Simple and rapid high-performance liquid chromatographic method for determination of celecoxib in plasma using UV detection: Application in pharmacokinetic studies

A. Zarghi [a,*], A. Shafaati [a], S.M. Foroutan [b], A. Khoddam [c]

[a] *Department of Pharmaceutical Chemistry, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, No. 10, Shams Alley, Across from Tavanir St., Vali-E-Asr Ave., Tehran, Iran*
[b] *Department of Pharmaceutics, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran*
[c] *Noor Research and Educational Institute, Tehran, Iran*

Abstract

A rapid, sensitive and reproducible HPLC method was developed and validated for the analysis of celecoxib in human plasma. The analysis was carried out on a monolithic silica column using UV detection at 254 nm. The assay enables the measurement of celecoxib for therapeutic drug monitoring with a minimum quantification limit of 10 ng ml^{-1}. The method involves simple, one-step extraction procedure, and analytical recovery was $100.5 \pm 1.3\%$. The calibration curve was linear over the concentration range of 10–800 ng ml^{-1}. The coefficients of variation for inter-day and intra-day assay were found to be less than 8%. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Celecoxib; Plasma; HPLC; Monolithic column

1. Introduction

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene sulfonamide is a cyclooxygenase-2 (COX-2) selective inhibitor that is used for treatment of osteoarthritis and rheumatoid arthritis. It is also used in the management of acute pain and dysmenorrhoea [1]. Celecoxib is readily absorbed from the gastro-intestinal tract with peak concentrations in plasma occurring about 2 h after administration by mouth. It is metabolized in the liver by the cytochrome P450 and is eliminated mainly as metabolites in the faeces and urine [2]. Several analytical methods have been presented for quantification of celecoxib in plasma including HPLC with UV [3–9] or fluorescence [10,11], MS [12,13] and MS–MS [14]. For the sample preparation, most of these methods require tedious extraction procedures, which involve liquid–liquid extraction [4–6,8,12,13], protein precipitation followed by liquid–liquid extraction [9], solid-phase extraction [7,10,14] and protein pre-

cipitation followed by solid-phase extraction [3]. Therefore, sample preparation is time-consuming, complex or both. Gurguis et al. [4] used isooctane–isopropanol as the extraction solvent and C18 column as stationary phase. However, it has been reported that this extraction solvent gave a noisy chromatogram, which decreased the sensitivity of the method [8]. Moreover, the mean recovery obtained for celecoxib with this method was low (70%). Altering the extraction solvent from isooctane–isopropanol to chloroform increased the mean recovery of celecoxib from 70 to 89% [8]. The extraction procedure described by Schonberger et al. [11] using large volume of chloroform for low amount of plasma sample practically caused some difficulties during mixing of samples. In addition, in some cases creation of emulsion prevented the separation of aqueous and organic phase. The extraction method of Chow et al. [7] involves solid phase extraction of celecoxib from plasma using C18 extraction cartridges. However, this extraction method is not suitable for the processing of multiple samples in a limited amount of time for bioequivalence and pharmacokinetic studies. A normal-phase HPLC method using column switching with a nitrophenyl column and UV detection for the determination of celecoxib in human plasma has been also reported by

---

* Corresponding author. Tel.: +98 21 88773521.
E-mail address: azarghi@yahoo.com (A. Zarghi).

Rose et al. [3]. Limit of quantification (LOQ) of this method is 25 ng ml^{-1} and a large volume of plasma samples is needed for analysis. Moreover, most of the aforementioned methods need long chromatographic elution time for analysis of celecoxib in plasma and were not suitable in all conditions. HPLC methods based on MS or MS–MS [12–14] as the detection system for the analysis of celecoxib in plasma are very sensitive, having low quantitation limits. However, these methods are not available for most laboratories because of their specialty requirement and financial reasons. The present study describes a rapid and sensitive HPLC method using a monolithic column with UV detection, which enables the determination of celecoxib with good accuracy at low drug concentrations in plasma using single-step extraction procedure. Separation was performed on a reversed-phase monolithic column, which has lower separation impedance compared with the particulate packings, and therefore, it allows easy optimizing chromatographic conditions to obtain desirable resolution in a short time. The sample preparation only involves a simple one-step protein precipitation and no evaporation step is required. Also, the use of a smaller sample volume provides an advantage as compared with some previous methods that require large sample volume for analysis of celecoxib. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

