Simplified Solid-Phase Extraction Procedure and Liquid Chromatographic Determination of Celecoxib in Rat Serum

M. H. Guermouche1,2, A. Gharbi2

1 Faculté de Chimie, USTHB, B.P. n°32, El-Alia, Bab-Ezzouar, Alger, Algeria, E-Mail: hguermouche@voila.fr
2 LNC, Département de Pharmacie, Faculté mixte de Medecine et Pharmacie, Université d’Alger, 2, rue Didouche Maurad, Alger, Algeria

Received: 23 October 2003 / Revised: 26 January and 23 April 2004 / Accepted: 28 April 2004
Online publication: 5 August 2004

Abstract

A simplified solid phase extraction method, eliminating a preliminary protein precipitation has been developed for the determination of celecoxib in rat plasma. The technique included a solid phase extraction of the serum samples on a [poly (divinylbenzene-co-N-vinylpyrrolidone)] sorbent. After conditioning, the cartridge was loaded with 0.5 mL of acidified serum containing internal standard. Elution was made with 1 mL of a mixture of acetonitrile and methanol (1/1, v/v). After evaporation of the eluate to dryness and reconstitution with methanol, the samples were analyzed on an octadecyl bonded phase with several mobile phases containing acetonitrile and a phosphate buffer. Detection was carried out using a Photodiode Array Detector. Full validation of the proposed method was provided (linearity range: 0.01–2 mg. L−1, average extraction efficiency: 92.4%; average intra-day variability: 4.6% with an accuracy of 94.8%; average interday variability: 5% with an accuracy of 95.3%, limit of detection: 0.005 mg. L−1, limit of quantification: 0.002 mg. L−1). The proposed method was successfully utilised to quantify celecoxib in rat plasma for a pharmacokinetic study.

Keywords

Column liquid chromatography
Solid phase extraction
Photodiode array detector
Celecoxib in rat serum

Introduction

Celecoxib (Celebrex), (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide), (Fig. 1) is a cyclooxygenase inhibitor that exhibits anti-inflammatory, analgesic and antipyretic activities. The mechanism of its action is the inhibition of prostaglandin synthesis by cyclooxygenase type 2 (COX-2) without inhibiting cyclooxygenase-1 (COX-1) [1]. Celecoxib provides analgesic/anti-inflammatory effects comparable to conventional COX-1 non-steroidal anti-inflammatory agents, but with a reduced side effects [2]. The pharmacokinetics of celecoxib in man [3] and animals [4, 5] has been extensively studied and a number of analytical methods have been used for the quantification of celecoxib such as micellar electrokinetic chromatography [6], radioactivity methods [3,4] and UV spectroscopy [7] but liquid chromatography is the most commonly employed. Rose et al. [8] proposed a column switching technique and a normal phase column after a long extraction procedure. Liquid chromatographic-mass spectrometry has also been carried out to determine Celecoxib in plasma using single-ion monitoring [9] or to follow its pharmacokinetics [10, 11]. To improve sensitivity, fluorescence detection combined with HPLC was used by Schönberger et al. [12]. Prior to Celecoxib analysis, different sample treatments were used such as solvent extraction [9, 10, 13, 14] and microdialysis [15]. Solid phase extraction (SPE) was also proposed [3, 4, 8] using a C-18 SPE cartridge. The proposed SPE procedures were difficult to use and time consuming. Recently, Stormer et al. suggested the use of a poly (divinylbenzene-co-N-vinylpyrrolidone) SPE cartridge (Oasis HLB) to extract celecoxib and its metabolites [16], but these authors suggested a prior protein precipitation with acetonitrile, evaporation to dryness of the organic phase and dissolution of the residue in water prior to solid phase extraction.

In this work, a poly (divinylbenzene-co-N-vinylpyrrolidone) SPE cartridge was used to directly extract celecoxib from rat serum without any supplementary step. The optimal chromatographic conditions for the Celecoxib determination were investigated.


**Experimental**

**Reagents**

Solvents (chromatographic grade) were from Fluka (Switzerland). Celecoxib was purchased from Pfizer (USA), Ibuprofen (internal standard) was from Sigma (USA).

