Journal of Pharmaceutical and Biomedical Analysis 78–79 (2013) 123–128

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

A liquid chromatography method with single quadrupole mass spectrometry for quantitative determination of indomethacin in maternal plasma and urine of pregnant patients

Xiaoming Wang, Daria I. Vernikovskaya, Tatiana N. Nanovskaya, Erik Rytting, Gary D.V. Hankins, Mahmoud S. Ahmed*

Department of Obstetrics & Gynecology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0587, USA

ARTICLE INFO

Article history:
Received 30 October 2012
Received in revised form 10 January 2013
Accepted 7 February 2013
Available online 15 February 2013

Keywords:
Indomethacin
LC–MS
Plasma
Urine
Method validation

ABSTRACT

A liquid chromatography with single quadrupole mass spectrometry method was developed for the quantitative determination of indomethacin in the maternal plasma and urine of pregnant patients under treatment. A deuterium-labeled isotope of indomethacin (d_4-indomethacin) was used as an internal standard. The maternal plasma and urine samples were acidified with 1.0 M HCl then extracted with chloroform to achieve the extraction recovery range of 94–104% with variation less than 11%. Chromatographic separation was achieved by a Waters Symmetry C_{18} column with isocratic elution of 0.05% (v/v) formic acid aqueous solution and acetonitrile (47:53, v/v). An in-source fragmentation was applied on the single quadrupole mass spectrometer equipped with an electrospray ionization source at positive mode. The LC–ESI-MS quantification was performed in the selected ion monitoring mode targeting ions at m/z 139 for indomethacin and m/z 143 for its internal standard. The calibration curves were linear in the concentration ranges between 14.8 and 2.97×10^3 ng/mL for plasma samples and between 10.5 and 4.21×10^3 ng/mL for urine samples. The relative standard deviation of this method was less than 8% for intra- and inter-day assays, and the accuracy ranged between 90% and 108%.

1. Introduction

Preterm birth is a major cause of neonatal mortality and morbidity worldwide. In the United States, the rate of preterm delivery is approximately 11% of live births [1,2]. Indomethacin, a prostaglandin synthetase inhibitor, is considered as a first-line tocolytic agent, beside magnesium sulfate, for the management of preterm labor [3]. However, indomethacin is classified by the FDA of the United States as pregnancy category C because of inadequate data on its safety and efficacy in this population. Therefore, one of the aims of the recent investigation by the NICHD Obstetric-Pharmacology Research Network (OPRU) is to determine the pharmacokinetics (PK) of indomethacin in pregnant patients.

A number of analytical methods for the quantitative determination of indomethacin in human biological samples have been reported. These methods have included liquid chromatography with UV (LC–UV) [4–12] and fluorometric detection [13] as well as liquid chromatography–mass spectrometry (LC–MS/MS) [14]. The reported HPLC–UV methods either required larger volumes (>0.4 mL) of plasma or urine samples [4–8] or had inadequate sensitivity (LLOQ > 60 ng/mL) [9–12], which prevented us from adopting these methods for routine clinical analysis. Taylor et al. [14] reported an LC–MS/MS method for indomethacin coupled with solid-phase extraction (SPE) which achieved good sensitivity (LLOQ = 5 ng/mL) and smaller volume of plasma (0.1 mL). However, the extraction recovery with this method was only 74% [14]. In addition, the SPE method is time-consuming and expensive considering the large number of patient samples in clinical trials. On the other hand, a liquid–liquid extraction method achieved a higher extraction recovery of indomethacin from human plasma (95%) [7].

Therefore, the goal of the current investigation was to develop and validate a sensitive, selective and rapid LC–MS method coupled with simple liquid–liquid extraction for quantitative determination of indomethacin in plasma and urine samples of pregnant patients under treatment.

2. Experimental

2.1. Chemicals and reagents

Chemicals were purchased from the following companies: indomethacin and chloroform from Sigma Chemical Co. (St. Louis,

* Corresponding author. Tel.: +1 409 772 0977; fax: +1 409 772 5297.
E-mail address: maahmed@utmb.edu (M.S. Ahmed).

0731-7085/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2013.02.006

MO); d_4-indomethacin from Toronto Research Chemicals Inc. (North York, Canada); HPLC-grade acetonitrile, methanol, formic acid and hydrochloric acid from Fisher Scientific (Fair Lawn, NJ).

2.2. Biological samples

Pooled blank human plasma containing heparin sodium as the anticoagulant was purchased from Innovative Research Inc. (Novi, MI). The urine samples and individual plasma samples used as blanks were donated by colleagues. Maternal plasma and urine samples were obtained from pregnant patients enrolled in a pilot PK study of indomethacin according to a protocol approved by the Institutional Review Board of The University of Texas Medical Branch at Galveston. The blood samples were collected into BD Vacutainer® tubes containing heparin lithium and the plasma was separated by centrifugation. Urine samples were collected at intervals and the volume for each was recorded. All biological samples were kept frozen at −70 °C until analysis.

