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Short communication

Determination of fentanyl in human plasma and fentanyl and norfentanyl in human urine using LC–MS/MS

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Abstract

Fentanyl, a potent analgesic drug, has traditionally been used intravenously in surgical or diagnostic operations. Formulations with fentanyl in oral transmucosal delivery system and in transdermal depot-patch have also been developed against breakthrough pain in cancer patients. In this report, LC–MS/MS methods to determine fentanyl in human plasma as well as fentanyl and its main metabolite, norfentanyl, in human urine are presented together with validation data. The validation ranges were 0.020–10.0 and 0.100–50.0 ng/ml for fentanyl in plasma and urine, respectively, and 0.102–153 ng/ml for norfentanyl in urine.

Liquid–liquid extraction of the compounds fentanyl, norfentanyl and the deuterated internal standards, fentanyl-d_5 and norfentanyl-d_5 from the matrixes was applied and separation was performed on a reversed phase YMC Pro C_{18}-column followed by MS/MS detection with electrospray in positive mode. The inter-assay precision (CV%) was better than 4.8% for fentanyl in plasma and 6.2% and 4.7% for fentanyl and norfentanyl, respectively, in urine.

The ruggedness of the methods, selectivity, recovery, effect of dilution and long-term stability of the analytes in plasma and urine were investigated. Effect of haemolysis and stability of fentanyl in blood samples were also studied.

The methods have been applied for the determination of fentanyl in plasma samples and fentanyl/norfentanyl in urine samples taken for pharmacokinetic evaluation after a single intra-venous (i.v.) dose of 75 μg fentanyl.

Keywords: Fentanyl; Norfentanyl; LC–MS/MS; Plasma; Urine; Validation

1. Introduction

Fentanyl is a potent, short-acting narcotic analgesic used as a surgical anaesthetic and for the treatment of pain in tumour patients. Therapeutic levels of fentanyl are as low as 1 ng/ml in plasma and methods with high sensitivity are required for the determination of fentanyl in biological fluids for pharmacokinetic studies. Fentanyl and/or its main metabolite, norfentanyl (Fig. 1) have been determined using GC [1], HPLC/UV [2–5], GC/MS [6–8], LC–MS/MS [9–12] and immunoassays [13,14]. HPLC and immunoassays did not offer the high sensitivity required for low dose studies of fentanyl, GC/MS give good sensitivity but requires long run times. LC–MS/MS offers often rapid and sensitive analysis with simple mobile phase compositions. Fentanyl in human plasma samples has earlier been determined by LC–MS/MS after sample preparation with mixed mode SPE [9,10] with a lower limit of quantification (LLOQ) of 0.05 ng/ml. An LC–MS/MS method for fentanyl and norfentanyl in primate plasma [11], with LLOQ at 0.025 and 0.05 ng/ml, respectively, has also recently been reported using repeated liquid–liquid extraction (LLE) for sample purification. Similarly, fentanyl and norfentanyl in human plasma [12] have been determined at a LLOQ of 0.05 ng/ml using LLE followed by LC–MS/MS. In this report, a rapid and robust LC–MS/MS method with atmospheric pressure ionization (API) technique, e.g. electrospray ionization in positive mode is reported. Sample preparation was performed with a one step LLE and the method, originally developed to analyse fentanyl in plasma samples, has been used with minor modifications to analyse fentanyl and norfentanyl in

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urine. The method has been validated to determine fentanyl in plasma in the range 0.020–10.0 ng/ml and to determine fentanyl and norfentanyl in urine in the range 0.100–50.0 and 0.102–153 ng/ml, respectively.

2. Experimental

2.1. Chemical and reagents

Fentanyl citrate (purity >99%) was from Sigma (St. Louis, MO, USA). Norfentanyl oxalate solution (1.0 mg/ml in methanol), fentanyl-d_5 (100 μg/ml in methanol), and norfentanyl-d_5 solution (100 μg/ml in acetonitrile), Fig. 1, were from Cerilliant (Pound Rock, TX, USA). Drug free urine and sodium and lithium heparin plasma were obtained within Quintiles AB (Uppsala, Sweden). Drug free citrate buffered plasma was from Uppsala University Hospital (Uppsala, Sweden). Acetonitrile, ethylacetate, *n*-heptane, 2-butanol, sodium hydroxide and formic acid were purchased from Merck (Damstadt, Germany), all were of either LC grade or analytical purity and used as received. Water was purified by passage through two Milli-Q purification systems from Millipore (Bedford, MA, USA).

