WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 11, 1505-1516. Research Article ISSN 2277–7105

DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF EPLERENONE AND TORSEMIDE FROM THEIR DOSAGE FORM

Monali R. Dakhole, Radheshyam T. Lohiya* and Milind J. Umekar

Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur 441002.

Article Received on
08 April 2025,

Revised on 28 April 2025,
Accepted on 18 May 2025

DOI: 10.20959/wjpr202511-36908

***Corresponding Author**
Radheshyam T. Lohiya
Smt. Kishoritai Bhoyar College of Pharmacy,
Kamptee, Nagpur 441002.

ABSTRACT

A simple, rapid, accurate, precise, and reliable UV-spectroscopic method for the simultaneous estimation of Eplerenone (EPL) and Torsemide (TSM) in their combined tablet dosage form has been developed. The UV-spectroscopic determination was performed at the absorption maxima of 245.0 nm for EPL, 286.0 nm for TSM, and 258.0 nm as the isoabsorptive point, using a methanol-distilled water (50:50) solvent mixture. The methods employed were the Simultaneous Equation Method and the Absorbance Ratio Method. Both methods exhibited linearity in the concentration ranges of 10-50 µg/mL for EPL and 4-20 µg/mL for TSM, with correlation coefficients between 0.9997 and 0.999. The accuracy of the methods was assessed by recovery studies, which showed results within acceptable limits for both Eplerenone and Torsemide. Additionally, the analysis demonstrated that both physical mixtures of the drugs and tablet formulations could be used for estimation. Among the various UV-spectroscopic methods available, the Simultaneous Equation Method and the Absorbance Ratio Method were found to be effective for the determination of these drugs in tablet dosage forms. The proposed UV-spectroscopic methods for the estimation of Eplerenone and Torsemide in their tablet formulations are suitable for routine quality control analysis

KEYWORDS: Eplerenone (EPL), Torsemide (TSM), UV- spectroscopy, Simultaneous equation method, Absorbance ratio method.

1. INTRODUCTION

Eplerenone is chemically Pregn-4-ene-7, 21-dicarboxylic acid, 9, 11-epoxy-17-hydroxy-3oxo-, γ-lactone, methyl ester, (7 α, 11 α, 17 α). A white to off white crystalline powder with molecular formula of $C_{24}H_{30}O_6$ and molecular weight 414.498 g/mol. It is slightly soluble in water. Eplerenone is an antihypertensive drug that is highly selective aldosterone receptor antagonist (SARA).[1] to effectively block aldosterone at receptor site in body tissue, aldosterone being a component of rennin angiotensin-aldosterone system. Eplerenone is used for treatment of hypertension and heart failure due to its cardio protective and renal protective effect.[2]

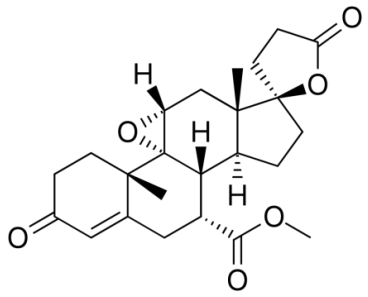


Fig 1: Structure of Eplerenone.

Torsemide is a loop diuretic drug chemically it is N- [(isopropyl amino) carbonyl]-4-[(3-methylphenyl) amino] pyridine-3-sulfonamide. White powder with molecular formula $C_{16}H_{20}N_4O_3S$ and molecular weight 348.4 g/mol. Torsemide is a Loop Diuretic drug. It is useful in the treatment of hypertension or edema associated with congestive heart failure, renal disease and hepatic disease. Structurally, it is a pyridine-sulfenyl urea used as an antihypertensive agent.[3]

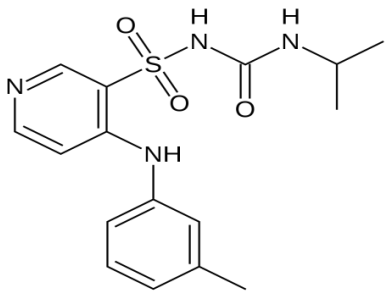


Fig 2: structure of Torsemide.