2.3. LC–MS conditions

LC–MS instrument consisted of a Waters 600E multi-solvent delivery system, 717 autosampler and a Waters EMD 1000 single-quadrupole mass spectrometer (Milford, MA) controlled by Empower™ 2 software (Waters, Milford, MA). Indomethacin was separated on a Waters Symmetry C_{18} column (150 mm × 4.6 mm, 5 μm) fitted with a Phenomenex C_{18} guard column (4 mm × 3.0 mm). The mobile phase consisted of 0.05% (v/v) formic acid aqueous solution and acetonitrile (47:53, v/v). Isocratic elution was performed for 11 min at a flow rate of 1.0 mL/min. The eluent from the HPLC column was split at 4:1 ratio and directed to the mass spectrometer at a flow rate of 200 μL/min.

The mass spectrometer was equipped with an electrospray ion source (ESI) and operated in positive mode. MS parameters were as follows: capillary voltage, 4.0 kV; cone voltage, 55 V; source temperature, 95 °C; desolvation temperature, 200 °C; desolvation gas flow rate, 150 L/h; cone gas flow rate, 150 L/h. Indomethacin and d_4-indomethacin (internal standard, IS) were monitored by selected ion monitoring (SIM) at m/z 139 for indomethacin, and m/z 143 for the IS.

2.4. Preparation of stock and working standard solutions, calibration standards and quality control (QC) samples

The stock solutions of indomethacin and its internal standard were prepared in 30% (v/v) methanol. Working standard solutions of Indomethacin were prepared in the range of 0.148–29.7 μg/mL for the analysis of maternal plasma samples, and 0.105–42.1 μg/mL for urine samples. The final concentration of the IS working solution was 2.00 μg/mL for analysis of plasma and urine samples. These solutions were stored at 4 °C.

The calibration standards were prepared by adding 20 μL of working standard solution into 200 μL of either plasma or urine to achieve a final indomethacin concentration range of 14.8–2.97 × 10^3 ng/mL for plasma samples and 10.5–4.21 × 10^3 ng/mL for urine samples. Quality control (QC) samples were prepared using blank plasma or urine at high, medium, and low concentrations as well as at the lower limit of quantification (LLOQ) of indomethacin.

2.5. Preparation of plasma and urine samples

An aliquot of the IS working solution (20 μL) was added to 200 μL of untested patient samples (plasma or urine), calibration standards or QC samples. The mixture was vortexed for 30 s, then 50 μL of 1.0 M hydrochloride acid and 900 μL of chloroform were added and vortexed for 3 min. The solution was centrifuged at 12,000 × g for 5 min, and the organic layer transferred to a clean tube. The remaining aqueous layer was re-extracted with 900 μL of chloroform as described above. The two organic layers were combined and dried under a stream of air at 40 °C. The dry residues were reconstituted in 100 μL of the mobile phase and centrifuged at 12,000 × g for 15 min. An aliquot (80 μL) of the supernatant from each sample was injected into the HPLC system.

2.6. Method validation

The method was validated according to the guidelines issued by the FDA for bio-analytical method validation [15]. The selectivity was determined by analyzing pooled and individual blank plasma and urine samples from six individual donors. The calibration curves were fitted using weighted least-squares linear regression of the internal ratio (peak area of the analyte/peak area of the IS) versus concentration. The limit of detection (LOD) was determined at a signal-to-noise (S/N) ratio of 3:1 by comparing test results from samples with known concentrations of indomethacin to blank samples. The lower limit of quantification (LLOQ) was defined as the lowest concentration that produced an S/N > 10 and could be quantified with an RSD < 20% and accuracy in the range of 80–120% [15].

The extraction recovery of indomethacin from plasma and urine samples was evaluated by comparing the peak area of indomethacin in QC samples to its peak area in the post-extracted samples at high, medium and low concentrations. The matrix effect of indomethacin and the IS was evaluated quantitatively by calculating the matrix factor, which is defined as the ratio of the analyte peak area of post-extraction samples to the analyte peak area of pure standards [16].

Intra-day and inter-day accuracy and precision of indomethacin in plasma and urine samples were evaluated by the analysis of six replicate QC samples at high, medium and low concentrations. For the intra-day assay accuracy and precision, QC samples were analyzed using a calibration curve prepared on the same day; for the inter-day assay accuracy and precision, the QC samples were analyzed on three consecutive days. Accuracy was expressed as [mean of measured concentration/nominal concentration] × 100% and the precision was represented by the relative standard deviation (RSD).

The stability of indomethacin in human plasma and urine samples was assessed by analyzing replicate QC samples at high and low concentrations. Freeze–thaw stability was determined after three freeze–thaw cycles. Each cycle consisted of freezing at −70 °C for 12 h and then thawing under the room temperature (22–25 °C). For short-term stability and long-term stability investigations, the unprocessed QC samples were kept at room temperature for 4 h and at −70 °C for 30 days, respectively. Then these QC samples were processed and analyzed. The concentration of indomethacin was calculated using a freshly prepared calibration curve.

3. Results and discussion

3.1. Method development

3.1.1. Mass spectrometric conditions

The ionization of indomethacin was investigated with the ESI interface in both positive and negative ion modes. The positive ion mode was chosen for analysis of indomethacin because of higher sensitivity of all its fragmentation ions than the negative ion mode. The quasi-molecular ion $[M+H]^+$ of indomethacin at m/z 358 could not be observed at low cone voltage most likely due to the weak amide bond of indomethacin that fragments easily into two ions at m/z 139 and m/z 174 (Fig. 1). Increasing the cone voltage from 15 V