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DEVELOPMENT OF DIFFERENCE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF LEFLUNOMIDE IN TABLET DOSAGE FORM

A new simple, accurate, precise, highly sensitive and reproducible difference spectrophotometric method for the determination of leflunomide in bulk and pharmaceutical dosage form is described. The difference spectroscopic method is based on the principle that leflunomide exhibit two different forms; in acidic and basic medium which differs in their absorption spectra. The difference spectra were obtained by reading the absorbance of leflunomide in 0.1 N HCl in the reference cell and the absorbance of leflunomide in 0.1 N NaOH in the sample cell and vice versa; in the difference spectrum maxima and minima were seen at 293.5 and 261.5 nm, respectively. The amplitude values were calculated, which was plotted against concentration. The method is found to be linear in the concentration range of 2-12 μg/ml. The percentage recovery was found to be between the ranges from 98.92 to 99.08%. The proposed method was statistically validated and successfully applied for analysis of tablet dosage forms.

Keywords: leflunomide; difference spectrophotometric method; validation; amplitude.

Leflunomide (LEF) is a novel isoxazole derivative possessing both anti-inflammatory and immune suppressive properties. LEF has been used to reduce the signs and symptoms of arthritis and to retard joint damage in patients with active rheumatoid arthritis. LEF is a pro-drug, which is rapidly and non-enzymatically converted to its active metabolite, A77 1726, after oral administration. Active metabolite A77 1726 possesses immunomodulator effects of the drug by reversible inhibition of the enzyme dihydroorotate dehydrogenase and inhibits cell proliferation of lymphocytes [1-6].

Several analytical methods for determination of LEF and its active metabolite by chromatographic techniques [7-16] are reported. LEF undergoes degradation not more than 4% on heating at 60 °C for 30 min in acidic and basic medium [17]. However, to date, no difference spectrophotometric method has been reported for the determination of this drug in pharmaceutical formulations.

The difference method is more sensitive than a simple UV method because the absorbances of the same concentration solutions at different maxima are added to each other. Thus, the amplitude obtained in the difference method is higher compared to the absorbance by the simple method for the same concentration of chromogen, which makes the method more sensitive. The objective of this study was to develop a simple, accurate, precise, highly sensitive and reproducible difference spectrophotometric method for estimation of LEF in pharmaceutical formulations.

MATERIALS AND METHODS

Chemicals

LEF was obtained as a gift sample from Cipla Ltd., India. Double distilled water was used throughout the study. Methanol, hydrochloric acid and sodium hydroxide used in the study were of analytical grade. 0.1 N HCl and 0.1 N NaOH were prepared and standardized as per IP-1996.

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Instrumentation

A Shimadzu UV/Vis spectrophotometer model 1601 (Japan) was employed with spectral bandwidth of 0.1 nm and wavelength accuracy of ±0.5 nm with automatic wavelength correction with a pair of 3 mm quartz cells were used for all the spectral and absorbance measurement. Commercially available tablets were procured from the local market.

Preparation of standard solution

Stock solution of 100 μg/ml in methanol was prepared from standard drug. Suitable sample volumes were taken in 10 ml volumetric flasks, and volume was made up with 0.1 N NaOH and 0.1 N HCl to prepare a series of standard solutions. Difference spectrum was obtained by keeping LEF in 0.1 N NaOH in the reference cell and LEF in 0.1 N HCl in the sample cell and vice versa.

Method validation

Preparation of calibration curve

Different volumes of stock solutions were suitably diluted with corresponding media (2, 4, 6, 8, 10 and 12 μg/ml) to get the desired concentrations. Each solution was analyzed in triplicate. The amplitude values were plotted against the corresponding concentrations to obtain the linear calibration curve.

Precision

For the determination of intra-day and inter-day accuracy and precision of the assay, samples containing LEF (6, 8 and 10 μg/ml) were analyzed six times a day (intra-day) and for three consecutive days. Precision was calculated as inter-day and intra-day coefficient of variation.

Selectivity

The selectivity of the method was assessed by analyzing standard drug, pharmaceutical product and placebo (composition of lactose monohydrate, crospovidone, starch, colloidal silicon dioxide, magnesium stearate and talc) and comparing the maxima and minima of the standard with that of the sample to determine whether the pharmaceutical product and placebo lead to interfere in the estimation.

Recovery studies

The recovery studies of the method were assessed by spiking the standard LEF with the preanalyzed samples and the mixtures were analyzed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulation.

Procedure for tablet dosage form

Tablet powder equivalent to 10 mg LEF (Lefumide, 10 mg LEF per tablet) was taken into a clean 50 ml volumetric flask and 20 ml of methanol was added and sonicated for 10 min. The final volume was made up to the mark. Then it was filtered through Whatman filter paper No. 42. Suitable samples were taken in 10 ml volumetric flasks, and volumes were made up with 0.1 N NaOH and 0.1 N HCl. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

RESULTS AND DISCUSSION

The development of simple method for the determination of drugs has received considerable attention in recent years because of their importance in quality control of drugs and drug products. There were no literatures reported for the determination of LEF by difference spectrophotometric method for routine analysis.

The suitability of the diluents was decided on the basis of the various trials. Large variation in the amplitude with change in normality of NaOH and HCl for the same concentration of LEF was observed. Difference spectrum was obtained by keeping LEF in 0.1 N NaOH in the reference cell and LEF in 0.1 N HCl in the sample cell and *vice versa*. LEF exists in two different forms - in acidic and basic media, which differ in their UV spectra. LEF showed two characteristic peaks at 261.5 and 293.5 nm with negative and positive absorbance, respectively (Figure 1).