Literature survey reveals that several papers RP-HPLC(1,4–12)HPTLC(13–15) and spectrophotometric(16–19) methods are available for the determination of Eplerenone and

Torsemide. The review of literature reveals that no method yet reported in Methanol: distilled water (50:50) for the simultaneous determination of both the drugs in combined dosage forms. This paper describes simple, rapid, accurate and reliable UV-Spectroscopy method for the simultaneous estimation of Eplerenone and Torsemide in their tablet dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents

The pharmaceutical dosage form used in study was **EPTUS T-10 Tablet** (Label claim 25mg EPL, 10mg TSM) manufactured by **GLENMARK PHARMACEUTICALS**. All chemicals and reagents used were analytical grade.

Instruments

Jasco V-630 double beam UV-visible spectrophotometer was used along with 1.0 cm path length matched pair of quartz cell for Spectrophotometer method.

A Shimadzu (AUX 220) electronic analytical balance was used for weighing the sample.

Preparation of Standard Solution

Torsemide standard stock solution (A1)

An accurately weighed quantity of TSM (~10mg) was transferred in 100.0ml volumetric flask, dissolved in sufficient quantity of Distilled water: Methanol (50:50). The volume was made up to the mark with Methanol: Distilled water (Concentration: 100µg/mL)

Working standard solution (A2)

1mL solution A1 was pipetted out and transferred in 10.0 mL volumetric flask and volume was made up to the mark with Methanol: Distilled water. (Concentration: 10µg/mL)

Eplerenone standard stock solution (B1)

An accurately weighed quantity of EPL (~10 mg) was transferred in 100.0 ml volumetric flask, dissolved in sufficient quantity of methanol: Distilled water (50:50). The volume was made up to the mark with Methanol: Distilled water. (Concentration: 100ug/mL).

Working standard solution (B2)

1mL solution B1 was pipetted out and transferred in 10.0 mL volumetric flask and volume was made up to the mark with Methanol: Distilled water. (Concentration: 10µg/mL).

Selection of Wavelength

The standard stock solution of A2 and B2 were scanned in UV rang 200-400 nm in 1.0 cm cell against solvent blank and spectrum was recorded.

The overlain spectrum of drugs so recorded is depicted in Figure No.3

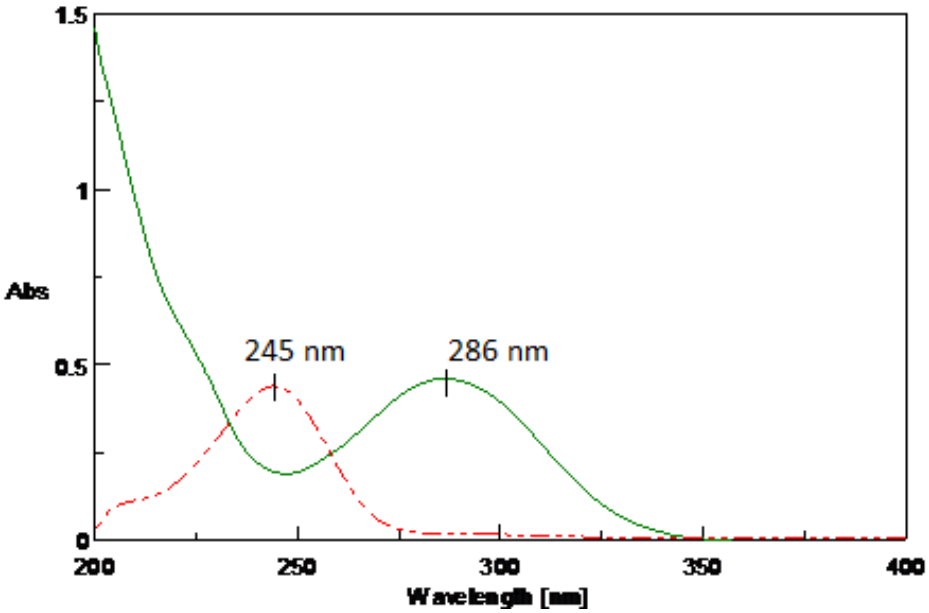
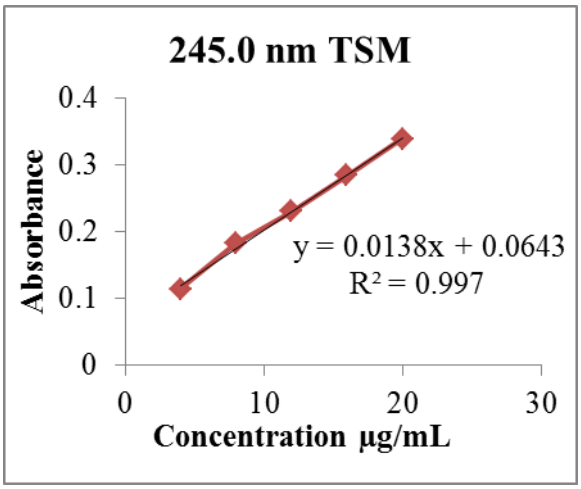


