International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 14, Issue 9 2022

Original Article

BIOANALYTICAL METHOD FOR ESTIMATION OF TERIFLUNOMIDE IN HUMAN PLASMA

POOJA GURAV, MRINALINI DAMLE*

All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Quality Assurance, Kennedy Road, Near RTO, Pune 411001
Email: damle_mc@aissmscop.com

Received: 26 May 2022, Revised and Accepted: 06 Jul 2022

ABSTRACT

Objective: Teriflunomide is used for the treatment of multiple sclerosis and is available in 7 mg and 14 mg tablets. This study aimed to develop and validate a simple and economical HPTLC method for the estimation of Teriflunomide in human plasma.

Methods: HPTLC method was developed using toluene: ethyl acetate: acetic acid as the mobile phase and the stationary phase was a TLC plate precoated with silica gel 60 F_{254}. The detection wavelength set was 294 nm. The sample preparation involved a simple protein precipitation technique with Acetonitrile as a precipitating protein agent; the internal standard selected was Rilpivirine. The validation was carried out as per bio-analytical method guidelines.

Results: The Rf value for Teriflunomide was found to be 0.46±0.04. The linearity range was observed from 10-60 µg/ml with a regression coefficient value of 0.9819. The developed method was validated for various parameters like specificity, linearity, accuracy, precision, recovery, and stability.

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

Keywords: Teriflunomide, HPTLC, Protein precipitation, Rilpivirine, Bioanalytical

DOI: https://dx.doi.org/10.22159/ijpps.2022v14i9.45151. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps.

INTRODUCTION

Teriflunomideis the active metabolite of Leflunomide which is a pyrimidine synthesis inhibitor used for the treatment of relapsing forms of multiple sclerosis. Also, it has anti-inflammatory and immunomodulatory properties. Chemicallyit is (Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl) phenyl] but-2-enamide [1].

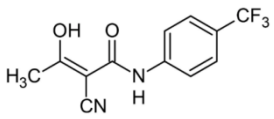

Fig. 1: Chemical structure of Teriflunomide

Teriflunomide selectively and reversibly inhibits dihydro-orotate dehydrogenase, which is a key enzyme in the pyrimidine synthesis pathway. As Teriflunomide inhibits this enzyme leads to a reduction in the proliferation of activated T and B lymphocytes without causing cell death [2]. The oral bioavailability of Teriflunomide is 100%, with peak plasma levels achieved within 1-2 h of intake and Cmax ranging from 19-45µg/ml. Teriflunomide has a mean plasma half-life of 10-18 d [3].

The literature survey revealed that there are many papers [4-12] on the bioanalytical method of Teriflunomide using LC-MS/MS, LC-UV, and UPLC. But there is no paper reported on the bioanalytical method for Teriflunomide using HPTLC. Bioanalytical methods involve the determination of analytes of interest in various biological matrix-like plasma, serum, urine, etc [13]. Some of the advantages of HPTL Conclude, a short analysis time, cost-efficient analysis, prior treatments for solvents like filtration and degassing can be evaded, and a fresh stationary phase and mobile phase are used for analysis which helps to prevent carryover.

MATERIALS AND METHODS

Chemicals and reagents

Teriflunomide was obtained as a gift sample from Natco Pharmaceuticals, Hyderabad, India. Rilpivirine was received as a gift sample from Mylan laboratories, Hyderabad, and was used as an internal standard. Methanol and ethyl acetate was of HPLC grade purchased from Merck Lifesciences, Pvt. Ltd., Mumbai, and toluene and acetic acid used were of AR grade purchased from Loba Chemie, Pvt. Ltd., Mumbai, India. Pooled plasma was obtained as a gift sample from Sassoon hospital, blood bank, Pune, India.

Instrumentation

Camag HPTLC system with win CATS software version 1.4.2, Shimadzu UV 1780 UV-Visible spectrophotometer, Japan, Shimadzu AY 120 Analytical balance, Japan, Remi Cyclo-mixer and Remi Centrifuge R-302.

