Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 7
Open Access

Validated RP-HPLC Bioanalytical Method for Determination of Torsemide in Rabbit Plasma

Swati Srungarapati[1]*, Girija Shankar Guntuku[2], Venkata Ramana Murthy Kolapalli[3]

[1] Associate Professor, Nirmala College of Pharmacy, Atmakur – 522503, Guntur District, Andhra Pradesh, India; Research Scholar, Department of Pharmaceutics, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530003, Andhra Pradesh, India.

Email: swathi0854@gmail.com

[2] Professor & Principal, Department of Biotechnology, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530003, Andhra Pradesh, India.

[3] Honorary Professor, Department of Pharmaceutics, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530003, Andhra Pradesh, India.

Corresponding Author:

Swati Srungarapati

Associate Professor, Nirmala College of Pharmacy, Atmakur – 522503, Guntur District, Andhra Pradesh, India

Email: swathi0854@gmail.com..

Cite this paper as: Swati Srungarapati, Girija Shankar Guntuku, Venkata Ramana Murthy Kolapalli (2024) Validated RP-HPLC Bioanalytical Method for Determination of Torsemide in Rabbit Plasma .*Frontiers in Health Informatics*, *Vol.13, No.7*, 1486-1497

ABSTRACT:

Torsemide, a loop diuretic used for the primary treatment for hypertension, heart failure and pulmonary oedema, requires a reliable and validated bioanalytical method for accurate quantification in biological matrices during pharmacokinetic and preclinical studies. While reversed phase high performance liquid chromatography (RP-HPLC) bioanalytical methods have been reported earlier in human plasma for the quantification of torsemide, no RP-HPLC method has been reported for quantification of torsemide in rabbit plasma, a matrix essential for the preclinical pharmacokinetics and bioavailability studies of new dosage form, human plasma validated methods cannot assumed to transfer directly to species-specific differences in plasma matrix composition. while LC-MS method in rabbit plasma has been reported earlier, they require complex and sophisticated operations. In this work, a simple, sensitive, and reproducible RP-HPLC method was developed and validated for the estimation of torsemide in rabbit plasma using azosemide as the internal standard. Chromatographic separation was achieved using a Intersil ODS (250 × 4.6 mm,5 µm), with a mobile phase composed of acetonitrile and 0.1% trifluoroacetic acid buffer in a ratio of 35:65 v/v, delivered at a flow rate of 1.0 mL/min. The detection wavelength was set at 272.4 nm, with a retention time of 5.198 minutes and 3.415 minutes for torsemide and azosemide respectively. The method exhibited linearity over the concentration range of 0.15-9 µg/mL (R^2 = 0.9999) in accordance with ICH Q2 (R1) guidelines. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.1582 µg/mL (15.82 ng/mL) and 0.4794 µg/mL (47.94 ng/mL), respectively, confirming the sensitivity of the assay making it suitable for quantification of torsemide in rabbit plasma. The validated bioanalytical method was developed for preclinical pharmacokinetic evaluation and bioavailability studies including chronotherapeutic drug delivery systems..

Keywords: Reverse phase HPLC, Torsemide, Bioanalytical estimation, Rabbit plasma, PDA detector.

INTRODUCTION

Torsemide, a pyridine-3-sulfonylurea derivative1 belonging to the loop diuretic class, is widely prescribed for the management of oedema associated with congestive heart failure, renal disease, and hepatic cirrhosis, as well as for the treatment of hypertension2. It is typically administered at doses ranging from 5 to 20 mg once daily, depending on therapeutic requirements3. Even though torsemide is an established loop diuretic, it is widely used in treatment of hypertension, pulmonary oedema, heart failure and post-operative care because of its pharmacokinetic profile with shorter half life of 2 hours and reaches the peak plasma concentration within about

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1 hour after oral administration. Torsemide remains a primary therapeutic choice of drug for treating pulmonary oedema and its effective management4,5. The early morning surge (3- 4 a.m.) in blood pressure which associated with natural circadian rhythm in hypertensive patients increase the organ damage, cardiovascular risks which leads to sudden cardiac death6..

According to the literature, different analytical approaches for torsemide determination in human plasma have been reported, for torsemide metabolite analysis and bioequivalence studies including HPLC method coupled with electrospray ionization tandem mass spectrometry[7], HPLC with UV/Vis intelligent detectors[8-10], UV-based quantification plasma purification[11], LC/MS based quantification[12] in rabbit plasma as shown in table no 1. Although the HPLC is considered and selected as a robust approach for torsemide bioanalysis. All these methods are used for the determination of torsemide in human plasma and some of these methods are not cost effective and requires high end instrumentation. According to the ICH M10 guidelines[13], the matrix used for validation only be used for study the samples, so the bioanalytical method developed in human plasma is not considered for pharamacokinetic evaluation.

Table no.1. Literature comparison of bioanalytical methods reported for torsemide quantification in biological matrices

Matrix	Method	Extraction	Remarks	Reference
Human plasma	HPLC-UV	Liquid–liquid extraction	Torsemide and metabolites.	Besenfelder (1987)
Human plasma	HPLC-UV	Solid-phase extraction (SPE)	Improved extraction.	Engelhardt et al. (2006)
Human plasma	RP-HPLC-UV	Protein precipitation	Simple bioequivalence method.	Khan et al. (2008)
Human plasma	HPLC-UV	Solid-phase extraction (SPE)	Improved recovery.	Aragón et al. (2011)
Human plasma	HPLC-ESI-MS/MS	Liquid–liquid extraction	Highly sensitive LC-MS/MS for bioequivalence.	Zhang et al. (2016)
Rabbit plasma	LC-ESI-MS	Protein precipitation	complex operation and high maintenance.	Hu et al. (2012)
Rabbit plasma	RP-HPLC-UV	Protein precipitation	Simple, economical isocratic method.	Present study

Though torsemide is an established drug in clinical practice, due to its importance in the treatment of cardiovascular risk, it attracts the research interests for development of novel drug delivery systems like chronotherapeutic formulations, sustained and controlled release formulations which should be subjected for preclinical evaluation. Although one LC-MS method for its quantification in rabbit plasma is reported, but its widespread use is restricted by the high cost of instrumentation, maintenance requirements, and limited availability of skilled personnel.

