e- ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr

RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF TORSEMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM.

Mohammad Neha Chandini* Rajitha S

KLR Pharmacy College, Paloncha, Bhadradri Kothagudem, Telangana- 507511, India.

Article Info

Received 29/01/2025
Revised 16/02/2025
Accepted 18/03/2025

Key words: -
RP-HPLC, Torsemide, Pharmaceutical Analysis, ICH Guidelines.

ABSTRACT

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is employed in this study to develop a method for estimating Torsemide in tablet dosage forms that is accurate and reliable. The quality control of pharmaceutical preparations of torsemide, a loop diuretic commonly prescribed for the treatment of edema and hypertension, requires precise analytical methods. An optimized mobile phase of acetonitrile and water in specific proportions was used to achieve chromatographic separation, monitored at an optimal UV detection wavelength using a C18 column. Validation of the method was conducted in accordance with International Conference on Harmonisation (ICH) guidelines, including assessment of parameters such as specificity, linearity, accuracy, precision, and robustness.

INTRODUCTION

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is a widely used analytical technique for the separation, identification, and quantification of components in complex mixtures [1]. The technique is called "reverse phase" because it uses a non-polar stationary phase (hydrophobic) and a polar mobile phase (hydrophilic), which is the reverse of traditional normal-phase chromatography [2, 3].RP-HPLC is popular for pharmaceutical analysis due to its precision, high resolution, reproducibility, and ability to handle both hydrophilic and hydrophobic compounds [4].

Principle of Separation in RP-HPLC

The separation of analytes in RP-HPLC is based on hydrophobic interactions between the analyte and the non-polar stationary phase [5, 6]. The key steps in the separation process include:

Corresponding Author
Mohammad Neha Chandini

Email: - mohdneha30@gmail.com

Sample injection

The analyte mixture is injected into the mobile phase stream.

Partitioning of analytes

As the mobile phase carries the sample through the stationary phase, components in the mixture interact with the hydrophobic surface of the column.

Retention behavior

Polar analytes elute first as they interact more with the polar mobile phase. Non-polar analytes are retained longer because of stronger interaction with the non-polar stationary phase.

Gradient or isocratic elution

Isocratic elution

The mobile phase composition remains constant throughout the run.

Gradient elution

The polarity of the mobile phase is gradually changed, helping to separate complex mixtures efficiently [7].

Hydrophobic Interactions in RP-HPLC

In RP-HPLC, the analytes' hydrophobicity (non-polar nature) plays a critical role in retention. Compounds with more non-polar character have stronger interactions with the hydrophobic stationary phase, leading to longer retention times. Conversely, polar compounds interact more with the mobile phase and elute faster [8, 9].

Factors Affecting Separation in RP-HPLC

Column Type

C18 (octadecyl) columns are commonly used for a wide range of compounds. C8 columns are used for slightly more polar compounds.

Mobile Phase Composition

A mixture of organic solvents (like acetonitrile or methanol) and water or buffer influences retention and separation.

pH of the Mobile Phase

pH adjustments are crucial for ionizable compounds to ensure consistent retention behavior.

Flow Rate

Faster flow rates can reduce retention times but may compromise resolution.

Temperature

Increasing the temperature can decrease retention times by reducing the viscosity of the mobile phase.

Advantages of RP-HPLC

- High precision and reproducibility.
- Can handle a wide variety of analytes (both hydrophilic and hydrophobic).
- Suitable for stability-indicating studies to detect degradation products.
- Can be scaled for preparative as well as analytical purposes [10].

Applications of RP-HPLC

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is widely used in various fields due to its ability to separate, identify, and quantify components in complex mixtures with high precision and reproducibility. Below is an overview of the major application areas for RP-HPLC [11].

MATERIALS AND METHODS

List of chemicals/Reagents

Water, Distilled water, Acetonitrile, Potassium dihydrogen Orthophosphate, Dipotassium hydrogen Orthophosphate, Potassium dihydrogen Phosphate, Dipotassium hydrogen Phosphate, Ortho-phosphoric acid, Ethanol, Methanol.

