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DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TORSEMIDE AND EPLERENONE IN BULK AND COMBINED DOSAGE FORM

Shraddha V. Tathe[1]*, Akshay Suryavanshi[2], Shubhangi Gaikwad[3], Shivangi Gupta[4], Bharat Dawra[4], Unnati Amale[4], Shivani Jadhav[4] and Ajit Gaiki[4]

[1]Department of Pharmaceutical Chemistry, Sinhgad Institute of Pharmacy, Narhe, Pune-411041, Maharashtra, India.
[2]Department of Pharmaceutical Quality Assurance, Sinhgad Institute of Pharmacy Narhe, Pune-411041, Maharashtra, India.
[4]Department of Pharmaceutical Chemistry, Sinhgad Institute of Pharmacy, Narhe, Pune 411041, Maharashtra, India.
[4]Sinhgad Institute of Pharmacy, Narhe, Pune-411041, Maharashtra, India.

***Corresponding Author: Shraddha V. Tathe**
Department of Pharmaceutical Chemistry, Sinhgad Institute of Pharmacy, Narhe, Pune-411041, Maharashtra, India.

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ABSTRACT
A simple, precise, accurate and rapid UV spectrophotometric method has been developed for simultaneous estimation of Torsemide and Eplerenone. A completely newly developed analytical method validation has been developed as per ICH Guidelines. The UV-spectrophotometric method, estimation of Torsemide and Eplerenone was carried at 244 nm and 262 nm by Q- Absorbance Ratio method. The ratio of absorbances are at two wavelengths, one which is an isoabsorptive point and other is the λ max of one of the two components. From the overlay spectra of two drug, it is evident that Torsemide and Eplerenone show an isoabsorptive point at 262 nm. The second wavelength used was 244 nm, which is λ max of Eplerenone.Therefore the method developed for Torsemide and Eplerenone is suitable for simultaneous estimation by UV spectrophotometry.

KEYWORDS: Torsemide, Eplerenone, Q-Absorbance Ratio, Simultaneous Estimation, Method Validation.

1. INTRODUCTION
Torsemide is a loop diuretic and chemically known as 3-pyridine sulphonamide n-(1-methylethyl amino)-carbonyl)-4-(3-methylphynyl) amino). It acts by inhibiting the $NA^+/k^+/2CI^-$ carrier system in the lumen of the thick ascending portion of the loop of Henle, resulting in the decrease in reabsorption for congestive heart failure and kidney disease.[3] It has a molecular formula of $C_{16}H_{20}N_4O_3S$ and a molecular mass of 348.4 g/mol. It is used in the treatment of hypertension or oedema associated with congestive heart failure.[3] Torsemide was developed and first introduced by the company Teva Pharmaceuticals and FDA approved in 2002.[1] However, Torsemide was first approved for clinical use by the FDA on 1993.[2]

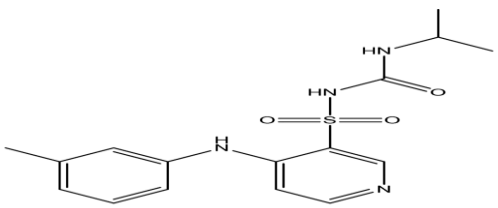

Fig. 1: Structure of torsemide.

Eplerenone, is an aldosterone receptor antagonist and chemically known as Pregn-4-Ene7, 21-Di-Carboxylic Acid, 9, 11-Epoxy-17-Hydroxy-3-Oxo, Y-Lactone Methyl Ester. It is used in the treatment of hypertension.[4&5] It has molecular formula of $C_{24}H_{30}O_6$ and a molecular mass of 414.5 g/mol.[6-9] Eplerenone is Slightly Soluble in water and Sparingly Soluble in methanol. Eplerenone is official in IP 2014[28] and analysed by Liquid chromatography. Various analytical methods have been reported for the estimation of Eplerenone as alone. They include stability indicating RP-HPLC,[18-20] UV method[21&22] HPTLC.[23]

Eplerenone binds to the mineralocorticoid receptor and thereby blocks the binding of aldosterone. This is used alone or in combination with other medicines to treat Oedema associated with Congestive Heart Failure, lowering high blood pressure which helps to prevent strokes, heart attacks and kidney problems.[6-9]