Hence, there is a need for development of simple, cost effective, robust bioanalytical method using RP-HPLC. so, the present method was developed and validated for the analysis of torsemide in rabbit plasma.

Particular emphasis is placed on achieving high recovery, low detection and quantification limits, and adherence to current bioanalytical method validation guidelines[14,15]. The proposed method is expected to serve as a reliable analytical tool for preclinical pharmacokinetic studies, thereby facilitating rational dosage optimization and advancing the therapeutic development of torsemide.

Chemicals and reagents

Torsemide was generously provided by Aurobindo Pharma Ltd., Hyderabad. HPLC-grade trifluoroacetic acid was sourced from Merck Limited, Mumbai, India. Other reagents were acquired from Sigma Aldrich Chemicals Private limited, Bangalore, India. The water for the study was produced from a Millipore Milli-Q Plus water purification system (Millipore Bedford Corp., Bedford, MA, USA).

Experimental animals

The study adhered to institutional animal ethics guidelines (protocol no. 1205/PO//RcBi/S/09/CPCSEA New Zealand rabbits (2-3 kg) were housed at 20-22°C with 65% RH on a light/dark cycle, with *ad libitum* feed and water.

Instrumentation

Method development and analysis were performed using a Waters Alliance HPLC system (e2695) with a PDA detector (2998) and an Intersil ODS, stainless steel analytical column (150 mm x 4.6 mm, 3.5 µm).

Method development

5.1 Preparation of standard solutions

Torsemide (24 mg) was precisely weighed and solubilized in acetonitrile and made up to 100 mL with the same solvent to obtain 240 µg/mL concentration (TSS-1). Subsequently, 2 mL of TSS-1 was diluted to 20 mL to get 24 µg/mL solution (TSS-2).

5.2 Preparation of internal standard

A 24 mg quantity of azosemide was solubilized in 100 mL of acetonitrile for obtaining 240 microgram/mL solution (ASS-1) and 2 mL of this was diluted to 20 mL for yielding a 24 µg/mL solution (ASS-2).

5.3 Preparation of working standard solutions of torsemide

An aliquot of 500 µL of TSS-2 of torsemide (24 µg/mL) was added to 200 µL of rabbit plasma along with 500 µL of azosemide (internal standard, 24 µg/mL) and 300 µL of acetonitrile. The mixture was finally diluted to 2 mL with mobile phase to obtain a working standard solution of torsemide at a concentration of 6 µg/mL. Using this concentration the optimisation of chromatographic conditions was done.

The mixtures was vortexed for 2 minutes and then subjected to centrifugation (REMI C-24 Plus, REMI Instruments Ltd., India) at 10,000 rpm for 10 minutes. The supernatant was collected using a micropipette, and a 10 µL was injected into the HPLC column using a Hamilton microsyringe. Each sample was analyzed in triplicate, and the average was used for reporting. Data analysis was conducted using MassLynx™ software.

Method validation[15]

6.1 Optimization of chromatographic conditions

The isosbestic point was determined for torsemide and the internal standard, azosemide, using a photodiode array (PDA) detector (Figure 1).

An isosbestic point is the wavelength at which two compounds exhibit the same absorbance, indicating a reliable reference wavelength for simultaneous analysis. In this study, the spectra of torsemide and azosemide were recorded in the UV range, and the intersection point was observed at 272.4 nm. This wavelength was selected as the isosbestic point for accurate and reproducible analytical determination.

Preliminary chromatographic studies were performed to optimize the separation conditions for torsemide in plasma by evaluating different stationary phases and mobile phase compositions. Initially, chromatographic trials were carried out using a Waters Xterra RP18 Column with acetonitrile and 0.1% trifluoroacetic acid in water as the mobile phase in the ratios of 70:30 (Trial 1) and 60:40 (Trial 2) to assess the effect of higher organic phase composition on analyte elution. Based on the chromatographic performance, further optimization was undertaken by replacing the stationary phase with an Inertsil ODS Column. Using this column, additional trials were performed with acetonitrile and 0.1% trifluoroacetic acid in the ratios of 10:90 (Trial 3), 20:80 (Trial 4) and 35:65 (trail 5).

In each trial, chromatographic parameters including retention time, theoretical plate count, peak shape and tailing factor were evaluated as part of system suitability assessment.

Method optimization was carried out by maintaining 10 µL of injection volume and 1mL/min flow rate at constant values to ensure consistency in system performance.

6.2 Construction of calibration curve

TSS-2 of torsemide (24 µg/mL) was added in volumes of 12.5, 25, 37.5, 75, 125, 250, 375, 500, 625, and 750 µL to 200 µL of rabbit plasma along with 500 µL AZ (containing 24 µg/mL) and 300 µL acetonitrile. This was further diluted to 2 mL volume with mobile phase (35:65 v/v of acetonitrile and trifluoroacetic acid) to get the working standard solution of torsemide with concentrations in triplicate: 0.15, 0.3, 0.45, 0.9, 1.5, 3, 4.5, 6, 7.5, and 9 µg/mL

respectively. The quantification was based on the peak area ratio of torsemide to the internal standard AZ. All the trials were done in triplicate and average values were reported. A calibration curve was plotted using these ratios against the respective concentrations of torsemide spiked in plasma.

