

Development and Validation of a RP-HPLC Method for the Quantitation and Dissolution Studies of Valdecoxib

Marcio FRONZA, Liberato Brum JUNIOR, Micheli WRASSE,
Thiago BARTH & Sérgio Luiz DALMORA*

Department of Industrial Pharmacy, Health Science Center,
Federal University of Santa Maria, 97.105-900 - Santa Maria-RS, Brasil

SUMMARY. An isocratic high-performance liquid chromatography (HPLC) procedure was developed for the dissolution rate studies and quantitative determination of valdecoxib in solid dosage forms and in active pharmaceutical ingredient. HPLC separation was carried out by reversed phase chromatography on a Synergi® fusion C₁₈ column (150 mm x 4.6 mm i.d.; 4 µm particle size), held at 30 °C. The mobile phase consisted of water, pH 7.0/acetonitrile (52:48, v/v), run at a flow rate of 1.0 mL/min and with UV detection at 210 nm. Method validation investigated parameters such as the linearity ($r^2=0.9999$), range, precision, accuracy, robustness and specificity. The method yielded good results with a quantitation limit of 50 ng/mL and a detection limit of 10 ng/mL. The dissolution test conditions and the dissolution medium was chosen as 0.5% of sodium lauryl sulfate in water at a stirring rate of 75 rpm. The described method can be successfully applied for the analysis of tablets, active pharmaceutical ingredient and drug dissolution studies.

RESUMEN. “Desarrollo y Validación de Método por HPLC para la Cuantificación y Estudios de Disolución de Valdecoxib”. En el presente trabajo se desarrolló y validó un método por cromatografía líquida de alta eficacia (CLAE) para los estudios de disolución y la estimación cuantitativa de valdecoxib en tabletas y en el ingrediente farmacéutico activo. En la técnica de CLAE se utilizó una columna cromatográfica Synergi® fusión de fase reversa RP-18 (150 x 4,6 mm) de 4 µm a 30 °C. La fase móvil estuvo compuesta por agua de pH 7,0-acetonitrilo (52:48, v/v), velocidad de flujo fue de 1,0 mL/min y se utilizó un detector ultravioleta con longitud de onda fijada a 210 nm. En la validación del método analítico se determinaron los siguientes parámetros de validación: linealidad ($r^2=0.9999$), curva de calibración, precisión, exactitud, robustez y especificidad; el método rindió buenos resultados con un límite de cuantificación de 50 ng/mL y un límite de detección de 10 ng/mL. El perfil de disolución se realizó en agua conteniendo 0,5% de lauril sulfato de sodio como medio de disolución a 75 rpm. El método desarrollado se empleó con éxito para el análisis de las tabletas, del ingrediente farmacéutico activo y para los estudios de disolución.

INTRODUCTION

Valdecoxib is chemically described as 4-(5-methyl-3-phenylisoxazol-4-yl) benzenesulfonamide, a novel highly selective cyclooxygenase-2 (COX-2) inhibitor investigated for the treatment of many inflammatory diseases such as rheumatoid arthritis, osteoarthritis and pain, causing fewer gastrointestinal complications than conventional non-steroidal anti-inflammatory drugs (NSAIDs) ¹.

Dissolution test has emerged in the pharmaceutical field as a very important tool based on the fact that for a drug to be absorbed and available to the systemic circulation, it must previously be solubilized. Therefore the dissolution studies are used not only to assess batch-to-batch consistency of drug release from solid dosage forms, but they are also essential in several stages of formulation development, for

screening and proper assessment of different formulations. Moreover, the *in vitro* dissolution profiles obtained from dissolution rate studies have also been used for the successful characterization of the *in vivo* behavior of drugs ²⁻⁴.

A liquid chromatography-tandem mass spectrometry assay was developed and validated after solid phase extraction for estimation of valdecoxib and its hydroxylated metabolite in human plasma ⁵. The RP-HPLC method with UV detection was also described for the pharmacokinetic study and determination of valdecoxib in human plasma ^{6,7}. The recent findings related to the adverse cardiovascular events or serious skin reactions, showed that additional pharmaceutical and clinical studies and the safety monitoring will be important to understand the risk and evaluate the safe dosage level for this class of drugs ^{8,9}.

