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Quantification of levornidazole and its metabolites in human plasma and urine by ultra-performance liquid chromatography–mass spectrometry

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ABSTRACT

We developed and validated an ultra-performance liquid chromatographic (UPLC) method coupled with atmospheric pressure chemical ionization (APCI) mass spectrometry for simultaneous determination of levornidazole and its first-pass metabolites, l-chloro-3-(2-hydroxymethyl-5-nitro-l-imidazolyl)-2-propanol (Ml), 2-methyl-5-nitroimidazole (M2) and 3-(2-methyl-5-nitro-1-imidazolyl)-1,2-propanediol (M4), in human plasma and urine. The biological samples were pretreated by protein precipitation and liquid–liquid extraction and analyzed using an ACQUITY UPLC CSH C_{18} column (2.1 × 50 mm, 1.7 μm) and a QTRAP mass spectrometer in multiple reaction monitoring mode via APCI. Acetonitrile and 0.1% formic acid in water was used as the mobile phase in gradient elution at a flow rate of 0.6 mL/min. The lower limit of quantification of this method was 0.0100, 0.00500, 0.0200 and 0.00250 μg/mL for levornidazole, M1, M2 and M4, respectively. The linear calibration curves were obtained for levornidazole, M1, M2, and M4 over the range of 0.0100–5.00, 0.00500–2.50, 0.0200–10.0 and 0.00250–1.25 μg/mL, respectively. The intra- and inter-batch precision was less than 12.2% in plasma and less than 10.8% in urine. The intra- and inter-batch accuracy was 87.8–105.7% in plasma and 92.8–109.2% in urine. The mean recovery of levornidazole, M1, M2 and M4 was 91.1–105.1%, 95.8–103.8%, 87.8–96.8%, 96.8–100.6% from plasma and 96.0–100.9%, 96.9–107.9%, 95.1–102.7%, 103.7–105.9% from urine respectively. This method was validated under various conditions, including room temperature, freeze–thaw cycles, long-term storage at −40 ± 5 °C, after pretreatment in the autosampler (at 10 °C), and 10- and 100-fold dilution. This newly established analytical method was successfully applied in a pharmacokinetic study following single intravenous infusion of levornidazole in 24 healthy Chinese subjects.

1. Introduction

Ornidazole, 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole, was developed by Roche (Switzerland) in 1970s and has already been on market in China. Ornidazole is soluble in chloroform (solubility >50%), its solubility in water is 12% and pK_a is 2.3. Ornidazole has obvious antimicrobial activity on anaerobes, *Trichomonas vaginalis* and *Amoebae*. Levornidazole is the levo isomer of ornidazole, which is a new 5-nitroimidazole antimicrobial drug. Levornidazole has shown anti-anaerobic activity, which is similar to ornidazole in general, but slightly stronger than ornidazole on some strains. Levornidazole has significantly lower central neurotoxicity than ornidazole. It has been reported that ornidazole is metabolized to five phase I metabolites

Abbreviations: UPLC, ultra-performance liquid chromatography; MS, mass spectrometry; MRM, multiple reaction monitoring; APCI, atmospheric pressure chemical ionization; HPLC, high performance liquid chromatography; LLOQ, lower limit of quantification; IS, internal standard; QC, quality control; RSD, relative standard error; AUC, area under the plasma concentration–time curve; $T_{1/2}$, half-life; C_{max}, maximum plasma concentration; MR, metabolic ratio; A_e, cumulative urinary excretion.