To determine the reproducibility and robustness of the process, the method was validated for accuracy, precision, specificity and recovery. The LOD and LOQ of the method was also determined.

6.3 Precision and accuracy[15,16]

Precision and accuracy are essential parameters in evaluating the reliability of a bioanalytical method. Precision reflects the consistency of repeated measurements of the analyte in the same biological matrix, while accuracy represents the closeness of the measured values to the true concentration. Intra-day and inter-day performance was assessed at four quality control (QC) concentrations of torsemide (0.15, 0.9, 6 and 9 µg/mL denoted as LLOQ, LOQ, MOQ, and HOQ respectively). Each QC level was analyzed in quintuplicate across multiple runs. Precision was expressed as the coefficient of variation (%RSD) of the concentrations calculated from the peak area ratios, whereas accuracy was calculated as the percent relative error (%RE) compared to the nominal values.

$$\%RSD = \left[\frac{s.d.}{mean}\right] \times 100$$

$$\%RE = \frac{\text{Mean concentration-Actual concentration}}{\text{Actual concentration}} \times 100$$

6.4 Specificity/Selectivity[16,17]

To establish the specificity of the developed method, multiple lots of blank rabbit plasma were analyzed to ensure no endogenous interference at the retention times corresponding to torsemide or AZ. The acceptance threshold was defined such that any interfering response should not exceed 20% of the signal at the lower limit of quantification (LLOQ).

6.5 Recovery[16,17]

Recovery evaluates the extraction efficiency of an analyte from plasma. It is determined by comparing the detector response of an extracted plasma sample to that of an equivalent unextracted standard solution. The mean recovery values were calculated as the average of replicates. The QC levels LOQ, MOQ and HOQ were utilized for the determination.

6.6 Limit of quantification (LOQ) and limit of detection (LOD)[18]

The (LOD) represents the lowest analyte concentration that can be reliably distinguished from baseline noise, although it cannot be quantified with precision. In this study, the LOD was established based on a signal-to-noise (S/N) ratio of 3:1 by comparing chromatographic responses of torsemide standards with those of blank plasma samples. The LOQ, defined as the minimum analyte concentration that can be measured with acceptable accuracy and precision, was determined using plasma standards and mobile phase solutions containing known torsemide concentrations, ensuring reproducible quantification at low levels.