2. Experimental

2.1. Chemicals

Celecoxib and mefenamic acid were supplied by Kimidarou Pharmaceuticals (Tehran, Iran). Celecoxib is available as oral capsule containing 200 mg of celecoxib and other inactive ingredients such as lactose monohydrate, sodium lauryl sulphate, povidone K30 and magnesium stearate. HPLC-grade acetonitrile and all other chemicals were obtained from Merck (Darmstadt, Germany). Water was obtained by double distillation and purified additionally with a Milli-Q system.

2.2. Instrumentation

The chromatographic apparatus consisted of a model Wellchrom K-1001 pump, a model Rheodyne 7125 injector and a model K 2501 UV detector connected to a model Eurochrom 2000 integrator, all from Knauer (Berlin, Germany).

2.3. Chromatographic conditions

The separation was performed on Chromolith Performance (RP-18e, 100 mm × 4.6 mm) column from Merck (Darmstadt, Germany). The wavelength was set at 254 nm. The mobile phase consisted acetonitrile, methanol and distilled water (45:10:45, v/v/v) containing 0.2% acetic acid (pH 3.5) at a flow rate of 2 ml min^{-1}. The mobile phase was prepared daily and degassed by ultrasonication before use. The mobile phase was not allowed to recirculate during the analysis.

2.4. Standard solutions

Stock solutions (16 mg ml^{-1}) of celecoxib were prepared in methanol and 1600 and 16 μg ml^{-1} solutions were made by dilution in methanol. Then 0.1, 0.2, 0.5, 1, 2, 4, 6 and 8 μg ml^{-1} working standards were prepared from the 16 μg ml^{-1} solutions and stored at +4 °C.

The solution of mefenamic acid, internal standard was prepared by dissolving 10 mg mefenamic acid in 10 ml methanol to obtain a concentration of 1 mg ml^{-1}. The final solution was obtained by diluting this solution with methanol to give concentration of 5 μg ml^{-1} of mefenamic acid and stored at +4 °C.

2.5. Sample preparation

To 450 μl of plasma in a glass-stoppered 15-ml centrifuge tube were added 50 μl of mefenamic acid as internal standard (5 μg ml^{-1}), 500 μl of acetonitrile and 100-mg NaCl. After mixing (30 s), the mixture centrifuged for 15 min at 8000 rpm. Then 20 μl of supernatant was injected into liquid chromatograph.

2.6. Biological samples

Twelve healthy male volunteers were included in this study. The study protocol was approved by the Ethics Committee of Shaheed Beheshti University of Medical Sciences, and a written informed consent was obtained from the volunteers. Celecoxib was administered in a single dose of 200 mg to the volunteers after overnight fasting. Plasma samples were collected at several intervals after dosing and then frozen immediately at −20 °C until assayed.

2.7. Stability

The stability of celecoxib was assessed for spiked plasma samples stored at −20 °C for up to 2 months, +4 °C for at least 1 month and at ambient temperature for 12 h. The stability of stock solutions stored at above mentioned temperatures was determined by injecting appropriate dilutions of stocks in distilled water at different days and comparing their peak areas with fresh stock prepared on the day of analysis. Samples were considered to be stable, if the assay values were within the acceptable limits of accuracy and precision.

2.8. Plasma calibration curves and quantitation

Blank plasma was prepared from heparinized whole-blood samples collected from healthy volunteers and stored at −20 °C. After thawing, 50 μl of one of the above-mentioned celecoxib working standards were added to yield final concentrations of 10, 20, 50, 100, 200, 400, 600 and 800 ng ml^{-1}. Internal standard solution was added to each of these samples to yield a concentration of 500 ng ml^{-1}. The samples were then prepared for analysis as described above.