RP-HPLC Method Development and Validation for the estimation of Torsemide in bulk and its tablet formulation

Preparation of 0.1 M Dipotassium Hydrogen Phosphate Buffer:

17.4 mg of Dipotassium hydrogen phosphate was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. The pH was adjusted to 6.8 with Ortho phosphoric acid.

Preparation of mobile phase

500 mL (50%) of dipotassium hydrogen phosphate buffer of pH 6.8 and 500 mL of ACN (50%) was mixed in a 1000 ml volumetric flask and kept for sonication in an ultrasonic water bath for 5 minutes. The solution was filtered through 0.45 µ filter under vacuum filtration. Mobile phase was used as diluent.

Standard preparation

100 mg of Torsemide was accurately weighed, transferred to a 50 ml volumetric flask, dissolved in the diluent and final volume was made upto the mark with the same to get a standard stock solution of 2 mg/ml.

Preparation of working standard solution

5 ml of the standard stock solution was diluted to, in a 25 ml volumetric flask to get 400 µg/ml working standard solution.

Determination of wave length

The working standard solution of Torsemide (400 µg/ml) was scanned in the range of 200-400 nm using mobile phase as blank. The drug showed maximum absorbance at 241 nm, the same was selected for the further analysis.

Assay of Tablet dosage form

Average weight of 20 tablets was determined. A tablet powder equivalent to 100 mg was weighed accurately and transferred to a volumetric flask of 50 ml capacity. 25 ml of mobile phase was transferred to volumetric flask and sonicated for 10 mins. The final volume was made upto the mark with the same to get the sample stock solution of 2000 µg/ml. The resulting solution was filtered through the membrane filter of 0.45µ. 5 ml was transferred to a 25 ml volumetric flask and diluted upto the mark with mobile phase to get a final concentration of 400 µg/ml. Chromatogram was recorded by injecting 20 µl of the resulting solution into the chromatograph. The peak area was determined and the amount of Torsemide was calculated.

Method development and optimization

Trial 1:
Column: Inertsil ODS C18 Column with 250 mm × 4.6 mm i.d and 5 µm Particle size
Mobile Phase: 0.1 M Dipotassium Phosphate buffer (pH 5.2): MeOH 50:50 v/v
Flow rate: 1.0 ml/min
Wavelength: 241.0 nm
Temperature: 45 °C
Injection Volume: 10 µl
Run time: 10 mins
Result: Chromatogram was shown in figure 1
Remarks: No proper peak was seen. Irregular base line.

Trial 2:
Column: Inertsil ODS C18 Column with 250 mm × 4.6 mm i.d and 5 µm Particle size
Mobile Phase: Triethyl amine (pH 7.5): MeOH 50:50 v/v
Flow rate: 1.0 ml/min
Wavelength: 241.0 nm
Temperature: Ambient
Injection Volume: 10 µl
Run time: 10 mins
Result: Chromatogram was shown in figure 2
Remarks: Peak was completely destroyed due to triethyl amine (base).

Trial 3:
Column: Waters C18 Column with 250 mm × 4.6 mm i.d and 5 µm Particle size
Mobile Phase: 0.1 M Dipotassium Phosphate buffer (pH 6.8) and acetonitrile in the ratio of 40:60 v/v Flow rate: 1.0 ml/min
Wavelength: 241.0 nm
Temperature: Ambient
Injection Volume: 10 µl
Run time: 10 mins
Result: Chromatogram was shown in figure 3
Remarks: Peak split with irregular base line

Trial 4:
Column: Waters C8 Column with 250 mm × 4.6 mm i.d and 5 µm particle size
Mobile Phase: 0.1 M Dipotassium Phosphate buffer (pH 6.8) and acetonitrile in the ratio of 60:40 v/v Flow rate: 1.2 ml/min
Wavelength: 241.0 nm
Temperature: Ambient
Injection Volume: 10 µl
Run time: 14 mins
Result: Chromatogram was shown in figure 4

Trial 5:
Column: Waters C8 Column with 250 mm × 4.6 mm i.d and 5 µm Particle size
Mobile Phase: 0.1 M Dipotassium Phosphate buffer (pH 6.8) and acetonitrile in the ratio of 70:30 v/v Flow rate: 1.2 ml/min
Wavelength: 241.0 nm
Temperature: 45 °C
Injection Volume: 10 µl
Run time: 5 mins
Result: Chromatogram was shown in figure 5
Remarks: Peak shape was good. But tailing factor was more.