2.2. LC–MS/MS method

The liquid chromatographic system consisted of two Shimadzu LC-10AD *VP* pumps (Tokyo, Japan), a Gilson 231 sampling injector equipped with a Gilson 832 temperature regulator (CEDEX, France) kept at 12 °C and a column oven, model 7990, from Jones Chromatography (Mid Glamorgan, UK) or a Hot Pocket from Keystone Scientific, Inc. (Bellefonte, PA, USA).

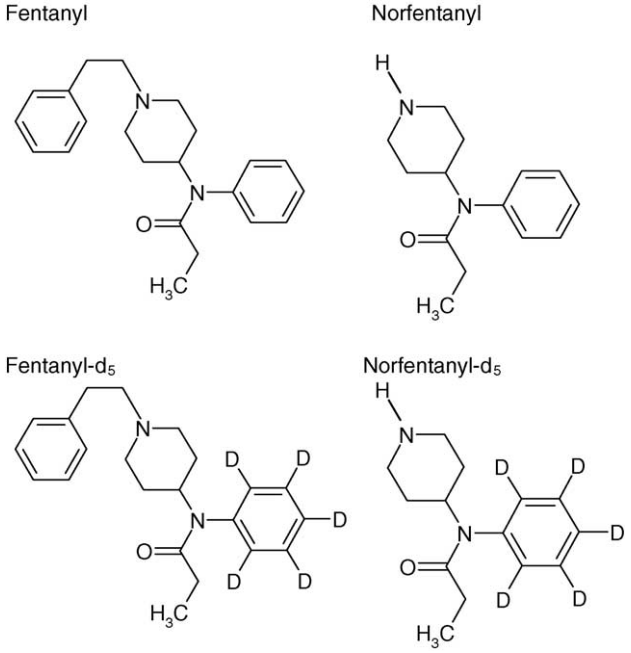

Fig. 1. Fentanyl, fentanyl-d_5, norfentanyl and norfentanyl-d_5.

Gradient elution was used for the separation of fentanyl and norfentanyl in urine samples with a linear gradient from 2 to 30% acetonitrile in 5 mM formic acid for 4 min and maintained at 30% for 1 min. For the determination of fentanyl in plasma, isocratic elution using 18% acetonitrile in 5 mM formic acid aqueous solution was applied. The column used in both methods was a YMC Pro C_{18}-column 50 mm × 2 mm i.d. (Weselerwald, Germany) protected by either an OptiGuard C_{18}, 1 mm pre-column from Optimize Technologies (Oregon City, OR, USA) or a SecurityGuard C_{18}, 4 mm × 2 mm, from Phenomenex® (Torrance, CA, USA).

A triple quadrupole mass spectrometer, Quattro II, with z-spray interface from Micromass (Manchester, UK) was operated in positive-ion mode. The capillary voltage was maintained at 3.5 kV. The cone voltage was 34 and 25 V for fentanyl and norfentanyl, respectively. The source and desolvation temperature was 80 and 250 °C, respectively. The cone gas flow was kept at approximately 80–90 l/h and the desolvation gas flow at 450–500 l/h. The collision gas pressure was (1.0–1.5) × 10^{-3} mbar and the collision energies (in-house frame of reference) were 23 and 18 V for fentanyl and norfentanyl, respectively. Multiple reaction monitoring (MRM), with one channel for plasma analysis and two channels for urine analysis was applied. The transitions were *m/z* 337 → 188 for fentanyl, 342 → 188 for fentanyl-d_5, 232 → 84 for norfentanyl and 237 → 84 for norfentanyl-d_5 with the dwell time 0.3 s for each pair. The software MassLynx, Ver. 3.4 (Micromass, Manchester, UK) was used for data acquisition and processing. A weighted (1/*x*) linear least squares regression was used to establish the calibration curve from the calibration samples and the concentration of the quality control samples was calculated using the calibration curve generated in each analytical run.

2.3. Sample preparation

2.3.1. Preparation of standard and quality control samples

2.3.1.1. Urine. Calibration and quality control (QC) samples were made from two separate stock solutions of fentanyl (5.00 μg/ml in methanol:5 mM formic acid aqueous solution, 5:95, v/v) and norfentanyl (10.0 μg/ml in methanol:5 mM formic acid aqueous solution, 1:99, v/v). Calibration samples 0.1–50 and 0.1–153 ng/ml for fentanyl and norfentanyl, respectively, were prepared in blank urine. QC samples at levels of 0.250, 5.00 and 40.0 ng/ml for fentanyl and 0.255, 20.4 and 122 ng/ml for norfentanyl were prepared as well as the samples for determination of lower and upper limits quantification (0.100 and 50.0 ng/ml for fentanyl and 0.100 and 153 ng/ml for norfentanyl, respectively). All spiked urine samples were aliquoted into polypropylene vials and stored frozen at −20 °C.