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Journal of Chromatography B, 830 (2006) 245–248
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of celecoxib in human plasma and breast milk by high-performance liquid chromatographic assay

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Received 13 May 2005; accepted 30 October 2005
Available online 16 November 2005

Abstract

A rapid and simple HPLC assay was developed for the determination of celecoxib in human plasma and breast milk. After proteins were precipitated with acetonitrile, celecoxib was resolved on a C18 column and detected by UV detection at 254 nm. Standard curves were linear over the concentration range 10–2000 μg/L ($r^2 > 0.99$). Bias was ≤±15% from 20 to 2000 μg/L in both matrices, intra- and inter-day coefficients of variation (imprecision) were <10%, and the limit of quantification was 10 μg/L.

Keywords: Celecoxib; Plasma; Milk; HPLC

1. Introduction

Breast milk is the optimal food for babies, but the sucking infant may be exposed to drugs during maternal pharmacotherapy. It is important to know the extent of drug transfer into human breast milk in order to assess the likely 'dose' ingested by the infant and therefore quantify risk.

Celecoxib (Fig. 1) was the first member of a subgroup of non-steroidal anti-inflammatory drugs (NSAIDs) known as selective cyclooxygenase-2 (COX-2) inhibitors and has been approved by the US Food and Drug Administration (FDA) for indications such as rheumatoid arthritis and osteoarthritis [1–2]. This group of drugs has been the subject of recent scrutiny with the voluntary withdrawal of rofecoxib (Vioxx) because of increased cardiovascular risk. Safety concerns are therefore of paramount importance in the use of these agents, including their safety during breast feeding.

The pharmacokinetic properties of celecoxib in healthy volunteers and patients have been described previously [3–5]. In addition, two reports describing the distribution of celecoxib into human breast milk have been published [6,7]. While these suggest low infant exposure, a limitation of the larger study ($n = 5$) [7] was assay sensitivity which resulted in inability to measure celecoxib concentrations in milk beyond 8 h. The other was a single case report [6] and measured celecoxib concentrations in milk only. More studies are therefore needed.

Various analytical methods for measuring celecoxib in human plasma have been developed [4,8–15] including HPLC with fluorescence [8] or UV detection [9,10,13–15], LC–MS [4,11] and LC–MS–MS [12]. It appears that only one method for measuring celecoxib in human breast milk has been reported, which employed HPLC with UV detection [7]. For the sample preparation, all of these methods require a relatively complex extraction procedure, which involves liquid–liquid extraction [4,10,11,13], protein precipitation followed by liquid–liquid extraction [7], solid-phase extraction [8,12] and protein precipitation followed by solid-phase extraction [9,14]. The complex extraction procedure is reagent and time consuming.

This paper presents a rapid, simple and sensitive HPLC method for measuring celecoxib in human plasma and breast milk that could be more convenient for clinical use. In this method, the sample preparation involves a simple one-step protein precipitation.

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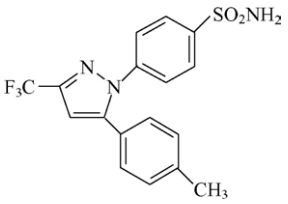
doi:10.1016/j.jchromb.2005.10.048



Celecoxib

Fig. 1. Chemical structure of celecoxib.

2. Experimental

2.1. Materials

Celecoxib was kindly donated by Pfizer (New York, NY, USA). HPLC grade acetonitrile was purchased from BDH (Poole, UK). Distilled, deionised water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA). The human plasma used as the assay blank and for the preparation of standards was obtained from New Zealand Blood Services (Christchurch, New Zealand). The human breast milk used as the assay blank and for the preparation of standards was donated by healthy volunteers.

2.2. Chromatography

HPLC analysis was performed on the Agilent 1100 Series system equipped with a quaternary pump, a variable wavelength detector set at 254 nm and an autosampler (Hewlett-Packard, Waldbronn, Germany). An Aqua C18 5 μm, 4 mm × 3.0 mm internal diameter guard column and an Aqua C18 5 μm, 75 mm × 4.6 mm internal diameter analytical column (Phenomenex, Torrance, CA, USA) were used for separation. Data were collected and analysed using the Agilent ChemStation (Hewlett-Packard, Waldbronn, Germany). The mobile phase was a mixture of acetonitrile and 0.01 M phosphate buffer pH 3.5 (50:50, v/v) containing 0.1% triethylamine. The mobile phase was filtered through a 0.45 μm filter and degassed under vacuum before use. The flow rate was 1.0 ml/min and the system was operated at ambient temperature.

