Journal of Chromatography B 1092 (2018) 51–57

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Bioanalysis of sulprostone, a prostaglandin E_2 analogue and selective EP_3 agonist, in monkey plasma by liquid chromatography-tandem mass spectrometry

Yifan Shi[a], Matthew M. Rankin[b], Lisa D. Norquay[b], Naidong Weng[a], Shefali Patel[a,*]

[a] Department of Pharmacokinetics, Dynamics, and Metabolism, Janssen Research & Development, Johnson & Johnson, 1400 McKean Road, Spring House, PA 19477, USA
[b] Department of Cardiovascular and Metabolism, Janssen Research & Development, Johnson & Johnson, 1400 McKean Road, Spring House, PA 19477, USA

ARTICLE INFO

Keywords:
Sulprostone
Prostaglandin E_2 analogue
Adduct formation
LC-MS/MS
Esterase inhibitor

ABSTRACT

Sulprostone is a potent prostaglandin E_2 (PGE_2) analogue and one of the first identified selective G-protein-coupled receptor 3 (EP_3) agonists. It has been investigated as a potential antiulcer agent and frequently used in the research of EP_3 antagonist. To assist pharmacokinetic and pharmacodynamic studies, a rapid and sensitive LC-MS/MS method was developed and qualified for the quantitation of sulprostone in monkey plasma. Using electrospray ionization mass spectrometry, an ammonium adduct in positive mode was chosen for analysis which had seven times of the sensitivity of the depronated ion in negative mode. Latanoprost, a prostaglandin $F_{2\alpha}$ analogue, was used as the internal standard while good sensitivity and chromatography were obtained on a 2.6 μm core-shell column with pentafluorophenyl stationary phase. An assay dynamic range of 2 to 4000 ng/mL was achieved with a sample volume of 25 μL plasma on a Sciex API4000 instrument with simple protein precipitation. Several esterase inhibitors including sodium fluoride (NaF), phenylmethanesulfonyl fluoride (PMSF), diisopropylfluorophosphate (DFP), paraoxon and dichlorvos as well as wet ice conditions were explored for the stabilization of sulprostone in monkey plasma. The developed method was successfully applied for the evaluation of pharmacokinetics of sulprostone after intravenous administration of 0.5 mg/kg to cynomolgus monkey.

1. Introduction

Prostaglandins (PGs) of the E class are cyclooxygenase (COX) metabolites of arachidonic acid, a C20-unsaturated fatty acid. Among the prostanoids, PGE_2 is the most widely produced in the human body and animal species [1]. It has diverse biologic activities in cell permeability, inflammation, fever and regulation of gastrointestinal mucosa [2–4]. PGE_2 mediates physiological and pathophysiological processes by binding to four different G-protein-coupled receptors (GPCRs): EP_1, EP_2, EP_3 and EP_4 [5]. The EP receptor subtypes differ in distribution of expression as well as second messenger signaling cascades.

EP_1 is a G_q-coupled receptor that activates phospholipase C to mediate the elevation of intracellular Ca^{2+} concentrations and promote phosphatidylinositol hydrolysis [6]. EP_2 and EP_4 couple to G_s proteins resulting in the activation of adenylate cyclase and an increase in cyclic adenosine monophosphate (cAMP) levels [1]. In opposition to EP_2 and EP_4 signaling, EP_3 is a G_i-coupled receptor, and results in an inhibition of cAMP-dependent pathways. Of the four PGE_2 receptor subtypes, EP_3 has the highest binding affinity. EP_3 has also been observed to have a widely distributed expression pattern and EP_3-mediated effects have been reported in the central nervous system, cardiovascular system, reproductive system, kidneys and urinary bladder [7]. As a result, modulators of EP_3 pharmacology are of broad interest in both academia and industry to potentially impact diseases such as diabetes mellitus [8], overactive bladder [9], atherothrombosis [10] and colon cancer [11].