KEY WORDS: Valdecoxib, Dissolution, RP-HPLC, Validation.

PALABRAS CLAVE: Valdecoxib, disolución, CLAE, validación

* Author to whom correspondence should be addressed. E-mail: sdalmora@ccs.ufsm.br

The aim of the present work was to develop and validate a sensitive RP-HPLC method to be applied to the analysis and dissolution rate studies of valdecoxib in tablets, contributing therefore for the quality control and safety of this type of pharmaceutical preparation.

MATERIALS AND METHODS

Chemicals and reagents

The valdecoxib reference standard was generously supplied by Pfizer Laboratories (Kalamazoo, USA). Valdecoxib tablets containing 10, 20 and 40 mg of active substance were obtained from commercial sources and used within their shelf life period. The HPLC-grade acetonitrile was purchased from Tedia (Fairfield, USA). Analytical grade hydrochloric acid, sodium hydroxide and potassium dihydrogen phosphate monobasic were obtained from Merck (Darmstadt, Germany). All chemicals used were of pharmaceutical or special analytical grade. For all analyses, double-distilled water filtered through a 0.45 μm membrane filter was used.

Apparatus and chromatographic conditions

A Vankel VK7010 (VanKel Technology Group, Cary, USA), a paddle-stirrer type of apparatus, was used integrated with a VK 8000 dissolution sampling station, VK type bidirectional peristaltic pump. A Shimadzu HPLC system (Shimadzu, Kyoto, Japan) was used, equipped with an SCL-10AVP system controller, LC-10 AD_{VP} pump, DGU-14A degasser, SIL-10AD_{VP} autosampler and an SPD-M10A_{VP} photodiode array (PDA) detector. The detector was set at 210 nm and peak areas were integrated automatically by computer using a Shimadzu Class VP[®] V 6.12 software program. The experiments were carried out on a reversed phase Phenomenex[®] (Torrance, USA) Synergi fusion C18 column (150 mm x 4.6 mm I.D., with a particle size of 4 μm and pore size of 80 Å). A security guard holder (4.0 mm x 3.0 mm I.D.) was used to protect the analytical column. The HPLC system was operated isocratically and the column was operated at 30 °C using a mobile phase of water, pH 7.0 adjusted with NaOH 0.1M/acetonitrile (52:48, v/v). Before use the mobile phase was filtered through a 0.45 μm membrane filter (Millipore[®]) and run at a flow rate of 1.0 mL/min. The injection volume was 10 μl for both standard and samples and all determinations were carried out in five replicates. For the robustness test a reversed phase Shimadzu (Kyoto, Japan) C₁₈ Shim-Pack CLC-ODS

column (150 mm x 4.6 mm i.d.; 5 μm particle size and pore size of 100 Å) and an Ace (Aberdeen, Scotland) C18 column (150 mm x 4.6 mm i.d.; 5 μm particle size and pore size of 80 Å), were also used.

Valdecoxib reference standard solutions

The stock solution of valdecoxib was prepared by weighing 10 mg of the reference standard which was transferred into a 10 mL volumetric flask and diluting to volume with methanol. The concentration of this solution was 1 mg/mL and was stable for at least two months stored in refrigerator at 4 °C. Working standard solutions of valdecoxib were prepared daily by diluting the stock solution to an appropriate concentration in water/methanol (60:40, v/v).

Valdecoxib sample solutions

Twenty tablets of each sample containing 10, 20 and 40 mg of valdecoxib were separated, accurately weighed and crushed to a fine uniform particle size powder. An appropriate amount of each concentration was transferred into an individual 50 mL volumetric flask, diluted to volume with methanol and sonicated for 10 minutes, obtaining concentrations of 1 mg/mL (stock solution). Working sample solutions of valdecoxib were prepared daily by diluting the stock solution to an appropriate concentration in water/methanol (60:40, v/v).