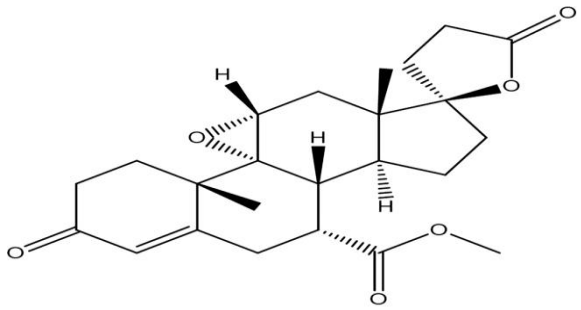

Fig. 2: Structure of eplerenone.

2. MATERIALS AND METHODS

2.1 Instruments

Spectrophotometric measurements were performed on Shimadzu UV-visible double spectrophotometric (model-V630 Jasco). All weighing was done on electronic balance (CB-50)

2.2 Chemicals and Reagents

The bulk drug Torsemide was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). and Eplerenone was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Fixed dose of combined dosage form of Torsemide 10 mg and Eplerenone 25 mg were used in this study.

2.3 Selection of solvents

Methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) was selected as solvents for studying spectral characteristics of drugs.

2.4 Preparation of solutions

2.4.1 Preparation of standard stock solution of torsemide

The standard stock solution was prepared by weighing accurate quantity of Torsemide (10 mg) and was transferred to 10 ml volumetric flask, 5 ml of methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) was dissolved with it and diluted up to the mark with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) to give a stock solution (A) having strength of 1000 µg/ml.

Then 1 ml of 1000 µg/ml standard stock solution was pipette out and transferred to 10 ml volumetric flask, 5 ml of methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) was dissolved with it and diluted up to the mark with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) to give a standard stock solution (B) having strength of 100 µg/ml.

2.4.2 Preparation of stock solution of torsemide

From the above standard stock solution (B) 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml and 3.5 ml of solutions were pipette out and transferred to 10 ml volumetric flask and made the volume up to 10 ml with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) to produce solutions of concentrations 10, 15, 20, 25, 30 and 35 µg/ml respectively.

2.4.3 Preparation of standard stock solution of eplerenone

Accurately weighed quantity of Eplerenone (10 mg) was transferred to 10 ml volumetric flask, 5 ml of methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) was dissolved with it and diluted up to the mark with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) to give a stock solution (A) having strength of 1000 µg/ml.

Then 1 ml of 1000 µg/ml standard stock solution were pipette out and transferred to 10 ml volumetric flask, 5 ml of methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) was dissolved with it and diluted up to the mark with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) to give a standard stock solution (B) having strength of 100 µg/ml.

2.4.4 Preparation of stock solution of eplerenone

From the above standard stock solution (B) 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml and 3.5 ml of solutions were pipette out and transferred to 10 ml volumetric flask and made the volume up to 10 ml with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) to produce solutions of concentrations 10, 15, 20, 25, 30 and 35 µg/ml respectively.

3. Selection of analytical wavelength

To determine the wavelength for the measurement of standard spectra of the selected drugs, Torsemide and Eplerenone were scanned between 200-400 nm against methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%).

Absorbance maxima of Eplerenone and Torsemide were obtained at 244 nm and 290 nm respectively and isoabsorptive point was obtained at 262 nm.

4. Preparation of sample solution

Twenty tablets were weighed and crushed to powder. The quantity of the powder equivalent to 25 mg Eplerenone and 10 mg Torsemide were transferred to a 50 ml volumetric flask. The content was mixed with methanol 50%, potassium dihydrogen phosphate (2pH) 30% and water 20% (30 ml) and sonicated for 15 min to dissolve the drug as completely as possible.

The solution was then filtered through a Whatman filter paper no. 41. The volume was made up to the mark with methanol 50%, potassium dihydrogen phosphate (2 pH) 30% and water 20% (30 ml) for making Torsemide (200 µg/ml) and Eplerenone (500 µg/ml)

The aliquot of this solution (0.5 ml) was transferred in to a 10 ml volumetric flask and volume was adjusted up to the mark with methanol 50%, potassium dihydrogen

phosphate (2 pH) 30% and water 20% (30 ml) to make final concentration of Torsemide (10 µg/ml) and Eplerenone (25 µg/ml).

