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NOTE

Spectrophotometric Determination of Leflunomide in Bulk and Pharmaceutical Formulations

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A simple, sensitive and precise spectrphotometric methods have been developed for the estimation leflunomide in bulk and dosage forms. Methanol was used as solvent for the estimation of the entire drug 2 with apparent molar absorbitivity of 55277 L mol^{-1} cm^{-1}. Beer's law was obeyed in the concentration range of 2-30 μg/mL. Results of the analysis were validated statistically and by recovery studies.

Key Words: Spectrophotometric determination, Leflunomide.

Leflunomide (LEF), [N-(4'-trifluoromethylphenyl)-5-methyl-isoxazole-4-carboxamide] is a novel isoxazol derivative with both antiinflammatory and immunosuppressive properties[1-3]. It 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 prodrug, which is rapidly and non-enzymatically converted to its active metabolite, A77 1726 after oral administration. It is reported that A77 1726 possesses immunomodulator effects of the drug by reversible inhibition of the enzyme dihydro dehydrogenase and inhibits cell proliferation of lymphocytes[1-3]. Since the conversion of LEF to A77 1726 *in vivo* is essentially complete, most pharmakokinetic studies have been focused to measure A77 1726. Several high performance liquid chromatography (HPLC) methods have been published for the kinetic monitoring and determination of A77 1726 in human blood and plasma[4-9]. A pharmaceutical determination of LEF by FIA-UV detection has also been reported[10].

Spectral and absorbance measurements were made with Elico SL 164 Double beam UV-Visible spectrophotometer with 1 cm path length quartz cells.

Standard and sample solutions: Methanol was used to prepare the stock solutions of 1 mg/mL. The drug was scanned initially for its absorption maxima and a calibration curve was plotted.

Method: Leflunomide was weighed accurately and dissolved in methanol so as to give a stock solution of concentration of 1000 μg/mL. Aliquots of 100 μg/mL solution were transferred into 10 mL volumetric flasks and volume was adjusted with distilled water to give final concentrations of 2, 5, 10, 20, 30 μg/mL. The absorbance was measured at 261 nm against distilled water as a blank. The proposed method was applied to the analysis of commercially available lefra tablets. A quantity of mixed contents of 20 tablets equivalent to 20 mg of leflunomide was transferred into a 50 mL volumetric flask. A small quantity of methanol was added to disolve the drug. It was made up to volume with methanol and the solution was finally filtered. The filtrate was further diluted with distilled water to 20 μg/mL concentrations and the absorbance was measured at 261 nm against methanol as a blank. Recovery studies were carried out by adding a known quantity of pure drug to the pre-analyzed solutions and the proposed method was followed. From the amount of drug found, percentage recovery was calculated.

The proposed method of determination of leflunomide showed molar absorptivity of 2.781666×10^4 $Lmol^{-1}cm^{-1}$. Linear regression of absorbance of concentration gave the equatin Y = 0.09860x + 0.0050 with a correlation coefficient of 0.9991. Relative standard deviation of < 0.5% (0.345) was observed for analysis of five replicate samples, indicating precision and reproducibility. Luflunomide exhibits its maximum absorption at 261 nm and obeyed Beer's law in the concentration range of 2-30 μg/mL. The results of analysis and recovery studies are presented in Table 1 and 2. The percentage recovery value indicates that there is no interference from the excipient(s) present in the formation. The developed method is found to be sensitive, accurate, precise and reproducible and can be used for the routine quality control analysis of leflunomide in bulk drug and formulations.

TABLE-1
RESULTS OF ASSAY

Formulation	Label claim (mg)	Amount found*		C.V. (%)
		(mg)	%	
Lefra	10.0	10.15±0.027	101.2	0.346
Lefra	20.0	18.973±0.040	99.46	0.819

*Mean of five determinations.

TABLE-2
RECOVERY STUDIES

S.No.	Label claim (mg/tablet)	Amount of standard added (mg)	Total amount recovered (mg)	% Recovery
1	10	0	9.89	98.8
2	20	20	21.33	102.2

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