Validation of Single Isocratic HPLC Method for the Assay of Valdecoxib and Determination of Metaisomer Impurity

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Abstract

A sensitive and accurate isocratic high-performance liquid chromagraphic method is developed for the determination of both metaisomer impurity and assay of valdecoxib drug substance. This method uses a Phenomenex Luna C18 (2) column, a mobile phase of 60:30:10 (v/v) 20mM NaH_2PO_4, methanol, and tetrahydrofuran, respectively, with UV detection. This method is validated and its stability-indicating capability is established by performing stress studies under acidic, basic, oxidation, light, humidity, and thermal conditions. Valdecoxib is well separated from its metaisomer impurity with a resolution of more than 2.0. The limit of detection of 0.007% is obtained for the metaisomer impurity, and the relative response factor is also determined. Repeatability is good, with a relative standard deviation of not more than 0.2% and 0.8% for the assay and impurity methods, respectively. A system suitability test is developed with acceptance criteria and the requirements are met throughout the method validation. The method is validated as robust to variations in chromatographic conditions.

Introduction

Valdecoxib, 4-(5-methyl-3-phenyl-4-isoxazolyl) benzene sulfonamide, is a non-steroidal anti-inflammatory drug reported to be a highly selective inhibitor (1) of the inducible form of cyclooxygenase (COX-2). It is administrated orally in the treatment of rheumatoid arthritis, osteoarthritis, and primary dysmenorrhoea. Cyclooxygenase exists as two isoforms: a constitutive COX-1 form and an inducible COX-2 form. The constitutive COX-1 appears to be responsible for most of the physiological prostaglandin production associated with gastric lining cytoprotection. In contrast, the inducible COX-2 is involved in acute inflammatory response, including joint inflammation. The selective inhibition of COX-2 while preserving COX-1 function provides an anti-inflammatory and analgesic effect without compromising the gastrointestinal tract (2,3). Clinical studies have demonstrated that COX-2 inhibitors lead to a significant reduction in joint pain, joint tenderness, and joint swelling with a statistically-significantly lower incidence of gastric ulceration.

Valdecoxib is not official in any pharmacopoeia; however, a few reports are available (4–9). Gradient high-performance liquid chromatography (HPLC) and packed-column sub/supercritical fluid chromatography (PCSFC) methods have been reported for impurity determination in valdecoxib drug substance (4). Valdecoxib and its metabolites have been investigated in human urine (5) as well as in human plasma (6) using solid-phase extraction–liquid chromatography–tandem mass spectrometry. Methods for the quantitation of valdecoxib in human plasma by HPLC with UV detector using liquid–liquid extraction have been described (7). Also, a liquid chromatography–mass spectrometry (LC–MS) method was developed for the simultaneous estimation of valdecoxib and etoricoxib in human plasma (8). A validated HPLC method for the separation of valdecoxib and its SC-77852 impurity in film coated tablet has been presented (9). Even though several methods were reported in the literature for the quantitation of valdecoxib, its metabolites, and its process impurity in the dosage forms and/or drug substance, to the best of our knowledge, there is no single HPLC published so far method for the assay as well as process impurity determination in valdecoxib drug substance.

An important aspect about valdecoxib is the formation of metaisomer as an impurity during its preparation from

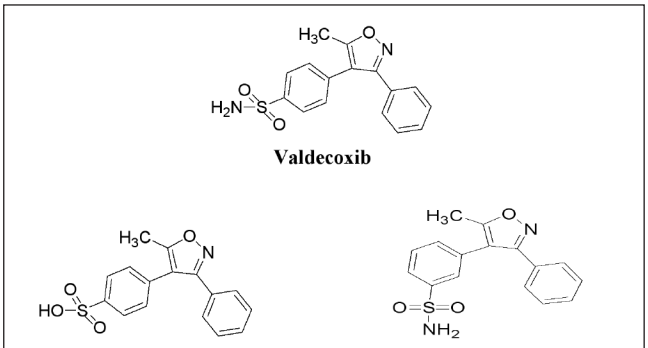

Figure 1. Chemical structures of valdecoxib and its process impurities.