Calibration curves were constructed by plotting peak area ratio (y) of celecoxib to the internal standard versus celecoxib concentrations (x). A linear regression was used for quantitation.

2.9. Selectivity and specificity

Control human plasma, obtained from 12 healthy volunteers, was assessed by the procedure as described above and compared with respective plasma samples to evaluate selectivity of the method. Aspirin, naproxen, indomthacin and codeine were also tested for potential interferences.

2.10. Precision and accuracy

The precision and accuracy of the method were examined by adding known amounts of celecoxib to pool plasma (quality control samples). For intra-day precision and accuracy six replicate quality control samples at each concentration were assayed on the same day. The inter-day precision and accuracy were evaluated on three different days.

2.11. Limit of quantification and recovery

For the concentration to be accepted as LOQ, the percent deviation from the nominal concentration (accuracy) and the relative standard deviation must be ±10% and less than 10%, respectively, considering at least five times the response compared with the blank response. The analytical recovery for plasma at four different concentrations of celecoxib (10, 50, 200 and $500 \, ng \, ml^{-1}$) was determined. Known amounts of celecoxib were added to drug-free plasma and the internal standard was then added. The relative recovery of celecoxib was calculated by comparing the peak areas for extracted celecoxib from spiked plasma and a standard solution of celecoxib in methanol containing internal standard with the same initial concentration (six samples for each concentration level).

2.12. Pharmacokinetic analysis

Celecoxib pharmacokinetic parameters were determined by non-compartmental methods. Elimination rate constant (K_{el}) was estimated by the least-square regression of plasma concentration-time data points in the terminal log-linear region of the curves. Half-life was calculated as 0.693 divided by K_{el}. The area under the plasma concentration–time curve from zero to the last measurable plasma concentration at time t (AUC_{0-t}) was calculated using the linear trapezoidal rule. Peak plasma concentration (C_{max}) and time to peak concentration (T_{max}) were obtained directly from the individual plasma concentration versus time curves.

3. Results and discussion

Under the chromatographic conditions described, celecoxib and the internal standard peaks were well resolved. Endogenous plasma components did not give any interfering peaks. Separation was performed on a reversed-phase monolithic column, which has lower separation impedance compared with the particulate packings, and therefore, it allows easy optimizing chromatographic conditions to obtain desirable resolution in a short time. Owing to use of the monolithic column, much faster