Experimental

Chromatographic conditions

HPTLC method

The chromatographic resolution was performed on a Merck TLC plate precoated with silica gel 60 F_{254} using a Camag Linomat V sample applicator. Samples were applied on the plate in the form 6 mm band using a Camag 100 µl sample syringe. 20×10 twin trough chamber was used for ascending development with mobile phase toluene: ethyl acetate: acetic acid (6.5:3:0.5v/v/v). Saturation time was set at 20 min; the plate was allowed to develop up to70 mm of distance. Densitometric scanning was performed on a Camag TLC scanner III at 294 nm for all developments operated by Win cat's software version 1.4.2. Deuterium and tungsten lamps were used as the radiation source.

Method development

Selection of mobile phase

The mobile phase first tried was toluene: ethyl acetate: glacial acetic acid in the ratio of 7.5:2:0.5 v/v/v [14]. To obtain well-resolved peaks of internal standard and Teriflunomide, the mobile phase was optimized to toluene: ethyl acetate: glacial acetic acid in the ratio of 6.5:3:0.5 v/v/v.

Selection of internal standard

Based on the λmax of the drug, various internal standards like Ivabradine hydrochloride, Mifepristone, Nebivolol, Rilpivirine, and

Paliperidone palmitate were tried. Rilpivirine showed better resolution, so it was selected as the internal standard.

Preparation of stock solution of teriflunomide

The stock solution was prepared by dissolving 10 mg Teriflunomide in methanol in a volumetric flask of 10 ml to obtain a concentration of 1000 µg/ml. Further dilution was made to obtain the concentration of 10 µg/ml.

Preparation of stock solution of internal standard

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

Preparation of spiked plasma sample

In a test tube, 0.1 ml stock solution of Teriflunomide (10-60 µg/ml) and 0.1 ml of internal standard (1000 µg/ml) were added to 0.8 ml plasma to obtain the required QC (Quality Control) samples. The QC samples were vortexed and plasma proteins were precipitated using 1 ml of acetonitrile and centrifuged to obtain supernatant. This supernatant was spotted.

Selection of detection wavelength

The wavelength selected was 294 nm based on the UV spectrum taken at a concentration of 20 µg/ml. The UV spectrum is shown in fig. 2.

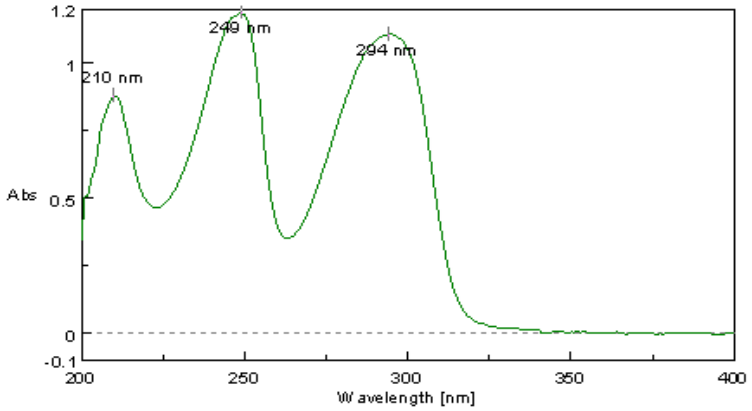

Fig. 2: UV spectrum of teriflunomide

Method validation

The developed method was validated as per the USFDA guidelines for bioanalytical methods [15-17].

QC samples

Different QC samples were prepared based on the Cmax of the drug and linearity range, namely, Low-Quality Control (LQC), Middle-Quality Control (MQC), and High-Quality Control (HQC) samples. These samples were used for the determination of method accuracy, precision, and stability of the sample.

Calibration curve

The calibration curve or linearity of the method exhibits direct proportionality between detector response and concentration of the analyte of interest. Three replicates of each QC sample were analyzed and peak areas were recorded. The response factor was calculated by taking the ratio of the area of Teriflunomide and the area of internal standard.

