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Quantitation of Valdecoxib in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection using liquid–liquid extraction

N.V.S. Ramakrishna*, K.N. Vishwottam, S. Wishu, M. Koteshwara

Biopharmaceutical Research, Suven Life Sciences Ltd, Serene Chambers, Road # 7, Banjara Hills, Hyderabad 500034, India

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Abstract

A simple, sensitive and specific HPLC method with UV detection (210 nm) was developed and validated for quantitation of Valdecoxib in human plasma, the newest addition to the group of non-steroidal anti-inflammatory drugs—a highly selective cyclooxygenase-2 inhibitor. The analyte and an internal standard (Rofecoxib) were extracted with diethyl ether/dichloromethane (70/30 (v/v)). The chromatographic separation was performed on reverse phase ODS-AQ column with an isocratic mobile phase of water/methanol (47/53 (v/v)). The lower limit of quantitation was 10 ng/ml, with a relative standard deviation of <20%. A linear range of 10–500 ng/ml was established. This HPLC method was validated with between-batch and within-batch precision of 1.27–7.45 and 0.79–6.12%, respectively. The between-batch and within-batch bias was 0.74–7.40 and −0.93 to 7.70%, respectively. Frequently coadministered drugs did not interfere with the described methodology. Stability of Valdecoxib in plasma was excellent, with no evidence of degradation during sample processing (autosampler) and 30 days storage in a freezer. This validated method is suitable for bioequivalence studies following single dose in healthy volunteers.

Keywords: Valdecoxib

1. Introduction

Valdecoxib [4-(5-methyl-3-phenyl-4-isoxazolyl)benzenesulfonamide] is the newest addition to the group of non-steroidal anti-inflammatory drugs (NSAIDs) known as selective cyclooxygenase-2 inhibitors (e.g., Celecoxib and Rofecoxib). This drug has been recently approved by the US FDA for treatment in rheumatoid arthritis, osteoarthritis and pain [1–5].

The NSAIDs exert their anti-inflammatory analgesic and antipyretic activities through the inhibition of cyclooxygenase (COX), a key enzyme for prostanoid synthesis. The enzyme exists as two isoforms: a constitutive form, COX-1, and an inducible form, COX-2 [6]. COX-1 is involved in prostaglandin synthesis and inhibition of this enzyme by non-selective NSAIDs is thought to be responsible for damage to the gastric mucosa and for antiplatelet activity, increasing the risk of bleeding. In contrast, COX-2 primarily synthesizes prostaglandins involved in inflammation. Selective inhibition of COX-2, while preserving COX-1 function, suppresses inflammation without causing the gastric adverse effects on increasing the risk of bleeding [1,7].

To date, no simple HPLC method has been reported for Valdecoxib quantitation in plasma. Zhang et al. presented a LC–MS/MS method with automated extraction for quantitation of Valdecoxib and its metabolites in human urine [8]. A sensitive analytical method is necessary for quantitation of the concentrations of Valdecoxib in human plasma in order to support pharmacokinetic and bioequivalence studies. The method reported in this paper is a simple and accurate HPLC–UV method to determine the plasma concentration of Valdecoxib using liquid/liquid extraction. Additionally, this method provides information about the stability of Valdecoxib in plasma and during sample processing (autosampler).

* Corresponding author. Tel.: +91-40-23556038/23541142; fax: +91-40-23541152.
E-mail address: nvsrk@suven.com (N.V.S. Ramakrishna).