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4-(5-methyl-3-phenyl-4-isoxazolyl) benzene sulfonyl chloride, which is evident from the process described in the patent WO 03/029230 A1 (10). Impurity profile of valdecoxib drug substance was studied by Roston et al. (4) using PCSFC, and its comparison with gradient HPLC revealed metaisomer and sulfonic acid (Figure 1) as synthetic process impurities. But a discrepancy in the quantitated level of metaisomer between PCSFC (0.22%) and HPLC (0.56%) was reported. This study indicated that resolution between the metaisomer and valdecoxib was poor for the accurate quantitation in PCSFC experiments where no baseline separation was achieved. On the other hand, the HPLC method [in which a Diphenyl LC-DP (Supelco) HPLC column was employed with the total run time of 63 min under gradient elution mode] was found to be better, but no method validation data was reported in the published paper (4). To overcome these issues, in the present work, a simple, accurate, and sensitive isocratic method utilizing a commonly used HPLC column (Phenomenex Luna C18) has been developed for the determination of process impurity and assay of valdecoxib drug substance. The developed method also leads to comparatively rapid measurement, with a run time of 50 min. The metaisomer impurity being a structural analogue and regioisomer of valdecoxib, the separation of this compound from valdecoxib posed practical difficulties under isocratic elution mode. By employing a methanol–tetrahydrofuran (THF) mixture as the organic phase (11), the resolution between metaisomer and valdecoxib has been established.

In the present investigation, a single isocratic HPLC method has been finalized with phosphate buffer, methanol, and THF as mobile phase for the assay as well as process impurity determination of valdecoxib. The proposed isocratic HPLC method is validated using United States Pharmacopoeia (12) and International Conference on Harmonisation guidelines as references (13–15).

Experimental

Chemicals and reagents

HPLC-grade methanol, acetonitrile, THF, and analytical reagent-grade NaH_2PO_4 were purchased from Merk (Merck Darmstadt, Germany), and water was from a Milli-Q purification system (Millipore, Billerica, MA). All drug and drug-related impurities were prepared and characterized by Shasun Chemicals and Drugs Ltd. (Chennai, India).

Equipment

A Shimadzu LC-2010AC quaternary HPLC system (Kyoto, Japan) equipped with a built-in UV–vis detector, auto sampler, and Class-VP (v. 6.01) software were used for the data acquisition and processing. A model SPDM-10Avp, photo diode array detector from Shimadzu was also used for the method validation and development purposes.

Chromatographic conditions

The HPLC column used was Phenomenex Luna C18 (2) (150 × 4.6 mm, 3 µm particle size, Torrance, CA). The column oven temperature was 30ºC. The mobile phase was 60:30:10 (v/v), 20mM NaH_2PO_4–methanol–THF, respectively. The injection volume for the assay and process impurity determination was 20 µL, and the flow rate was 1.0 mL/min. The run time for the assay was 35 min, and process impurity determination was 50 min. Chromatograms were obtained with UV detection at the wavelength of 240 nm. A mixture of water and acetonitrile in the ratio of 1:1 (v/v) was used as diluent.

Solution preparation

For process impurities

System suitability stock solution was prepared by dissolving 5 mg each of metaisomer impurity (VC2) and sulphonic acid impurity (VC1) as its sodium salt in 100 mL of diluent. System suitability solution was prepared by dissolving 25 mg of valdecoxib standard in a 50-mL volumetric flask using diluent by sonication, followed by the addition of 5 mL of system suitability stock solution. This solution was then diluted up to the volume with diluent. Resolution between valdecoxib and VC2 was evaluated as part of the system suitability, with the acceptance criteria of not less than 1.5. Standard preparation was made by dissolving 10 mg of valdecoxib standard in 100 mL of diluent. One milliliter of this solution was pipetted out into a 100-mL volumetric flask and diluted to volume with diluent. The relative standard deviation (RSD) for the valdecoxib peak from the five replicate injections was evaluated as part of system suitability with the acceptance criteria of not more than 2.0%. Sample preparation was made by dissolving 25 mg of valdecoxib sample in 50 mL of diluent.