Trial 6:
Column: Waters C8 Column with 250 mm × 4.6 mm i.d and 5 µm Particle size
Mobile Phase: 0.1 M Dipotassium Phosphate buffer (pH 6.8) and acetonitrile in the ratio of 50:50 v/v
Flow rate: 1.2 ml/min
Wavelength: 241.0 nm
Temperature: Ambient
Injection Volume: 5 µl
Run time: 5 mins
Result: Chromatogram was shown in figure 6
Remarks: Good peak with more than 4500 theoretical plate count and Rt was brought down to 2.5 mins.

RESULTS AND DISCUSSION

RP-HPLC Method Development and Validation for the estimation of Torsemide in bulk and its tablet formulation

The wavelength for the detection of TAP was found to be at 241 nm. Figure 7 shows the λ max curve of Torsemide. Table 7.1 shows the optimized chromatographic conditions of Torsemide.

When methanol and acetonitrile were used in initial scouting, it was observed that acetonitrile was found to be better in terms of resolution and peak shapes as compared to methanol. Therefore, acetonitrile was used as an organic modifier for method development. Strong acidic and basic buffer has been avoided, as they were non-volatile and nonamenable with PDA detector. Ammonium acetate buffer (10 mM) was chosen, but peak shapes and tailing factor were found to be unacceptable for analyte. The effect of different pH and mobile phase compositions were also tried to improve the resolution and peak symmetry. The peak shape and symmetry were found to be improved with dipotassium phosphate buffer. In addition to commonly using C18 column, C8 stationary phase was also selected. In an attempt to improve peak symmetry and resolution on C18 column, various combinations of Dipotassium hydrogen phosphate buffer and ACN was

used in different proportions. Buffer pH was adjusted to 6.8 with ortho phosphoric acid. But still the Torsemide peak was broader and analyte was eluting at 10 mins. The C8 column was found to be more suitable because of less tailing factor with improved peak shape and considerable reduction in the retention time (2.4 min) of Torsemide (polar compound). Therefore, C8 column with 0.1 M dipotassium hydrogen phosphate buffer (pH 6.8) and ACN at a ratio of 50:50 v/v was selected for further studies. Blank, standard and sample chromatograms of Torsemide were shown in figure 8 to 10.

Method Validation

The developed method was validated as per ICH guidelines Q2 (R1)22. The validation parameters studied were specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

System suitability:

Prior to the validation study, system suitability tests were performed by measurement of general characteristics such as peak symmetry, number of theoretical plates, retention time, tailing factor etc. The results obtained were satisfactory and in accordance with guidelines. System suitability data was shown in Table 1.

Specificity

Specificity of an analytical method is its capability to measure the analyte precisely and particularly in presence of parts that may be likely to be present in the sample matrix. Chromatograms of standard and sample prove that the method was specific.

Linearity

The linearity plot was constructed with five concentrations at the level of 50 150% (200, 300, 400, 500, 600 µg/ml of Torsemide). The response of the drug was found to be linear in the studied concentration range and the linear regression equation was y = 21349x + 32996. The correlation coefficient was found to be 0.9999. The linearity curve was shown in figure 11.

Precision

Intra and inter-day precision of the analytical method was determined by performing method precision for three times in same day and followed by three consequent days. %RSD was calculated and found to be within the specified limits.

Accuracy study

The accuracy of the method was assessed by standard addition method. % Recovery for three concentrations (corresponding to 50, 100 and 150 % of test solution concentration) were determined. For each concentration three replicates were prepared. The mean recovery of Torsemide was found to be 100 %. Chromatograms of accuracy were shown in Figure 12.

