Original Article

NOVEL HPTLC-DENSITOMETRIC METHOD FOR THE ESTIMATION OF TERIFLUNOMIDE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: The current work is intended towards the development of a novel, simple and precise high-performance thin layer chromatographic (HPTLC) method coupled with a densitometer for the estimation of teriflunomide (TEF) present in the marketed formulation.

Methods: The chromatographic development was performed on aluminum plates coated with silica gel 60 F_{254} using toluene: ethyl acetate: glacial acetic acid (7.5:2: 0.5 v/v/v) as the mobile phase. Densitometric scanning was achieved at the absorbance maxima, UV 284 nm.

Results: Well separated band was observed with Rf value 0.46. The calibration curve plotted in the concentration range 100-700ng/band exhibited an excellent linear relationship with the r^2 value of 0.9928. The method was found to comply with all the validation parameters as per the ICH guidelines.

Conclusion: The method ensures minimal use of mobile phase with minimal run time compared to other reported analytical methods. This validated method can be used by quality control laboratories for the routine quantitative analysis of tablets consisting of Teriflunomide.

Keywords: Densitometer, HPTLC, ICH guidelines, Marketed formulation, Teriflunomide, UV

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INTRODUCTION

Teriflunomide (fig. 1) (TEF) is an orally administered, second-generation immunosuppressive/immunomodulatory agent which is an active metabolite of Leflunomide. It acts by inhibiting dihydroorotate dehydrogenase, an enzyme which is responsible for pyrimidine de novo synthesis [1-4]. It is permitted by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as a disease-modifying therapy (DMT's) for adults with relapsing-remitting multiple sclerosis (RRMS). This drug, not official any pharmacopeias like IP, USP and BP.
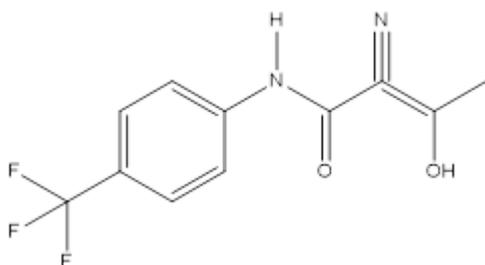
Fig. 1: Teriflunomide

The literature survey disclosed various methods for the estimation of TEF in API, marketed formulations and biological fluids. The detailed information on the various methods available are as follows; chromatographic methods such as HPLC [5, 6], UPLC [7], RP-HPLC [8, 9], LC-MS [10-13]. In all the reported techniques, the overall solvent consumption, cost per analysis and overall time required for analysis were much more. Furthermore, No HPTLC method has been reported so far, for the estimation of TEF in the marketed formulation. Therefore, the current work is directed

towards the development of a novel HPTLC method for the determination of TEF in the marketed formulation having sensitivity in terms of nanogram with minimal development time, minimal use of solvents and its validation according to ICH guidelines.

MATERIALS AND METHODS

Materials and reagents

Teriflunomide pure drug (API) was procured as a gift sample from Glenmark Ltd., Noida. All the other reagents used were of analytical grade and were procured from Merck specialties, private limited, Mumbai, India. Marketed Formulation (Denopsy tablets, Natco Pharma, Hyderabad) was purchased from a local pharmacy.

HPTLC instrumentation and chromatographic conditions

CAMAG HPTLC system equipped with Linomat 5 sample applicator operated under a gentle stream of nitrogen, coupled with a 100 μl HAMILTON syringe and a CAMAG TLC scanner 3controlled by winCATS software was used for the application and detection of spots respectively. Aluminum sheets having a dimension of 20×10 cm, coated with silica gel 60 F_{254} procured from Merck, Germany and a CAMAG twin trough TLC chamber having a dimension 27.0 cm width × 26.5 cm height × 7.0 cm was used for the chromatographic development.

For chamber saturation, 20 ml of the mobile phase was transferred into the development tank with the lid closed. The assembly was kept aside for 20 min under room temperature. The plates were air dried using an air dryer after the application of spots and after the development, prior to densitometric scanning. The chromatographic conditions were as described in table 1.