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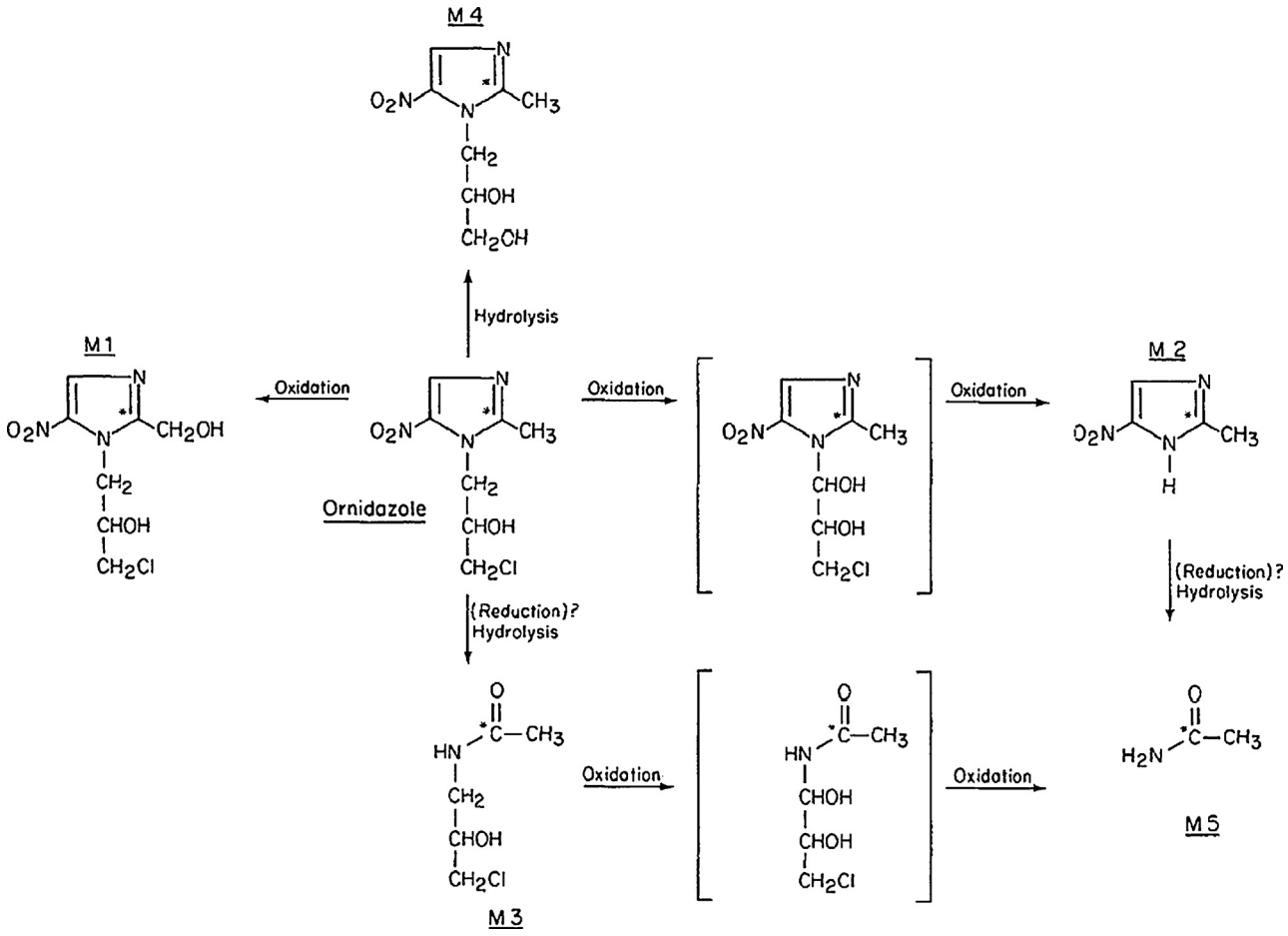

Fig. 1. Schematic diagram illustrating the metabolism of ornidazole. *^{14}C labeled, [] = postulated intermediates.

in human urine [1]: M1, l-chloro-3-(2-hydroxymethyl-5-nitro-l-imidazolyl)-2-propanol; M2, 2-methyl-5-nitroimidazole; M3, *N*-(3-chloro-2-hydroxypropyl)acetamide; M4, 3-(2-methyl-5-nitro-l-imidazolyl)-l, 2-propanediol, and M5, acetamide (Fig. 1).

A number of different methods have been reported for detection of nitroimidazoles in biological fluids [2–11]. However, no method is available for simultaneous determination of levornidazole and its metabolites. The purpose of this study was to develop and validate a method that can be used for the determination of levornidazole and its metabolites (M1, M2 and M4) in real human plasma and urine samples. In this paper, a simple, sensitive, precise, accurate and specific ultra-performance liquid chromatographic–tandem mass spectrometric (UPLC–MS/MS) method is described. The newly established method was successfully applied to a pharmacokinetic study, which required high sensitivity and selectivity.

2. Materials and methods

2.1. Chemicals and reagents

Levornidazole, M1 and M4 were all supplied by Sanhome Pharmaceutical Co., Ltd. (Nanjing, China). M2 and internal standard (IS, metronidazole) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products. Acetonitrile and methanol, both of HPLC grade were purchased from Sigma (Sigma, USA). Water was Milli-Q grade. All the other chemicals and solvents were of analytical grade.

2.2. Instrument and LC–MS/MS conditions

Determination was performed using a Waters ACQUITY UPLC system (Waters, USA) coupled with an API4000 QTRAP mass spectrometer (AB SCIEX, USA). Chromatographic separation was carried out on an ACQUITY UPLC CSH C_{18} column (2.1 × 50 mm, 1.7 μm) using a mobile phase composed of 0.1% formic acid in water and acetonitrile in gradient elution (Table 1) at a flow rate of 0.6 mL/min. The injection volume was 3 μL. Both analytes and IS were determined by atmospheric pressure chemical ionization (APCI) in positive ion mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 220.0 → *m/z* 127.5 for levornidazole, *m/z* 236.2 → *m/z* 170.9 for M1, *m/z* 127.9 → *m/z* 110.7 for M2, *m/z* 202.5 → *m/z* 127.7 for M4 and *m/z* 172.1 → *m/z* 127.8 for IS, respectively. The curtain gas and ion source gas 1 were set at 20 and 40 psi. The nebuilzer current was 4.0. The temperature was 500 °C. The optimized collision energies of 25,19, 20, 22 and 20 eV were used for levornidazole, M1, M2, M4 and IS, respectively.

Table 1
Gradient elution procedure.

Time (min)	0.1% Formic acid in water (%) (B)	Acetonitrile (%) (A)	Cure
0	5	95	–
0.1	5	95	10
2.5	70	30	10
3.6	5	95	1