Validation of the chromatographic method

Once the chromatographic and the experimental conditions were established, the method was validated by the determination of the following parameters: linearity, range, precision, accuracy, robustness, limit of detection (LOD), limit of quantitation (LOQ), specificity and system suitability tests, following the ICH guidelines¹⁰.

Linearity and range

The range of linearity was determined by constructing three calibration curves. For the construction of each calibration curve seven standard solutions of valdecoxib at different concentrations in the range of 0.05-150 $\mu\text{g/mL}$ were prepared. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Each measurement was carried out in five replicates of 10 μl injections for the standard solution to verify the reproducibility of the detector response at each concentration level.

The peak areas of the chromatograms were plotted against the concentrations of valdecoxib to obtain the calibration curve. The seven concentrations of the standard solution were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was determined by performing twelve repeated analysis of the same working solution of valdecoxib, on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on three different days (inter-day) and also by another analyst performing the analysis in the same laboratory (between-analysts).

Accuracy

To confirm the accuracy of the proposed method, a total of 15 determinations were performed using 3 concentration levels covering the specified range.

Robustness

The robustness was assessed by altering the following experimental conditions such as, by changing the flow rate from 0.8 to 1.2 mL/min, the mobile phase composition with water pH 7.0/acetonitrile (55:45; 50:50; 48:52; 45:55), the wavelength in the range of 200 to 370 nm, the temperature of the column between 25 °C to 40 °C and using different columns.

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ), were calculated based on the ICH guidelines¹⁰.

Specificity

Specificity of the method towards the drug was established through determination of purity peak of valdecoxib in the working standard solution using a PDA detector.

System suitability

To ensure the validity of the analytical procedure, a system suitability test was established. Data from five injections of 10 µL of the working standard solution containing 100 µg/mL were used for the evaluation of the system suitability parameters like asymmetry, number of theoretical plates, retention time and area, by the CLASS-VP® V 6.12 software.

Analysis of tablet dosage forms

The analysis of the pharmaceutical tablet dosage forms of valdecoxib, containing 10, 20 and 40 mg, was carried out by the proposed RP-HPLC method against the reference standard.

In vitro drug dissolution rate studies for tablets

The dissolution rate studies of valdecoxib from tablets were performed on a paddle-stirrer type of apparatus by a semi-automated system. Drug release tests were carried out according to conventional dissolution procedures recommended for single-entity products^{11,12} in 900 mL of different media of HCl 0.1 M (50 and 75 rpm), 0.25% and 0.5% of sodium lauryl sulfate in water (50 and 75 rpm), and distilled water (50 and 75 rpm), for 120 min. The temperature of the cell was maintained at 37 ± 0.5 °C by using a thermostatic bath. At each sample time interval, an exact volume of the sample was withdrawn from each flask and immediately replaced with an identical volume of fresh medium to maintain a dissolution sink condition. A correction factor was included in the calculations to account for the drug lost in the sampling. At predetermined time intervals (0, 5, 10, 15, 30, 60, 90 and 120 min) for the development of the methodology and (0, 3, 5, 7, 10, 15, 20, 30, 45 and 60 min) for the dissolution studies, the concentrations of valdecoxib in the dissolution medium were determined by the proposed RP-HPLC method. The cumulative percentage of drug released was plotted against time, in order to obtain the release profile and to calculate the *in vitro* dissolution data (n=12) by the linear regression equation¹¹.

RESULTS AND DISCUSSION

A reversed-phase HPLC method was proposed as a suitable method for the determination of valdecoxib in drug dissolution studies and tablet dosage form. The best chromatographic conditions were adequately selected. Figure 1 shows a typical chromatogram of valdecoxib standard solution with a symmetrical peak, well separated from the solvent front. The retention time observed (5.51 min) allows a rapid determination of the drug.