Limit of detection and Limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by using standard deviation of response and slope of the calibration curve. The LOD and LOQ of the proposed method were found to be 0.0023 and 0.0048 µg/ml respectively.

Robustness study

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions like flow rate (± 0.1 ml/min) and the column temperature (± 2°C). System suitability data was found to be satisfactory during variation of the analytical conditions. Results of system suitability show that the analytical method remained unaffected by slight but deliberate changes in the analytical conditions. The results obtained from robustness study.

Assay

The proposed method was applied for the tablet of Torsemide and the mean % assay was found to be 100 %. The chromatogram showed that no interference from excipients. The result of % assay was shown in table 2.

The proposed HPLC method was validated as per ICH guidelines. The developed HPLC method was less time consuming when compared with other reported methods. The Rt of the proposed method was 2.4 min. The linearity of the method was too wide (200-600µg/ml) when compared to reported methods. The method was found to be sensitive as the LOD and LOQ was found to be 0.0023 & 0.0048 µg/mL respectively. This proves that the method can able to detect and quantify Torsemide in very low concentrations. As the mobile phase (0.1 M K2HPO4 buffer (pH 6.8): ACN 50:50 v/v) was very simple and easy to prepare. The 50% of buffer content in the mobile phase reduces the organic content by 50%, as a result the column life will also get extended. The buffer is helpful for not only maintaining the pH of the medium but also it makes the drug stable for all kinds of chemicals reactions like dissociation or polymerization etc. Due to these advantages the proposed method was too simple and very less time consuming without any extra extraction process.

Table 1: System suitability data for Torsemide.

S. No	Parameter	Result	Acceptance Limit
1	Retention time (Rt)*	2.470	
2	Number of theoretical plates (N)*	6732	More than 4000
3	Tailing factor (T)*	1.10	Less than 2
4	Capacity factor (K)*		0.5<K<20
* Number of injections: 6 replicates			

Table 2: Assay data of Torsemide Tablets.

Sample No.	Sample Area	% Assay
1	8729689	99.10
2	8811325	99.25
3	8635493	98.32
4	8819726	100.91
5	8799017	100.41
6	8905936	101.03
	Average	99.83
	STD Deviation	1.04
	% RSD	1.04