Preparation of standard solutions

A stock solution containing 1000 ng/μl of TEF was prepared by using acetonitrile as a diluent. 0.5 ml of this solution was pipetted out into a 10 ml volumetric flask and the volume was made up, to get a working solution consisting of 50 ng/μl of TEF.

Table 1: HPTLC Chromatographic conditions

Parameters	Values
Mobile phase	Toluene: Ethyl acetate: Glacial acetic acid (7.5:2: 0.5 v/v/v)
The volume of mobile phase used	20 ml
Chamber saturation time	20 min
Band length	6 mm
Dosage speed	150 nl/s
Distance between bands	10 mm
Development time	7 min
Detector wavelength	284 nm
Rf value	0.46

Preparation of sample solution (Formulation)

As specified in the label, each tablet contains 14 mg of TEF. The weight of 10 tablets was noted and crushed using a mortar and pestle, the quantity of powder equivalent to 10 mg of teriflunomide was transferred to a 10 ml volumetric flask and the volume was made up with acetonitrile. The required dilutions were made to get a final concentration of 50 ng/µl.

Method validation

The method was validated according to ICH guidelines for the following parameters: Linearity, accuracy, precision, the limit of detection (LOD) and limit of quantification (LOQ), specificity and robustness [14-16].

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The ideal mobile phase of the method was selected after several experimental trials. Solvents like acetonitrile, methanol, toluene, ethyl acetate used initially; did not provide a clear spot. Therefore, various permutations and combinations of these solvents were used for the trials. Out of all the combinations, toluene and ethyl acetate was found to give a spot. For better resolution, 0.5% glacial acetic acid was added. Thus, the final chromatographic condition, toluene: ethyl acetate: glacial acetic acid in the ratio 7.5: 2: 0.5 (v/v/v) was able to achieve a clear spot with good R_f value. Increasing the volume of glacial acetic acid leads to an increase in the R_f value and vice versa. The chamber saturation time was found to show a similar effect.

The wavelength for the densitometric scanning of the spots was selected after obtaining the UV spectra of TEF (fig. 2), which showed the maximum absorbance at 284 nm.

Linearity

The calibration curve (fig. 3) was plotted between peak areas versus concentration. The linearity of TEF was constructed by applying 7 bands in the concentration range of 100-700 ng/band. The regression equation (r2= 0.9928) demonstrated the good linearity of the method. The slope and intercept of the regression equation were 15.471 and 3505.7 respectively for TEF. The linearity was found to be satisfactory and reproducible. The calibration curve of linearity and overlay densitogram of TEF is depicted in fig. 3 and 4 respectively.
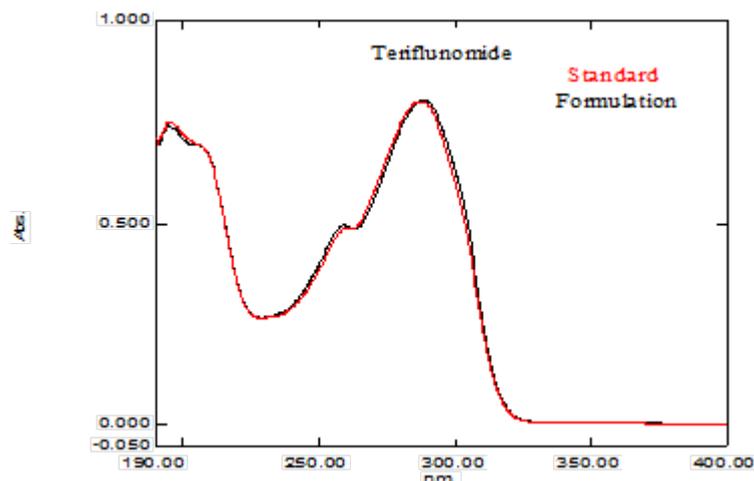