5. Validation

5.1 Preparation of calibration curve

5.1.1 Calibration curve of torsemide

Calibration curve of Torsemide consists of different concentrations of standard Torsemide solution ranging from 10-35 µg/ml. The solutions were prepared by pipetting out 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml and 3.5 ml of the working standard solution of Torsemide (100 µg/ml) and transferred into the series of 10 ml volumetric flasks and the volume was adjusted up to the mark with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%).

The absorbance of the solutions was measured at 244 nm and 262 nm against methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%).

Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

5.1.2 Calibration curve of eplerenone

Calibration curve of Eplerenone consists of different concentrations of standard Torsemide solutions ranging from 10-35 µg/ml. The solutions were prepared by pipetting out 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml and 3.5ml of the working standard solution of Eplerenone (100 µg/ml) and transferred into the series of 10ml volumetric flasks and the volume was adjusted up to the mark with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%).

The absorbance of the solutions was measured at 244 nm and 262 nm against methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%).

Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

5.2 Linearity and Range

Linear response was determined by analysing five independent levels of the calibration curve in the range of 10-35 µg/ml or 10 to 35 µm/ml (n=6) of Torsemide and Eplerenone.

Absorption correction curves were constructed for the correct concentrations, correlation coefficients and regression equations of Torsemide and Eplerenone were calculated.

5.3 Precision

5.3.1 Repeatability

The aliquot of 2 ml of standard Torsemide solution (100 µg/ ml) was transferred to a 10 ml volumetric flask. Aliquot of 2ml of a standard Eplerenone solution (100 µg/ml) was transferred to 10 ml volumetric flask. The volume was adjusted up to the mark with methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) for increasing 20 µg/ml Torsemide solution and 20 µg /ml Eplerenone solution.

The absorbance of the solutions was measured by spectrophotometry six times and % RSD was calculated.

5.3.2 Intraday

The aliquots of 2.0 ml, 2.5 ml and 3.0 ml of the standard Torsemide working solution (100 µg/ml) were transferred to 10 ml volumetric flasks. Aliquots of 2.0 ml, 2.5 ml and 3.0 ml of the standard Eplerenone working solution (100µg /ml) were transferred to 10 ml volumetric flask.

The volume is adjusted so that methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) were displayed at 20, 25 and 30 µg/ml Torsemide solutions or 20, 25 and 30 µg/ml Eplerenone solutions. Solutions were analysed on the spectrometry, 3 times on the same day.

5.3.3 Interday

The aliquots of 2.0 ml, 2.5 ml and 3.0 ml of the standard Torsemide solution (100 µg/ml) were transferred to 10 ml volumetric flasks. Aliquots of 2.0 ml, 2.5 ml and 3.0 ml of the standard Eplerenone working solution (100 µg/ml) were transferred to 10 ml volumetric flask.

The volume is adjusted so that methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) were displayed at 20, 25 and 30 µg/ml Torsemide solutions and 20, 25 and 30 µg/ml Eplerenone solutions. Solutions were analysed on 3 different days on the spectrometry and % RSD was calculated.

5.4 Accuracy (Recovery)

Accuracy of the developed method was confirmed by doing recovery study as per ICH norms at three different concentrations levels 50%, 100%, 120% and the values were measured at all wavelengths for Torsemide and Eplerenone. This operation was done in triplicate. From the recovery study it was clear that the method is very accurate for quantitative estimation of Torsemide and Eplerenone in Tablet dosage forms as the statistical results were within the acceptance range.

6. Q-Absorbance ratio method

The absorption coefficient method uses the absorption coefficient at two selected wavelengths. One is the absorption point, and the other is one of two components.

