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HPLC Determination of Valdecoxib from Pharmaceutical Formulation

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Present method describes high performance liquid chromatographic determination of valdecoxib from pharmaceutical formulations by using rofecoxib as an internal standard. The separation was carried out on cosmosil octadecyl silane (C_{18}) (150 mm × 4.6 mm, i.d.) 5 µ column. The mobile phase was comprised of ammonium acetate buffer:acetonitrile in the volume ratio of (55:45) (v/v) with 0.1 % triethylamine. The detection and quantification was carried out using a UV-visible detector at 239 nm. Linear concentration range of valdecoxib was observed to be 0.30-100.00 µg mL^{-1}. The developed method was validated to determine its accuracy, precision, specificity and stability by carrying out recovery, linearity, specificity and stability experiments. The method developed is simple, fast, accurate and precise and hence can applied for routine quality control analysis of valdecoxib from pharmaceutical formulation and for stability studies.

Key Words: Valdecoxib, HPLC determination.

INTRODUCTION

Valdecoxib is a non-steroidal antiinflammatory drug (NSAID) exhibiting anti-inflammatory, analgesic and antipyretic properties. Valdecoxib is a selective inhibitor of cyclooxygenase-2 and mainly used for the treatment of oestoarthritis, rheumatoid arthritis and primary dysmenorrhea. It's chemical name is 4-(5-methyl-3-phenyl-4-isoxazolyl) benzene sulfonamide[1,2].

In the light of earlier published work[2] on analysis of valdecoxib from plasma and pharmaceutical preparation, the present research work describes a simple, fast, accurate and precise high performance liquid chromatographic method for estimation of valdecoxib from its pharmaceutical formulation. The measurements were done at 239 nm, the wavelength of maximum absorbance. The observations from application of method were subjected to statistical validation to determine its linearity, accuracy, precision, specificity, ruggedness, robustness and stability[3,4].

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EXPERIMENTAL

Jasco, PU 980 HPLC isocratic pump, Jasco AS-1550 autosampler and Jasco, UV-970 UV-Visible detector were used. The integration was done using Borwin chromatographic software, version 1.21.

Acetonitrile used was of HPLC grade. Ammonium acetate, glacial acetic acid and triethylamine were of AR grade.

Preparation of drug solutions

Standard drug solution: Accurately weighed 100 mg pure standard of valdecoxib (99.92 %) was transferred to 100 mL volumetric flask. The drug was dissolved in methanol and diluted upto the mark and mixed well. This gave a standard stock solution of strength 1000 μg mL^{-1}, by making suitable dilution 10 μg mL^{-1} solution was also prepared.

Internal standard solution: Accurately weighed 100 mg pure standard of rofecoxib (99.98 %) was transferred to 100 mL volumetric flask. The drug was dissolved in methanol and diluted upto the mark and mixed well. This gave a standard stock solution of strength 1000 μg mL^{-1}, by making suitable dilution 100 μg mL^{-1} solution was prepared.

The separation was carried on cosmosil octadecyl silane (C_{18}) (150 mm × 4.6 mm, i.d.) 5 μ column. The mobile phase was comprised of ammonium acetate buffer:acetonitrile in the volume ratio of (55:45) (v/v) with 0.1 % TEA and pH of the phase was adjusted to 6.5 with glacial acetic acid. The flow rate of the mobile phase was maintained at 1.0 mL/min. Injection volume was 20 μL and the detector wavelength was set at 239 nm.

Validation of method[5-7]

System suitability test: The system suitability test was performed by injecting a mixture of valdecoxib and rofecoxib six times. Mean, standard deviation and coefficient of variance were calculated. Results given in Table-1 indicate conformity to all compendial requirements.

Preparation for calibration curve: Into a series of 100 mL standard volumetric flasks, varying volumes of standard solution equivalent to 0.30 to 100.00 μg mL^{-1} were taken. To each of these flasks 1.0 mL of 1000 μg mL^{-1} rofecoxib solution was added and diluted up to mark with mobile phase and mixed well. After setting all the chromatographic parameters 20 μL of each of the solutions prepared was injected into the chromatographic system. The peak area ratio of drug *i.e.*, valdecoxib to internal standard *i.e.*, rofecoxib were calculated. The data in this range was further considered for statistical validation and regression analysis. The linearity experiment was carried out in triplicate to ascertain the sensitivity, accuracy and precision of the method. A graph of concentration of valdecoxib in μg mL^{-1} *vs.* peak area ratio values on the ordinate was plotted. The regression analysis of the calibration data was carried out to determine the relationship between the absorbance and concentration.

The results showed a linear relationship between concentration and the detector response. The lowest level of quantification was observed to be 0.30 µg mL^{-1} which indicates the sensitivity of the method.