separations are possible as compared with traditional chromatographic columns packed with porous particles. Accordingly, the chromatographic elution step is undertaken in a short time (less than 6 min) with high resolution. A mobile phase consisted acetonitrile, water, and methanol in the ratio of 45:45:10 containing 0.2% acetic acid was optimum to achieve the best resolution between celecoxib and internal standard peaks. The increased percentage of acetonitrile or methanol reduced the retention time of mefenamic acid and celecoxib peaks resulting by the interference of latter and endogenous plasma peaks. Using less amount of acetonitrile or methanol gave tailing for celecoxib and internal standard peaks. Fig. 1 shows typical chromatograms of blank plasma in comparison with spiked samples analyzed for a pharmacokinetic study. The average retention times of celecoxib and mefenamic acid were 3.6 and 4.8 min, respectively. Celecoxib is metabolized to three inactive metabolites in humans including hydroxylated and carboxylated metabolites and the glucuronic acid conjugated of the carboxylated metabolite [15]. We did not have the authentic metabolite standards available to test for interference. However, these metabolites are more hydrophilic than celecoxib and unlikely to elute at the
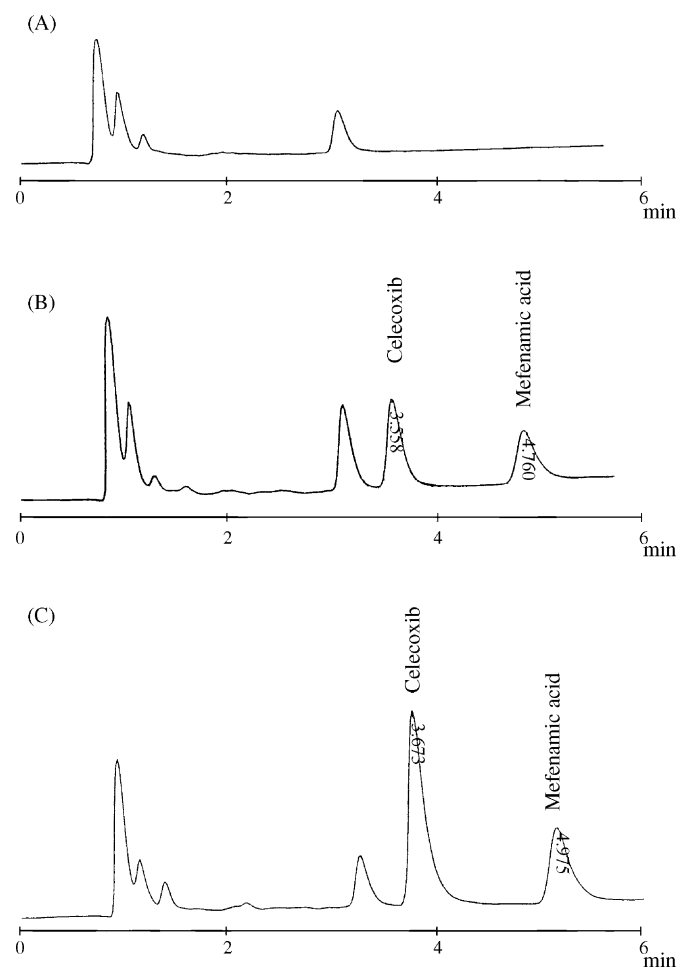

Fig. 1. Chromatograms of (A) blank plasma; (B) blank plasma spiked with $200 \, ng \, ml^{-1}$ celecoxib and $500 \, ng \, ml^{-1}$ mefenamic acid (internal standard); (C) plasma sample from a healthy volunteer 2 h after oral administration of 200 mg of celecoxib.

Table 1
Assay linearity

	Coefficient of the linear regression analysis ($r \pm$ S.D.)	Slope $\pm$ S.D.	Intercept $\pm$ S.D.
Intra-assay $n = 6$	$0.9998 \pm 6.60 \times 10^{-4}$ R.S.D. = 0.0660%	0.00328 ± 0.00005 R.S.D. = 1.52%	0.0147 ± 0.0006
Inter-assay $n = 9$	$0.9995 \pm 8.20 \times 10^{-4}$ R.S.D. = 0.0821%	0.00331 ± 0.00006 R.S.D. = 1.81%	0.0150 ± 0.0008

Table 2
Recovery data of celecoxib from plasma

Celecoxib spiked concentration (ng ml^{-1})	Celecoxib concentraion found ($n = 6$)	Recovery (mean $\pm$ S.D.)%
10	9.8	98.1 ± 1.1
50	50.2	100.5 ± 1.9
200	204.0	102.0 ± 0.6
500	508.0	101.6 ± 1.8

Table 3
Reproducibility of the analysis of celecoxib in human plasma ($n = 6$)

Concentration added (ng mL^{-1})	Concentration measured (mean $\pm$ S.D.)	
	Intra-day	Inter-day
10	9.8 ± 0.7 (7.2)	9.8 ± 0.8 (7.9)
20	21.0 ± 1.5 (7.1)	21.6 ± 1.1 (5.1)
200	208.5 ± 12.3 (5.9)	206.5 ± 11.9 (5.8)
500	495.4 ± 18.8 (3.8)	505.6 ± 19.2 (3.7)

Values in parentheses are coefficients of variation (%).