Sulprostone, a potent PGE_2 analogue, was identified as one of the first EP_3 selective agonists. While the in vivo half-life of PGE_2 is extremely short, sulprostone demonstrates an improved stability profile [12]. These properties permitted the use of sulprostone clinically for pre-operative cervical dilatation, as well as its investigation as a potential antiulcer agent [1]. Given its selectivity and stability characteristics, sulprostone is also widely used to investigate EP_3-mediated biology. The identification of novel EP_3 antagonists as potential antithrombotics used sulprostone as an EP_3 agonist [13]. To assist in evaluating safety and pharmacokinetic properties in the reported preclinical studies, accurate analytical methods to determine the plasma concentration of sulprostone were required. Initially, Falkner et al.

* Corresponding author.
E-mail address: SPatel31@its.jnj.com (S. Patel).

https://doi.org/10.1016/j.jchromb.2018.05.034
Received 23 February 2018; Received in revised form 21 May 2018; Accepted 24 May 2018
Available online 25 May 2018

performed metabolite and mass balance studies using tritium and carbon-14 labeled sulprostone with a radioactivity HPLC detector [14]. Later, Kuhnz et al. expanded the metabolite analysis using only the tritium labeled compound with GC/MS, NMR and IR for structure elucidation [15]. The requirement of radioisotopes limited the usefulness of these methods for definitive quantitation of preclinical and clinical samples. The complicated purification process was not only tedious and time-consuming, but also lacked sufficient specificity. In recent years, LC-MS/MS techniques have been extensively used for bioanalysis due to the unique advantages of sensitivity, selectivity, robustness, and linearity.

In this work, we developed and qualified an LC-MS/MS method for quantitation of sulprostone in monkey plasma. Sulprostone, as with other prostaglandin analogues, is difficult to ionize and is prone to matrix suppression. Multiple approaches were reported in the literature to enhance sensitivity for LC-MS/MS. For example, Ji et al. utilized an acetate adduct to dramatically improve the assay sensitivity for dapagliflozin in human plasma [16]. Dziadosz et al. developed a strategy using two different adducts to analyze valproic acid which has inefficient collision-induced dissociation fragmentation [17]. In our study, an ammonium adduct of sulprostone was identified in positive mode which had seven times of the sensitivity of the depronated ion in negative mode. Consequently, the reproducibility of adduct formation would need to be further investigated as suggested previously in literature [18]. The presence of a wide variety of additional eicosanoids in plasma posed analytical challenges in separation from interferences. Satisfactory chromatographic performance was achieved using a 2.6 µm core-shell column with pentafluorophenyl stationary phase. As compound instability was observed in monkey plasma at room temperature, wet ice conditions as well as several esterase inhibitors were evaluated to stabilize sulprostone in the plasma. The established lower limit of quantitation (LLOQ) was 2 ng/mL based on an extraction volume of 25 µL with protein precipitation extraction. To the best of our knowledge, this is the first reported LC-MS/MS assay for the quantitation of sulprostone in plasma. This simple, rugged, and sensitive method was successfully applied to a monkey study to evaluate pharmacokinetic (PK) parameters for sulprostone.

2. Experimental

2.1. Chemicals and reagents

Reference standard for sulprostone and internal standard latanoprost were obtained from Sigma-Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO), isopropanol (IPA), sodium fluoride (NaF), HPLC grade acetonitrile and methanol were purchased from EMD Millipore (Billerica, MA). Formic acid (reagent grade), ammonium formate (> 99%), phenylmethanesulfonyl fluoride (PMSF), diisopropylfluorophosphate (DFP), paraoxon and dichlorvos and were purchased from Sigma-Aldrich (St. Louis, MD). Deionized water was purified via Milli-Q system from EMD Millipore. Monkey plasma (K_2EDTA) was obtained from Bioreclamation (Westbury, NY).