For assay

System suitability solution was prepared by dissolving 10 mg of valdecoxib standard and 1 mg of metaisomer impurity (VC2) using 25 mL of acetonitrile followed by the addition of 25 mL of water into a 100-mL volumetric flask. This solution was then diluted to volume with diluent. Resolution between valdecoxib and VC2 was evaluated as part of the system suitability with the acceptance criteria of not less than 1.5. Standard preparation was made by dissolving 50 mg of valdecoxib standard using 25 mL of acetonitrile by sonication in a 50-mL volumetric flask followed by the dilution to volume with water. Five milliliters of this solution was pipetted out into a 50-mL volumetric flask and diluted to volume with diluent. The RSD for the valdecoxib peak from the five replicate injections was evaluated as part of the system suitability, with the acceptance criteria of not more than 2.0%. Sample preparation was made by dissolving 50 mg of valdecoxib sample using 25 mL of acetonitrile by sonication in a 50 mL volumetric flask, followed by the dilution to volume with water. Five milliliters of this solution was pipetted out into a 50-mL volumetric flask and diluted to volume with diluent.

Results and Discussion

Development of chromatographic method

In preliminary development work, experiments were performed using simple phosphate (NaH_2PO_4) buffer with

acetonitrile as organic phase at different compositions, but no separation between VC2 and valdecoxib was obtained. Because of this, weaker solvent (methanol) was tried. Separation resulted with the resolution (R) of 1.75 when 40% methanol was used, but the retention factor (k) values were high (> 20). Also, VC2 eluted just after the valdecoxib peak with no clear baseline separation. Therefore, a mixture of methanol and acetonitrile (ACN) at various compositions were used to have better separation with reasonable k values, but no baseline separation was obtained with the ACN–methanol mixtures. Considering the structural factors of VC2 and valdecoxib, being regioisomers, it was decided to introduce THF in the organic phase to utilize its unique selectivity for the isomer separation. Accordingly, ACN–THF and methanol–THF mixtures were investigated to achieve better resolution. The methanol–THF (3:1, v/v) mixture yielded a good resolution of more than 2.0 with reasonable k values (< 12), whereas the ACN–THF combination resulted in poor resolution. It is significant to note that THF played a vital role in shifting the retention time of valdecoxib after VC2 peak. From all the described experiments, it was concluded that the presence of THF in the organic phase, especially along with methanol, has a great influence on retention and separation; therefore, the methanol–THF (3:1, v/v) mixture was found to be an optimal organic phase.

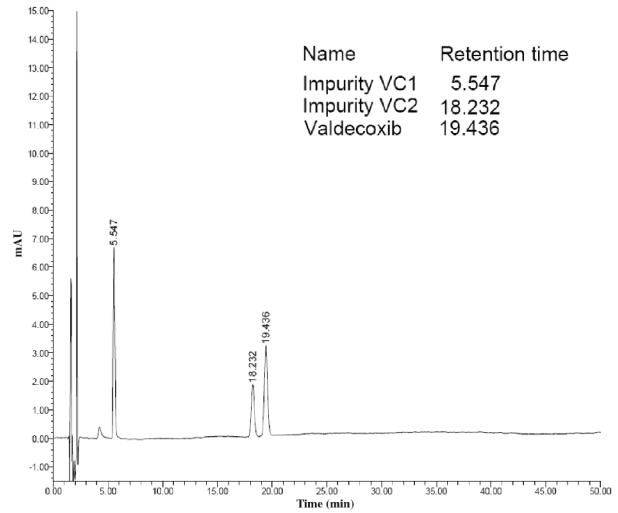

Figure 2. Chromatogram showing the separation between valdecoxib, VC1, and VC2 impurities at 0.2, 0.3, and 0.15% concentrations, respectively.