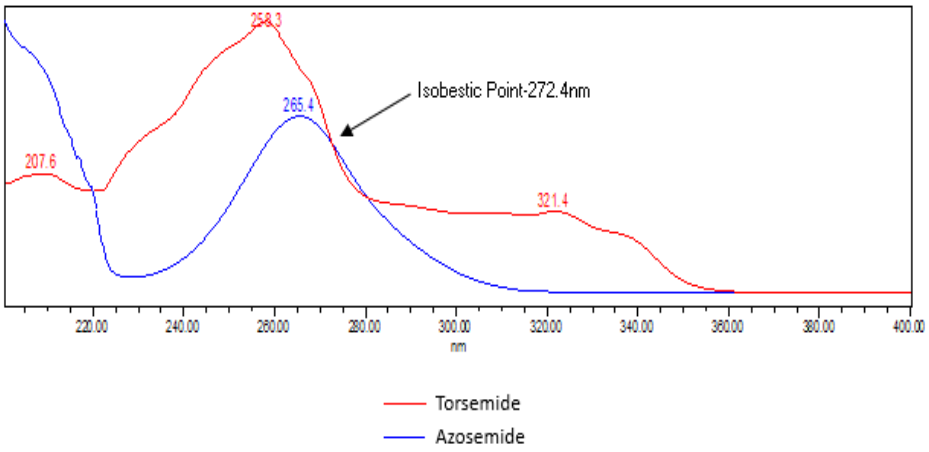


Figure 1: UV spectrum indicating isobestic point of torsemide and AZ

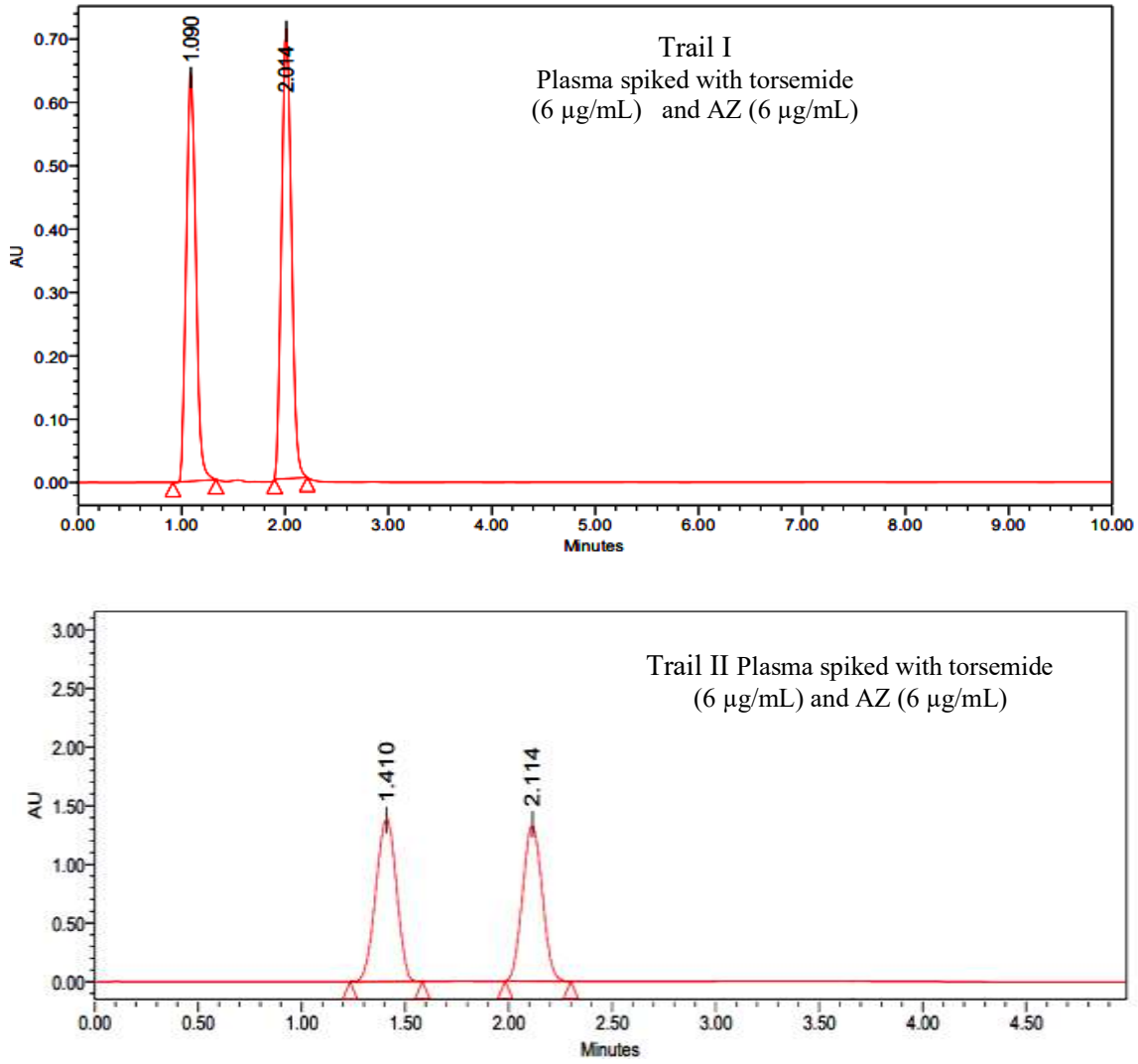
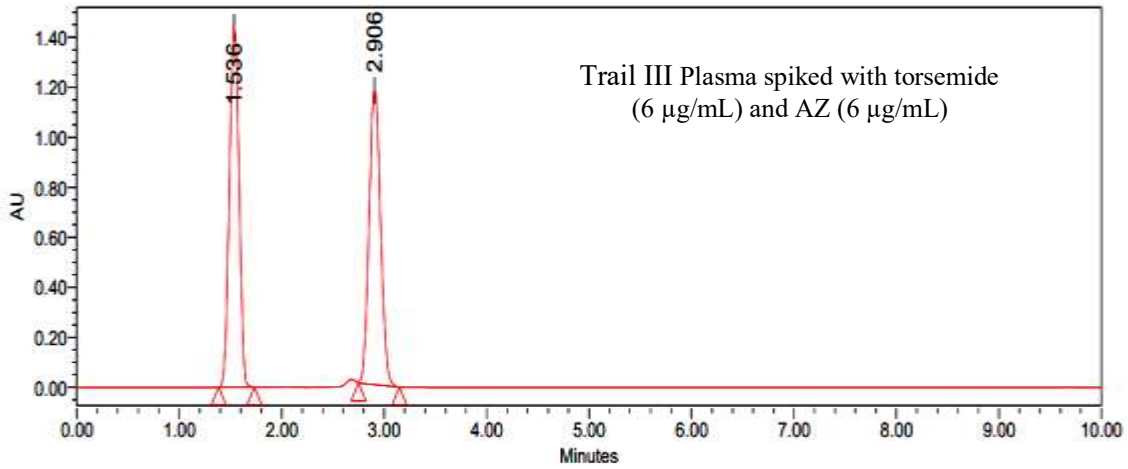