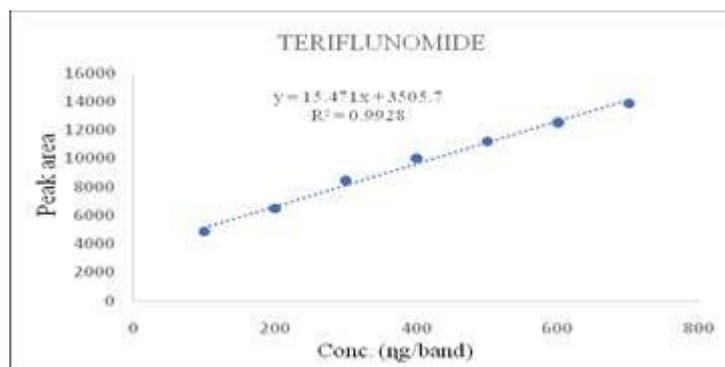**Fig. 2: Overlay UV absorption spectra of TEF standard and formulation**

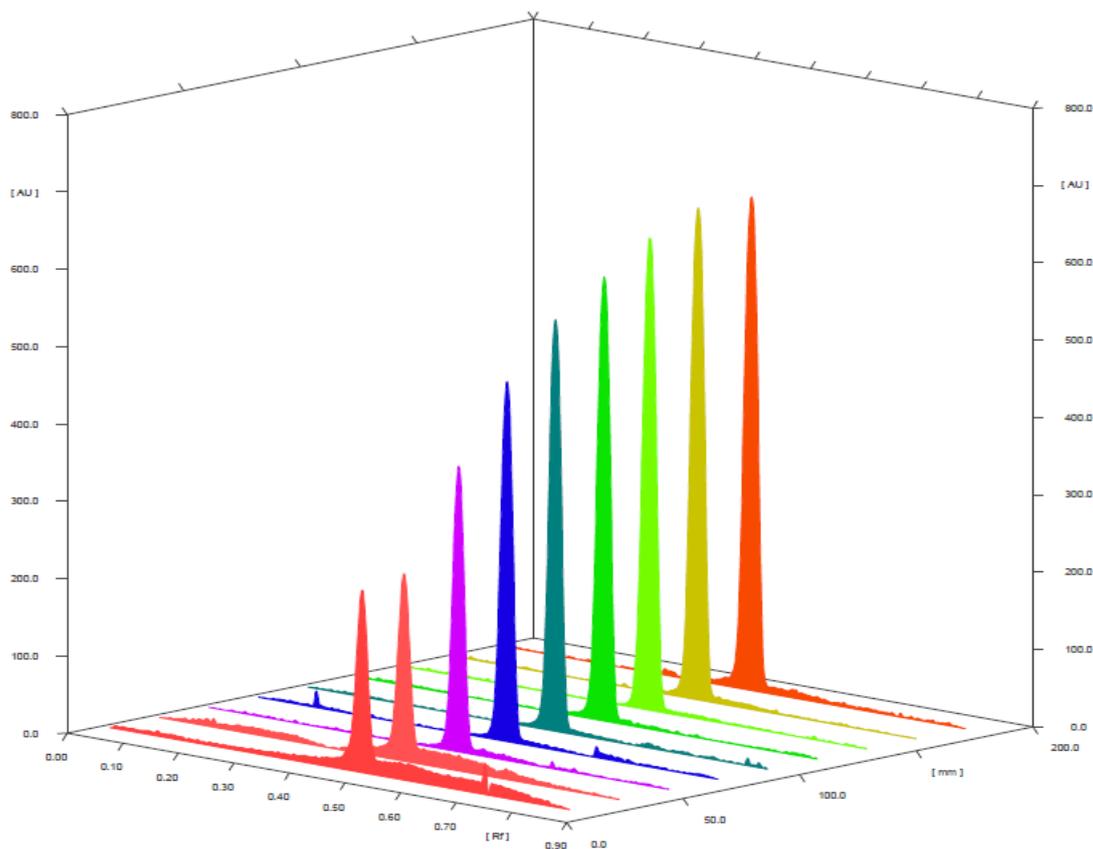
Fig. 3: Calibration curve of TEF

Fig. 4: Overlay densitogram of TEF

Accuracy

Accuracy was assessed in terms of percentage recovery, by spiking the band of the formulation with 50%, 100%, 150% of pure drug and then finding out the amount of the drug recovered. The mean percentage of recovery was found to be 99%, as depicted in table 2.