2.3. Standards

A standard stock solution of celecoxib (1.0 mg/ml) was prepared by dissolving 10 mg of celecoxib in 10 ml of methanol. The working standard solution of celecoxib (100 μg/ml) was prepared by diluting the stock solution with methanol. The plasma and milk calibration curves of celecoxib were constructed by spiking drug-free human plasma or breast milk with celecoxib working standard solution at concentrations of 10, 20, 100, 200, 500, 1000 and 2000 μg/L.

Bulk celecoxib plasma and milk standards for determination of freeze–thaw stability were prepared in single 10 ml aliquots in the concentrations of 20, 200, and 2000 μg/L. Celecoxib plasma and milk quality control (QC) standards were prepared in the same concentrations as the bulk standards and stored in multiple 0.2 ml aliquots for assay with each analytical run. Both bulk standards and QC standards were stored at −30 °C until analysed.

2.4. Sample preparation

Acetonitrile 0.2 ml was added to 0.2 ml each of blank, standard, QC or the samples of plasma or milk to precipitate the proteins. Each mixture was vortexed for 30 s, centrifuged at 15,000 × *g* for 10 min, and 40 μl of clear supernatant was injected into the HPLC system. All the samples were analysed in triplicate and the mean values used in subsequent analyses.

2.5. Validation

The standard curve was a plot of the peak area of celecoxib versus the corresponding concentrations of celecoxib. The linearity of the standard curve was evaluated using least-squares linear regression analysis. To determine recovery of celecoxib at concentrations of 20, 200 and 2000 μg/L from plasma or milk, an identical set of standards in water was prepared and one volume equivalent of acetonitrile was added to the standards just before injection. Absolute recoveries at each concentration were measured by comparing the response of pre-treated standards with the response of standards that had not been subjected to sample pre-treatment. The effects of freezing and thawing on celecoxib concentrations were studied using bulk celecoxib plasma and breast milk standards at 20, 200 and 2000 μg/L, which were subjected to four freeze–thaw cycles before analysis. Quality control was assessed by analysis of six samples at each concentration on the same day (intra-day) and of one sample at each concentration on 6 different days (inter-day). Bias was determined as the measured minus the actual concentration, expressed as a percentage of the actual concentration. Imprecision was measured as intra- and inter-day coefficients of variation. The limit of quantification for this assay was defined as the lowest concentration of celecoxib that could be detected with intra- and inter-day coefficients of variation <20% ($n = 5$) and a mean value <20% deviation from the spiked value.

3. Results and discussion

3.1. Chromatography

Under the chromatographic conditions employed, the retention time of celecoxib was 8.2 min. Blank plasma/milk samples from more than six different sources of the same matrix were tested for interference, and the celecoxib peak was free of interference from any other peaks present in the plasma or milk blanks (Fig. 2). Celecoxib is metabolised to three metabolites in humans: an hydroxylated metabolite, a carboxylated metabolite and a glucuronide conjugate of the carboxylated metabolite [3]. We did not have the analyte metabolites available to test for interference. However, these metabolites are more hydrophilic than celecoxib and unlikely to elute at the same retention time as the parent compound under our HPLC conditions. To investigate possible interference from commonly co-administered drugs,