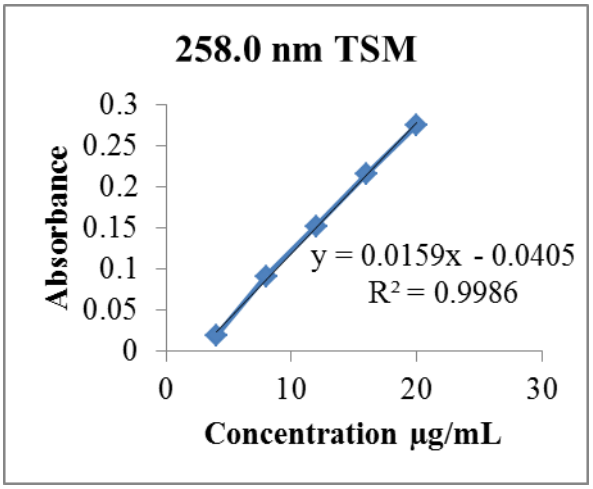
Fig 3: Overlain spectra of Eplerenone and Torsemide.

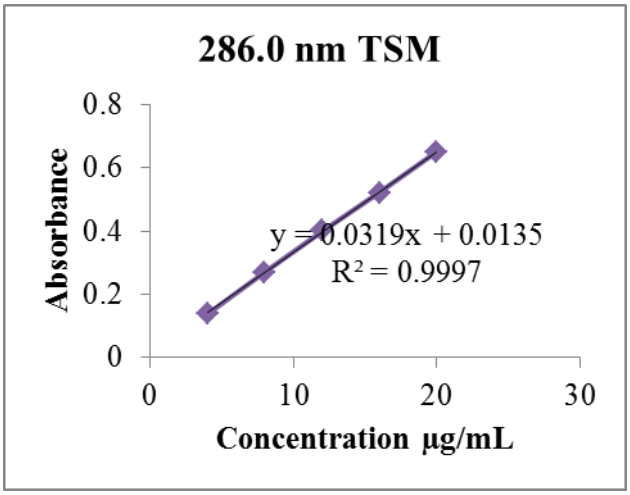
The study of spectrum reveals that EPL shows a well-defined λ max at 245.0 nm, TSM shows a well-defined λ max at 286.0 nm and isobestic point shows at 258.0 nm. The estimation of drugs by simultaneous equation method was developed using 245.0nm and 286.0 nm i.e. λ max values of two drugs. Similarly, the estimation by absorbance ratio method was done at 258.0 nm isosbestic point and 245.0nm λ max for Eplerenone i.e. λ max value of isosbestic wavelength.