Figure 1: Trial 1 Chromatogram of Torsemide

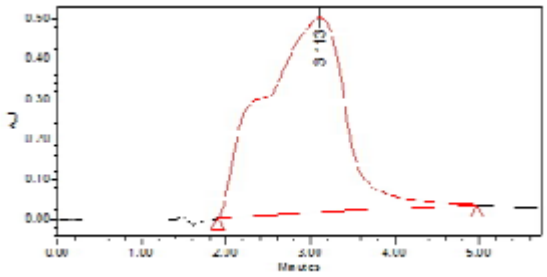


Figure 2: Trial 2 Chromatogram of Torsemide

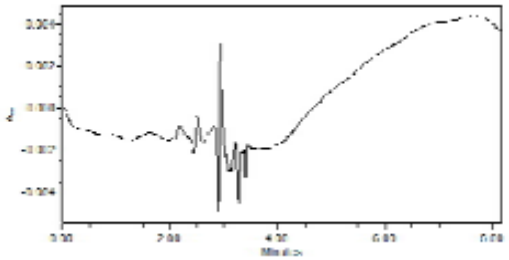


Figure 3: Trial 3 Chromatogram of Torsemide

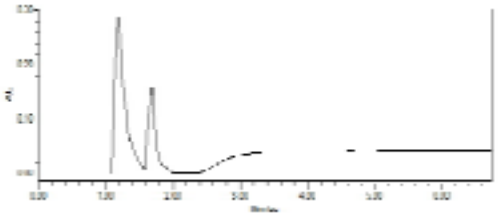


Figure 4: Trial 4 Chromatogram of Torsemide

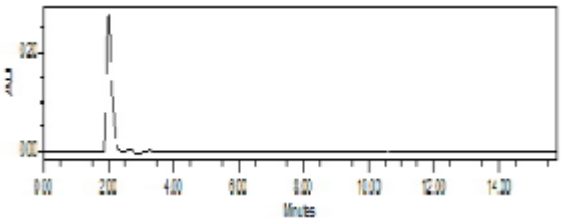


Figure 5: Trial 5 Chromatogram of Torsemide

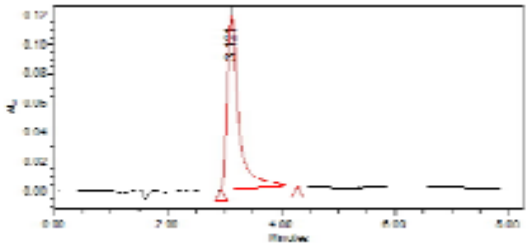


Figure 6: Trial 6 Chromatogram of Torsemide

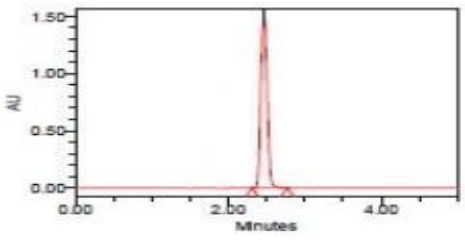


Figure 7: λmax spectra of Torsemide

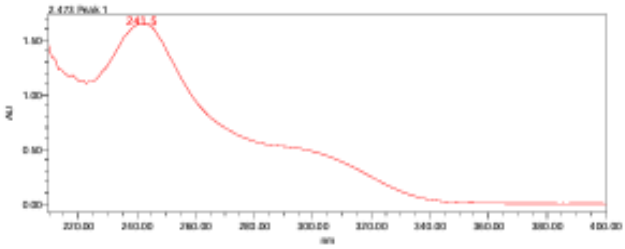


Figure 8: Blank Chromatogram of Torsemide

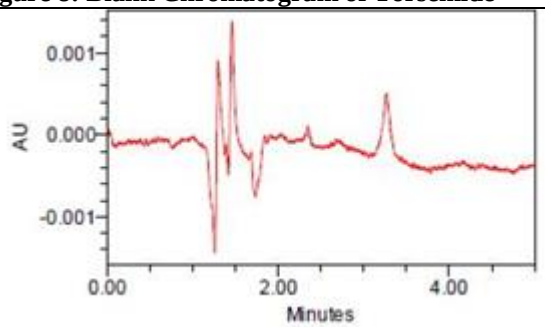


Figure 9: Standard Chromatogram of Torsemide

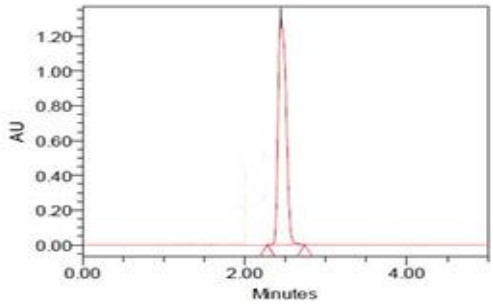


Figure 10: Sample Chromatogram of Torsemide

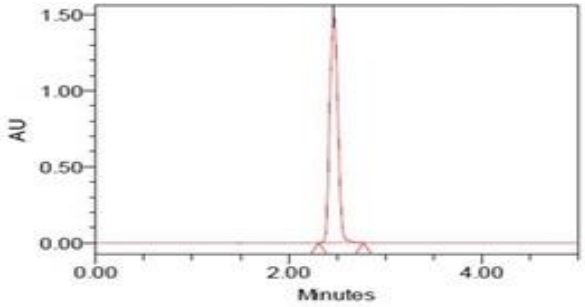


Figure 11: Linearity curve for Torsemide

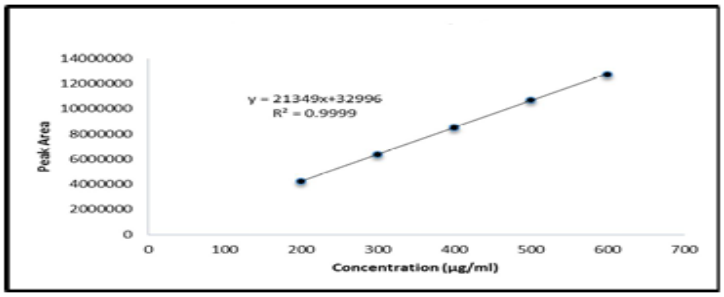
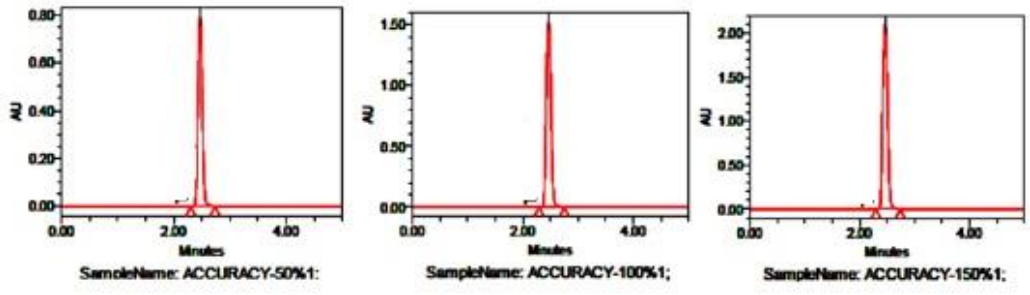


Figure 12: Accuracy Chromatogram of Torsemide at 50, 100 and 150%



CONCLUSION

The developed RP-HPLC method proved to be effective for the routine analysis of Torsemide in both active pharmaceutical ingredients (API) and pharmaceutical dosage forms. The method demonstrated high accuracy, precision, and robustness, making it suitable for quality control and routine analysis purposes.

REFERENCES

1. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2011). *Introduction to Modern Liquid Chromatography*. John Wiley & Sons.
2. ICH. (2005). Q2(R1): Validation of Analytical Procedures: Text and Methodology. International Council for Harmonisation.
