Rapid determination of methadone in post-mortem 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 post-mortem 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).

METHODS AND MATERIAL

Reference Material and Chemicals

Methadone and deuterated methadone-d9 (0.1mg/mL methanol), were obtained from Cerilliant (Round Rock, TX, USA) supplied by Promochem, Wesel, Germany.
Ethyl acetate p.a., methanol p.a., acetonitrile p.a., ascorbic acid, dichloromethane, and Extrelut NT (1mL) were obtained from Merck, Darmstadt, Germany, acetic anhydride from Fluka, Buchs, Switzerland.

Precipitation with acetonitrile

The precipitation with acetonitrile according to Stimpfl *et al.* [2] allows the use of small amounts of sample (200mg) and a rapid sample workup. Acetonitrile, alkalized with saturated disodium hydrogen phosphate solution (2mL, 10:1; v/v) is added to the sample material as well as d9-methadone as internal standard. Subsequently, the mixture is subjected to ultrasonic treatment, vortexed for 3 minutes and centrifuged for 5 minutes at 3000 rpm. 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 dichloromethane, which subsequently is evaporated, the residue reconstituted with methanol and subjected to GC-MS analysis. Where required, the sample is acetylated with acetic anhydride to remove interfering matrix components to enhance mass spectra interpretation.
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, prior to the basic extraction the aqueous solution is acidified and extracted once with 2mL ethyl acetate.

Instrumentation

Quantitative analyses of the basic extracts were performed on a Hewlett Packard 6890 gas chromatograph equipped with a HP 5973 mass selective detector, operated in the electron impact mode at 70eV, and a HP 6890 series injector. A MDN5S column (15 m x 250 µm, 0.25 µm film thickness, Supelco, Bellefonte, PA, USA) was installed and helium used as carrier gas at a constant flow of 1mL/min (average velocity 53 cm/sec). The following temperature program was used with a total running time of 18 min: the initial temperature was set to 100°C for 2 min, increased to 200°C at a rate of 20°C/min, then increase to 300°C at a rate of 25°C/min and held for 7 min. Splitless injections were performed with the injection inlet temperature set to 280°C. The GC/MS transfer line was heated to 300°C, ion source temperature was set to 230°C.
For SIM analyses the ions monitored were *m/z* 72, 223 and 294 for methadone and *m/z* 78, 226 and 303, d9-methadone, respectively.

RESULTS AND DISCUSSION

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.
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%.
A calibration curve for methadone in brain and whole blood matrix were found to be linear over the concentration range 125 to 2625ng/g (R^2 = 0.99) for brain and 150 to 1650ng/mL (R^2 = 0.99) for whole blood, respectively. Each calibration was established by adding methadone to a brain and blood sample (decayed material), which were negative for methadone and were added in the working range of 200 mg ± 50 mg.
The limit of detection (LOD) and limit of quantification (LOQ) for methadone in brain and blood were determined at a signal-to-noise ratio of 3:1 for the ion m/z 72 analyzed in SIM mode. The LOD for methadone in brain is 35ng/g, the LOQ is 105ng/g; the LOD for methadone in blood is 25ng/mL, the LOQ is 75ng/mL.
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 %. The blood concentration of seven cases, where only methadone was detected range from 154ng to 1381ng/mL, in the cerebellum concentrations from 579 to 2501ng/g were found (see Table 1). 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 (Table 1). 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.

Table 1: Comparison of methadone concentration in cerebellum and blood (* quantitative results obtained by deuterated internal standards)

case	sex	age	ng/mL blood	ng/g cerebellum	other drugs *
1	F	31	154	579	-
5	M	20	521	1246	-
6	F	17	431	1609	-
8	M	49	724	1824	-
9	M	40	1381	2501	-
11	M	16	371	877	-
2	M	38	265	435	morphine: 1.7µg/mL in blood, 0.2µg/g in cerebellum
3	F	43	n.a.	543	morphine: 0,13µg/g in cerebellum
4	M	43	n.a.	2357	morphine: 0.4µg/g in cerebellum; dihydrocodeine: 5µg/g in cerebellum; meprobamate
7	M	32	139	115	morphine: 0.5µg/mL in blood, 1.1µg/g in cerebellum; benzoylecgonine: 0.15µg/mL in blood, 0.15µg/g in cerebellum
10	M	22	447	918	benzoylecgonine, traces

CONCLUSION

The presented validated method allows a high sample throughput and the accurate determination of postmortem, in particular decayed brain and blood material. 11 cases were investigated showing that concentrations of methadone in brain are higher than in blood specimen. It is planned to investigate methadone positive cases thoroughly in future in order to make interpretation easier and more significant.

References

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