same retention time as the parent compound under described chromatographic conditions. To investigate possible interference from commonly co-administered drugs, aspirin, naproxen, indomethacin and codeine were analysed under our chromatographic condition. None of the drugs mentioned above interfered with analytes peaks as well. For the sample preparation, several tedious extraction methods have been used for analysis of celecoxib in biological fluids [3–6,8,9]. In the method of Gurguis et al. [4] isooctane–isopropanol as the extraction solvent and C18 column as stationary phase were used. However, it has been reported that this extraction solvent gave a noisy chromatogram, which decreased the sensitivity of the method [8]. Moreover, the mean recovery obtained for celecoxib with this method was low (70%). The method described by Schonberger et al. [11] using large volume of chloroform for 0.5 ml of plasma practically caused some difficulties during mixing of samples. In addition, in some cases creation of emulsion prevented the separation of aqueous and organic phase. The extraction method of Chow et al. [7] involves solid phase extraction of celecoxib from plasma using C18 extraction cartridges. However, this extraction method is expensive and is not suitable for processing of multiple samples in a limited amount of time for bioequivalence and pharmacokinetic studies. In our method, sample preparation involves protein precipitation, and no evaporation step is required. 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, a 1:1 ratio of acetonitrile to plasma was considered for sample preparation. Under this condition, the majority of protein was precipitated, and celecoxib and internal standard were free of inference from endogenous components in plasma. The calibration curve for the determination of celecoxib in plasma was linear over the range 10–800 ng ml^{-1}. The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (r) for calibration curves were either equal to or better than 0.999. The slops of plasma standard curves in the nine different preparations were practically the same (the CVs were less than 2% for the slopes of plasma standard curves). For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression curves (Table 1). The analytical recovery for plasma at four different concentrations of celecoxib was determined. Known amounts of celecoxib were added to drug-free plasma in concentrations ranging from 10 to 500 ng ml^{-1}. The internal standard was added and the absolute

recovery of celecoxib was calculated by comparing the peak areas for extracted celecoxib from spiked plasma and a standard solution of celecoxib in methanol containing internal standard with the same initial concentration. As shown in Table 2 the average recovery was $100.5 \pm 1.3\%$ ($n = 6$) which was considerably higher than some previous reported methods [4–8,10]. Using UV detection method, the limit of quantification obtained was 10 ng ml^{-1} by analyzing this amount of celecoxib in a drug-free plasma sample, which was comparable with those obtained by previous methods using LC–MS (LOQ = 5 ng ml^{-1}) [12] or fluorescence detector (LOQ = 10 ng ml^{-1})[10]. This is sensitive enough for drug monitoring and other purposes such as pharmacokinetic studies. We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of celecoxib. As shown in Table 3, coefficients of variation were less than 8%, which is acceptable for the routine measurement of celecoxib. Stability was determined for spiked plasma samples under the conditions as previously described. The results showed that the samples were stable during the mentioned conditions. Over 500 plasma samples were analyzed by
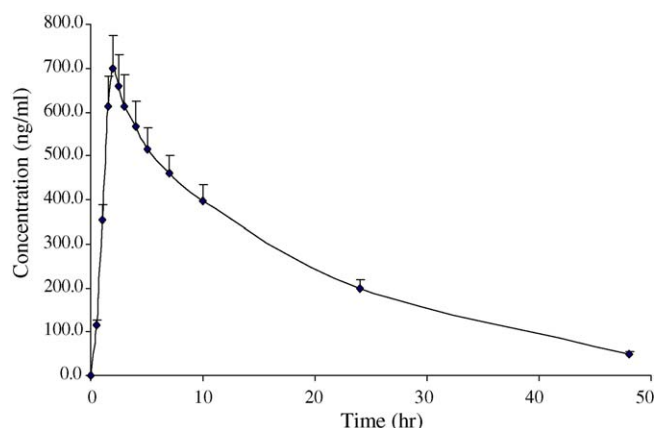
Fig. 2. Mean plasma concentration–time profile of celecoxib in healthy volunteers ($n = 12$) after a single 200 mg celecoxib.

