Journal of Chromatography B, 1000 (2015) 176–180

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Evaluating misoprostol content in pregnant women with hourly oral administration during labor induction by microElution solid phase extraction combined with liquid chromatography tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 25 April 2015
Received in revised form 1 July 2015
Accepted 5 July 2015
Available online 17 July 2015

Keywords:
Labor induction
Misoprostol acid
Liquid chromatography tandem mass spectrometry
highly-selective reaction monitoring
microElution solid phase extraction

ABSTRACT

Misoprostol is a widely used alternative of prostaglandin for labor induction. Based on previous studies, we envision that small and frequent oral dosage of misoprostol is an effective method for labor induction. To monitor the misoprostol content during labor induction, a rapid, sensitive, and selective microElution solid phase extraction (μElution SPE) combined with liquid chromatography tandem mass spectrometry (LC–MS/MS) was developed. Using μElution SPE could minimize the sample consumption and elution volume in order to maximize the sample enrichment and throughput. The misoprostol acid, a metabolite of misoprostol, was gradient separated in a Bidentate C18 column, then quantified by highly-selective reaction monitoring (H-SRM) in a total run time of 6 min. The developed method was optimized and validated in human plasma, and showed linear range of 0.01–10 ng/mL. The limit of detection (LOD) was 0.001 ng/mL. The recovery ranged from 89.0 to 96.0%, and no significant matrix effect or carryover was observed. The precision, accuracy and stability were met with the criteria of U.S. FDA guidance. The developed method was successfully applied to evaluate misoprostol concentration during labor induction in pregnant women. The concentration-time profiles approves that hourly oral administration of misoprostol is a safe and effective method without drug accumulation for labor induction.

1. Introduction

Labor induction is widely used over the world because continuation of pregnancy is harmful to a mother and her fetus. In World Health Organization (WHO) Global Survey, the delivery involved labor induction was as high as 35.5% in Asian countries [1]. Prostaglandins are the most effective choice for helping labor induction. The main disadvantages of prostaglandins are that they are expensive and sensitive to temperature changes. The affordable alternative, misoprostol, is a prostaglandin E1 analogue, which has some advantages, such as high stability at room temperature, cheap, and could be given in several routes [2,3]. It is a safe, effective, and promising method for both nulliparous and multiparous women, and it is more efficient with oral administration than vaginal administration [4,5]. From the results of clinical trials in previous studies, we envision that using small and frequent oral doses of misoprostol is an effective method for labor induction. To approve the hypothesis and establish a reliable protocol for labor induction, it is important to monitor the misoprostol concentration during the process of hourly administration.

Misoprostol was readily metabolized to its pharmacologically active form, misoprostol acid (MPA), after five minutes of oral administration. The peak concentration of misoprostol acid was achieved in 12 ± 3 min, and its half-life was 20–40 min, then declined rapidly thereafter [6]. Independent of the route of administration, the therapeutic dose does not usually exceed 0.8 mg per day. To assess safety of misoprostol, the total dosage and side effects were considered. The safety of misoprostol has been documented in dosage up to 1.6 mg per day [7] and the side effects of the drug are diarrhea, pyrexia and shivering. But even higher dosage of misoprostol applied, only mild side effects have been observed. Several published case reports [8,9,10] demonstrate that overdos-

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http://dx.doi.org/10.1016/j.jchromb.2015.07.012
1570-0232/

ing of misoprostol with single dosage more than 3.0 mg resulted in hyperthermia, hypoxia and rhabdomyolysis. In previous clinical pilot study [11], the maximum total dosage was 9.6 mg in nulliparous woman who only experienced the diarrhea and it is the most common side effect (50%) in the study. Because of recommended therapeutic dose is low, the highest concentration of the bioactive metabolite is very low [12]. Therefore, an analytical method with high sensitivity and selectivity is needed for determining the concentration of misoprostol acid in plasma.

Determination of misoprostol acid in biological matrices is usually accomplished by several analytical methods, such as radioimmunoassys [6], gas chromatography–negative ionization chemical ionization tandem mass spectrometry (GC–NICI–MS/MS) [13,14], and liquid chromatography-tandem mass spectrometry (LC–MS/MS) [12,15,16]. The drawback of GC–NICI–MS is that it requires complicated derivatization procedures, long chromatographic time, and sacrificed sensitivity with *syn* and *anti* isomer. Solid phase extraction (SPE) is one of the widely used preparation methods for LC–MS/MS, which could combine the cleanup and enrichment at the same time. Usually, SPE requires the large amount of sample and elution solvent, from milliliter to liter range, and needs to convert the eluent into the suitable solvent in order to achieve better LC separation. These steps are not only time-consuming but also cause experiment errors. microElution solid phase extraction (μElution SPE) has the unique design to allow the loading of 10–750 μL of sample and eluting of ultra-low elution solvent, which is 25 μL [17–21]. These properties help to avoid using large amount of sample, eliminate the evaporation and reconstruction step, hence increasing the sample throughput. The sorbent used in this study, Oasis HLB (Hydrophilic-Lipophilic Balance), is a divinylbenzene/*N*-vinylpyrrolidone polymer that has both lipophilic and hydrophilic groups and exhibit excellent wetting properties. Therefore, HLB could give maximum extraction efficiency for MPA in plasma sample without the loss of recovery or breakthrough problem, which is the major drawback of silica-C18 sorbent.