Method validation

Calibration curve

A set of six solutions of LEF at concentrations ranging from 2 to 12 μg/ml were prepared. Each sample was analyzed in triplicate; calibration curve was constructed by plotting the amplitude against concentration using linear regression analysis. The regression equation was found to be $y = 0.079x - 0.002$ with the correlation coefficient of 0.9990; indicating an excellent linearity of the developed method. At the range, the percent relative standard deviations of the amplitude of three replicate analyses were found to be less than 2.0%.

Precision

The results of intra-day precision and accuracy for LEF is shown in Table 1. The intra-day precision was determined by calculating the % RSD for six determinations at each concentration of three samples and was found to be between 0.585 and 0.92%. The mean precision was found to be 0.72.

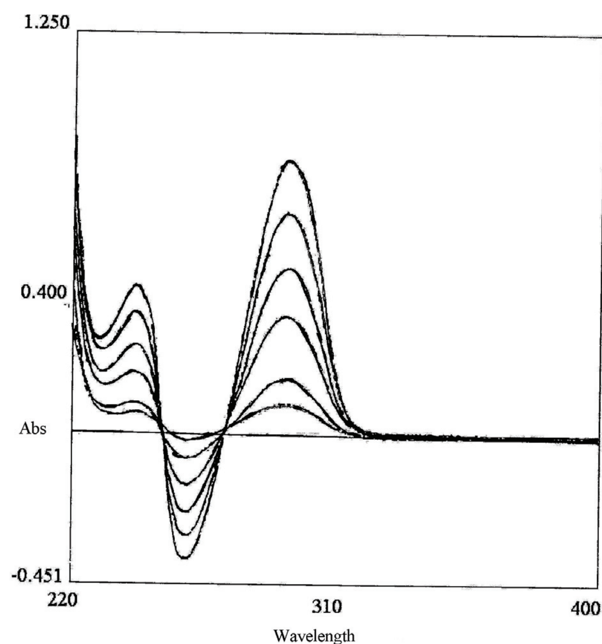

Figure 1. Difference spectra of leflunomide.

The result of inter-day precision and accuracy for LEF is shown in Table 1. The inter-day precision was determined by calculating the % RSD for six determinations at each concentration of three samples on three consecutive days and was found to be between 0.011 and 0.018%. The mean precision was found to be 0.15.

Table 1. Intra-day and inter-day precision of proposed method (n = 6)

Actual concentration µg/ml	Intra-day precision			Inter-day precision		
	Observed concentration, µg/ml	SD	RSD, %	Observed concentration, µg/ml	SD	RSD, %
4	3.98	0.038	0.92	3.98	0.004	0.11
6	5.96	0.034	0.58	5.97	0.010	0.17
8	7.95	0.052	0.65	7.97	0.014	0.18

Table 2. Recovery studies (n = 6, mean recovery ± SD = 99.0 ± 0.09)

Drug	Amount of standard spiked, µg/ml	Amount of standard recovered, µg/ml	Recovery, %	RSD, %
Leflunomide	4	3.963	99.08	0.79
	6	5.934	98.90	0.64
	8	7.92	99.0	0.60

Selectivity

The selectivity of the method was confirmed by comparing the wavelength of maxima and minima of standard with that of LEF in the marketed formulation. The difference spectroscopic UV spectrum obtained for drug is superimposable with the spectrum obtained with the market formulation and there is no change in the maxima and minima wavelength. The absence of interfering peaks from the excipients commonly present in the tablets indicated that degradation of LEF had not occurred in the formulation that was analyzed by this method. Hence the developed method is specific and selective.

Accuracy

The results listed in Table 2 showed the developed method is accurate and the recovery was found to be between 98.91 and 99.08%.

Analysis of pharmaceutical solid dosage form

A superimposable spectrum was observed for the leflunomide samples extracted from tablets, which indicates that there is no interference from the common excipients present in the tablet. The mean leflunomide content was found to 99.0%. The above results revealed that the developed method can be used for the determination of LEF in pharmaceutical formulation. The low % RSD value indicted the suitability of this method for routine analysis of LEF in pharmaceutical dosage forms.

CONCLUSION

The developed method was found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of LEF in bulk drug and marketed solid dosage formulation without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of LEF. Its advantages are low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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NAUČNI RAD

RAZVOJ DIFERENCIJALNE SPEKTROFOTOMETRIJSKE METODE ZA ODREĐIVANJE LEFLUNOMIDA U TABLETAMA

Razvijena je nova jednostavna, precizna, osetljiva i reproduktivna diferencijalno spektrofotometrijska metoda za određivanje leflunomida u rasutom stanju i u farmaceutskim dozirnim oblicima. Diferencijalno spektrofotometrijska metoda je zasnovana na principu da leflunomid postoji u dva različita oblika koji imaju različite apsorpcione spektre u kiseloj i u baznoj sredini. Diferencijalni spektar je dobijen uporednom analizom spektra leflunomida u 0,1 M HCl (referentna ćelija) i spektra leflunomida u 0,1 M NaOH (uzorak). Izračunate amplitudne vrednosti su grafički predstavljene u zavisnosti od koncentracije. Određena je lineranost u opsegu koncentracija od 2-12 μg/ml. Procentualne „recovery“ vrednosti su u opsegu od 98,92 do 99,08%. Predložena metoda je statistički validirana i uspešno je primenjena za analizu leflunomida u tabletama.

Ključne reči: katalizator; izomerizacija n-heksana; fosfatima modifikovan cirkonijum(IV)-oksid; sulfatima modifikovan cirkonijum(IV)-oksid.