An LC/MS-APCI method for screening and quantification of four fentanyl analogues, atropine and scopolamine in biosamples

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INTRODUCTION: Fentanyl (FL) and its analogues, atropine (AT) and scopolamnine (SC) are used for medical (anaesthetic and analgesic) often simultaneously and non-medical (as street drug, substitute of drug and chemical warfare agents) purposes. They act on human at very low doses, which lead to low concentrations in organism. These substances are difficult to detect by means of routinely used screening procedures.

AIM: A sensitive screening and quantification method was developed for FL, alfentanil (AF), sufentanil (SF), remifentanil (RF), AT and SC in biosamples.

METHODS: The method was based on LLE followed by LC-APCI-MS. The drugs were extracted with diethyl ether at pH 11 from blood and at pH 10 from urine. The compounds were separated on a Superspher 60 RP-select B column (125 × 4 mm) using gradient elution (0.1% (v/v) formic acid in acetonitrile and water). Internal standardisation was carried out using papaverine (PA) as internal standard and the target ions were monitored in the SIM mode. Quantitative (pseudomolecular, underlined) and qualitative ions were chosen using abundance and lack of baseline interference as the selection criteria. The following ions (m/z) was chosen: FL – 337 and 188, AF – 417 and 197, SF – 387 and 238, RF – 377 and 228, AT – 290 and 188, SC - 304 and 156), and PA – 340.

RESULTS: The LC-APCI-MS assay was found to be selective for FL, AF, SF, RF, AT and SC in blood and urine. No interfering peaks were observed in the extracts of seven different drug-free blood and urine samples. Interference with drugs typically taken in combination was tested and could be excluded due to different retention time and/or mass spectra. Using 1 mL of blood and urine samples, the methods had the validation parameters [ng/mL]:
LODs and LOQs ranged from 0.2 to 0.6 and from 0.6 to 2, respectively for six compounds. The LOQs corresponded to the lowest calibrator concentrations with a S/N≥10. Seven and fife-point calibration curves showed LOLs of 0.5-25 ng/mL for blood and 2.5-50 ng/mL for urine.
Linear regression correlation coefficients of the calibration curves were ≥ 0.97 for all compounds. Extraction recovery of the analytes from blood ranged from 65 to 100 % at 5 ng/mL and from urine 55-75 % at 20 ng/mL.
Within-day and between-day precision (expressed as RSD) was ≤13 and 20% for AT and SC, respectively, and ≤10% for four fentanyl analogues determined at 5 ng/mL for all analytes. Accuracy for the 5 ng/mL (n=4) in-house quality controls was between 88 and 96% of target amount. In reconstituted extracts the analytes were stable for a period of more than 24 hours at room temperature or for 3 days at –20oC. The procedure was applied for determination of FL with AT in 3 forensic cases and AT with SC in 7 hospital cases.

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The blood was taken during autopsy of persons who died shortly after surgical procedure in which FL and/or AT was used as an adjunct to general anaesthesia. The urine samples were taken from patients during hospitalisation three days after Datura stramonium poisoning in recreational use. The blood concentrations [ng/mL] were: FL - 1.7, 12.7 and 1,0, and AT - 19 and 68 and 13. AT (at concentrations of 28.5, 9, 2.3 and 54 ng/mL) and SC (of 1.5 ng/mL) were determined in urine in four and one out of seven patients.

CONCLUSIONS: Because increasing number of reports of illicit use of fentanyl class compounds and Datura stramonium have been observed, a sensitive and reliable LC-APCI-MS method was developed that was suitable for simultaneous detection and quantification of six compounds in biofluids for clinical and forensic toxicology purposes.

KEYWORDS: *LC/MS-APCI, Screening method, Fentanyl and its analogues, Atropine and scopolamine*

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