**Chromatographic Instrumentation**

A Waters chromatograph was used with a 600E pump, a 7625i Rheodyne injector with a 20 µL sample loop and a Waters diode array detector 991. Separations were carried out on a NovaPak C18 column (150 x 4.6 mm) from Waters (USA) preceded by a C18 home-made guard column (20 x 4.0 mm). Several mobile phases were tested. They were made from acetonitrile mixed with acetate buffers of different pH. Flow rate was fixed at 1 mL min⁻¹. Data were collected with a Millenium 32 program (Waters). Quantitation was at 254 nm.

**Collection of the Samples**

Serum samples were collected from Wistar rats (mean weight = 200 g) which received intraperitoneally 5 mg.Kg⁻¹ of Celecoxib suspension. After 0.5, 2, 3, 4, 6, 8, 11 and 24 hours, blood samples were collected from the orbital venous plexus. The samples which corresponded to 0.5, 2, 3, 4 and 6 h were collected six times. Serum samples were separated by centrifugation at 6,000 rpm for 15 min. They were stored at -20 °C and allowed to thaw at 25 °C prior to use.

**Sample Extraction Procedure**

The solid phase extractions of the samples were carried on poly (divinylbenzene-co-N-vinylpyrrolidone) cartridges (Oasis HLB, 60 mg) from Waters. The cartridges were conditioned with 1 mL of methanol and 1 mL of acetonitrile before being used for extraction. The acidified sample (0.5 mL of serum containing 10 mg.L⁻¹ Ibuprofen + 0.15 mL of H₃PO₄) was passed through the cartridge with minimal suction; the cartridge was washed with 1 mL of water, sucked dry and then eluted with 1 mL of methanol:acetonitrile (1:1 v:v). The eluate was evaporated to dryness under nitrogen at 30 °C and reconstituted with 100 µL of methanol. 20 µL aliquots were injected into the chromatograph.

**Extraction Recovery**

The extraction recoveries were determined by comparing the peak areas of the extracts of spiked serum samples with those obtained by direct injection of the same amount of Celecoxib.

**Calibration Graph**

Stock solutions of celecoxib (50 µg.mL⁻¹) and internal standard (200 µg.mL⁻¹) were prepared separately in methanol. Calibration samples of Celecoxib (0.01 - 2 µg.mL⁻¹) and ibuprofen (10 µg.mL⁻¹) were prepared by adding various volumes of stock solution of celecoxib and a constant volume of internal standard in appropriate volumes of pooled drug-free serum.

**Limit of Detection (LOD), Limit of Quantification (LOQ)**

The detection limit (LOD) of Celecoxib was estimated as the amount in serum which corresponded to three times the base line noise. The limit of quantification (LOQ) was determined as the lowest concentration of the calibration curve.

**Precision and Accuracy**

For spiked serum with different amounts of celecoxib and internal standard, the inter-run precision of the method was estimated by calculating the coefficient of variation (C.V.) of the concentrations measured on different days (n = 6), the intra-run precision was determined in the same way on the same day (n = 10). The accuracy was calculated as % bias.

**Selectivity, Specificity**

To evaluate possible endogenous interferences, five rat serum blanks were analyzed by the proposed procedure.

**Results and Discussion**

**Mobile Phase and Internal Standards Selection**

Various mobile phases with different compositions and pH were evaluated for elution of the Celecoxib and internal standard. The chromatograms were studied for peak shape and efficiency as shown in Fig. 2. The optimal mobile phase (resolution factor 3.8, plate number 3050, tailing factor 1.1) corresponded to 47% acetate buffer (pH 5, 0.075M) and 53% of acetonitrile (v:v).

Several internal standards were tested with UV detection: 5-methyl-2-nitrophenol (retention time t₉ 5 min) used in previous reports [17], Ibuprofen (t₉ 4.8 min), Ketoprofen (t₉ 2 min), Naproxen (t₉ 2.5 min). After pilot investigations, we choose Ibuprofen as the internal standard because it is well separated from Celecoxib (resolution 6.8 as shown in Fig. 2b) under the conditions used and no interfering peaks were detectable in blank serum samples.

**Validation Assay of the Method**

**Calibration Graph**

Calibration curves were obtained by least-squares linear regression analysis of the peak area ratio, y, of analyte/internal standard versus analyte concentration, x. The method was linear in the range 0.01 – 2 mg.L⁻¹; the corresponding equation is y = (0.09398 ± 0.00490) + (0.1739 ± 0.0099)x with a correlation coefficient R of 0.9985.

**Recovery, Precision and Accuracy**

The recoveries of Celecoxib from spiked serum samples were calculated by comparing peak areas at low, medium and