Figure 2: Mobile phase optimization Trail (I, II) chromatographs



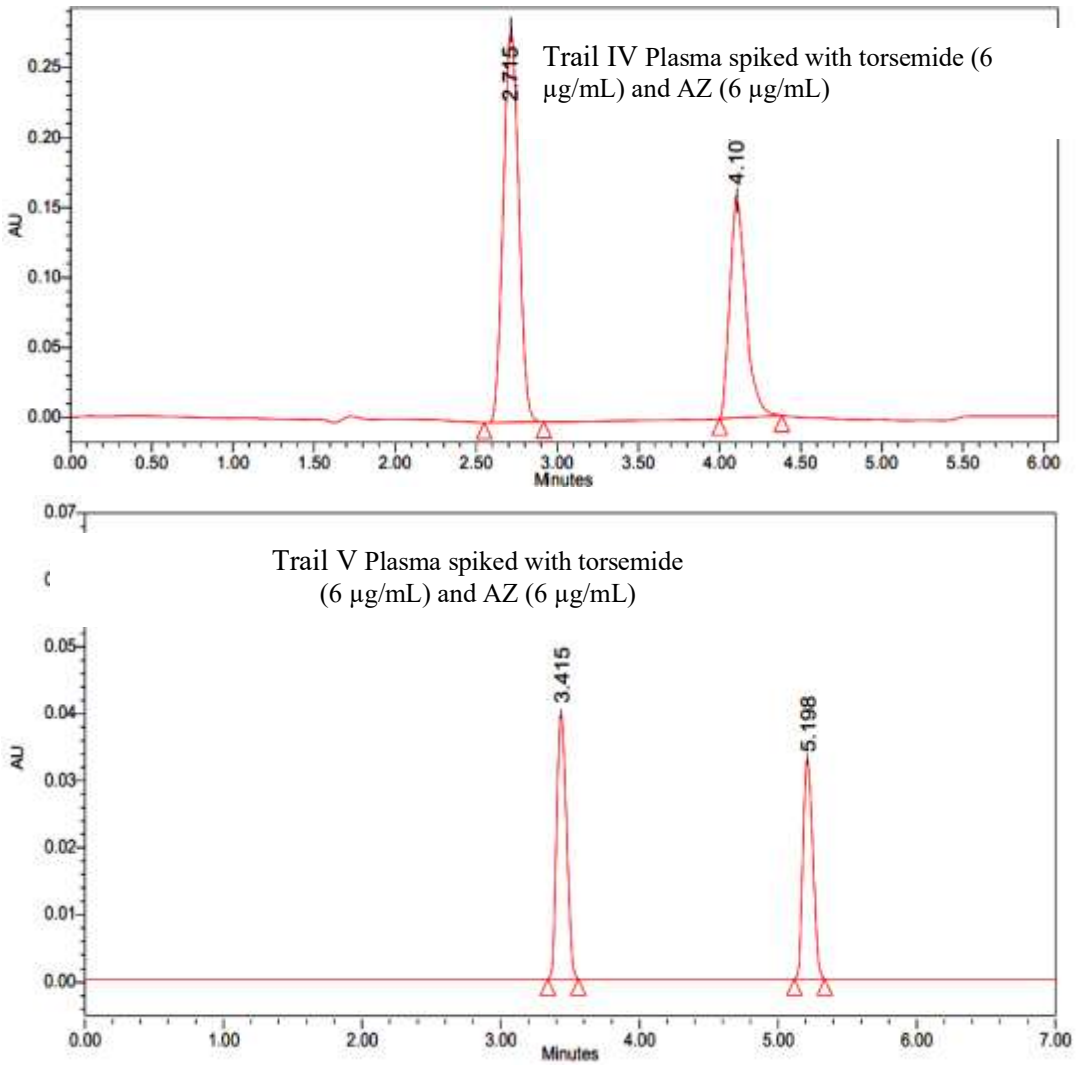
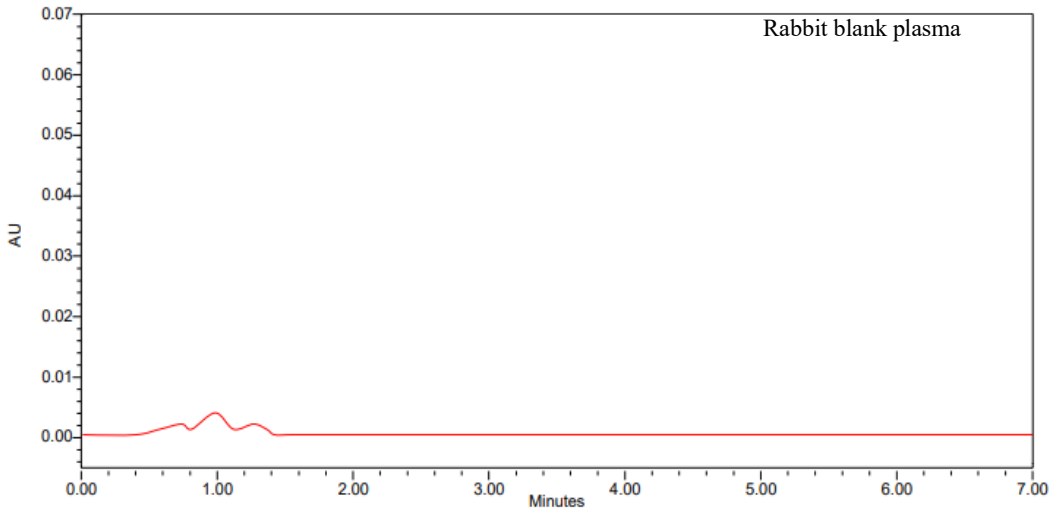