2.2. Instrumentation

The LC system used was a Shimadzu LC-20AD pump coupled with a Sil-20AC HT autosampler (Kyoto, Japan). A Kinetex PFP column (3 × 50 mm, 2.6 µm) from Phenomenex (Torrance, CA) was used for chromatographic separation. All analyses were conducted on a triple quadrupole API4000 mass spectrometer from Applied Biosystems, Inc. (Foster City, CA) with a Turbo Ionspray interface. The system was controlled by Analyst 1.6 software.

2.3. LC-MS/MS conditions

For the HPLC, mobiles phases used were 10 mM ammonium formate with 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B). The following gradient was applied at a flow rate of 0.6 mL/min: linearly increased from 20% from 90% of mobile phase B from 0 to 2.7 min, held for 1 min and decreased back to 20% in 0.1 min. The total run time was 4.5 min. The autosampler temperature was set at 4 °C, and the LC column was maintained at room temperature.

Sulprostone was monitored as an ammonium adduct using positive ion electrospray ionization (ESI) with selective reaction monitoring (SRM). The mass spectrometer conditions were optimized as listed: curtain gas and collision gas were set as 25 and 10; the turbo spray voltage was set at 5000 V and ion source gas 1 and gas 2 were both set at 50 psi. The ion source temperature was set at 400 °C. Entrance potential (EP) was maintained at 10 V. The transition monitored for sulprostone ammonium adduct was m/z 483 → 354 with declustering potential (DP) set at 54 V, collision energy (CE) at 17 eV. For internal standard latanoprost, the SRM transition was 433 → 105; and DP and CE were 51 V and 60 eV, respectively.

2.4. Solution, standards and sample preparation

Stock solutions of sulprostone and internal standard latanoprost were prepared at 0.5 mg/mL in 50:50 DMSO/methanol (v/v) and stored at 4 °C. Calibration standard curves in cynomolgus plasma (K_2EDTA) were prepared fresh daily with concentrations of 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 4000 ng/mL. Four levels of quality control (QC) samples were prepared at the concentrations of 6, 30, 800, and 3200 ng/mL. All unknown samples and quality controls were stored at −80 °C until analyzed. Latanoprost stock solution was diluted with acetonitrile to a final concentration of 2000 ng/mL which was used as the internal standard working solution.

Monkey plasma samples were extracted using protein precipitation. Unknown samples, standard and QC samples were kept on wet ice during extraction. A 25 µL aliquot of plasma was pipetted into a 96-well plate; 25 µL of internal standard working solution was added to each sample followed by an addition of 200 µL of acetonitrile. After mixing and centrifugation, a 100-µL portion of the supernatant was transferred to a new plate that contained 100 µL of deionized water.

2.5. Method qualification

The method qualification was carried out in accordance to Janssen internal bioanalytical guideline which consisted of a calibration curve, six replicates of QCs, benchtop and freeze-thaw stability QCs in one analytical batch. The linearity of the calibration curve was determined using a linear $1/x^2$ weighted regression model. The accuracy and precision were assessed by analyzing QC samples at four concentration levels. The intraday assay precision was evaluated by analyzing six replicates of QC samples per concentration level. The interday assay precision was not evaluated for method qualification. Assay accuracy and precision within 100% ± 20% were deemed acceptable. Stability of sulprostone in matrix was assessed using QC samples at two concentrations for three freeze-thaw cycles and four hours on wet ice. A percent deviation from the nominal concentration of ± 20% was deemed acceptable to consider the sample stable under the storage condition tested.

2.6. Stabilizing effect of esterase inhibitors

While the plasma stability under wet ice condition met our acceptance criteria for method qualification, the compound did show trends of degradation for both freeze-thaw and benchtop stability. Several esterase inhibitors, NaF, PMSF, DFP, paraoxon and dichlorvos, were added to monkey plasma to test their stabilizing effect for sulprostone. Inhibitor stock solutions were prepared at 0.4 M in water for NaF, 0.2 M in IPA for DFP and dichlorvos, and 0.4 M in DMSO for PMSF and paraoxon. Appropriate amount of inhibitor was spiked into monkey