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2. Experimental

2.1. Chemicals

Valdecoxib drug substance was from Hetero Drugs Ltd (Hyderabad, India). Rofecoxib (internal standard, I.S.) was obtained from Cadila Healthcare Limited (Ahmedabad, India). Chemical structures are presented in Fig. 1. A stock solution of Valdecoxib (1 mg/ml) was prepared in methanol, and a stock solution of the I.S. (1 mg/ml) was prepared in acetonitrile. HPLC-grade LiChrosolv methanol, diethylether and dichloromethane were from Merck (Darmstadt, Germany). HPLC Type I water from Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

2.2. Chromatography

The integrated high performance liquid chromatography system (LC 2010C, Shimadzu Corporation, Kyoto, Japan) was equipped with a quaternary pump, a degasser, an autosampler, an injector with a 100 μl loop, a column oven, a UV detector and a data system (Class VP version 6.12). The separation of compounds was made on a YMC ODS-AQ column (5 μm, 250 mm × 4.6 mm i.d.) at ambient temperature. The mobile phase was a mixture of water/methanol (47/53 (v/v)) pumped at a flow-rate of 1.0 ml/min. Detection was set at a wavelength of 210 nm.

2.3. Sample processing

A 1 ml volume of plasma was transferred to a 15 ml glass test tube, and then 50 μl of Rofecoxib working solution (6 μg/ml) was spiked. Next a 5 ml aliquot of extraction solvent, diethyl ether/dichloromethane (7/3), was added using Dispensette Organic (Brand GmbH, Postfach, Germany). The sample was vortex-mixed for 5 min using a Multi-Pulse Vortexer (Glas-Col, Terre Haute, USA). The sample was then centrifuged using Multifuge 3S-R (Kendro Laboratory Products, Sorvall-Heraeus, Germany) for 5 min at 800 × *g*. The organic layer was quantitatively transferred to a 6 ml glass tube and evaporated to dryness using a TurboVap LV Evaporator (Zymark, Hopkinton, MA, USA) at 40 °C under a stream of nitrogen. Then, the dried extract was reconstituted in 200 μl of water/methanol (80/20 (v/v); diluent) and a 100 μl aliquot was injected into chromatographic system.

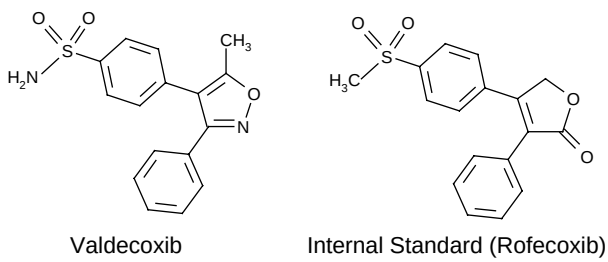

Fig. 1. Chemical structures of Valdecoxib and Internal Standard (Rofecoxib).

2.4. Bioanalytical method validation

2.4.1. Calibration and control samples

Working solutions for calibration and controls were prepared from the stock solution by an adequate dilution using diluent. The I.S. working solution (6 μg/ml) was prepared by diluting stock solution with diluent. 50 μl of working solutions were added to 950 μl of drug-free plasma to obtain Valdecoxib concentrations of 10, 20, 50, 80, 100, 200, 300 and 500 ng/ml. The quality control samples were prepared in pool, at concentrations of 10 ng/ml (LLOQ): 30 ng/ml (low), 250 ng/ml (medium) and 400 ng/ml (high), as a single batch at each concentration, and then divided in aliquots that were stored in the freezer at −70 °C until analysis.

A calibration curve was constructed from a blank sample (a plasma sample processed without an I.S.), a zero sample (a plasma processed with I.S.) and eight non-zero samples covering the total range (10–500 ng/ml), including lower limit of quantification (LLOQ). Such calibration curves were generated on seven consecutive days. Linearity was assessed by a weighted ($1/x^2$) least squares regression analysis. The calibration curve had to have a correlation coefficient (r^2) of 0.99 or better. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value except LLOQ, which was set at 20%.

2.4.2. Specificity

Randomly selected blank human plasma samples, which were collected under controlled conditions, were carried through the extraction procedure and chromatographed to determine the extent to which endogenous plasma components may contribute to interference with the analyte or the internal standard. The results were compared with the results to those obtained from a solution with a drug concentration nominally at 10 ng/ml of an extracted Valdecoxib plasma sample.