The calibration curves for valdecoxib were constructed by plotting the area of the peaks versus concentration. Linearity was observed in a concentration range from 0.05 to 150 µg/mL. This concentration range corresponds to 0.05 to 150% of the target concentration of 100 µg/mL. A linear regression by the least squares

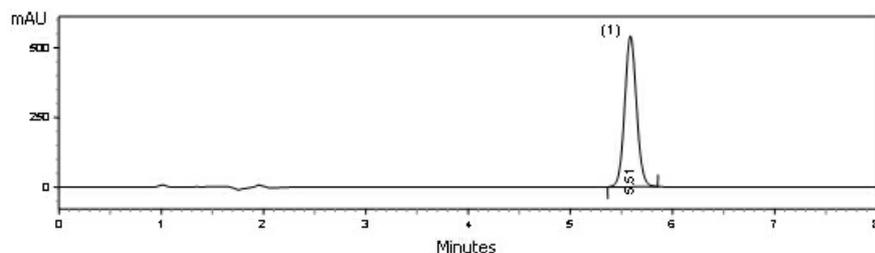


Figure 1.
RP- HPLC chromatogram
of valdecoxib reference
standard 100 µg/mL
Peak 1: valdecoxib.

Sample	Theoretical amount (µg/mL)	Experimental amount (µg/mL)	Purity ^a (%)	RSD (%)
1	100.0	100.08		
2	100.0	99.97		
3	100.0	100.08		
4	100.0	99.91		
5	100.0	99.95		
6	100.0	100.01		
7	100.0	99.98	100.01	0.06
8	100.0	99.99		
9	100.0	100.04		
10	100.0	99.98		
11	100.0	100.13		
12	100.0	100.02		

Table 1. Valdecoxib concentrations obtained in the analysis of twelve determinations by RP-HPLC to show method repeatability. ^a Mean of twelve determinations.

method was then applied. The value of the determination coefficient ($r^2 = 0.9999$) showed excellent linearity of the calibration curve for the method.

The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homologous sample. Repeatability was studied by calculating the relative standard deviation (RSD) for twelve determinations of the concentration of 100 µg/mL, performed on the same day and under the same experimental conditions. The results of valdecoxib determinations in the working standard solution with the relative standard deviation calculated as 0.06% are shown in Table 1.

Intermediate precision includes the estimation of variations in analysis when a method is used within laboratories, on different days, by different analysts, and on different equipments. The intermediate precision was assessed by analyzing two working standard solutions on three different days (inter-day, Table 2); the RSD values obtained were 0.22 and 0.19%, respectively. Between-analysts precision was determined by calculating the RSD for the analysis of three working standard solutions by two analysts; the

Sample	Day	Purity ^a (%)	Mean ^b	RSD (%)
1	1	100.03		
	2	100.27	100.04	0.22
	3	99.84		
2	1	99.70		
	2	100.04	99.92	0.19
	3	100.03		

Table 2. Between-day precision data of RP-HPLC for valdecoxib. ^a Mean of five replicates; ^b Mean of three days.

Sample	Analyst	Purity ^a (%)	Mean ^b	RSD (%)
1	A	100.07	99.92	0.21
	B	99.77		
2	A	99.89	100.12	0.33
	B	100.35		
3	A	99.80	99.99	0.27
	B	100.18		

Table 3. Between-analysts precision data of RP-HPLC for valdecoxib. ^a Mean of five replicates; ^b Mean of two analysts.

values were found to be 0.21, 0.33 and 0.27%, respectively (Table 3).

The accuracy of an analytical method is the closeness of the test results obtained using the proposed method and the true value. The accuracy was assessed from five replicates determinations of three different solutions containing 80, 100 and 120 µg/mL. The absolute means obtained were 99.90, 100.02 and 100.15% respectively, with a mean value of 100.03% and RSD of 0.12% as shown in Table 4. It is evident that the method is accurate within the desired range.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors considered were: variations in the flow rate, percentage of acetonitrile in the mobile phase, column temperature and wavelength. The results and the experimental range of the selected variables are given in Table 5, together with the optimized values.