Here, we designed a simple, rapid, and reliable method for evaluating misoprostol content in pregnant women during labor induction in order to prove the proposed hypothesis. To achieve the purpose, the microElution solid phase extraction combined with LC–MS/MS is developed to detect trace misoprostol acid in human plasma sample and validated to meet the criteria of the U.S. FDA guidance.

2. Experimental

2.1. Materials, reagents and chemicals

Misoprostol acid (MPA) and misoprostol acid-d_5 (MPA-d_5), as the internal standard (IS), were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Ultrapure water (>18 MΩ) was purified with a Milli-Q system (Millipore Simplicity®, Millipore, France). The quality of the solvents and chemicals used during this study were HPLC grade or better. The stock solution of MPA (1 mg/mL) and MPA-d_5 (50 μg/mL) were prepared in methanol and stored in the refrigerator at −30 °C. Standard working solutions were prepared daily by mixing stock solutions and methanol–water (1:1, v/v) to the required concentrations. MicroElution 96-well SPE (μElution SPE) with Oasis HLB sorbent (2 mg) were obtained from Waters (Milford, MA, USA).

Human plasma samples and drug-free plasma were obtained from China Medical University Beigang Hospital (Yunlin, Taiwan). These samples used in this pilot study were collected from three healthy pregnant women (27.5 ± 4.5 year-old) who did not have a disease of the heart, liver, and kidney and an allergy to misoprostol. No analgesic agent was used during labor induction and the common side effect of diarrhea happened to one of three pregnant women who relieved the symptom easily by anti-diarrhea agent. The formal ethic approval (IRB#DMR99-IRB-242, approved on 09 Dec. 2010) was obtained from the institutional review board of China Medical University Hospital, and volunteers provided with informed written consents. For the evaluation of concentration of misoprostol during labor induction, the subjects received a tablet of 200 μg misoprostol (Cytotec®, Pfizer Inc., Taiwan) by hourly oral administration more than 8 h. Human plasma samples were collected at 0 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, and 8 h in the clean K3EDTA-treated polypropylene tubes. After centrifuging, the samples were stored in the refrigerator at −30 °C until analysis. Drug-free plasma were used for method development and validation.

2.2. Instrumentation

The Thermo Scientific Accela LC system and autosampler (Thermo Scientific, San Jose, CA, USA) were used for chromatographic analysis. The gradient separation was performed by Cogent Bidentate C18 column (100 mm × 2.1 mm i.d., 4 μm, MicroSolv Technology, Eatontown, NJ, USA) with Bidentate C18 guard column (20 mm × 2 mm i.d.) at 30 °C and flow rate of 0.4 mL/min. The mobile phases were water and acetonitrile. Gradient separation began at 40% acetonitrile, isocratic for 1 min, then increased to 95% acetonitrile within 0.5 min, isocratic for 2 min. Afterward, the conditions returned to 40% acetonitrile and equilibrated for 2 min, resulting a total run time of 6 min. An injection volume of 5 μL was used.

The TSQ Quantum Ultra EMR triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) was equipped with heated electrospray ionization (H-ESI). The MS analysis was operated in negative ionization mode with H-ESI and quantified by using highly-selective reaction monitoring (H-SRM). The tuning parameters were optimized for MPA by using post-column T infusion method, which was infusing 10 μg/mL MPA through syringe pump at LC flow rate of 0.4 mL/min. The optimized H-ESI voltage and temperature were set at 4.5 kV and 300 °C, respectively. Sheath gas was set at 20 arbitrary unit and Aux gas at 10 arbitrary unit. In H-SRM, argon was used as the collision gas at the pressure of 1.5 mTorr with optimized collision energy of 20 eV for both MPA and IS. The mass transitions used were *m/z* 367 > 249 for MPA and *m/z* 372 > 249 for MPA-d_5. The resolution (FWHM, full width at half-maximum) of Q1 and Q3 were operated at 0.4 and 0.7, respectively. Raw data was acquired and processed by using Xcalibur 2.0.7 (Thermo Scientific).

2.3. Sample preparation

An aliquot of 200 μL sample containing IS solution (40 μL) was diluted with 200 μL of 0.1% formic acid, and then centrifuged at 14,000 rpm for 10 min. The supernatant was transferred to another clean Eppendorf tube. The μElution SPE plate was conditioned sequentially with 0.5 mL MeOH, 0.5 mL water and 0.5 mL 0.5% acetic acid. An aliquot of 300 μL supernatant was loaded into μElution plate, and then washed with 0.5 mL 0.5% acetic acid, 0.5 mL acetonitrile–water–acetic acid (100:900:0.5, v/v/v), and 0.5 mL water. After that, the elution step was done with 50 μL of acetonitrile–water (50:50, v/v). The eluent was directly analyzed by LC–MS/MS.

2.4. Method validation and real sample analysis

The method was validated to meet the criteria of the U.S. FDA guidance, Guidance for Industry: Bioanalytical Method Validation [22]. To evaluate method performance, such as calibration curve,