2.4.3. Recovery

Recovery of Valdecoxib was evaluated by comparing the mean peak areas of six extracted low, medium and high quality control samples to mean peak areas of six neat reference solutions (unprocessed). Recovery of Rofecoxib (I.S.) was evaluated by comparing the mean peak areas of ten extracted quality control samples to mean peak areas of ten neat reference solutions (unprocessed) of the same concentration.

2.4.4. Accuracy and precision

Within-batch accuracy and precision evaluations were performed by repeated analysis of Valdecoxib in human plasma. The run consisted of a calibration curve plus six replicates of each LLOQ, low, medium and high quality control samples. Between-batch accuracy and precision were assessed by analysis of samples consisting of a calibration

curve and six replicates of LLOQ, low, medium and high quality control samples for Valdecoxib on three separate occasions.

The overall precision of the method expressed as relative standard deviation and accuracy of the method expressed in terms of bias (percentage deviation from true value).

2.4.5. Stability

The short-term stability was examined by keeping replicates of the low and high plasma quality control samples at room temperature for approximately 24 h. Freeze–thaw stability of the samples was obtained over three freeze–thaw cycles, by thawing at room temperature for 2–3 h, refrozen for 12–24 h. Autosampler stability of Valdecoxib was tested by analysis of processed and reconstituted low and high plasma QC samples, which are stored in the autosampler tray for 24 h. Stability of Valdecoxib in human plasma was tested after storage at approximately −70 °C for 30 days. For each concentration and each storage condition, six replicates were analyzed in one analytical batch. The concentration of Valdecoxib after each storage period was related to the initial concentration as determined for the samples that were freshly prepared and processed immediately.

3. Results and discussion

3.1. Separation

Valdecoxib and I.S. were well separated from co-extracted material under the described chromatographic conditions at retention times of 11.9 and 7.9 min, respectively (Fig. 2). The peaks were of good shape, completely resolved one from another at therapeutic concentrations of Valdecoxib. No interference with constituents from the plasma matrix was observed.

3.2. Linearity and sensitivity of the assay

Linear least-square regression analysis of the calibration graph on seven different days demonstrated, linearity between the response and the nominal concentration of Valdecoxib over the range of 10–500 ng/ml. Table 1 shows the equations of the standard curves of Valdecoxib in plasma on 7 different days. The results of linear regression analysis show that the correlation coefficients of all standards curves were better than 0.993. The data show the excellent reproducibility of the sample analysis. Table 2 shows mean and descriptive statistics of back calculated concentrations in calibration curve.

3.3. Extraction

The extraction recovery of Valdecoxib was 79.7% on average, and the dependence on concentration is negligible.

Table 1
Standard curves for HPLC assay of Valdecoxib in plasma[a]

	Slope	Intercept	r
Day 1	209.275	−1.47846	0.99894
Day 2	208.433	1.44885	0.99627
Day 3	211.860	−2.62850	0.99660
Day 4	204.727	−0.40468	0.99924
Day 5	201.306	−3.10795	0.99921
Day 6	187.986	−0.60397	0.99377
Day 7	198.086	−1.44953	0.99945
Mean	203.096	−1.17489	0.99764
S.D.	8.192	1.51770	0.00216

[a] Eight calibration standards were included in each calibration curve.

The recovery of internal standard, Rofecoxib was 97.42% at the concentration used in the assay (300 ng/ml).

3.4. Specificity

There were no interfering peaks present in six different randomly selected samples of drug free human plasma used for analysis at the retention times of either analyte or internal standard. There was no interference of Valdecoxib and Rofecoxib analysis by other potentially co-administered drugs such as paracetamol, nicotinamide, ibuprofen, caffeine, aspirin, ampicillin, amoxicillin, loratadine, desloratadine, atorvastatin, cerivastatin, clopidogrel, metformin, glimepiride, venlafaxine, celecoxib, naproxen and nimuselide.

3.5. Accuracy of the assay

The accuracy values for between- and within-batch studies at the LLOQ and at low, medium and high concentrations of Valdecoxib in plasma were within acceptable limits ($n = 3$) (Table 3).