TABLE-1
RESULTS OF SYSTEM SUITABILITY EXPERIMENT

Obs. No.	Conc. of drug (µg/mL)	Peak area ratio	Capacity factor		Asymmetry		Theoretical plates		Resolution
			Drug	IS	Drug	IS	Drug	IS	
1	10	1.622	623	733	1.00	1.02	10398	10925	4.33
2	10	1.627	623	734	1.02	1.00	10326	10938	4.31
3	10	1.624	621	731	1.00	1.00	10279	10895	4.23
4	10	1.625	621	732	1.00	1.02	10356	10936	4.34
5	10	1.623	618	728	1.01	1.04	10389	10945	4.31
6	10	1.622	611	721	1.00	1.00	10256	10923	4.33
Mean		1.624	620	730	1.01	1.01	10334	10927	4.31
SD		0.002	4.550	4.792	0.008	0.016	57.928	17.720	0.040
COV		0.126	0.734	0.657	0.832	1.612	0.561	0.162	0.933

Robustness[8,9]: While developing the method robustness of the method was checked by varying the method parameters like the proportions of the mobile phase, concentration of the buffer, pH and flow-rate of mobile phase and wavelength of detection. The effect of changes was observed on chromatographic parameters like retention time, tailing factor, resolution, peak area and theoretical plates. Results obtained show no significant variation in above parameters indicating robustness of method.

Specificity study[7]: To check the developed method is specific and stabilty indicating the drug was subjected to stressed conditions like treatment with 0.1 M HCl, 0.1 M NaOH, 3 % H_2O_2, 110 ºC heat and ultraviolet light. The treated samples were analyzed by the above chromatographic conditions, an additional peak well resolved from the drug peak and internal standard peak was observed for solutions obtained from the degradation process by heating at 110 °C, ultraviolet light exposure and treating with the acid and oxidizing agent which confirmed that the method developed for assay of valdecoxib is specific and stability indicating method.

Assay of tablets[10,11]: Twenty tablets were accurately weighed and powdered finely in a mortar. Powder equivalent to 10 mg valdecoxib *i.e.* content of valdecoxib per tablet was weighed accurately and transferred to 100 mL volumetric flask. To this flask 1.0 mL of 1000.0 µg/mL internal standard was added. The contents were dissolved by sonicating in methanol for 10 min and made upto mark with methanol. This solution was filtered through Whatmann filter paper No. 41 and filtrate was collected.

One mL of the above sample was taken in a 10 mL volumetric flask and diluted up to the mark using mobile phase. The procedure was repeated 7 times by individually weighing tablet powder each time. After setting all the chromatographic parameters 20 µL of the sample solutions and standard were injected into the chromatographic system. The amount of valdecoxib per tablet was calculated by comparing the peak-area ratio values of standard and sample solutions.

The average of 7 observations was taken to determine the content of valdecoxib. Table-2 gives the peak area ratio values and amount of valdecoxib present in the pharmaceutical formulation as percentage assay.

TABLE-2
RESULTS OF ASSAY OF VALDECOXIB FORM TABLETS

Sr. No.	Weight of the sample taken for assay (mg)	Peak area ratio	Amount of valdecoxib found (mg/tablet)	Percentage assay
1	10.4	1.622	10.08	100.83
2	10.2	1.572	9.96	99.60
3	10.5	1.627	10.02	100.18
4	10.3	1.622	10.18	101.79
5	10.5	1.610	9.91	99.08
6	10.3	1.594	10.00	100.04
7	10.6	1.628	9.92	99.25
	Mean		10.01	100.11
	Standard deviation		0.10	0.95
	Coefficient of variation (%)		0.95	0.95

Recovery studies[10,11]: Recovery study was performed to study the accuracy, reproducibility (precision) and selectivity *i.e.* to check whether any positive or negative interference occurs from active ingredients, excipients, impurities, diluents and degradation products. The recovery study was conducted by addition of different amount of pure drug with known concentration to a pre analyzed sample solution. The recovery of added samples was studied at 4 different levels 0.0, 5.0, 10.0 and 15.0 mg. Each level was repeated 7 times. From the results obtained amount of drug found, standard deviation, coefficient of variation and percentage recovery was calculated. Results obtained are within the acceptance limit indicating accuracy, precision and selectivity of method. Results are tabulated in Table-3.

TABLE-3
RESULTS OF RECOVERY EXPERIMENT

Amount of the sample taken (mg)	Amount of the sample found (mg)	% Recovery	Standard deviation	Coefficient of variance
5.04	5.08	100.69	0.16	1.06
10.03	10.02	99.92	0.13	1.29
15.03	15.13	100.65	0.21	1.37

RESULTS AND DISCUSSION

The proposed method is based on the separation of the drug and the internal standard by high performance liquid chromatographic method using a octadecyl silane column. The measurement was done at 239 nm. The drug concentration was linear in the range of 0.30-100.0 μg mL^{-1}. The lowest level of quantification was observed to be 0.30 μg mL^{-1}, which indicates the sensitivity of the method.

The coefficient of variation for system suitablity experiment was observed to be less than 2 % for retention time, peak asymmetry, capacity factor, resolution, theoretical plates and peak area ratio indicating suitabilty of the system. The presence of peaks other than the drug peak and internal standard peak observed during stress stability study indicate that the method is sensitive and also stability indicating. The recovery experiment was carried out by standard addition method. The recovery of the added standard was found between 100.32-100.72 %, this confirms the absence of either positive or negative interference from excipients or any other ingredients present in the formulation indicating the selectivity of the method. The standard deviation and coefficient of variation, % assay and % recovery value indicates that the developed method is simple, fast, accurate, precise, specific, sensitive and selective, hence can applied for routine quality control analysis of valdecoxib from pharmaceutical preparation and stability studies.

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