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Research Article

BIOANALYTICAL METHOD FOR TERIFLUNOMIDE ESTIMATION BY HPLC

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ABSTRACT

Objective: The aim of this study was to develop and validate simple and economical HPLC method for estimation of Teriflunomide in human plasma.

Methods: HPLC method was developed using AgilentEclipse XBD C8 (4.6 mm×150 mm) as stationary phase and mobile phase used was ammonium acetate buffer: methanol (40: 60 v/v) The detection was carried at wavelength 294 nm. A simple protein precipitation technique was used with acetonitrile as protein precipitating agent and Paliperidone palmitate was chosen as internal standard. Validation was carried out as per USFDA guidelines for bio-analytical method.

Results: The linearity range set was 10–60 μg/ml. The value of regression coefficient was found to be 0.9953. Retention time for Teriflunomide was found to be 4.8 min. The developed method was validated for various parameters such as specificity, linearity, accuracy, precision, recovery, and stability.

Conclusion: The developed method is simple, rapid, and economical for estimation of Teriflunomide in human plasma.

Keywords: HPLC, Teriflunomide, Paliperidone palmitate, Bio-analytical, Validation.

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INTRODUCTION

Teriflunomide is active metabolite of leflunomide.chemically teriflunomide is (Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl) phenyl]but-2-enamide [1]. The chemical structure of Teriflunomide is given in Fig. 1.

It is reported that Teriflunomide has bothanti-inflammatory and immunosuppressive properties [2]. It shows immunomodulator effects by reversible inhibition of enzyme dihydro-orotate dehydrogenase and inhibits cell proliferation of lymphocytes. It is used to treat rheumatoid arthritis.

In vitro studies with human plasma show that 99.4% of added Teriflunomide is bound to plasma proteins, which is consistent with a low volume of distribution. Teriflunomide shows nearly 100% of bioavailability and the plasma half-life is approximately 15 d [3]. Teriflunomide reaches a maximum plasma concentration in the time period of 6–12 h following oral administration [4].

As per literature survey, there are few reported papers showing work in various biological matrix such as urine, plasma, serum, whole blood for estimation of Teriflunomide, and its metabolite using different analytical technique such as HPLC, UPLC, and LC-MS/MS [2-10]. Few of these reported papers use gradient system of elution and complex mobile phase [3-6], some of the reported methods require adjustment of pH and maintenance of column temperature [4-10]. Hence, a simple method with isocratic elution was developed for estimation of Teriflunomide in human plasma.

METHODS

Chemicals and reagents

Teriflunomide was obtained as gift sample from Natco Pharmaceuticals, Hyderabad, India. Paliperidone palmitate was used as internal standard which was received as gift sample from Wockhardt Limited, Aurangabad, India. Acetonitrile of HPLC grade was purchased from Merck Lifesciences, Pvt. Ltd., Mumbai, India. Methanol was bought from Thomas Baker Chemicals Pvt. Ltd., Mumbai, India andammonium acetate was of AR grade purchased from Loba chemie, Pvt. Ltd., Mumbai, India. Water of HPLC grade was generated using Water purification system LabLink Xtra Pure.Pooled plasma was obtained as a gift sample from Sassoon hospital, blood bank, Pune, India.

Instrumentation

HPLC method

Jasco HPLC coupled with Jasco PU-2080 Plus pump and UV-2075 Plus UV/Vis detector operated with Borwin software version 1.50, Shimadzu UV 1780 UV- Visible spectrophotometer, Japan, Shimadzu AY 120 Analytical balance, Japan, Remi Cyclo-mixer, Prama SM15 US Sonicator and Remi Centrifuge R-302.

Chromatographic conditions

The stationary phase used was AgilentEclipse XBD C8 (4.6 mm×150 mm). The elution was achieved using mobile phase 0.1 M Ammonium acetate buffer: Methanol in ratio of 40: 60 v/v. The injection volume used for analysis was 50 μl and detection was carried out at wavelength of 294 nm. The run time per injection was 10 min with a flow rate of 1 ml/min. Retention time for Teriflunomide observed was 4.8 min and retention time for internal standard was 2.4 min.

Method development

Selection of mobile phase

Different mobile phases were tried to obtain a proper resolution of Teriflunomide from internal standard and plasma proteins. The optimized mobile phase was 0.1M Ammonium acetate buffer: Methanol (40:60 v/v).

