P-153. Highly sensitive UFLC-MS/MS analysis of fentanyl and norfentanyl in different post-mortem matrices: seven forensic case studies

Ruth Verplaetse, Eva Cuypers, Coralie Silvestre, Dieter Waumans, Jan Tytgat
Laboratory of Toxicology, University of Leuven, Leuven (Belgium)

Introduction. Fentanyl a synthetic narcotic analgesic with high potency and its major metabolite norfentanyl often occur in low concentrations in biological samples. **Aims.** A highly sensitive UFLC-MS/MS method was used for the analysis of seven post-mortem case studies including different matrices: whole blood, urine, bile, liver and hair. The necessity of matrix-matched calibration curves and dilution of samples containing high concentrations of analytes was also evaluated. **Methods.** Washed, pulverized hair was extracted with methanol. Blood, urine, bile, liver and extracted hair samples were applied on mixed-mode cation exchange Bond Elut Plexa PCX SPE cartridges followed by UFLC-MS/MS analysis (Shimadzu Prominence UFLC coupled to 3200 QTRAP, Applied Biosystems) with H_2O + 10 mM ammonium bicarbonate at pH 9.0 and methanol as mobile phase on a Acquity C18 column (1.7 µm particle size, 2.1 mm x 50 mm). The method was fully validated for blood and urine resulting in limits of detection of 5 pg/ml fentanyl and norfentanyl in whole blood and 0.25 pg/ml fentanyl and 2.5 pg/ml norfentanyl in urine. **Results.** Based on the behavior of the internal standards, the limits of detection of this method for other matrices were estimated: 5 pg/ml fentanyl and norfentanyl in bile, 0.5 pg/g fentanyl and 5 pg/g norfentanyl in liver and 5 pg/g fentanyl and 50 pg/g norfentanyl in hair. Because of the use of deuterated internal standards with identical behavior as the analytes, matrix-matched calibration curves were not necessary. Dilution of samples containing levels of fentanyl and norfentanyl outside the calibration range was needed because at these concentrations the analytes can suppress the ionization of the internal standards. Concentrations of fentanyl and norfentanyl had varying ranges in the different matrices: 1.5-24.2 ng/ml fentanyl and 0.0075-3.0 ng/ml norfentanyl in blood, 5.8 - 224.8 ng/ml fentanyl and 9.8-613.5 ng/ml norfentanyl in urine, 25.3-49.4 ng/ml fentanyl and 17.6-21.8 ng/ml norfentanyl in bile, 8.9-10.2 ng/g fentanyl and 4.1 - 17.6 ng/g norfentanyl in liver and 13.9-64.6 ng/g fentanyl and 0.74-11.1 ng/g norfentanyl in hair. **Conclusions.** Considering all the results from the different matrices, the death of the victim was (partly) caused by the use of fentanyl in four cases.

P-154. Quantification in post-mortem blood, and identification in urine, of tramadol and its two main metabolites in two cases of lethal tramadol intoxication

Benjamin De Backer, Françoise Renardy, Raphaël Denooz, Corinne Charlier
Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology, CIRM, CHU Sart-Tilman and Forensic Institute, University of Liège, Liège (Belgium)

Introduction. Tramadol is an opioid analgesic inducing fewer side effects than other compounds of this class and has been extensively prescribed for the treatment of moderate to severe pain. Although few fatal overdoses due to tramadol alone are reported, it is well demonstrated that this analgesic can cause fatal complications when it is ingested solely. We report here two cases of tramadol-related fatalities which involved a 17-year-old man and a 75-year-old female which probably both commit suicide. None the decedents were prescribed tramadol but a blood screening revealed the presence of the drug in both cases. **Aims.** Tramadol and its two main metabolites, O-desmethyltramadol (ODT) and N-desmethyltramadol (NDT), were quantitatively and qualitatively determined in post-mortem peripheral blood and urine, respectively. **Methods.** An HPLC method coupled with fluorescence detection was developed for the analysis of tramadol, ODT and NDT in whole blood. The method was validated following an approach using accuracy profiles based on β-expectation tolerance intervals for the total error measurement, and assessing the measurements uncertainty. The method was then adapted for the identification of the compounds in urine. **Results.** Tramadol and NDT were validated for concentration between 10 and 600 µg/L, ODT was between 5 and 300 µg/L. The relative standard deviations (precision) were lower than 11.6% and the relative biases (trueness) were smaller than 12.5%. The relative expanded uncertainty was lower than 25.7%. The following concentrations were found in peripheral blood of case 1: 7.7 mg/L in tramadol, 1.3 mg/L in ODT and 0.6 mg/L in NDT. In case 2, tramadol, ODT and NDT concentrations reached 48.3 mg/L, 2.4 mg/L and 10.1 mg/L, respectively. Norfluoxetine was detected in sub-therapeutic levels in case 2 and cannot have directly contributed to death. **Conclusions.** Tramadol concentration found in case 2 is one of the highest described in the literature. The differences in ODT:NDT ratios between the cases may be explained by pharmacokinetic interactions and quantitative differences in the activity of the cytochrome-P450 2D6, which converts tramadol to ODT.

P-155. Concentrations of morphine, codeine and 6-monoacetyl morphine in femoral blood in heroin-related deaths compared with apprehended drivers

Anita Holmgren, Johan Ahlner, Alan Wayne Jones
Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping (Sweden)

Introduction. Heroin (diamorphine) is the most dangerous recreational drug in terms of acute toxicity, intensity of pleasure and the social harm it causes. Heroin is rapidly metabolised to 6-monoacetyl morphine (6-MAM) and then to morphine. The presence of codeine in blood of heroin-users arises from the metabolism of 6-acetyl codeine, which is an impurity in illicit heroin. **Methods.** We used an in-house database (TOXBASE) to locate heroin-related deaths using 6-MAM as a biomarker. The concentrations of 6-MAM, morphine and codeine in femoral blood were determined by GC-MS in autopsy cases and compared with the concentrations in venous blood from impaired drivers. **Results.** Of 766 heroin-related deaths 88% were men and 12% were women although their mean age was about the same (35 y). In traffic cases 91% of offenders were men (mean age 33 y) and 9% were women (mean age 35 y). The concentrations of 6-MAM, free-morphine and free-codeine in blood samples from the living and the dead are compared and contrasted in the table.

Opiate	Type of case (N)	Mean blood conc. mg/L	Median blood conc. mg/L	90th, 95th and 97.5th percentiles, mg/L
MAM	Autopsy (766) Traffic (125)	0.018 0.021	0.01 0.008	0.03, 0.04, 0.06 0.03, 0.09, 0.11
COD	Autopsy (747) Traffic (947)	0.04 0.01	0.02 0.01	0.07, 0.10, 0.21 0.02, 0.03. 0.04
MOR	Autopsy (766) Traffic (1950)	0.34 0.05	0.24 0.03	0.65, 0.86, 1.09 0.12, 0.16, 0.22