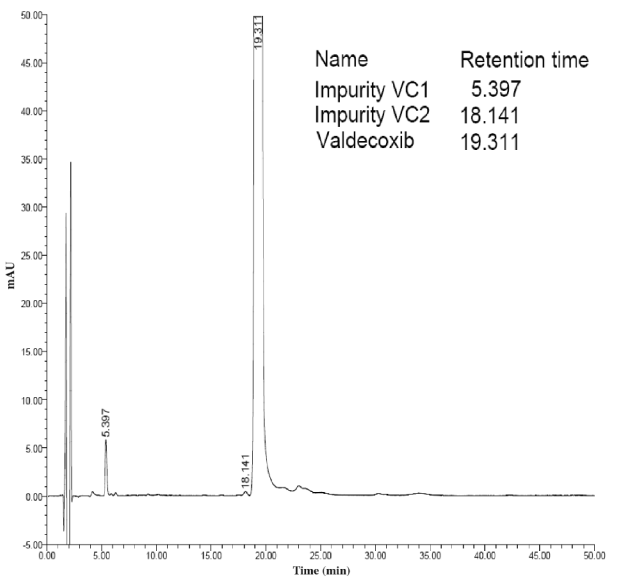

Figure 3. Typical sample chromatogram of valdecoxib for process impurities.

In the described experiments, NaH_2PO_4 buffer (pH 4.5) without any adjustment in pH was used; however, the effect of pH on retention and resolution was studied. When, the pH of the buffer solution was reduced to 4.0 and 2.5 with ortho phosphoric acid, a 1–2 min increase in the retention was observed with no significant change in the resolution. At pH 5 and 6.5, adjusted using NaOH, no remarkable variation in retention or resolution was noticed. It was concluded that the pH of the buffer solution does not have any significant effect on the retention and resolution; hence, NaH_2PO_4 buffer without any pH adjustment was found to be ideal. The effect of column oven temperature was also studied by keeping the temperature at 25°C, 30°C, and 35°C. No significant change in resolution was evidenced at 25°C and 30°C, but a slight decrease in the resolution was noticed during an increase of temperature to 35°C. Hence, it was concluded that the best results were achieved when the temperature was 30°C. From all the earlier investigations carried out, the optimal chromatographic conditions determined for the separation of VC2 and valdecoxib was 60:30:10 (v/v), 20mM NaH_2PO_4–methanol–THF, respectively, with the column oven temperature of 30°C. Figure 2 represents a typical chromatogram of valdecoxib spiked with impurities VC1 and VC2. It is significant that valdecoxib is completely separated from its process impurities. Also, VC1 and VC2 are well resolved from each other. The typical retention times of valdecoxib, metaisomer, and sulfonic acid was 19, 18, and 5 min, respectively. Retention factor for VC1, VC2, and valdecoxib were 2.7, 11.2, and 11.9, respectively. A typical sample chromatogram of valdecoxib is also presented in Figure 3.

The proposed isocratic HPLC method was validated for

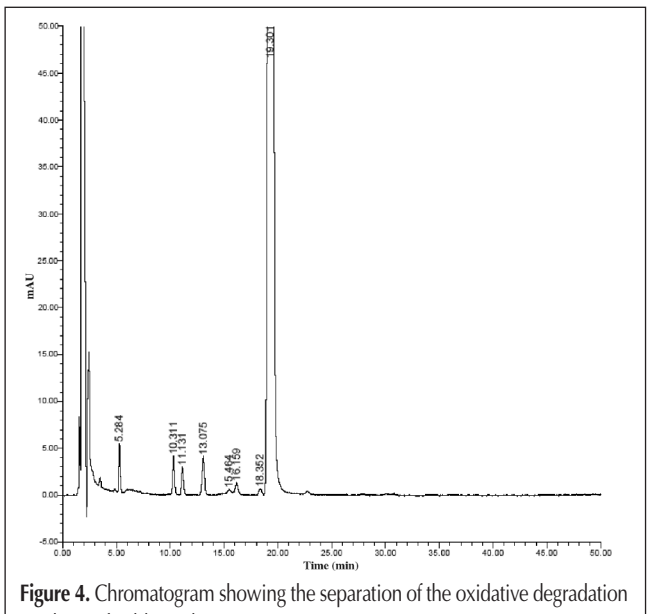

Figure 4. Chromatogram showing the separation of the oxidative degradation products of valdecoxib.

specificity, repeatability, linearity, accuracy, detection limit, quantitation limit, stability of analyte solution, intermediate precision, and robustness.