Selection of internal standard

Various internal standards were tried such as Ivabradine hydrochloride, Paliperidone palmitate, Mifepristone, Nebivolol, and Rilpivirinebased on the λmax of drug. Better resolution was observed with Paliperidone palmitate hence, selected as internal standard.

Preparation of stock solution of Teriflunomide

Accurately weighed 10 mg Teriflunomide was dissolved in Methanolin a 10 ml volumetric flask to obtain a concentration of 1000 μg/ml stock solution. Further by dilution 10 μg/ml of concentration was obtained.

Preparation of stock solution of internal standard

Weighed 10 mg of Paliperidone palmitate was dissolved in methanol in a 10 ml volumetric flask to get concentration of 1000 µg/ml.

Preparation of spiked plasma sample

In test tube, to 0.8 ml of plasma, 0.1 ml of Teriflunomide (100–600 µg/ml) and 0.1 ml of internal standard (1000 µg/ml) was added to obtain required quality control samples. These samples were vortexed and 1 ml acetonitrile was added to precipitate plasma proteins. It was subjected to centrifugation to obtain supernatant which was injected to the system.

Selection of detection wavelength

Based on UV spectrum taken at concentration of 20 µg/ml, the selected wavelength was 294 nm. The UV spectrum of Teriflunomide is shown in Fig. 2.

Method validation

The developed method was validated for following parameters according to USFDA guidelines for bioanalytical method [11,12].

QC samples

For performing validation parameters, different quality control samples such as low quality control (LQC), mid quality control (MQC), and high quality control (HQC) are prepared. The QC samples are prepared based on Cmax of drug and linearity range.

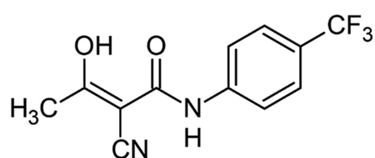

Fig. 1: Chemical structure of teriflunomide

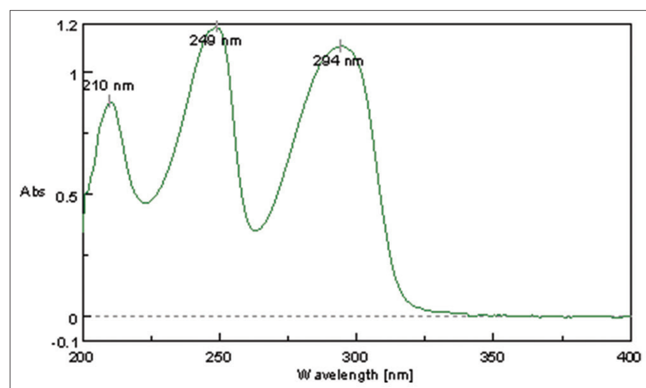

Fig. 2: UV spectrum of teriflunomide

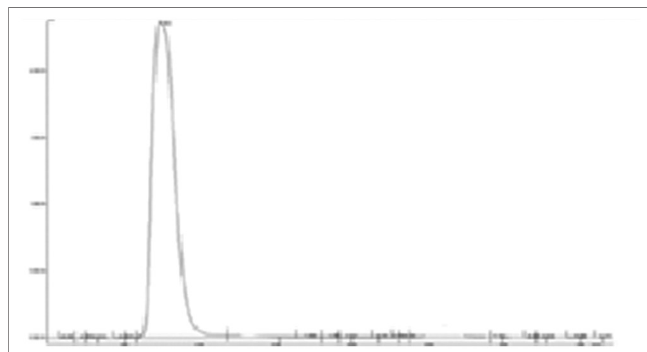

Fig. 3: Representative chromatogram of blank plasma

Calibration curve

Linearity of bioanalytical method represents direct relation between detector response and concentration of analyte of interest. The linearity range chosen was 10–60 µg/ml as the Cmax of Teriflunomide lies between the range of 19–45 µg/ml [13]. Five sets of each quality control samples were analyzed and peak areas were recorded.

Selectivity

Ability to differentiate and quantify analyte in presence of other interfering substance refers to selectivity of bioanalytical method. The specificity of method is demonstrated by analyzing blank plasma, zero plasma and spiked plasma. There should not be any interfering peak at the same retention time of Teriflunomide.