The overlap spectrum of the two active ingredients shows that the Torsemide and Eplerenone isotopic point is 262 nm. The second wavelength used was 244 nm, which is λ max in Eplerenone. (Fig. 9)

Six effective pattern solutions having concentrations 10, 15, 20, 25, 30 and 35 µg/ml for Torsemide and 10, 15, 20, 25, 30, and 35 µg/ml for Eplerenone were all set in

methanol (50%), potassium dihydrogen phosphate (2 pH) (30%) and water (20%) and the absorbance at 262 nm (isoabsorptive point) and 244 nm (λ max of Eplerenone) were slow and absorptivity coefficients were calculated.

The absorbance of the test elucidation (10 µg/ml of Torsemide and Eplerenone) i.e. A_1 and A_2 were recorded at 262 nm (isoabsorptive point) and 244 nm (λ max of Eplerenone) respectively, and ratios of absorbance was calculated, i.e. A_2/A_1.

Relative concentrations of two drugs in the appraise was calculated by means of next equations.

$C_X = [(Q_M - Q_Y)/ (Q_X - Q_Y)] \times A_1/a_{x1}$ (i)

$C_Y = [(Q_M - Q_X)/ (Q_Y - Q_X)] \times A_1/a_{Y1}$ (ii)

The Q-values and absorptivity for equally drugs were calculated as follows:

Q_M = Absorbance of section elucidation at 244 nm (A_2)/Absorbance of model explanation at 262 nm (A_1)

Q_X = Absorptivity of Torsemide at 244 nm (a_{x2})/Absorptivity of Torsemide at 262 nm (a_{x1})

Q_Y = Absorptivity of Eplerenone at 244 nm (a_{y2})/Absorptivity of Eplerenone at 262 nm (a_{y1})

Where, A_1 and A_2 are absorbances of mixture at 262 nm and 244 nm.

Q_X and Q_Y are Q-values of Torsemide and Eplerenone respectively.

a_{x1} and a_{y1} are absorptivities of Torsemide and Eplerenone at 262 nm.

a_{x2} and a_{y2} are absorptivities of Torsemide and Eplerenone at 244 nm.

The laboratory analysis process was constant 3 times with check out solutions.

7. RESULTS AND DISCUSSION

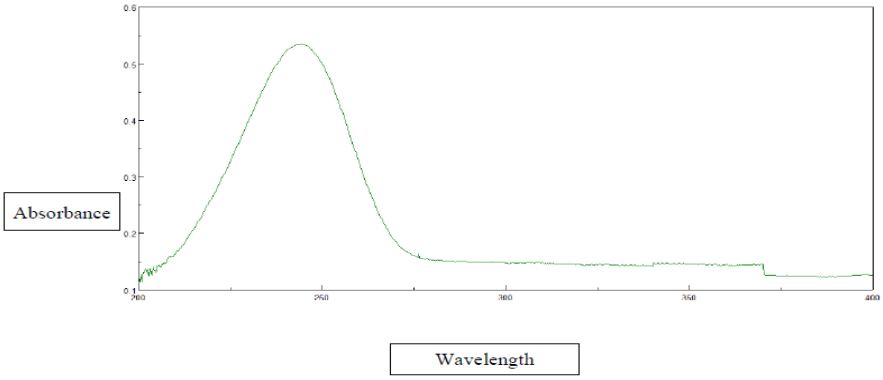

Fig. 1: Spectra of eplerenone (10µg/ml).

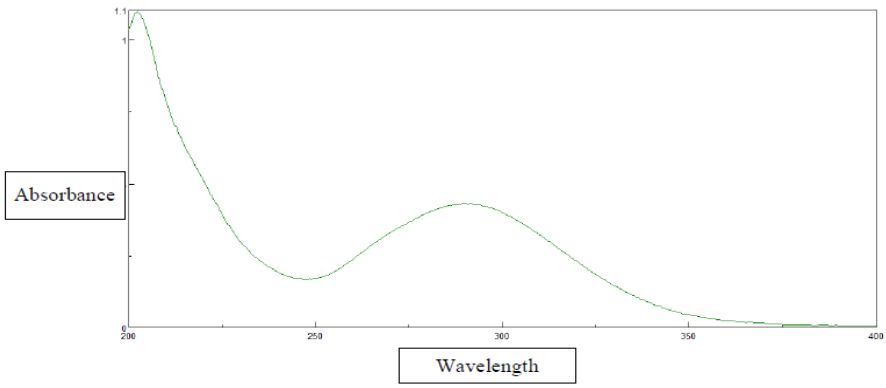

Fig. 2: Spectra of torsemide (10 µg/ml).

