. As a consequence such a method would allow a thorough investigation of methadone cases and the comparison of methadone concentrations in whole blood and brain tissue (cerebellum).

METHOD: The precipitation with acetonitrile according to Daldrup [2] allows the use of small amounts of sample (200mg) and a rapid sample workup. Acetonitrile, alkalized with saturated disodium hydrogen phosphate solution, is added to the sample material as well as d9-methadone as internal standard. Subsequently, the mixture is subjected to ultrasonic treatment and vortexed. The supernatant is decanted and concentrated in vacuo.
The aqueous solution is adjusted to pH 9 and transferred to an Extrelut NT1 column. The basic fraction is eluted with chloroform. Chloroform is evaporated, the residue reconstituted with ethylacetate and subjected to GC-MS analysis.
However, for the analysis of brain samples it is necessary to remove fatty acids and products of putrefaction since these compounds deteriorate the GC-MS analysis considerably. Hence, the aqueous solution is acidified and extracted with ethylacetate.

RESULTS: The above described method was used to investigate 11 cases where methadone was found either as the only drug or in combination with other drugs.
Each sample was extracted and analyzed twice in SIM mode, relative standard deviations (RSD) for both, blood and brain, were between 2.6 and 14.0 %.
In order to investigate sample homogeneity for brain specimen, six samples from various parts of the cerebellum were taken and analyzed. No significant difference was found within the cerebellum, RSD for all samples was 8.4%. Calibration curves for both, blood and brain were established with perfect linearity within the working range ($R^2 = 0.99$).
The blood concentration of seven cases, where only methadone was detected range from 154ng to 1381ng/ml, in the cerebellum concentrations from 579 to 2500ng/g were found. In four cases where other drugs (morphine, dihydrocodein, benzoylecgonine, meprobamate) were detected, concentration ranges from 139 to 265ng/ml blood and 115ng to 2357ng/g cerebellum were found. In general methadone concentrations in the cerebellum were higher than in whole blood, except in one case where methadone was present in low amounts, which could be a sign of a methadone intake some time before death.

CONCLUSION: The presented validated method allows a high sample throughput and the accurate determination of post-mortem, in particular decayed brain and blood material. 11 cases were investigated showing that concentrations of methadone in brain are higher than in blood specimen.

REFERENCES:

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