3. Meyer, V. R. (2010). *Practical High-Performance Liquid Chromatography*. John Wiley & Sons.
4. Swartz, M. E., & Krull, I. S. (2012). *Handbook of Analytical Validation for HPLC and Related Techniques*. CRC Press.
5. Kazakevich, Y., & LoBrutto, R. (2007). *HPLC for Pharmaceutical Scientists*. John Wiley & Sons.
6. Patel, D., Patel, N., & Patel, C. (2014). RP-HPLC method development and validation for the simultaneous estimation of Torsemide and Spironolactone. *International Journal of Pharmaceutical Sciences Review and Research*, 28(2), 80-85.
7. Desai, S., & Kachhadia, P. (2018). Development and validation of an RP-HPLC method for estimation of Torsemide. *Journal of Pharmaceutical Analysis*, 10(1), 45-52.
8. Kumar, V., Singh, M., & Sharma, P. (2020). Stability-indicating RP-HPLC method development and validation for Torsemide in pharmaceutical dosage forms. *Asian Journal of Research in Chemistry*, 13(6), 301-306.
9. Shah, D., Patel, A., & Mehta, S. (2019). Stability-indicating RP-HPLC method for simultaneous estimation of Torsemide and Spironolactone in combined tablet dosage form. Journal of Applied Pharmaceutical Science, 9(2), 45-53.
10. Kumar, V., & Singh, H. (2018). Development and validation of RP-HPLC method for the estimation of Torsemide in pharmaceutical formulations. Journal of Pharmaceutical Research, 15(3), 120-126.
11. Gupta, R., & Kumar, S. (2020). Development and validation of a sensitive RP-HPLC method for the estimation of Torsemide in biological samples. Biomedical Chromatography, 34(5), e4783.