Specificity and selectivity

Selectivity of an analytical method is the ability of the method to differentiate and quantify the drug sample in the presence of other interfering substances. Specificity is the ability to discriminate and quantify the analyte of interest in presence of various other matrix components in the sample. The specificity of the method is demonstrated by analyzing blank plasma, zero plasma, and spiked plasma. No interfering peaks should be observed at the same Rf value of Teriflunomide.

Accuracy

The accuracy was estimated by analyzing three different runs of freshly prepared Quality Control samples. The accuracy was determined by analyzing peak areas and the % mean accuracy was calculated.

Precision

Precision includes the determination of Intermediate precision as well as repeatability. Intermediate precision represents within laboratory variation [18]. Precision should include three independent runs and the % CV was determined.

Recovery

Recovery of method expresses the measures of the efficiency of extraction procedure within variation limit. It is performed by comparing the results obtained from the analyte or internal standard from the extracted samples at different concentrations with standard solutions [19].

Stability

The purpose of determining stability is to detect any degradation of the analyte that occurred during the entire process of sample collection, storage, extraction, and analysis. It is recommended to determine stability during short-term storage, long-term storage as well as freeze-thaw cycles. Stability samples should be compared with freshly prepared QC samples.

RESULTS AND DISCUSSION

Method development

In the current work, the HPTLC method was optimized for the estimation of Teriflunomide from human plasma. This method involves use of less solvent as a mobile phase when compared to HPLC method. Also, the optimized method does not involve any preconditioning like maintenance of column temperature as reported in HPLC methods [4-12]. HPTLC has several advantages over the HPLC technique, which involves the use of a renewed stationary phase (a new TLC plate for every run), and time-cost saving because many samples can be analyzed in one run on a single plate using a few milliliters of the mobile phase.

Method validation

Calibration curve

The method was found to be linear over the range of 10–60 µg/ml when concentration was plotted against the response factor. The regression coefficient was found to be 0.9819.

Table 1: Linearity

Plasma concentration (µg/ml)	Response factor*	% CV
10	0.15±0.0075	5.05
20	0.24±0.0198	8.25
30	0.31±0.0008	0.26
40	0.45±0.0049	1.10
50	0.62±0.0091	1.48
60	0.72±0.0240	3.34

*Data given in mean±SD, n = 3.

Specificity and selectivity

The method was found to be specific as no interfering peaks were observed at the Rf of Teriflunomide.

Accuracy

The method was found to be accurate as the % mean accuracy at each concentration was found to be in the acceptable range, as shown in table 2.

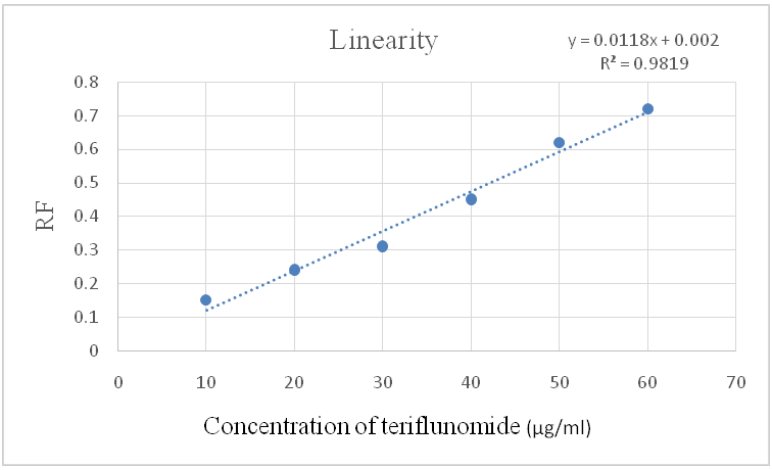

Fig. 3: Calibration curve of teriflunomide spiked plasma

Table 2: Accuracy

Concentration(µg/ml) (n=5)	LQC (4.5)	MQC (27)	HQC (50)
Spotted amount(ng/band)	90	540	1000
Amount found (mean±SD)	100.72±2.96	549.56±10.03	939.37±0.29
% CV	2.93	1.82	0.03
% Mean accuracy	111.91	101.77	93.94