Specificity

Stress studies

To demonstrate the stability indicating capability of the method, a valdecoxib sample was subjected to stress by acid, alkali, hydrogen peroxide, UV, fluorescent light, thermal, and humidity. The stressed samples were assayed to determine the percentage of degradation. Major degradation happened under oxidative conditions, whereas no significant degradation observed in all other stress conditions. It was concluded that valdecoxib is sensitive to oxidation but quite stable in other conditions. A representative chromatogram of valdecoxib, showing the oxidative degration product, is presented (Figure 4). The homogeneity of the valdecoxib peak in each stressed sample was examined by peak purity testing. Peak purity values are calculated by Shimadzu Class-VP software by comparing the UV spectrum at the apex of the peak with the spectra at the upslope and downslope of the peak and similarity indices are generated, relative to the apex spectra (17). Peak purity value of more than 0.999 was considered as acceptance criteria to demonstrate the spectral homogeneity of the peak. Table I summarizes the stress conditions along with the percentage degradation and peak purity results. It is evident that at all the stress conditions, the valdecoxib peak purity is greater than 0.999, proving spectral homogeneity. Also, degradation products formed during the stress conditions are well resolved from VC1, VC2, and valdecoxib, which proves that the adopted method is specific and stability indicating. Hence, the proposed method shall be used for the stability monitoring of valdecoxib drug substance.

Table I. Stress Studies

Stress conditions	Degradation (%)	Peak purity
Oxidation (25% H_2O_2 at 80°C for 1 h)	12	0.9999
Base Hydrolysis (2N NaOH at 80°C for 2 h)	≤ 0.5	0.9998
Acid Hydrolysis (4N HCl at 80°C for 2 h)	< 0.5	0.9998
Heat (At 105°C for 72 h)	< 0.5	0.9998
Humidity (95% RH at 25° C for 72 h)	< 0.5	0.9998
UV light (Solid exposure) (for 72 h)	< 0.5	0.9998
UV light (Solution exposure) (for 72 h)	< 0.5	0.9998
Fluorescent Light (Solid exposure) (for 72 h)	< 0.5	0.9998
Fluorescent Light (Solution exposure) (for 72 h)	< 0.5	0.9999

Table II. Accuracy for Process Impurities

Impurity (VC1)		Impurity (VC2)	
% Concentration*	% Recovery	% Concentration*	% Recovery
	99.83		99.42
0.15	100.23	0.075	93.83
	100.34		93.37
	99.86		96.46
0.30	99.49	0.15	96.15
	100.58		95.15
	97.03		93.40
0.36	97.69	0.18	93.04
	98.31		92.95
Mean	99.26	Mean	94.20
RSD (%)	1.28	RSD (%)	1.44

*Concentration level was relative to sample concentration.

Validation for the determination of process impurities

Repeatability

Valdecoxib and VC1, VC2 impurities were prepared at 0.20%, 0.30%, and 0.15% concentrations (relative to sample preparation), respectively, and injected in six replicates. The RSD (n = 6) values obtained for the area of valdecoxib, VC1, and VC2 were 0.57, 0.58, and 0.71%, respectively, indicating a high degree of repeatability.

Linearity and relative response factor

Linearity was validated by measuring area responses for each impurity and valdecoxib over the range of 0.01 to 0.36%, relative to sample concentration. Each preparation (n = 7) was injected in triplicate, and the mean area (n = 3) calculated was plotted against the concentration. The squared regression coefficients obtained for valdecoxib, VC1, and VC2 were 0.9994, 0.9996, and 0.9992, respectively. The results revealed an excellent linearity. The slope of the calibration curve for valdecoxib was ~ 1.3 times the slope values for the impurities. Relative response factors (RRF) were calculated for impurities versus valdecoxib from the linearity data. RRF obtained for VC1 and VC2 were 0.797 and 0.774, respectively.

Accuracy

Accuracy was established through recovery experiments by spiking known amounts of each impurity (VC1 and VC2) with a valdecoxib sample at the concentrations specified in Table II. Each preparation was injected in triplicate, and the percent recovery was calculated and tabulated. For each preparation, the area response of the matrix interference was subtracted, and the corrected area response was used in the recovery calculation. The recovery obtained between 93% and 100.6% for all the impurities proves that the proposed method is accurate.