Accuracy

The accuracy of a bioanalytical method was determined by analyzing three different concentration of quality control samples and the peak areas were recorded. % Mean accuracy was calculated.

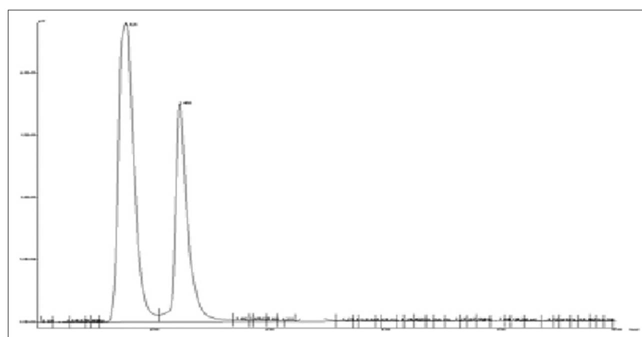

Fig. 4: Representative chromatogram of zero plasma (internal standard 50 µg/ml at Rt=2.4 mins)

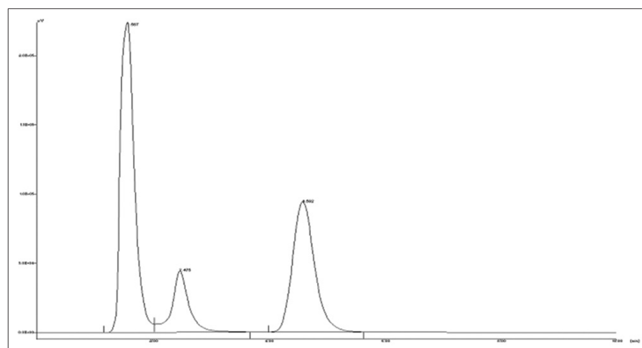

Fig. 5: Representative chromatogram of spiked teriflunomide (20 µg/ml) at Rt=4.8 mins+Internal standard (50µg/ml) at Rt=2.4 mins

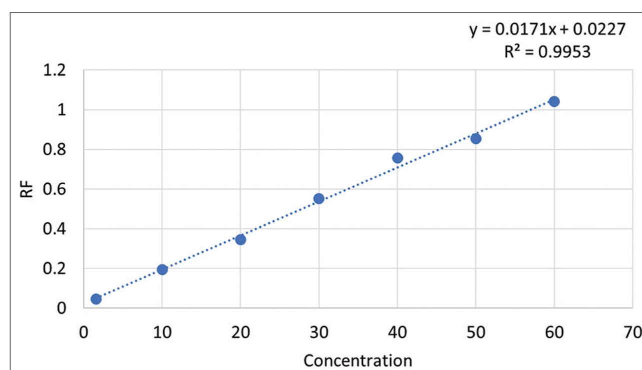

Fig. 6: Calibration curve of Teriflunomide spiked plasma

Table 1: Linearity

Concentration (μg/ml) (n=5)	1.6	10	20	30	40	50	60
Mean Response factor	0.045	0.192	0.346	0.553	0.755	0.852	1.04
SD	0.0033	0.0028	0.0023	0.0023	0.0037	0.0023	0.0188
% CV	7.27	1.47	0.68	0.42	0.50	0.27	1.82

SD: Standard deviation, CV: Coefficient of variation, n: Number of samples

Table 2: Accuracy

Concentration (n=5)	LQC (4.5 μg/ml)	MQC (27μg/ml)	HQC (50μg/ml)
Mean concentration	4.48	26.02	45.95
SD	0.38	0.13	0.39
% CV	8.53	0.52	0.86
% Mean accuracy	99.71	96.4	91.9

LQC: Low quality control, MQC: Mid quality control, HQC: High quality control, SD: Standard deviation, CV: Coefficient of variation, n: number of samples

Table 3: Precision

Intermediate precision	Concentration (n=5)	LQC (4.5 μg/ml)	MQC (27 μg/ml)	HQC (50 μg/ml)
	Mean Concentration	4.78	25.91	46.14
	SD	0.05	0.37	0.63
	% CV	1.21	1.45	1.38
Repeatability	Concentration (n=5)	LQC (4.5 μg/ml)	MQC (27 μg/ml)	HQC (50 μg/ml)
	Mean Concentration	4.79	25.95	46.12
	SD	0.06	0.38	0.65
	% CV	1.30	1.47	1.41