Table 2: Accuracy study of TEF

Level of recovery (%)	Amount of formulation (ng/band)	Amount of pure drug (ng/band)	% Recovery	Mean	% RSD[a]
50	100	50	99.20		
	100	50	98.04	98.94±0.9	0.668
	100	50	99.60		
100	100	100	100.10		
	100	100	99.80	99.7±0.4	0.287
	100	100	99.40		
150	100	150	99.60		
	100	150	98.00	98.6±1.0	0.721
	100	150	98.20		

[a]Mean for three independent analyses, SD=Standard deviation, RSD=Relative standard deviation

Precision

Intraday and interday precision of the method was assessed by developing the plate after application of 6 replicates of the highest concentration on the TLC plate on the same day and the consecutive day respectively. Precision was reported in terms of %RSD. The %RSD values were found to be less than 2% as depicted in table 3, indicating the high precision of the developed method.

Table 3: Precision study of TEF

Precision studies	Intraday	Interday
Conc. (ng/band)	700	700
Average peak area (n=6)	13830.5	13734.17
Standard deviation	101.8361	124.8431
% RSD[a]	0.7363	0.9089

[a]Mean for six independent analyses, SD=Standard deviation, RSD=Relative standard deviation.

Limit of detection and limit of quantitation

LOD and LOQ were calculated with the aid of standard deviation (σ) and slope (s) from the calibration curve (n=3), by using the formula LOD = 3.3 σ/s and LOQ = 10 σ/s. LOD and LOQ of TEF were found to be 21 ng/band and 65 ng/band respectively, which indicates the good sensitivity of the method towards the analyte.

Specificity

The specificity of the method was assessed by comparing the UV absorption spectra (fig. 2) and densitogram of standard (fig. 4) TEF with the formulation (fig. 5). The densitogram of both standard and formulation showed the same R_f value indicating the good specificity of the method.

Robustness

The robustness was assessed by making small but deliberate changes in the method parameters such as; mobile phase ratio, chamber saturation time, scanning wavelength and finding out its effect on the peak area by calculating %RSD. The % RSD was found to be within 2%, which indicates the reliability of the method. The robustness of the method are depicted in table 4.

Table 4: Robustness study of TEF

Parameters	% RSD[a]
Mobile phase composition (±2 ml)	1.35
Chamber saturation time (±2 min)	0.98
Scanning wavelength (±2 nm)	1.72

[a]Mean for five independent analyses, SD=Standard deviation, RSD=Relative standard deviation.