Study of Beer-Lambert's Law

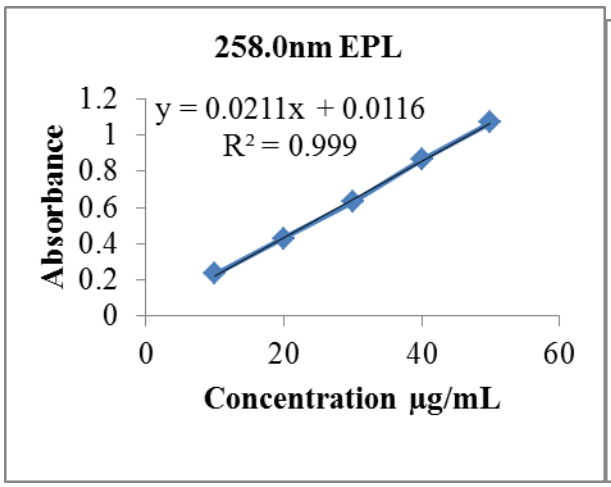
Standard stock solution of TSM and EPL were diluted with Distilled water: Methanol to get concentration in range 4-20 µg/mL for TSM and 10-50 µg/mL EPL respectively. Absorbances of each of the resulting solutions were measured at 245.0, 258.0 and 286.0 nm in 1.0 cm cell using solvent blank. The standard curves for TOR and EPL are shown in Fig. 4,5 and 6.