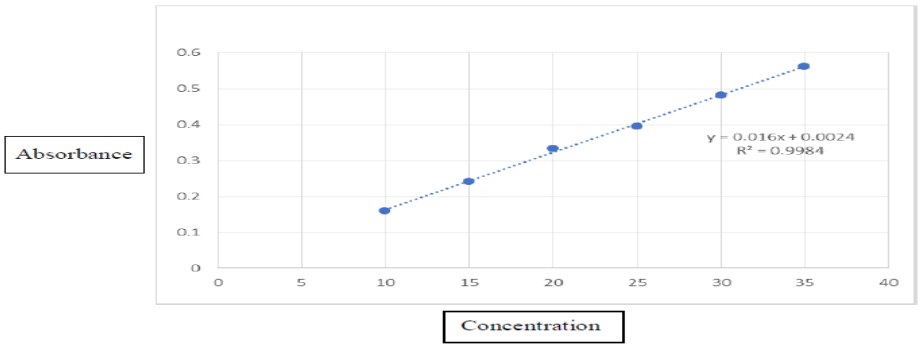

Fig. 3: Calibration curve for torsemide at 244 nm (λ max of torsemide).

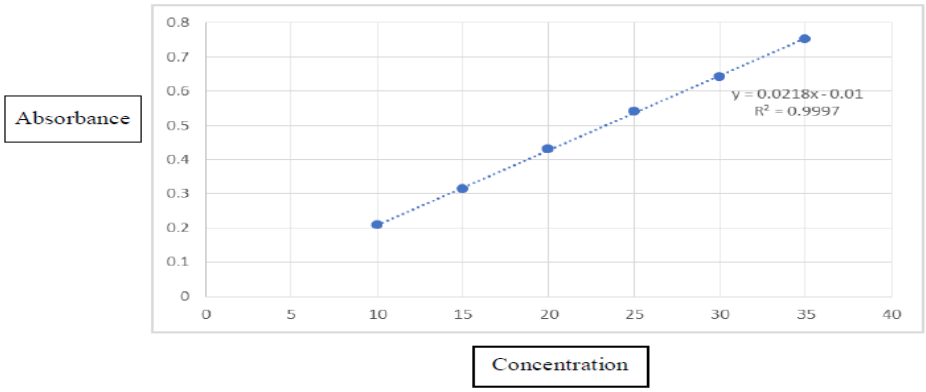

Fig. 4: Calibration curve for torsemide at 262 nm (λ max of torsemide).

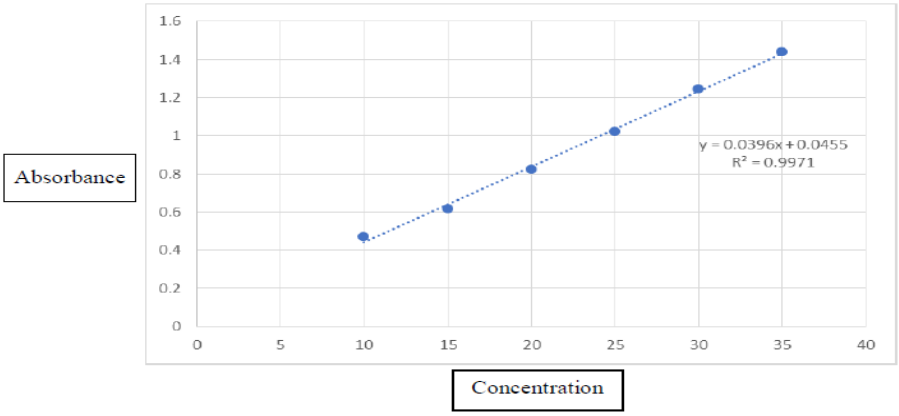

Fig. 5: Calibration curve for eplerenone at 244 nm (λ max of eplerenone).