Table 4
Pharmacokinetic parameters of celecoxib in healthy volunteers following a single oral dose of 200 mg of celecoxib

Parameter	Result (mean ± S.D.)
T_{max} (h)	18.1 ± 0.3
C_{max} (ng ml^{-1})	699.1 ± 78.6
AUC$_{0-t}$ (ng h ml^{-1})	11942.1 ± 1422.9
K_{el} (h^{-1})	0.055 ± 0.002
$T_{1/2}$ (h)	11.85 ± 0.61

this method without any significant loss of resolution. No change in the column efficiency and back pressure was also observed over the entire study time, thus proving its suitability. In this study plasma concentrations were determined in 12 healthy volunteers, who received 200 mg of celecoxib each. Fig. 2 shows the mean plasma concentration–time profile of celecoxib. The derived pharmacokinetic parameters of 12 healthy volunteers are summarized in Table 4. These pharmacokinetic parameters are in good agreement with that found previously [11,15].

4. Conclusions

A rapid and simple HPLC method using UV detector has been described for analysis of celecoxib in human plasma. Using reversed-phase monolithic column, the chromatographic elution step is undertaken in a short time (run time < 6 min) with high resolution. Sample preparation involves protein precipitation using acetonitrile and no evaporation step is required. Moreover, due to low LOQ (10 ng/ml), good accuracy and precision this method is suitable for pharmacokinetic and bioequivalence studies of celecoxib in humans.

References

[1] N.E. Lane, J. Rheumatol. 24 (Suppl. 49) (1997) 20.
[2] D. Clemett, K.L. Goa, Drugs 59 (2000) 957.
[3] N.M. Davies, A.J. McLachlan, R.O. Day, K.M. Williams, Clin. Pharmacokinet. 38 (2000) 225;
    M.J. Rose, E.J. Woolf, B.K. Matuszewski, J. Chromatogr. B 738 (2000) 377.
[4] M.S. Guirguis, S. Sattari, F. Jamali, J. Pharm. Pharm. Sci. 4 (2001) 1.
[5] G. Jayasacar, M.K. Kumar, K. Chandrascknar, P.S. Prasad, Y.M. Rao, Pharmazie 57 (2002) 619.
[6] E. Stromer, S. Bauer, J. Kirchheiner, J. Brockmoller, I. Roots, J. Chromatogr. B 783 (2003) 207.
[7] H.H.S. Chow, N. Anavy, D. Salazar, D.H. Frank, D.S. Alberts, J. Pharm. Biomed. Anal. 34 (2004) 167.
[8] H. Jalalizadeh, M. Amini, V. Ziaee, A. Safa, H. Farsam, A. Shafiee, J. Pharm. Biomed. Anal. 35 (2004) 665.
[9] T.W. Hale, R. McDonald, J. Boger, J. Hum. Lact. 20 (2004) 397.
[10] S.K. Paulson, T.A. Kaprak, C.J. Gresk, D.M. Fast, M.T. Baratta, E.G. Burton, A.P. Breau, A. Karim, Biopharm. Drug Dispos. 20 (1999) 293.
[11] F. Schonberger, G. Heinkele, T.E. Murdter, S. Brenner, U. Klotz, U. Hofmann, J. Chromatogr. B 768 (2002) 255.
[12] U. Werner, D. Werner, A. Pahl, R. Mundkowski, M. Gillich, K. Brune, Biomed. Chromatogr. 16 (2002) 56.
[13] M. Abdel-Hamid, L. Novotny, H. Hamza, J. Chromatogr. B 753 (2001) 401.
[14] L. Brautigam, G. Vetter, I. Tegeder, G. Heinkele, G. Geisslinger, J. Chromatogr. B 761 (2001) 203.
[15] N.M. Davies, A.J. McLachlan, R.O. Day, K.M. Williams, Clin. Pharmacokinet. 38 (2000) 225.