LQC: Low quality control, MQC: Mid quality control, HQC: High quality control, SD: Standard deviation, CV: Coefficient of variation, n: number of samples

Table 4: Recovery

Conc. (μg/ml) (n=6)	Mean area	SD	% CV	% Mean recovery	% Overall mean recovery	Overall SD (recovery)	Overall % CV (recovery)
1.6	55512.6	22.77	0.0410	71%	73.93%	2.16	2.92
	78186.7	3.58	0.0045				
4.5	88519.8	46.83	0.0529	72.13%			
	122722.5	3.59	0.0029				
10	239523.1	2580.24	1.0772	72.6%			
	329921.6	2.25	0.0006				
16	338401.4	3886.46	1.1484	76.4%			
	442933.8	2.97	0.0006				
27	527525.5	7280.39	1.3801	73.6%			
	716746.6	2.83	0.0003				
38	819208.6	1121.11	0.13	74.3%			
	1110039.0	3.58	0.0003				
50	1120926	3543.95	0.3161	77.5%			
	1446356.1	3.05	0.0002				

SD: Standard deviation, CV: Coefficient of variation, n: number of samples

Precision

For estimating method precision, both intermediate precision and repeatability are to be performed. Precision was estimated by analyzing runs of three different concentration and %CV was calculated.

Recovery

In recovery, the efficiency of extraction procedure within variation limit is determined. It is calculated by comparison of spiked samples with standard samples.

Stability

To check the degradation of analyte that may occur during entire process of sample collection, storage, extraction, and analysis at different time and conditions such as short-term storage, long-term storage as well as during freeze thaw cycles. Stability samples should be compared with freshly prepared QC samples.

RESULTS

Selectivity

The method was found to be selective as no any interfering peaks were observed at theRtof Teriflunomide. The representative chromatogram of blank plasma, zero plasma and spiked Teriflunomide is shown in Figs. 3-5 respectively.

Calibration curve

The method was proven to be linear over the selected range that is 10–60 μg/ml and 1.6 μg/ml is LLOQ (Lowest limit of Quality Control), the regression coefficient obtained after plotting concentration against response factor was 0.9953, as shown in Fig. 6. The response factor was calculated by taking ratio of area of Teriflunomide and area of internal standard (Table 1).

Table 5: Stability

S. No.	Stability	% Mean stability		% CV
1	Freeze thaw stability (Three cycles) (n=12)	LQC	95%	0.86
		HQC	95.6%	0.06
2	Short-term stability (for 4h at RT) (n=12)	LQC	96%	1.01
		HQC	97.4%	0.10
3	Long-term stability (for 14 d at 4ºC) (n=12)	LQC	94.8%	0.89
		HQC	95.9%	0.11
4	Stock solution stability (for 5 d) (n=12)	MQC	95.1%	0.21
		IS	98.7%	0.18
5	Post-preparative stability (for 5h at RT) (n=12)	MQC	96.7%	0.26
		IS	98.9%	0.12

LQC: Low Quality Control, MQC: Mid Quality Control, HQC: High Quality Control, IS: Internal standard, RT: Room temperature, CV: Coefficient of variation, n: number of samples

Accuracy

The three independent runs of QC samples were analyzed for estimation of the accuracy, it was expressed in terms of % mean accuracy. The method was found to accurate as the % mean accuracy at each concentration was found to be in acceptable range as shown in Table 2.

Precision

After analyzing the QC samples of different concentration, the %CV was calculated and it was found to be within limit and indicate that the method is precise based on result given in Table 3.

Recovery

The recovery was determined by taking ratio of spiked sample to that of standard sample and the % mean recovery was calculated (Table 4).

Stability

The peak areas of stability QC samples were compared with peak areas of freshly prepared QC samples and the % mean stability was calculated. The results are summarized in Table 5.

DISCUSSION

The bio-analytical method developed by us has certain advantages over the techniques reported in the literature. The bio-analytical methods reported in the literature for Teriflunomide involve use of LC-MS/MS technique [2-8]. There are some HPLC methods reported [6,9], but these methods involve use of mobile phase component acetonitrile which is costlier than methanol and these methods also need maintenance of column temperature. These reported methods include use of gradient elution system. Whereas, in our developed method, a simple economic mobile phase as ammonium acetate buffer: methanol in ratio of 40:60 v/v was used in isocratic mode. The developed method was validated as per USFDA guidelines and data obtained were within acceptance criteria of guidelines.