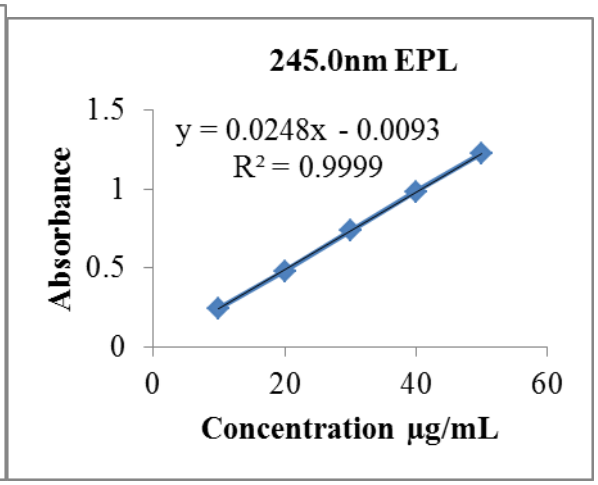


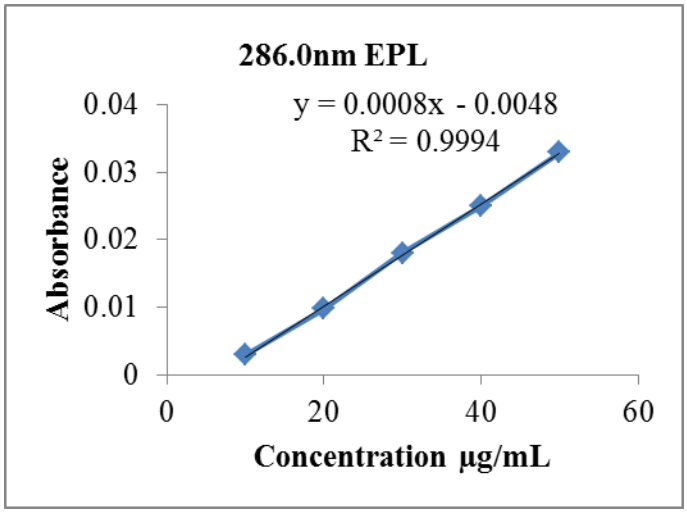




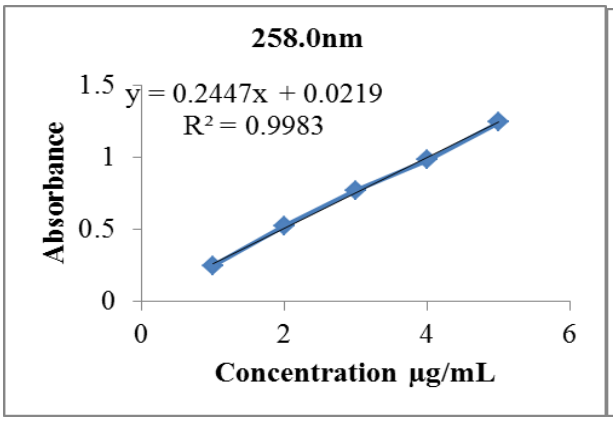
Study of Beer- Lambert's law of TSM.

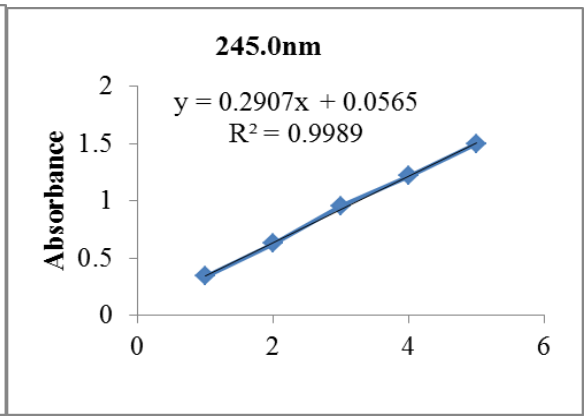


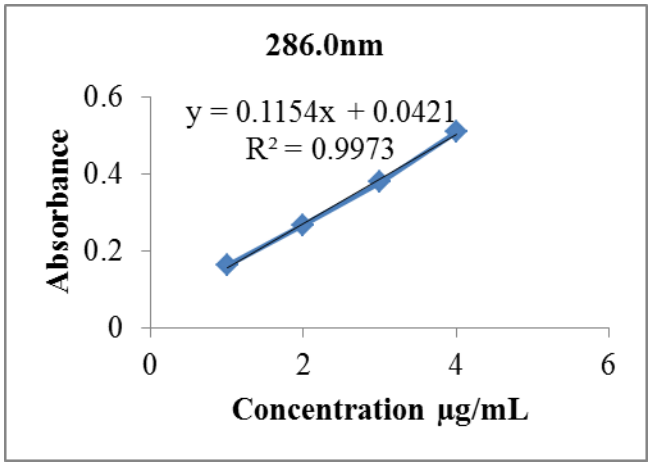




Study of Beer- Lambert's law of EPL.







Study of Beer- Lambert's law of TSM and EPL.

Determination of Absorptivity value

The absorbance of each of the final dilution were measured in triplicate in 1.0 cm cell against blank at 245.0, 258.0 and 286.0 nm and A (1% 1cm) values were calculated using below formula.

$$\text{A (1\% 1cm)} = \frac{\text{Absorbance}}{\text{Concentration } (\frac{g}{100}mL)}$$

Procedure

Estimation of EPL and TSM in marketed formulation

For estimation of formulation, twenty tablets of brand EPTUS T-10 Tablet were weighed and finely powdered. An accurately weighed quantity of tablet powder containing (~10mg TSM and 25mg EPL) was transferred to 100.0 mL volumetric flask, shaken for 15 min with sufficient quantity of Distilled water: methanol and volume was made up to mark. The content was filtered through Whatman filter paper. A 1.0 mL portion of this solution was further diluted up to 10.0 mL with Distilled water: methanol to get final concentration of about (10.0 µg/mL of TSM, 25µg/mL EPL).