Figure 3: Mobile phase optimization Trail (III, IV,V) chromatographs



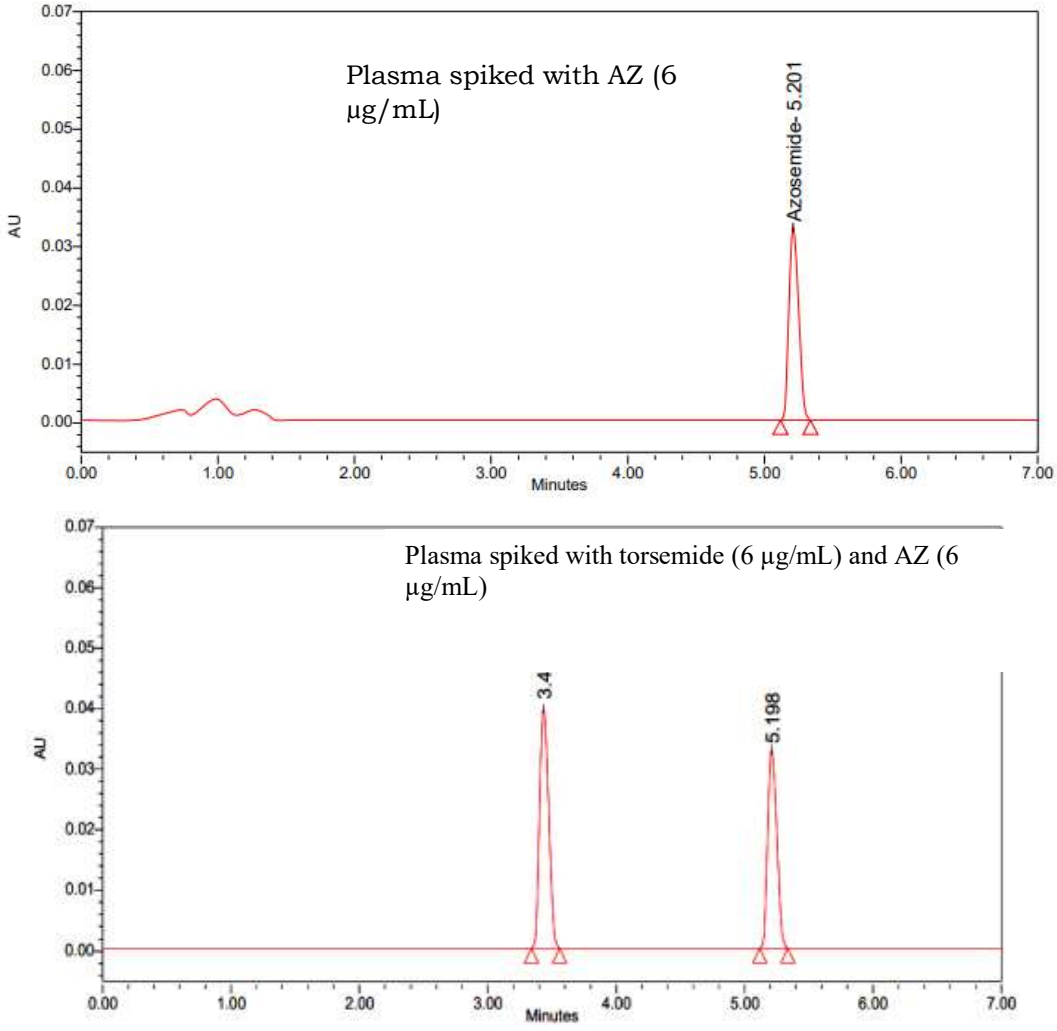


Figure 4: HPLC chromatogram of torsemide and AZ in rabbit plasma

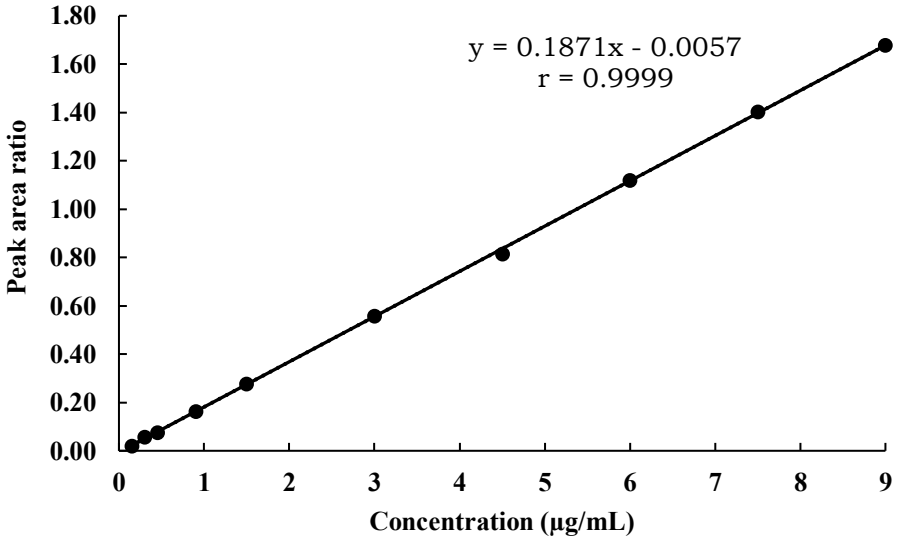


Figure 5: Peak area ratio vs concentration curve of torsemide in rabbit plasma

Table 1: Concentration vs. peak area ratios for torsemide in rabbit plasma (n=6)

Concentration of torsemide (µg/mL)	Peak area ratio	%RSD
0.15	0.02	0.87
0.3	0.06	0.51
0.45	0.08	0.35
0.9	0.16	0.13
1.5	0.28	0.17
3	0.56	0.06
4.5	0.81	0.10
6	1.12	0.07
7.5	1.40	0.25
9	1.68	0.11

Table 2: Intra-assay and inter-assay accuracy and precision of torsemide in rabbit plasma (n=3)

Assay type	Level*	Actual conc. (µg/mL)	Mean measured conc. (µg/mL)	s.d.	Precision (%RSD)	Accuracy (%RE)
Intra assay	LLOQ	0.150	0.1504	0.0015	0.99	0.27
	LQC	0.9	0.902	0.0011	0.116	0.22
	MQC	6	6.006	0.0054	0.90	0.10
	HQC	9	9.008	0.081	0.90	0.09
Inter assay	LLOQ	0.150	0.1508	0.0020	1.32	0.53
	LQC	0.900	0.903	0.0014	0.155	0.33
	MQC	6	6.007	0.0072	0.120	0.12
	HQC	9	9.011	0.0108	0.120	0.12

Table 3: Peak Specificity of torsemidein rabbit plasma (n=6)

S.No.	LLOQ		% interference
	Peak Area	RT	
1	2896	3.423	Nil
2	2875	3.420	Nil
3	2839	3.427	Nil
4	2855	3.423	Nil
5	2860	3.427	Nil
6	2887	3.422	Nil

Table 4: Recovery of torsemide from rabbit plasma drug solution (n=3)

Drug	QC level	Conc. of drug added (µg/mL)	% Recovery
Torsemide	Low	0.900	98.58
	Medium	6	99.09
	High	9	99.14

Results and discussion

The successful development of any new dosage form will be complete only with proper in vivo pharmacokinetic studies in either a suitable animal model or human subjects. For the measurement of torsemide, a number of analytical techniques have been documented in human plasma.