Table 3: Precision

Intermediate precision (n=5)	Concentration(µg/ml)	LQC (4.5)	MQC (27)	HQC (50)
	Spotted amount (ng/band)	90	540	1000
	Amount found (mean±SD)	100.22±3.18	538.21±9.12	937.22±3.18
	% CV	3.17	1.69	0.33
Repeatability	Concentration (µg/ml)	LQC (4.5)	MQC (27)	HQC (50)
(n=5)	Spotted amount (ng/band)	90	540	1000
	Amount found (mean±SD)	100.72±2.96	528.84±8.88	936.24±3.62
	% CV	2.93	1.67	0.38

Table 4: Recovery

Concentration (ng/band) (n=5)	Peak area (mean±SD)	% CV	% Mean recovery	% Overall mean recovery	Overall SD (Recovery)	Overall % CV (Recovery)
32	460.22±3.4539	0.75	76.85			
	598.8±4.5782	0.76		75.5	3.42	4.53
90	675.54±10.7123	1.58	74.19			
	910.54±10.9680	1.20				
200	1000.26±7.9060	0.79	72.71			
	1375.64±17.0468	1.23				
320	1101.8±5.0275	0.45	73.53			
	1498.26±6.5435	0.43				
540	1714.38±54.1313	3.15	71.68			
	2391.6±6.0573	0.25				
760	2308.62±9.4414	0.40	76.95			
	2999.98±5.1878	0.17				
1000	2956.76±8.2432	0.27	82.59			
	3579.74±17.5449	0.49				

Table 5: Stability

S. No.	Stability (n=6)	% Mean stability		% CV
1	Freeze-thaw stability (Three cycles)	LQC	96	2.79
		HQC	96.33	1.77
2	Short-term stability (for 4h at RT)	LQC	96.99	3.98
		HQC	98.92	1.02
3	Long-term stability (for 14 d at 4 °C)	LQC	95.67	1.88
		HQC	98.20	2.14
4	Stock solution stability (for 5 d)	MQC	96.60	2.01
		IS	98.53	0.43
5	Post preparative stability (for 5hat RT)	MQC	97.61	0.71
		IS	99.09	0.26

n = 6.

Precision

The %CV for each concentration was calculated and was found to be within the limit based on the result given in table 3.

Recovery

The recovery was determined by comparison of standard samples with that of spiked plasma samples and the overall % CV was calculated, which was found within the limit.

Stability

By comparing the peak areas of spiked stability QC samples with peak areas of freshly prepared QC samples, the % mean stability was calculated and the results are summarised in table 5.

CONCLUSION

The optimized method was found to be simple, economic, and linear over a wide concentration range. A simple, easy protein precipitation technique was used for sample pre-treatment. The high throughput advantage of HPTLC makes the method rapid. It may be used for therapeutic drug monitoring.

ACKNOWLEDGEMENT

The authors are thankful to AISSMS College of Pharmacy, Pune, for providing the necessary analytical instruments to carry out this work.

FUNDING

No funds were received from any agency.

AUTHORS CONTRIBUTIONS

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.

CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest.