METHOD I: Simultaneous equation method

The simultaneous equation method of analysis is based on the absorption of the drugs Eplerenone and Torsemide at their wavelength maxims. Two wavelengths selected for the method are 245.0 and 286.0 nm that are absorption maxima of Eplerenone and Torsemide. The stock solutions of both the drugs were diluted with Distilled water: Methanol to get concentration in range 4-20 µg/mL for TSM and 10-50 µg/mL EPL respectively. The absorbance was measured at the selected wavelength and concentrations in the sample were obtained by using following equations 1 and 2.

$$Cx = \frac{A_2\, ay1 - A_2\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \qquad Cy = \frac{A_1 ax_2 - A_2 ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where, A_1 and A_2 are absorbances of diluted mixture at 245.0 and 286.0 nm respectively. Cx and Cy are the concentration of EPL and TSM respectively (g/100mL). ax_1, ax_2, ay_1, ay_2 are absorptivities of EPL and TSM at 245.0 and 286.0 nm respectively.

Method II: Absorbance ratio method[20,21]

The absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one is an isosbestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Figure 4), wavelength 258.0 nm (isosbestic point) and 245.0 nm. The absorptivity coefficient of both drugs was

determined and the individual concentration of EPL and TSM was determined using the following equations 1 and 2:

$$Cx = \frac{Qm-Qy}{Qx-Qy} X \frac{A1}{ax} \quad Cy = \frac{Qm-Qx}{Qy-Qx} X \frac{A1}{ay}$$

Where, Qm = Ratio of absorbance of laboratory mixture at 245.0 nm and 258.0 nm Qx and Qy are Ratio of absorptivity of EPL at 245.0 and 258.0 nm and TSM at 245.0 and 258.0 nm. ax and ay are Absorptivity of EPL at 245.0 nm and TSM at 286.0 nm. A is Absorbance of mixture at isobestic point at 258.0 nm. Cx and Cy are Concentration of EPL and TSM.

METHOD VALIDATION

The UV spectrophotometric method was validated. The performance parameters like accuracy, precision and ruggedness were evaluated.

Accuracy

Recovery studies performed by standard addition method. Results are shown in **Table no. 2**

Precision

Precision of any analytical method is expressed as SD and RSD of series of measurements. Precision of estimation of EPL and TSM by proposed method was ascertained by replicate analysis. Results are shown in **Table no.1**

Ruggedness

Different analyst

The tablet content was analyzed by proposed method by two different analysts. Results are shown in **Table No.3**

Interday and Intraday variation

An accurately weighed quantity of tablet content equivalent to about 25 mg EPL and 10 mg of TSM was transferred to 100.0 mL volumetric flask, sonicated for 15 min with sufficient quantity of Methanol: distilled water up to the mark. The content in the flask were filtered through Whattman filter paper. A 1.0 ml of filtrate was diluted to 10.0 ml in a volumetric flask using solvent. The absorbance of the final solution was read after 0hr, 3hr and 5hr in 1.0 cm cell at selected wavelength. Similarly, the absorbance of the same solution was measured

on 1st day, 2nd day and 3rd day and % label claim were calculated using formulae as described under marketed formulation. The results are shown in **Table No. 4 & 5**

RESULT AND DISCUSSION

The tablet formulation containing EPL and TSM can be successfully analyzed by UV Spectroscopic using Simultaneous equation, Absorbance ratio method which can be routinely used for quality control analysis of EPL and TSM in marketed formulation. Hence, accurate, reliable UV Spectroscopy methods were developed. These methods can be utilized for routine quality control analysis of Eplerenone and Torsemide in their formulation.

Table No. 1: Results of simultaneous estimation of marketed formulation for Method I&II.

Sr. No	Wt. of tablet powder taken in (mg)	% Label claim			
		Method (I)		Method (II)	
		EPL	TSM	EPL	TSM
1	181.2	97.86	99.08	96.08	100.2
2	181.1	96.99	100.8	97.74	100.4
3	181.2	97.24	99.08	96.48	99.08
4	181.3	97.6	99.19	97.42	100.5
5	181.2	97.9	99.51	97.34	99.08
Mean		**97.51**	**99.53**	**97.01**	**99.85**
±SD		**0.39**	**0.73**	**0.69**	**0.71**
%RSD		**0.41**	**0.73**	**0.72**	**0.71**

Table No. 2: Recovery studies.

