Determination of Fentanyl in Post Mortem Blood by LC/MS/MS

Analytical Unit, St George’s - University of London, UK

Malgorzata Puchnarewicz, Karoliina Laamanen, Terry D Lee, Jennifer Button and David W Holt.

Introduction

Fentanyl is a synthetic opioid analgesic with a potency 80 times higher than morphine. The drug acts in the central nervous system (CNS) by agonising mu (µ) receptors, which are highly concentrated in the areas of the brain involved in nociception.

Chemicals and Reagents

Fentanyl citrate (purity >99%) and Fentanyl-d5 (0.1mg/mL in methanol) were obtained from Sigma and LGC Promochem respectively. HPLC grade methanol and methyl-tert-butyl-ether (MTBE) were purchased from Rathburns Chemicals Limited (Walkerburn, Scotland). Sodium hydroxide (40% solution) was obtained from BDH (Poole, Dorset, England). Deionised water was prepared on site (ELGA Limited).

Method

The HPLC system consisted of Perkin Elmer PE200 series autosampler, pump and column oven. Chromatography was achieved using a silica column (Supercosil LC-Si, 10cm x 4.6mm, 5µm) maintained at 50°C. The mobile phase consisting of acetonitrile/de-ionised water/formic acid (50/50/0.2, v/v/v) was pumped at 1mL/min. The output from the column was split 10:1 before entering the mass spectrometer. The volume of injection was 10µL.

Detection was by tandem mass spectrometry (LC/MS/MS), using a Sciex API2000 triple quadrupole equipped with a turbo-ion spray interface. The method was run in positive ionisation mode and was monitoring precursor and product ions of fentanyl (m/z: 337.1/105.1) and fentanyl-d5 (m/z: 342.1/105.1).

Extraction

100µL of whole blood calibrator/sample, 25µL of 1mg/mL fentanyl-d5 (internal standard), 100µL of 1 molar sodium hydroxide and 1mL of MTBE were added to 2mL tubes and mixed for 15 minutes. Following centrifugation the organic phase was transferred to a 4.5mL polypropylene tube and evaporated to dryness. The residue was reconstituted in 250µL of 80% methanol and injected onto the LC/MS/MS system.

Conclusion

The use of fentanyl is steadily increasing as the biological effects of fentanyl are identical to heroin; with the exception that fentanyl is more potent. The drug is very “attractive” within the narcotic market and with its analogues is sold under the names; synthetic heroin or “China white”. The drug is also occasionally abused by medical personnel who have easy access to the drug.

The increased use of fentanyl requires an easy and reproducible method for quantification. Fentanyl was measured using LC/MS/MS, which offers rapid and sensitive analysis with simple mobile phase composition and small sample volume. The assay can be used to measure the more potent analogues: alfentanil, sufentanil, lofentanil, carfentanil and remifentanil.

References:
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