Detection and quantitation limit

The detection limit (DL) and quantitation limit (QL) for valdecoxib, VC1, and VC2 impurities were determined by signal-to-noise (S/N) ratio method. The minimum concentration at a 3:1

S/N ratio was established as the DL, and the concentration at 10:1 S/N ratio was taken as the QL. A solution containing impurities and valdecoxib was prepared at their QL concentration and injected in six replicates. Actual QL (%) values obtained were 0.010, 0.020, and 0.016 for VC1, VC2, and valdecoxib, respectively. The RSD of the area at QL for VC1, VC2, and valdecoxib were 2.58, 2.57, and 2.04, respectively. DL (%) of 0.003, 0.007, and 0.005 for VC1, VC2, and valdecoxib, respectively, indicated that the proposed method is very sensitive.

Stability of analyte solution

The stability of VC1 and VC2 was monitored (using the solution containing VC1 and VC2 impurities at 0.3% and 0.15% concentration level) by measuring area responses of injections made over a period of 24 h. Figure 5 represents the relationship between area response and time. The RSD values for the area responses of VC1 and VC2 were 0.34% and 1.0%, respectively, which concludes the significant solution stability at 25 ± 2°C for 24 h.

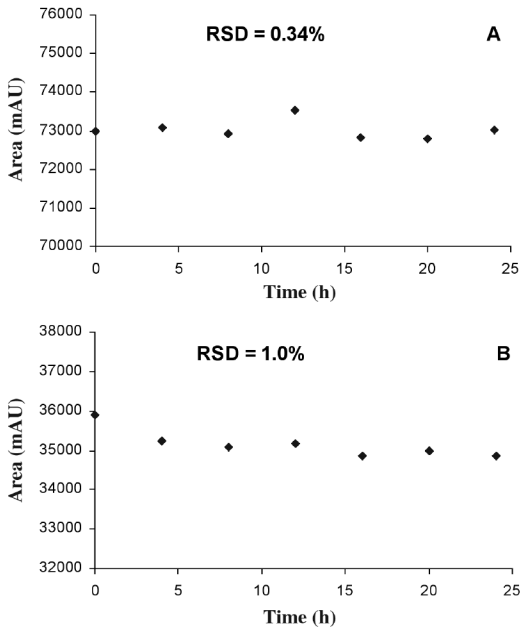

Figure 5. Stability of 0.3% (w/w) VC1 impurity solution (A) and 0.15% (w/w) VC2 impurity solution (B) at 25 ± 2°C.

Table III. Ruggedness for Process Impurities

Impurity VC1 (%)		Impurity VC2 (%)		Total impurities (%)	
Set 1*	Set 2†	Set 1*	Set 2†	Set 1*	Set 2†
0.179	0.192	0.024	0.029	0.203	0.221
0.179	0.183	0.024	0.028	0.203	0.211
0.179	0.183	0.024	0.027	0.203	0.210
0.176	0.186	0.024	0.028	0.200	0.214
0.179	0.188	0.023	0.028	0.202	0.216
0.178	0.186	0.023	0.027	0.201	0.213
RSD (%)					
0.68	1.82	2.17	2.68	0.62	1.86

* Set 1 = analyst 1, column 1, system 1, and day 1.
† Set 2 = analyst 2, column 2, system 2, and day 2.

Intermediate precision

Ruggedness of the method was evaluated by performing the sample analysis in six replicates using two different columns, different HPLC instruments, and different analysts on different days, and the results are summarized in Table III. The RSD values of less than 2.7% for individual and total impurities reveal that the method adopted is rugged.

Robustness

This study was performed by making small but deliberate variations in the method parameters. The effect of variations in flow rate, wavelength of detection, mobile phase composition, and column oven temperature was studied, and the results pertaining to system suitability test and impurity levels are presented in Table IV. Under all the variations, system suitability requirements were found to be within the acceptance criteria and hence the proposed method is robust.

Assay Validation

Specificity

Assay of valdecoxib was determined in the sample spiked with impurities (VC1 and VC2) and also for the unspiked sample. Percentage difference in the assay between spiked and unspiked samples was found to be 0.27, and the peak purity for valdecoxib peak in the spiked sample was 0.9999, which proves that the assay method is specific.

Repeatability

Valdecoxib standard preparation was injected in six replicates. The RSD calculated for the area of valdecoxib peak from the replicate injections was 0.09%, which indicates very good repeatability.