Torsemide and its metabolites were analysed simultaneously using early HPLC techniques created by Besenfelder (1987) and Engelhardt et al. (2006), although they required time-consuming extraction processes, such as liquid-liquid and solid-phase extraction. Aragón et al. (2011) used solid phase extraction (SPE) in conjunction with UV detection to produce better recoveries, while Khan et al. (2008) reported a simpler RP-HPLC method for human plasma and showed its utility in bioequivalence investigations. HPLC-ESI-MS/MS was used more recently by Zhang et al. (2016), offering significantly higher sensitivity and lower quantification limits appropriate for clinical bioequivalency studies. As there are no reported methods for RP-HPLC in rabbit plasma, no comparative study was done.

Though torsemide is having many bioanalytical methods in humans, despite these developments, species-specific variations in plasma composition may affect selectivity, recovery, and analytical performance, hence techniques established in human plasma cannot be simply transferred to rabbit plasma without further validation. Although an LC-MS quantification of torsemide in rabbit has been reported previously with evidenced by its good sensitivity because of high instrumentation costs and maintenance, its routine use is not possible. Despite of the superior sensitivity, it is highly susceptible to matrix effects caused by endogenous biological components[19] that co-elute with the analyte. These components can alter ionising efficiency in the mass spectrophotometer, leading to ion suppression or ion enhancement, which effect accuracy, precision and robustness.

In contrast, RP-HPLC with UV detection is not susceptible to ion suppression, as detection is based on UV absorbance rather than ionization, and is therefore a simpler and more robust option for routine bioanalytical applications where adequate sensitivity can be achieved. Therefore, as there is no validated RP-HPLC is available for quantification of torsemide in rabbit plasma. In the present study, a RP-HPLC bioanalytical method was developed by using azosemide as an internal standard which is also a loop diuretic with structural similarity with the drug instead of midazolam reported in LC-MS method[12] because of its favourable ionising properties. This structural similarity and pharmacological similarity to torsemide provides chromatographic behavior more comparable to the analyte than midazolam and optimized to enhance analytical reliability facilitates a cost-effective and practical alternative for routine bioanalysis.

The incorporation of an internal standard was considered essential to improve accuracy and reproducibility in quantitative plasma analysis. Furthermore, a volatile acidic mobile phase was selected to minimize the risk of buffer-related precipitation and to ensure better column performance and system robustness.

In the development of a robust bioanalytical method for the estimation of torsemide, acetonitrile was selected as the organic component of the mobile phase owing to its low UV cut-off, excellent miscibility with aqueous modifiers, and superior elution strength compared to methanol, which ensures sharp peak shapes and reduced run times. Azosemide was chosen as the internal standard due to its structural similarity to torsemide, comparable chromatographic behavior, and stability under analytical conditions, thereby ensuring accurate and reproducible quantification. Both compounds belong to the loop diuretic class and contain sulfonamide functional groups, resulting in similar chromatographic behavior under reversed phase conditions. The use of a structurally related compound ensures comparable extraction efficiency and retention characteristics, thereby improving method precision and accuracy.

7.1 Optimization of chromatographic conditions

Initial chromatographic trials were carried out using acetonitrile and 0.1% trifluoroacetic acid as the mobile phase on a Waters X-Terra RP-18 column at ratios of 70:30 and 60:40 (trials 1 and 2). These conditions resulted in suboptimal chromatographic performance. Although the tailing factors were within acceptable limits, the theoretical plate counts were considerably lower than the system suitability requirements (≤2681 for torsemide). In addition, very short retention times (1.09-2.14 minutes) were observed, indicating inadequate retention and insufficient resolution of torsemide from endogenous plasma components (Figure 2).

Subsequently, the column was changed to an Inertsil ODS column and trials were conducted using acetonitrile and 0.1% trifluoroacetic acid at ratios of 10:90 and 20:80 (trials 3 and 4). Under these conditions, chromatographic efficiency improved markedly, as evidenced by higher theoretical plate counts (>6000) and acceptable tailing factors (<1.2). However, Trial 3 showed the presence of an unidentified peak, suggesting possible interference from matrix components. In Trial 4, the internal standard exhibited an asymmetric peak shape, which could adversely affect quantification accuracy and reproducibility (Figure 3).

Based on these observations, trials 1-4 were rejected due to poor efficiency, inadequate retention, or unsatisfactory peak characteristics. With an objective of optimizing the conditions, trial 5 was taken up and finalized.

Final optimization was achieved using acetonitrile and 0.1% trifluoroacetic acid in 35:65 ratio (trail 5) on the Inertsil ODS column (150 mmx4.6 mm, 3.5 µm). This condition provided the best chromatographic performance, with system suitability parameters well within the acceptance criteria. High theoretical plate counts were obtained for both torsemide (9745) and the internal standard (8376), along with excellent peak symmetry (tailing factor <1.08). Adequate retention times of 3.415 minutes for torsemide and 5.198 minutes for the internal standard ensured sufficient separation from endogenous interferences. A resolution of 9.76 between the analyte and internal standard further confirmed the suitability of the optimized conditions (Figure 2). The process was found to produce same retention times of 3.4 and 5.2 minutes for torsemide and azosemide on repetition (Figure 3). Every sample was spiked thrice, and the retention time was consistent across all injections. The total analysis time for both the test compound and the internal standard was approximately 5 minutes, providing a rapid and efficient analysis.