3.6. Precision of the methods

3.6.1. Within-batch variability of the assay

The results shown in Table 3 indicate that the assay method is reproducible for replicate analysis of Valdecoxib in human plasma within the same day.

Table 2
Statistical evaluation of the analysis results for Valdecoxib in standard curves during seven days

Concentration added (ng/ml)	Concentration found (mean ± S.D. $n = 7$) (ng/ml)	Precision (%)	Bias (%)
10	9.93 ± 0.29	2.89	−0.70
20	20.07 ± 0.96	4.77	0.35
50	50.21 ± 0.74	1.47	0.42
80	81.37 ± 3.60	4.43	1.71
100	102.40 ± 2.69	2.63	2.40
200	197.70 ± 3.86	1.95	−1.15
300	286.97 ± 5.93	2.07	−4.34
500	498.03 ± 13.75	2.76	−0.39

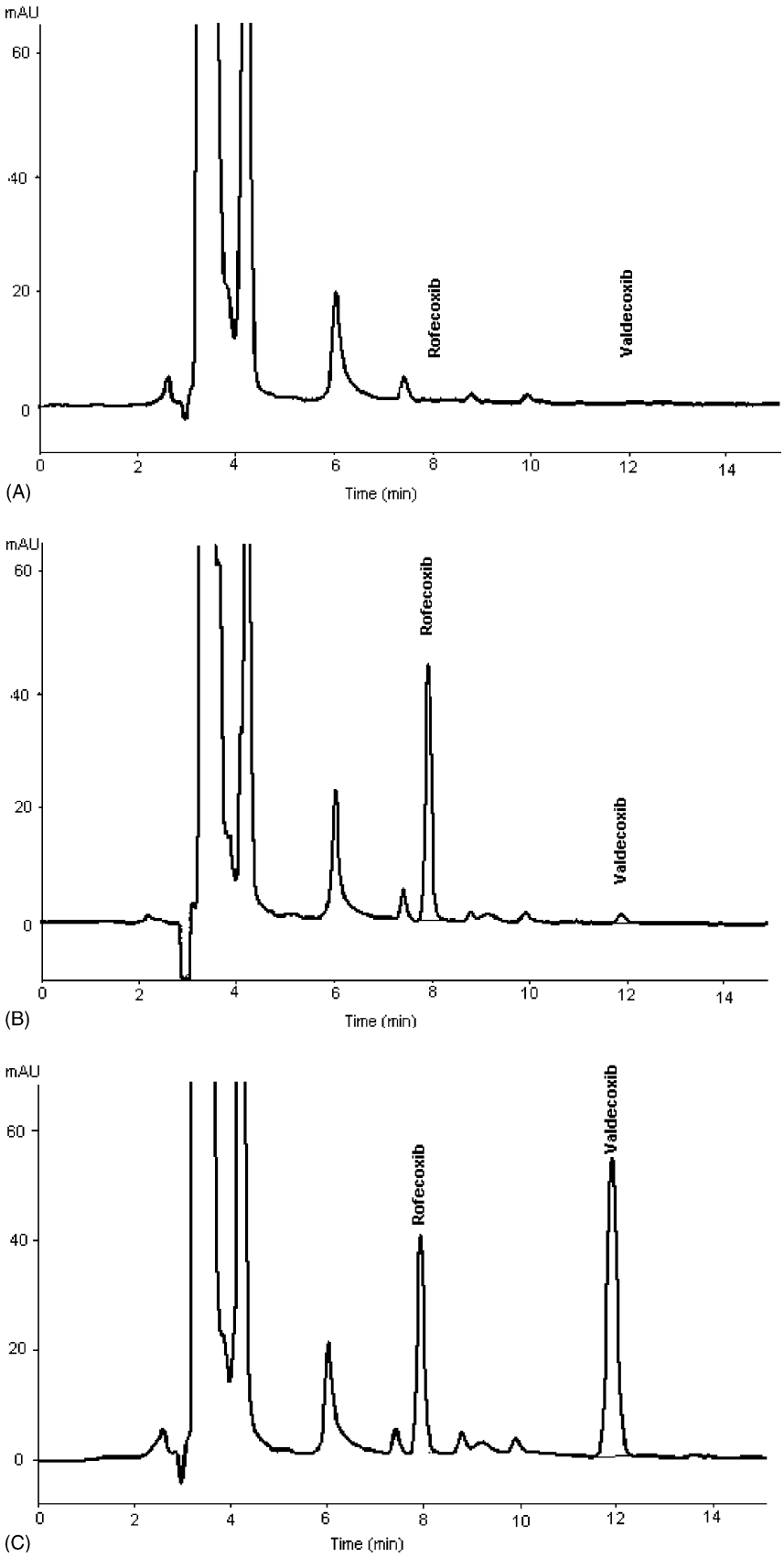

Fig. 2. Chromatograms resulting from (A) the analysis of blank human plasma, (B) human plasma spiked with 10 ng/ml of Valdecoxib and 300 ng/ml of I.S. and (C) human plasma spiked with 400 ng/ml of Valdecoxib and 300 ng/ml of I.S., respectively. Approximate retention times: Valdecoxib: 11.9 min; Rofecoxib: 7.9 min.

Table 3
Accuracy and precision of the HPLC method for determining Valdecoxib concentrations in plasma samples

Concentration added (ng/ml)	Within-batch precision ($n = 6$)			Between-batch precision ($n = 3$)		
	Concentration found (mean ± S.D.) (ng/ml)	Precision (%)	Bias (%)	Concentration found (mean ± S.D.) (ng/ml)	Precision (%)	Bias (%)
10	10.77 ± 0.66	6.12	7.70	10.74 ± 0.41	7.45	7.40
30	32.22 ± 0.90	2.81	7.40	30.38 ± 1.59	4.98	1.26
250	252.08 ± 2.91	1.15	0.83	251.81 ± 0.29	1.49	0.72
400	396.28 ± 3.14	0.79	−0.93	397.02 ± 1.21	1.27	0.74

3.6.2. Between-batch variability of the assay

The results shown in Table 3 indicate that the assay method is reproducible on different days.

3.7. Stability