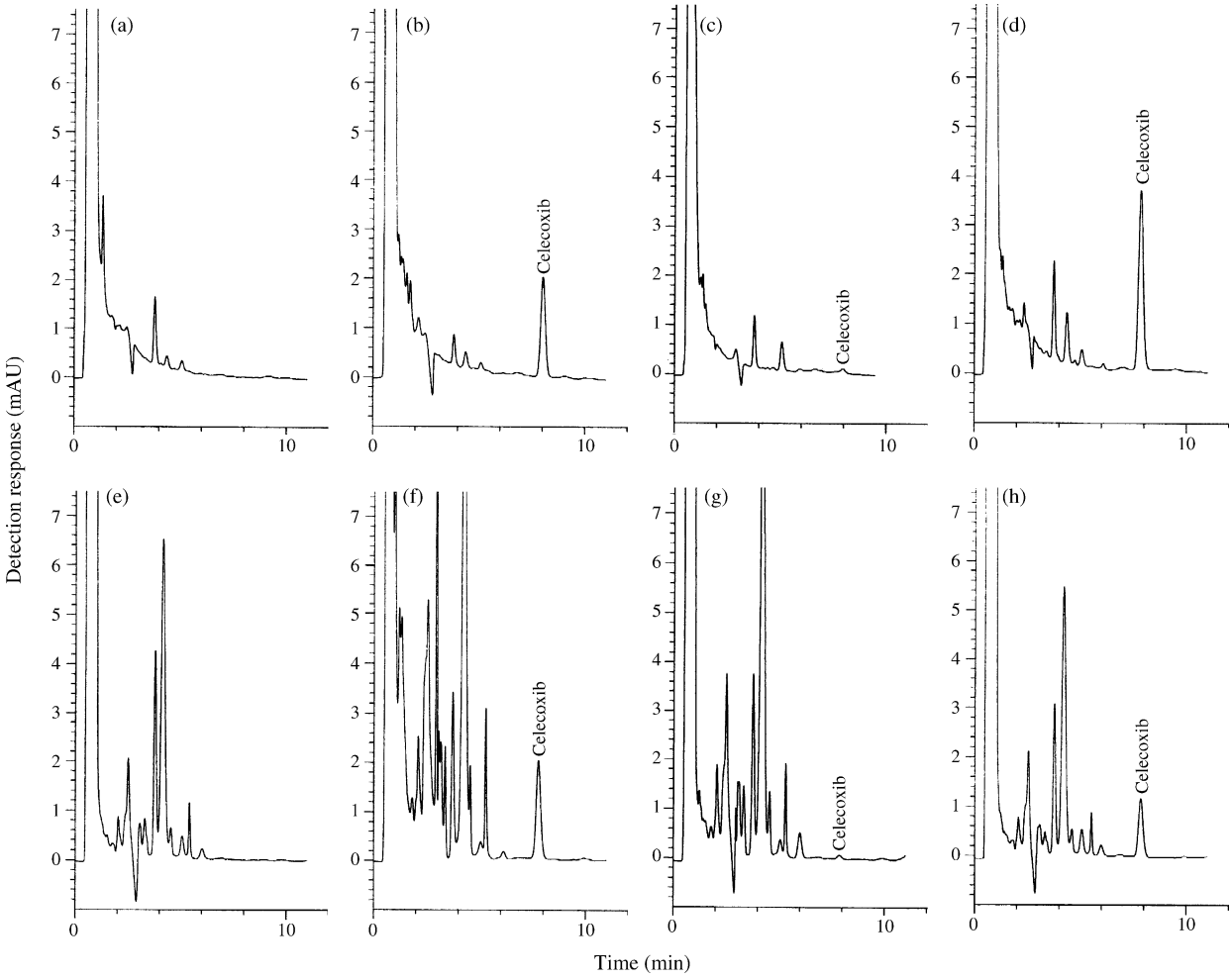


Fig. 2. HPLC chromatograms obtained from (a) blank plasma sample; (b) plasma sample spiked with celecoxib to give a concentration of 500 μg/L; (c) plasma sample spiked with celecoxib to give a concentration of 10 μg/L; (d) plasma sample taken from a healthy lactating volunteer 4 h after administration of a single oral dose of celecoxib 200 mg (celecoxib concentration = 910 μg/L); (e) blank breast milk sample; (f) breast milk sample spiked with celecoxib to give a concentration of 500 μg/L; (g) breast milk sample spiked with celecoxib to give a concentration of 10 μg/L; and (h) breast milk sample from a healthy lactating volunteer taken 4 h after administration of a single oral dose of celecoxib 200 mg (celecoxib concentration = 300 μg/L).

paracetamol, codeine, cimetidine, prednisone, sulphasalazine, omeprazole and methotrexate were analysed under our assay condition. No interference was observed.

3.2. Sample preparation

Protein precipitation became more efficient with increasing volumes of acetonitrile. However, greater volumes of acetonitrile diluted the sample, thereby affecting the sensitivity of the assay. To improve the sensitivity, the volume of acetonitrile was reduced, with a 1:1 ratio of acetonitrile to plasma or milk being optimal. Under this condition, the majority of protein was precipitated and celecoxib was free of interference from endogenous compounds in the plasma and milk. For long term performance, the guard column cartridge was changed every 150–200 injections. The analytical column demonstrated no deterioration of performance after more than 800 injections.