Analysis of formulation

The % of a drug found in the formulation of the currently developed method was found to be 98.9%. The densitogram obtained from the formulation (fig. 5) exhibited a single spot at R_f 0.46 without any interference of the excipients. The close agreement of the percentage of the drug found with label claims depicted the application of this method for the routine analysis of TEF present in its formulation.
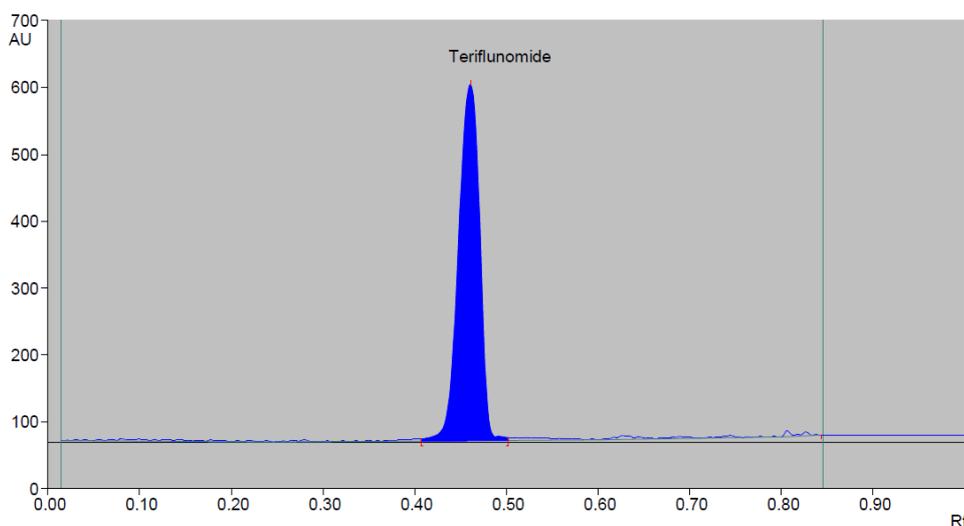
Fig. 5: Densitogram of the formulation

DISCUSSION

A novel, simple and precise high-performance thin layer chromatographic (HPTLC) method coupled with densitometer was developed for the estimation of TEF present in the marketed formulation. There are some HPLC methods [7, 8] reported in the literature, but they have certain restrictions like require a large quantity of sample and/or organic solvents or sensitive to microgram concentration. Few methods like Liquid chromatography-mass spectroscopy for determination TEF in human or animal plasma [9-12] etc. are found in the literature, but these methods are not preferred for routine analysis of TEF in bulk and formulation studies, because of the high cost of analytical technique and the skilled requirement for sample treatment. A detailed evaluation of the analytical method procedures discussed with the present method was given in table 1. Accuracy results displayed good reproducibility with % RSD values below 2. This was found to be accurate as percent recovery observed was high i.e. within the range of 98.6-98.94 %, suggesting that the proposed method showed good agreement between the standard and the observed values and demonstrate an adequate accuracy within the specified limits as shown in the table 2. Furthermore, detection limit depends upon the instrument sensitivity as low detection limits give high sensitivity. The LOD and LOQ of developed for HPTLC method was found to be 21ng/ml and 65ng/ml respectively confirms method is sensitive. Results of intra and inter-day precision studies were expressed as %RSD, the results were given in table 3. The low values of %RSD showed that the method is precise. The results of robustness are tabulated in table 4, the method is not altered by changing the method parameters like mobile phase composition, chamber saturation time and scanning wavelength which proves that the method is robust. In addition, the estimation of the marketed preparation of TEF with the validated methods showed that the drug contents separated with no interfering peaks generated by the excipients in the marketed formulation as shown in the fig. 5. The method is versatile and simple for the analysis TEF in pure and pharmaceutical formulations. The method was found to obey all the validation parameters as per the ICH guidelines. The method confirms minimal use of the mobile phase with a short run time compared to other reported analytical methods. This validated method can be used for quality control laboratories for the routine quantitative analysis of tablets consisting of TEF.

CONCLUSION

A novel, simple, rapid and precise HPTLC method coupled with densitometer scanning has been developed for the determination of TEF in the marketed formulation. This validated method can be used by quality control laboratories for the routine quantitative analysis of tablets consisting of TEF. The additives used in this formulation were also not interfering with the analysis. The method ensures minimal use of mobile phase with minimal run time compared to other reported analytical methods. Non-requirement of skilled personnel to operate the instruments involved is an added advantage of this method.

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AUTHORS CONTRIBUTIONS

The submitted research work is a part of the Master of Pharmacy in Pharmaceutical Analysis specialization. This project was guided by Dr. BM Gurupadayya and laboratory research work and validation of the method was carried out by Mr. T S Vishwas with the coordination of Dr. Rupshee Jain.

CONFLICT OF INTERESTS

The authors report no conflicts of interest

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