There were no significant changes in the chromatographic pattern when the above modi-

Theoretical amount (µg/mL)	Experimental amount ^a (µg/mL)	Accuracy ^b (%)	RSD (%)
80	79.92		
100	100.02	100.03	0.12
120	120.18		

Table 4. Recovery of valdecoxib standard solution added to sample solution analyzed by RP-HPLC for valdecoxib. ^a Mean of five replicates analyses; ^b Accuracy: obtained concentration expressed as % of the nominal concentration.

fications were made in the experimental conditions, showing thus that the method is robust. The equivalence of the columns evaluated by the system suitability tests demonstrated also non significant column-to-column variability.

The LOD and LOQ were obtained from the slope and the standard deviation of the intercept from three calibration curves determined by a linear regression line as defined by ICH. The LOD and LOQ were found to be 10 and 50 ng/mL, respectively. These values were also used in an experimental assay confirming the calculation.

The studies with the PDA detector showed that the valdecoxib peak was free from any co eluting peak, demonstrating thus that the proposed method is specific for the analysis of valdecoxib.

The system suitability tests were also carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed. Results of system suitability tests are given in Table 6 showing that the parameters are within the suitable range.

The RP-HPLC method validated in this paper was used to evaluate the potency of valdecoxib in the same batches of tablet forms used for the dissolution studies, with the labelled claim of 10, 20 and 40 mg. The results obtained in the analysis were expressed as percentage of the drug and were: 100.45%, 99.81% and 100.75%, respectively.

The *in vitro* studies were performed using eight different dissolution conditions. As valdecoxib is a poorly water-soluble drug with rela-

Variable	Range investigated	Valdecoxib ^a (%)	Optimized value
Flow rate (mL/min)	0.8	100.91	
	1.0	100.07	1.0
	1.2	100.33	
Column temperature (°C)	25	100.44	
	30	100.06	30
	35	99.83	
Injection volume (µL)	40	99.70	
	5	99.62	
	10	100.04	10
Percent acetonitrile	20	99.45	
	45	99.75	
	48	100.19	48
Wavelength (nm)	50	101.09	
	55	99.35	
	200 - 370	-	210

Table 5. Chromatographic conditions and range investigated during robustness testing. ^a Mean of five replicates.

tively low bioavailability, a surfactant agent sodium lauryl sulfate (SLS), was added in the media to improve the solubility of the drug. As shown in Figure 2 the best dissolution rate profile was achieved with water as the medium containing 0.5% of sodium lauryl sulfate with a paddle rotating at 75 rpm, that was chosen to carry out the dissolution tests of the batches of valdecoxib containing 10, 20 and 40 mg. The concentrations of valdecoxib in the dissolution medium were evaluated using the proposed RP-HPLC method and the coefficients of variation at the earlier time points, up to 15 minutes, were within 7.99-12.92% (acceptable < 20%) and the other time points within 0.99-3.8% (acceptable < 10%) (n=12)¹².

CONCLUSION

The data validation shows that the RP-HPLC method is accurate, robust and possesses excellent linearity and precision characteristics. This method can be successfully used for the quantitation of valdecoxib as active substance, in dissolution studies and in tablet dosage forms. The dissolution data are reliable and precise. This approach is valid not only for the valdecoxib,

Parameter	Minimum	Maximum	RSD (%)	Status
Asymmetry	1.19	1.20	0.376	Passed
Theoretical plates	9493	9828	1.207	Passed
Retention time	5.510	5.529	0.159	Passed
Area	4192705	4221212	0.315	Passed

Table 6. Results of the system suitability test.