CONCLUSION

The optimized method was found to be simple and economic. The method was found to be linear over wide range. Here, simple and easy protein precipitation technique was used for sample pre-treatment and simple isocratic elution was adopted.

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AUTHOR CONTRIBUTION

Pooja Gurav studied, carried out the research work and drafted the manuscript under the guidance of Mrinalini Damle. The manuscript was checked and approved by Mrinalini Damle.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR FUNDING

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REFERENCES

1. PubChem. PubChem Compound Summary for CID 54684141, Teriflunomide. Bethesda: National Library of Medicine, US. National Center for Biotechnology Information; 2004. Available from: https://www.pubchem.ncbi.nlm.nih.gov/compound/teriflunomide [Last accessed on 2022 Apr 24].
2. Parekh JM, Vaghela RN, Sutariya DK, Sanyal M, Yadav M, Shrivastav PS. Chromatographic separation and sensitive determination of teriflunomide, an active metabolite of leflunomide in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2217-25. doi: 10.1016/j.jchromb.2010.06.028, PMID 20643586
3. Rakhila H, Rozek T, Hopkins A, Proudman S, Cleland L, James M, *et al*. Quantitation of total and free teriflunomide (A77 1726) in human plasma by LC-MS/MS. J Pharm Biomed Anal 2011;55:325-31. doi: 10.1016/j.jpba.2011.01.034, PMID 21349677
4. Rule GS, Rockwood AL, Johnson-Davis KL. LC-MS/MS method for the quantification of the leflunomide metabolite, teriflunomide. In: Langman LJ, Snozek CL, editors. Human Serum/Plasma. LC-MS in Drug Analysis-Part of Methods in Molecular Biology Book Series. Berlin: Springer; 2018. p. 75-83.
5. Yao X, Liu Y, Song L, Jiang J, Xiao F, Liu D, *et al*. Development of a simple HPLC-MS/MS method to simultaneously determine teriflunomide and its metabolite in human plasma and urine: Application to clinical pharmacokinetic study of teriflunomide sodium and leflunomide. Biomed Chromatogr 2018;33:e4420.
6. Suneetha A, Raja RK. Comparison of LC-UV and LC-MS methods for simultaneous determination of teriflunomide, dimethyl fumarate, and fampridine in human plasma: Application to rat pharmacokinetic study. Biomed Chromatogr 2016;30:1371-7. doi: 10.1002/bmc.3694, PMID 26849839
7. Filali-Ansary A, Lunven C, Turpault S, Beyer YJ, O'Brien A, Delfolie A, *et al*. Dried blood spot methodology in combination with liquid chromatography/tandem mass spectrometry facilitates the monitoring of teriflunomide. Ther Drug Monit 2016;38:471-82.
8. Rule GS, Rockwood AL, Johnson-Davis KL. Quantitation of teriflunomide in human serum/plasma across a 40,000-fold concentration range by LC/MS/MS. Methods Mol Biol 2016;1383:195-203.
9. Suneetha A, Rajeshwari RK. Prescriptive oriented drug analysis of multiple sclerosis disease by LC-UV in whole human blood. J Chromatogr Sci 2016;54:165-74.
10. Raouf AA. Development and Validation of Analytical Methods for the Determination of Some Drugs Used for Musculoskeletal System Disorders and Osteoporosis Therapy [Thesis]. Egypt: Cairo University; 2019.
11. Kadian N, Raju KS, Rashid M, Malik MY, Taneja I, Wahajuddin M. Comparative assessment of bio analytical method validation guidelines for the pharmaceutical industry. J Pharm Biomed Anal 2016;126:83-97.
12. U.S. Department of health and human Services food and drug administration. In: Bio Analytical Method Validation Guidance for Industry. US Food and Drug Administration; 2018. Available from: https://www.fda.gov/files/drugs/published/bioanalytical-method-validation-guidance-for-industry.pdf
13. Oh J, O'Connor PW. An update of teriflunomide for treatment of multiple sclerosis. Ther Clin Risk Manag 2013;9:177-90. doi: 10.2147/TCRM.S30947, PMID 23761970