Sr. No.	Amt. of pure Drug added in (mg)		% Recovery			
			Method I		Method II	
	EPL	TSM	EPL	TSM	EPL	TSM
1	20.0	8.0	97.55	97.70	98.7	98.87
2	24.9	9.6	97.91	97.41	99.72	98.9
3	29.8	12.3	96.71	98.37	99.43	99.16
Mean			**97.39**	**97.82**	**99.38**	**98.97**
±SD			**0.98**	**0.49**	**0.52**	**0.15**
%RSD			**0.99**	**0.50**	**0.53**	**0.16**

Table No. 3: Results of different analysts.

Analyst	Wt. of powder taken in (mg)	% label claim			
		Method I		Method II	
		EPL	TSM	EPL	TSM
Analyst I	181.2	97.24	99.08	96.48	99.8

Analyst II	181.0	98.69	99.04	97.25	98.17
Mean		**97.96**	**99.06**	**96.86**	**98.98**

Table No. 4: Result of Intraday study.

Time	Wt. of powder taken in (mg)	% label claim			
		Method I		Method II	
		EPL	TSM	EPL	TSM
0hr	181.1	97.98	99.44	97.33	99.14
3hr		97.90	98.64	97.93	101.0
5hr		98.27	98.90	98.05	99.94
Mean		**98.05**	**98.99**	**97.77**	**100.0**

Table No. 5: Result of Interday study.

Day	Wt. of powder taken in (mg)	% label claim			
		Method I		Method II	
		EPL	TSM	EPL	TSM
Day 1	181.1	97.98	99.44	97.33	99.14
Day 2		96.69	98.94	97.81	99.0
Day 3		97.84	99.27	97.9	98.40
Mean		**97.50**	**99.21**	**97.68**	**99.18**

CONCLUSION

The tablet formulation containing EPL and TSM can be successfully analyzed by UV Spectroscopic using Simultaneous equation and Absorbance ratio method which can be routinely used for quality control analysis of EPL and TSM in marketed formulation. Since no official method was available for determination of TSM and EPL from their combined dosage form. Hence, accurate, reliable UV Spectroscopy and method was developed. These methods can be utilized for routine quality control analysis of Eplerenone and Torsemide in their formulation.

ACKNOWLEGMENT

The author is thankful to Smt. Kishoritai Bhoyar college of Pharmacy, Kamptee, Nagpur (MS), India for providing necessary help for the work.

REFERENCES

1. Rane VP, Patil KR, Sangshetti JN, Yeole RD, Shinde DB. Stability-indicating RP-HPLC method for analysis of eplerenone in the bulk drug and in a pharmaceutical dosage form. Acta Chromatogr, 2009 Dec; 21(4): 619–29.
2. National Center for Biotechnology Information. “PubChem Compound Summary for CID 443872, Eplerenone” PubChem,

https://pubchem.ncbi.nlm.nih.gov/compound/Eplerenone. Accessed 9 May, 2025; 2025 May 9.