The stability experiments were aimed at testing all possible conditions that the samples might experience after collecting and prior the analysis. These were performed as described in Section 2.4.5. All stability results are summarized in Table 4. Three freeze–thaw cycles and 24 h room temperature storage for low and high quality controls samples indicated that Valdecoxib was stable in human plasma under these conditions. QC samples were stable for at least 30 days if stored frozen at approximately −70 °C. Testing of autosampler stability of quality control samples (Table 4) indicated that Valdecoxib is stable when kept in the autosampler for up to 24 h.

Table 4
Stability of the samples

Sample concentration (ng/ml)	Concentration found (ng/ml)	Precision (%)	Bias (%)
Short-term stability for 24 h ($n = 6$) in plasma			
30	27.27	5.03	−9.1
400	415.82	2.06	3.95
Three freeze and thaw cycles ($n = 6$)			
30	30.13	4.02	0.43
400	404.02	2.43	1.01
Autosampler stability for 24 h ($n = 6$) (after extracting and reconstitution)			
30	27.82	5.43	−7.2
400	399.37	0.58	0.15
30-days stability at −70 °C ($n = 6$)			
30	28.38	6.66	−5.40
400	403.90	0.52	0.97

4. Conclusions

This is the first method describing the liquid/liquid extraction of Valdecoxib from human plasma and subsequent quantitation by HPLC with UV detection. The method has shown acceptable precision, accuracy and adequate sensitivity for use in bioequivalence studies of Valdecoxib in healthy volunteers. The method described here is simple, sensitive, specific and fully validated as per FDA guidelines [9]. The validated method allows quantification of Valdecoxib in the 10–500 ng/ml range.

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