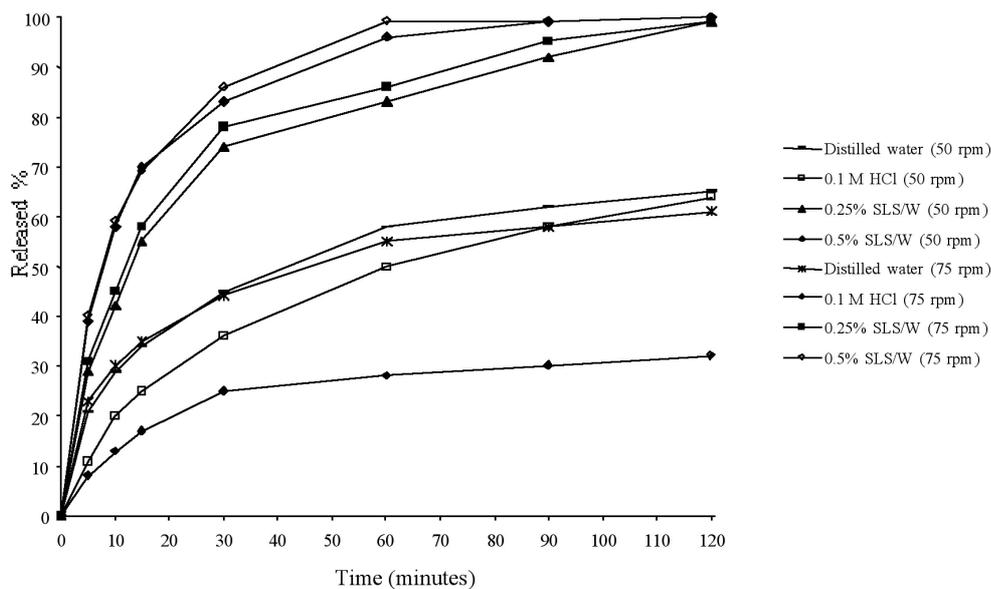


Figure 2. Comparison of dissolution profiles of valdecoxib tablets in different dissolution media with paddles (Apparatus 2) using the proposed RP-HPLC.

but also for other solid dosage forms, therefore, contributing for the establishment of procedures to assure the quality, safety and efficacy of the developed drug product.

Acknowledgements. The authors wish to thank Pfizer Laboratories for supplying valdecoxib and FAPERGS for the support.

REFERENCES

1. Talley, J.J., D.L. Brown, J.S. Carter, M.J. Granelto, C.M. Koboldt, J.L. Masferrer, W.E. Perkins, R.S. Rogers, A.F. Shaffer, Y.Y. Zhang, B.S. Zweifel & K. Seibert (2000) *J. Med. Chem.* **43**: 775-7.
2. Furlanetto, S., F. Maestrelli, S. Orlandini, S. Pinzauti & P. Mura (2003) *J. Pharm. Biomed. Anal.* **32**: 159-65.
3. Ambike, A.A., K.R. Mahadik & A. Paradkar (2004) *Int. J. Pharm.* **282**: 151-62.
4. Shah, V.P., A. Noory, C. Noory, B. McCullough, S. Clarke, R. Everett, H. Naviasky, B.N. Srinivasan, D. Fortman & J.P. Skelly (1995) *Int. J. Pharm.* **125**: 99-106.
5. Zhang, J.Y., D.M. Fast & A.P. Breau (2003) *J. Pharm. Biomed. Anal.* **33**: 61-72.
6. Sane, R.T., S. Menon, A.Y. Deshpande & A. Jain (2005) *Chromatographia* **61**: 137-41.
7. Ramakrishna, N.V.S., K.N. Vishwottam, S. Wishu & M. Koteswara (2004) *J. Chromatogr. B* **802**: 271-5.
8. Fitzgerald, G.A. (2004) *N. Engl. J. Med.* **351**: 709-11.
9. Reddy, L.R. & E.J. Corey (2005) *Tetrahedron Lett.* **46**: 927-9.
10. International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceutical for Human Use, Validation of Analytical Procedures: Methodology (ICH-Q2B), November, 1996, 1-8.
11. Moore, J.W. & H.H. Flanner (1996) *Pharm. Technol.* **20**: 64-74.
12. FDA 1997a, Center for Drug Evaluation and Research, Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, August 1997.