3. National Center for Biotechnology Information. “PubChem Compound Summary for CID 41781, Torsemide” PubChem, https://pubchem.ncbi.nlm.nih.gov/compound/Torsemide. Accessed 9 May, 2025; 2025 May 9.
4. Zhang L, Wang R, Tian Y, Zhang Z. Determination of torasemide in human plasma and its bioequivalence study by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. J Pharm Anal., 2016 Apr; 6(2): 95–102.
5. Gide P, Sonawane S, Chitnis A. Development and validation of RP-HPLC method for estimation of eplerenone in spiked human plasma. J Pharm Anal., 2012 Oct; 2(5): 390–3.
6. Bihola U, Prajapati P, Agrawal YK. HPLC – Quality by Design Approach for Simultaneous Detection of Torsemide, Spironolactone and Their Degradant Impurities. Pharm Technol Hosp Pharm., 2018 Aug 28; 3(3): 123–34.
7. Patel RB. STABILITY INDICATING RP-HPLC METHOD DEVLOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF EPLERENONE AND TORSEMIDE IN TABLET DOSAGE FORM. World J Pharm Pharm Sci., 2017 May 1; 1397–406.
8. Vijaya Kumar Meher1* GKGSKPSKD. Method Development And Validation For The Torsemide In Tablet Dosage Form By Rp-Hplc. J Pharm Negat Results, 2022; 13(4): 2365–70.
9. Hinge MA, Patel D. Optimization of HPLC method using central composite design for estimation of Torsemide and Eplerenone in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences, 2022; 58.
10. Krupali S. Patel1 ABSVLSBN. Development and Validation OF RP-HPLCMethod for the Simultaneous Estimation of Eplerenone and Torsemide in Pharmaceutical Dosage Form. J Pharm Sci Bioscientific Res., 2016; 6(3): 283–90.
11. Rajesh Shukla1* NBVPDGAPJ. RP-HPLC Determination of Torsemide in Pharmaceutical Formulation by Liquid Chromatography. Asian Journal of Biomedical and Pharmaceutical Sciences, 2012; 2(15): 45–8.
12. Ganji S, Dhulipala S, Nemala AR. Development and validation of RP HPLC method for the estimation of Sofosbuvir and related impurity in bulk and pharmaceutical dosage form. Futur J Pharm Sci., 2021 Dec 28; 7(1): 154.
13. Agbaba D, Čarapić M. Applications of thin-layer chromatography in the pharmaceutical industry. In: Instrumental Thin-Layer Chromatography. Elsevier, 2023; p. 519–73.

14. Younis SE, El-Nahass SA, Soliman SA, Youssef RM. Simultaneous micro-determination of eplerenone and torsemide in their combined tablets using HPTLC-Dual wavelength spectrodensitometric and spectrophotometric methods. Microchemical Journal, 2020 Jul; 156: 104861.
15. Tathe S V., Deshmukh PR, Kashid AM, Gaikwad S. Development and Validation of a High-Performance Thin-Layer Chromatographic Method for the Simultaneous Estimation of Torsemide and Eplerenone by Quality by Design Approach. JPC - Journal of Planar Chromatography - Modern TLC., 2019 Dec; 32(6): 495–500.
16. Jatin Guptaa GKVYSRW. Development and validation of a UV spectrophotometric method for the estimation of torsemide in bulk and in tablet dosage form. Journal of Chemical and Pharmaceutical Research, 2010; 2(4): 513–7.
17. P. Saifulla Khan*1 PRRVKR and NA. Extractive Spectrophotometric estimation of Eplerenone using Acid dyes in Tablet dosage forms. Der Pharma Chemica., 2012; 4(5): 1883–6.
18. A M, Patel D. Chemometric Assisted Spectrophotometric Method Development for Evaluation of Torsemide and Eplerenone in their Combined Tablet Dosage Forms. Indian Journal of Pharmaceutical Education and Research, 2022 Jan 12; 56(1): 255–63.
19. Jagtap R, Patel SK, Toppo J, Karthikeyan C, Minz S, Tiwari A. Validated Novel Green Uv Spectrophotometric Method for the Simultaneous Estimation of Torsemide and Eplerenone in Pharmaceutical Formulation, 2025.
20. Madhuri A. Hinge 1 DrDP 2, DrAP. Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Torsemide and Eplerenone in Bulk Drugs and Combined Dosage Form. International Journal of Pharmaceutical Sciences Review and Research, 2019; 63–7.
21. Shraddha V. Tathe1* ASSGSGBDUASJ and AG. DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TORSEMIDE AND EPLERENONE IN BULK AND COMBINED DOSAGE FORM. European Journal of Biomedical AND Pharmaceutical sciences, 2021; 8(6): 556–63.