It is usually considered desirable to use an internal standard when performing HPLC quantitation to control for extraction and HPLC injection variability. This method did not utilize an internal standard as a simple sample preparation of precipitation was used, which was not suspected to result in large variability in the extraction recoveries. In addition the use of an autosampler improves the injection-volume precision and provides low HPLC injection variability [16]. The absence of significant bias (≤15%) and low intra- and inter-day coefficients of variation (all <10%) showed that our procedure was acceptable without an internal standard.

3.3. Linearity and limit of quantification

Nine lots of plasma/milk standard curves were freshly prepared using nine independent sources of the same matrix. In all cases, the plasma and milk standard curves of celecoxib were linear ($r^2 > 0.99$) over the concentration ranges 10–2000 μg/L. The intercept with the *y*-axis was not significantly different from zero. The slopes of plasma/milk standard curves in the nine different preparations were practically the same (the CVs were 1.03 and 2.43% for the slopes of plasma and milk standard curves, respectively). The limit of quantification of celecoxib

Table 1
Intra- and inter-day assay variance of the determination of plasma celecoxib

Type of variance	Sample	Concentration spiked (μg/L)	Concentration found (μg/L) (mean ± S.D.)	Bias (%)	Imprecision CV (%)
Intra-day ($n = 6$)	QC1	20.0	22.4 ± 0.7	+12.0	3.1
	QC2	200	192 ± 1.5	−4.0	0.8
	QC3	2000	2001 ± 24.3	+0.04	1.2
Inter-day ($n = 6$)	QC1	20.0	23.0 ± 2.2	+15.0	9.6
	QC2	200	196 ± 2.5	−2.0	1.3
	QC3	2000	2005 ± 19.3	+0.25	1.0

Table 2
Intra- and inter-day assay variance of the determination of milk celecoxib

Type of variance	Sample	Concentration spiked (μg/L)	Concentration found (μg/L) (mean ± S.D.)	Bias (%)	Imprecision CV (%)
Intra-day ($n = 6$)	QC1	20.0	21.8 ± 0.9	+9.0	4.1
	QC2	200	209 ± 4.2	+4.5	2.0
	QC3	2000	2004 ± 14.5	+0.2	0.7
Inter-day ($n = 6$)	QC1	20.0	19.3 ± 1.5	−3.5	7.8
	QC2	200	208 ± 1.8	+4.0	0.9
	QC3	2000	1987 ± 82.3	−0.65	4.1

was around 10 μg/L in both plasma and milk, at which the mean values were within ±15% of the spiked values and the intra- and inter-day coefficients of variation were <5%.

3.4. Recoveries

The absolute recoveries of celecoxib from plasma and breast milk determined at concentrations of 20, 200 and 2000 μg/L were similar and consistent. The mean ± S.D. absolute recoveries of celecoxib were 96.0 ± 3.3% from plasma ($n = 6$ plasma extracts at each concentration) and 98.3 ± 5.4% from breast milk ($n = 6$ breast milk extracts at each concentration).

3.5. Stability of celecoxib

Celecoxib was found to be stable in plasma and breast milk for at least four freeze–thaw cycles when stored at −30 °C. The mean values ($n = 4$) measured at each concentration deviated ≤5.0% from the spiked values for both plasma and breast milk samples. The standard stock solution of celecoxib was shown to remain stable for at least four months at 4 °C.

3.6. Bias and imprecision (Tables 1 and 2)

The bias of the method decreased as concentrations increased and there was no constant direction to the bias (i.e. + or −). The maximum bias observed was 15%, which related to the inter-day measurement of the 20 μg/L sample. Imprecision was small, as indicated by intra-day coefficients of variation of <5.0% and inter-day coefficients of variation of <10% for both plasma and milk assays at all concentrations.

4. Conclusions

A validated HPLC method has been described that is simpler than previous methods largely through the use of a simple one step protein precipitation instead of more complex extraction procedures. The method has proven to be rapid, sensitive, specific, accurate and precise, and is currently being used to analyse celecoxib concentrations in human plasma and breast milk after oral administration of celecoxib in lactating mothers.

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