Linearity

Five preparations of valdecoxib at the concentration (%) of 80, 90, 100, 110, and 120 of assay level were made, injected in triplicate, and the mean area calculated was plotted against the concentration. The correlation coefficient of 0.999 obtained for valdecoxib represented excellent linearity in the specified concentration range.

Table IV. Robustness for Process Impurities

Variation	R*	RSD (%)	Impurities (%)		
			VC1	VC2	Total
No variation	2.31	0.76	0.178	0.024	0.202
Flow rate (1.10 mL/min)	2.20	0.65	0.170	0.024	0.194
Flow rate (0.90 mL/min)	2.22	0.69	0.170	0.024	0.194
Column temp. (35°C)	2.04	0.38	0.179	0.022	0.201
Column temp. (25°C)	2.39	0.43	0.173	0.027	0.200
Wavelength (245 nm)	2.31	0.54	0.174	0.022	0.196
Wavelength (235 nm)	2.31	0.87	0.183	0.027	0.210
Mobile phase A†	2.40	0.63	0.178	0.023	0.201
Mobile phase B†	2.15	0.96	0.171	0.023	0.194
Mobile phase C†	2.22	0.98	0.185	0.020	0.205
Mobile phase D†	2.04	0.62	0.180	0.021	0.201
RSD (%)			2.95	9.43	2.53

* Resolution between valdecoxib and VC2.
† Composition of buffer–methanol–THF for A (61.5:29.0:9.5); B (59.5:31.0:9.5), C (60.5:29.0:10.5), and D (58.5:31.0:10.5).

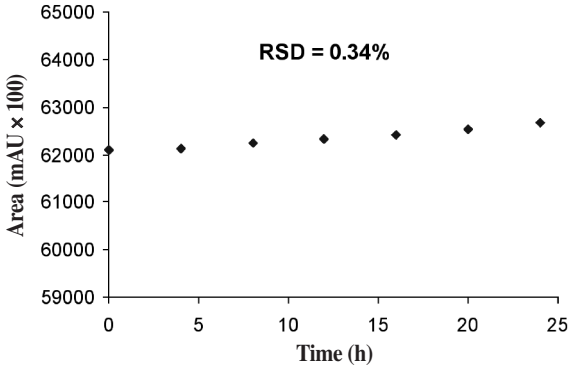

Figure 6. Stability of valdecoxib assay preparation at 25 ± 2°C.

Stability of analyte solution

The stability of valdecoxib sample preparation was monitored by measuring the area response of injections made over a period of 24 h. Figure 6 represents the relationship between area response and time. The RSD of 0.34% for valdecoxib concludes its stability at 25 ± 2°C for 24 h.

Intermediate precision

The assay of valdecoxib sample was determined in six replicates using two different columns, different HPLC instruments and different analysts on different days. The RSD obtained with the two chromatographic systems were 0.25 and 0.17%, respectively, which reveals that the method adopted is precise.

Robustness

The assay of valdecoxib was determined by making small but deliberate variations in the method parameters. The effect of variations in flow rate of 1.1 and 0.9 mL/min, wavelengths of 245 and 235 nm, and column oven temperature of 35°C and 25°C on assay values were studied. Under all the variations, the assay values were consistent with the RSD of 0.25%, proving the robustness of the method.

Conclusion

The single isocratic HPLC method described in this study resolved metaisomer from valdecoxib with suitable resolution (> 2.0) for pharmaceutical applications. Method validation data has proved that the developed method is very sensitive as well as accurate for the estimation of process impurities (metaisomer and sulfonic acid), and robust to minor variations in the chromatographic conditions. The proposed method is also simple and cost-effective, as it utilizes a commonly used C18 HPLC column under isocratic elution with moderate run time. Moreover, the specificity and stability-indicating capability of this method has also been demonstrated through stress studies. Hence, the proposed isocratic method can be used conveniently for the determination of both assay and process impurity of valdecoxib drug substance.

Acknowledgments

The authors would like to acknowledge Shasun's Management for providing facility to perform research and take this opportunity to thank Generics team for the supply of drug substance and impurities.

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Manuscript recieved July 30, 2007;
revision received January 28, 2008.