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TECHNICAL NOTE

Sensitive determination of pethidine and its metabolite in human hair by liquid chromatography-tandem mass spectrometry

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Hair;
LC-MS/MS

Summary A method for the quantitative determination of pethidine (meperidine) and it metabolite norpethidine (normeperidine) in human hair by LC-MS-MS is presented. After acid incubation of 50 mg hair powder with pethidine-d4 and norpethidine-d4 used as internal standard, a liquid-liquid extraction to pH 9.5 was achieved. Hair extracts were separated on an X-Terra MS C18 column using a gradient of acetonitrile and formate buffer pH 9.5 and were detected by tandem mass spectrometry in the positive electrospray ionisation mode. The ions transitions monitored in multiple reaction monitoring were m/z 248 > 174; m/z 252 > 178; m/z 234 > 160; m/z 238 > 164 for pethidine, pethidine-d4, norpethidine and norpethidine-d4, respectively. The recoveries were above 70% for both compounds. Linear responses were observed for pethidine concentrations ranging from 1 to 5000 pg/mg ($r^2 > 0.999$) and for norpethidine concentrations ranging from 2 to 5000 pg/mg ($r^2 > 0.999$), respectively. The quantification limits were estimated to be 2 pg/mg for pethidine and 5 pg/mg for norpethidine. The intraday and interday precision (measured by coefficient of variation) was less than 12%. The accuracy was observed ranging from 88% to 113% for both compounds. In the hair of an addict, concentrations were 0.85 to 2.32 and 0.29 to 3.25 ng/mg for pethidine and norpethidine, respectively, in 4 × 3 cm segments.

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Introduction

Pethidine (meperidine) is a narcotic analgesic drug, which is used in the management of moderate to several pain. It is a phenyl-piperidinic synthethic drug. The pharmacological effects of pethidine are similar to those of morphine.

After administration, pethidine is mostly metabolized in liver to norpethidine (Fig. 1).

Even if the therapeutic use of pethidine is decreasing due to the toxicity of one of its metabolites, its analysis can be of interest to detect drug addict or doping practice.

Several chromatographic methods were developed for the determination of pethidine in blood and urine. These assays were based on gas chromatography with various non-specific detectors [1—4]; gas chromatography/mass spectrometry [2,5]; gas chromatography/surface ionization organic mass spectrometry [6]; gas chromatography/tandem mass spectrometry [7]; capillary electrophoresis [8] and liquid chromatography secondary ion/tandem mass spectrometry [9].

The detection of pethidine in body fluids documents recent exposure. To obtain long-term information concerning drug consumption, hair analysis is the alternative matrix. The advantage of hair is the larger detection window, from weeks to months against a few days for urine. At this time, very few specific methods have been published for the determination of pethidine in hair [10,11].

The aim of our work was to develop and validate a sensitive liquid chromatography/tandem mass spectrometry method for the quantification of pethidine and its metabolite in human hair.

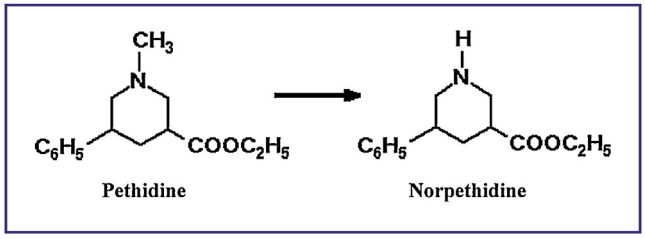

Figure 1. Chemical structures of pethidine and norpethidine.

Experimental

Standards and reagents

Pethidine, norpethidine, pethidine-d4 and norpethidine-d4 were purchased from Promochem (Molsheim, France).

Acetonitrile HPLC grade, hydrochloric acid, sodium hydroxyde and ammonium chloride were from Merck (Darmstadt, Germany). Methanol HPLC grade, methylene chloride, isopropanol, n-heptane, ammonium hydroxyde (28%) were obtained from Prolabo (Paris, France). Ammonium formate was purchased from Fluka (Saint-Quentin Fallavier, France).

Extraction

After decontamination of the hair strand with methylene chloride (2 × 5 ml for 2 min), hair was pulverized in a ball mill. Then, 50 mg hair powder were overnight incubated at 56 °C in 1 ml 0.1 M HCl in the presence of 25 ng of pethidine-d4 and norpethidine-d4, used as internal standards.

After neutralization with 1 ml of 0.1 M NaOH, the homogenate was extracted with 2 ml of saturated ammonium buffer 28% (NH_4Cl adjusted to pH 9.5 with ammonium hydroxide) and 10 ml methylene chloride/isopropanol/n-heptane (25/10/65, v/v).

After agitation and centrifugation, the organic phase was purified by an additional acid extraction (5 ml of 0.2 M HCl) and the aqueous layer was re-extracted with 2 ml of ammonium buffer pH 9.5, 1 ml of 1 M NaOH and 5 ml of methylene chloride/methanol (9/1, v/v). After agitation and centrifugation, the organic phase was removed and evaporated to dryness at 45 °C in a Speed Vac Concentrator. The dry extract was dissolved in 50 μl of methanol and transferred to microvials for analysis.

Instrumental procedure

The chromatographic separation was carried out with an HPLC Alliance 2695 (Waters) on a X-Terra MS C18 (3.5 μm, 100 × 2.1 mm id) column heated at 30 °C.

The mobile phase was composed of formate buffer (2 mM, pH 9.5) and acetonitrile. A gradient was run at 0.2 ml/min, consisting of 20% acetonitrile (t = 0 min), increased to 60% acetonitrile at t = 10 min, and then hold at 60% acetonitrile

Table 1 MRM transitions for the detection of pethidine, norpethidine and internal standards by LC-MS/MS.

Compound	Retention time (min)	Parent ion (m/z)	Daughter ions (m/z)	Cone (V)	Collision (eV)
Pethidine	12.1	248.3	<u>174.2</u> 220.3	43	3434
Pethidine-d4	12.1	252.4	<u>178.3</u> 224.3	43	3434
Norpethidine	9.2	234.3	<u>160.2</u> 55.9	33	2525
Norpethidine-d4	9.2	238.3	<u>164.2</u> 57.9	33	2525

The transition for quantification is underlined.