The isocratic mode was maintained with 1 mL/min flow rate, using 35:65 volume/volume of acetonitrile and 0.1% trifluoroacetic acid solvent system as the mobile phase with an Intersil ODS, stainless steel analytical column, with a 10 µL injection volume.

7.2 Calibration curve linearity

The calibration curve for torsemide was constructed over the concentration range of 0.15-9 µg/mL (Table 1). A strong linear correlation was observed between the peak area ratio of torsemide to azosemide and the respective concentrations. Linear regression analysis yielded a correlation coefficient (r) of 0.9999, with the equation y=0.1871x-0.0057 (Figure 5). Precision across the calibration range was confirmed by low %bias values of less than 0.9%, which is within the standard limit of 5% in accordance with ICH (M10) guidelines.

7.3 Accuracy and precision

The intra-assay precision and accuracy results demonstrated high reliability and reproducibility of the method across all quality control levels (Table 2). At the lower limit of quantification (LLOQ, 0.15 µg/mL), the mean measured concentration was 0.1504 µg/mL with a standard deviation (SD) of 0.0015, yielding a %RSD of 0.99 and %RE of 0.27%. These findings confirm the ability of the method to accurately quantify analyte concentrations even at the lowest quantifiable level. At higher concentrations (LQC, MQC, and HQC), the intra-assay %RSD values were extremely low (≤0.99), and the %RE remained below 0.27, indicating minimal intra-day variability and strong analytical robustness. Such results reflect that the method is both precise and accurate across the entire calibration range.

Inter-assay evaluations, which assess day-to-day reproducibility, also revealed excellent performance. At LLOQ, the mean concentration was 0.1508 µg/mL (150.8 ng/mL with a %RSD of 1.32 and %RE of 0.53. Although slightly higher than intra-assay variability, these values still fall within the acceptable bioanalytical method validation criteria, confirming consistent sensitivity over multiple runs. Similarly, for LQC, MQC, and HQC, %RSD values were ≤0.155 and %RE values ≤0.33, underscoring negligible inter-day deviations. The close agreement between actual and measured concentrations across all QC levels further highlights the reliability of the assay. Overall, the precision and accuracy results from both intra- and inter-assay validations confirm that the developed method is highly reproducible and capable of providing reliable quantification throughout the tested concentration range. The lowest calibration level corresponding to a peak area ratio of 0.02 was evaluated as LLOQ. The level was included in the calibration curve as it met the bioanalytical acceptance criteria for accuracy, precision, and signal-to-noise ratio (≥10), in accordance with regulatory ICH (M10) guidelines.

7.4 Specificity

Blank plasma samples analyzed under the developed conditions exhibited no interfering peaks at the retention times of torsemide or azosemide (Table 3). The observed responses at the analyte retention time were consistently less than 20% of the LLOQ peak area, fulfilling the acceptance criteria with ICH (M10) guidelines. The retention time of torsemide was found to be stable and reproducible across samples with very low fluctuations, ranging between 3.420-3.427 min.

7.5 Recovery

The extraction efficiency of the developed method was evaluated by assessing the recovery of torsemide from plasma samples at three different quality control (QC) levels. The mean recovery values ranged between 98.58% and 99.09% (Table 4), indicating that the procedure provides consistent and reproducible recovery across the

studied concentration range. Recovery values close to 100% suggest minimal loss of analyte during the sample preparation process, highlighting the robustness of the extraction procedure. These results confirm that the method is free from significant matrix interferences and does not introduce systematic bias during plasma processing. High recovery rates also demonstrate that the extraction procedure is capable of effectively isolating torsemide from plasma components without compromising the integrity of the analyte. The recovery results obtained in the present study satisfied the regulatory requirements in accordance with ICH (M10) guidelines demonstrating the suitability of the extraction procedure for quantitative determination of torsemide in rabbit plasma.

7.6 LOQ and LOD

The method validation demonstrated an LOD of 0.01582 µg/mL (15.82 ng/mL) and an LOQ of 0.04794 µg/mL (47.94 ng/mL) for torsemide in plasma. The LOD value indicates the method's high sensitivity, enabling reliable detection of trace amounts of the drug, which is critical for pharmacokinetic and bioavailability studies. The LOQ value confirms that concentrations as low as 47.94 ng/mL can be quantified with adequate precision and accuracy, ensuring the method is robust for therapeutic drug monitoring and analytical applications meeting the acceptance criteria in accordance with ICH (M10) guidelines.

Conclusion

The new RP-HPLC method was developed to support the preclinical investigation which is sensitive, accurate, and cost-effective tool with mobile phase of acetonitrile-0.1% trifluoroacetic acid (35:65) for quantifying torsemide in rabbit plasma. The high ratio of aqueous portion in mobile phase assures low cost execution and eco-friendly nature. Its successful validation in accordance with bioanalytical guidelines ensures reliability and reproducibility, making it well suited for pharmacokinetic applications. By offering high recovery, low detection limits, and minimal matrix interference, this method can serve as a valuable alternative to existing method, particularly in resource limited laboratories. Overall, the established method contributes to preclinical research on torsemide and may support future investigations aimed at optimizing therapeutic strategies.

Acknowledgements

No acknowledgements to declare.

Conflict of interest:

Authors declare no conflict of interest...

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