REFERENCES

1. PubChem [internet]. (Us). Bethesda: National Library of Medicine; 2004. PubChem Compound Summary for CID 54684141, Teriflunomide. National Center for Biotechnology Information. Available from: https://pubchem. ncbi.nlm.nih.gov/compound/Teriflunomide. [Last accessed on 24 Apr 2022].
2. Bar-Or A, Pachner A, Menguy Vacheron F, Kaplan J, Wiendl H. Teriflunomide and its mechanism of action in multiple sclerosis. Drugs. 2014 Apr;74(6):659-74. doi: 10.1007/s40265-014-0212-x, PMID 24740824.
3. Oh J, O'Connor PW. Teriflunomide. Neurol Clin Pract. 2013 Aug;3(3):254-60. doi: 10.1212/CPJ.0b013e318296f299, PMID 23914327.
4. Rakhila H, Rozek T, Hopkins A, Proudman S, Cleland L, James M. Quantitation of total and free teriflunomide (A77 1726) in human plasma by LC-MS/MS. J Pharm Biomed Anal. 2011 Jan 26;55(2):325-31. doi: 10.1016/j.jpba.2011.01.034, PMID 21349677.
5. 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 Jun 25;878(24):2217-25. doi: 10.1016/j.jchromb.2010.06.028, PMID 20643586.
6. Xueting Y, Yang L, Ling S, Ji J, Fei X, Dongyang L. Development of a simple HPLC-MS/MS method to simultaneously determine teriflunomide and its metabolite in human plasma and urine: application to the clinical pharmacokinetic study of teriflunomide sodium and leflunomide. Biomed Chromatogr. 2018;33(3):e4420. doi: 10.1002/bmc.4420
7. 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 Feb 1;30(9):1371-7. doi: 10.1002/bmc.3694, PMID 26849839.
8. Rule GS, Rockwood AL, Johnson Davis KL. LC-MS/MS method for the quantification of the leflunomide metabolite teriflunomide. In: Human Serum/Plasma Langman LJ, Snozek CLH, editors. LC-MS in Drug Analysis–Part of Methods in Molecular Biology book series. Springer; 2018. p. 75-83.
9. Filali AA, Lunven C, Turpault S, Beyer YJ, Amy O, Astrid D. Dried blood spot methodology in combination with liquid chromatography/tandem mass spectrometry facilitates the monitoring of teriflunomide. Therapeutic drug monitoring (lippincott Williams and Wilkins open access); 2016.
10. Xia Y, Zhang F, Wang W, Guo Y. Analysis of volatile compounds from Siraitia grosvenorii by headspace solid-phase microextraction and gas chromatography-quadrupole time-of-flight mass spectrometry. J Chromatogr Sci. 2015;53(1):1-7. doi: 10.1093/chromsci/bmu012. PMID 24668041.
11. 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. LC-MS/MS method for the quantification of the leflunomide metabolite, teriflunomide Human Serum/Plasma. Methods Mol Biol. 2016:195-203.
12. 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.
13. Ramya SS, Subrahmanyam CVS, Shyam SR. Bioanalytical RP-HPLC method development and validation of clopidogrel bisulfate in Wistar rat plasma and its application to pharmacokinetic study. Int J Appl Pharm. 2021 Nov 17;14(1):106-11.
14. Patil PK, Damle M. Stability indicating HPTLC method for analysis of teriflunomide. Int J Pharm Sci Nanotechnol. 2020 May 31;13(6):4926-31.
15. Kadian N, Raju KS, Rashid M, Malik MY, Taneja I, Wahajuddin M. Comparative assessment of bioanalytical method validation guidelines for the pharmaceutical industry. J Pharm Biomed Anal. 2016;126:83-97. doi: 10.1016/j.jpba.2016.03.052. PMID 27179186.
16. U. S. Department of Health and Human Services food and drug administration. Bioanalytical Method Validation Guidance for industry [internet]. US FDA; 2018. Available from:

https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. [Last accessed on 18 Jul 2022].

17. Shah SK, Dey S, De S. Simultaneous determination of atorvastatin and atenolol in rabbit plasma by RP-HPLC method and its application in a pharmacokinetic study. Int J Curr Pharm Sci. 2022 Jan 10:72-8. doi: 10.22159/ijcpr.2022v14i2.1968.
18. Tijare LK, Rangari NT, Mahajan UN. A review on bioanalytical method development and validation. Asian J Pharm Clin Res. 2016 Aug 24;9(Suppl 3):6-10.
19. Pravallika KE, Prameela RA, Ratna KM. Bioanalytical method development and validation of Entrectinib in rat plasma by liquid chromatography-tandem mass spectrometry. Asian J Pharm Clin Res. 2020 Sep 22;13(11):155-63.