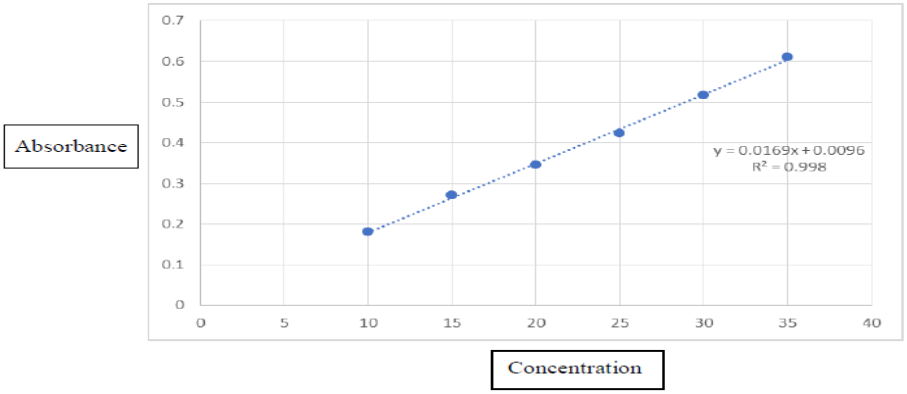

Fig. 6: Calibration curve for eplerenone at 262 nm (λ max of eplerenone).

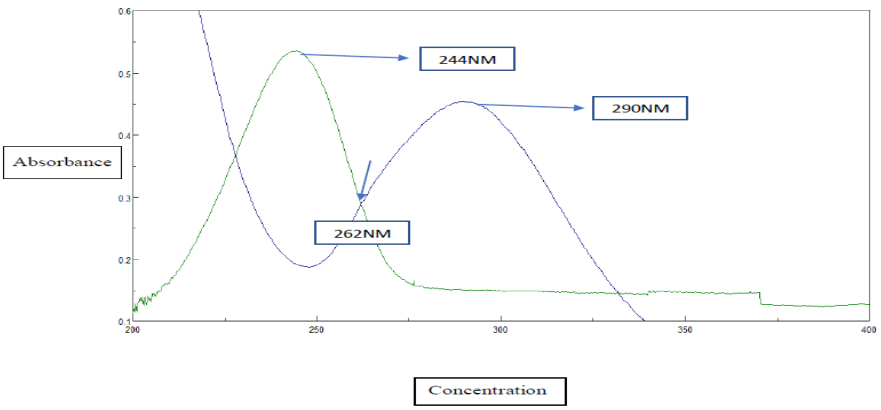

Fig. 7: Overlain absorption Spectra of Eplerenone and Torsemide showing isoabsorptive point (262 nm).

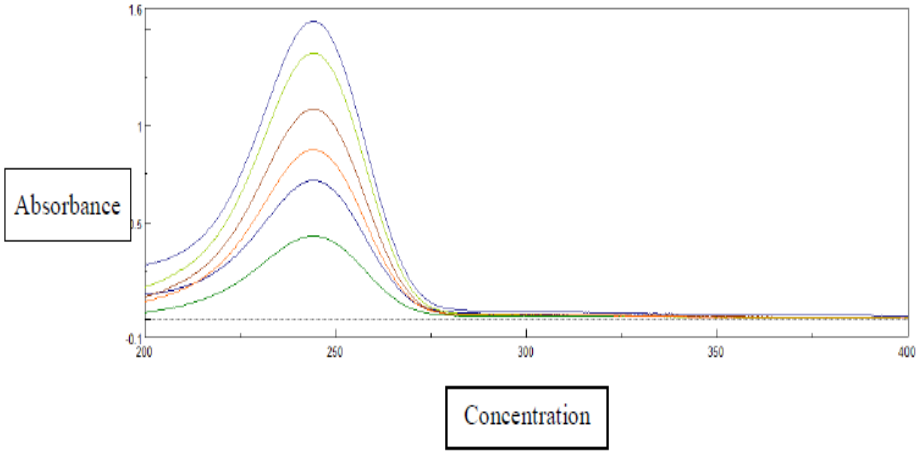

Fig. 8: Overlay spectra of eplerenone (10-35 µg/ml).

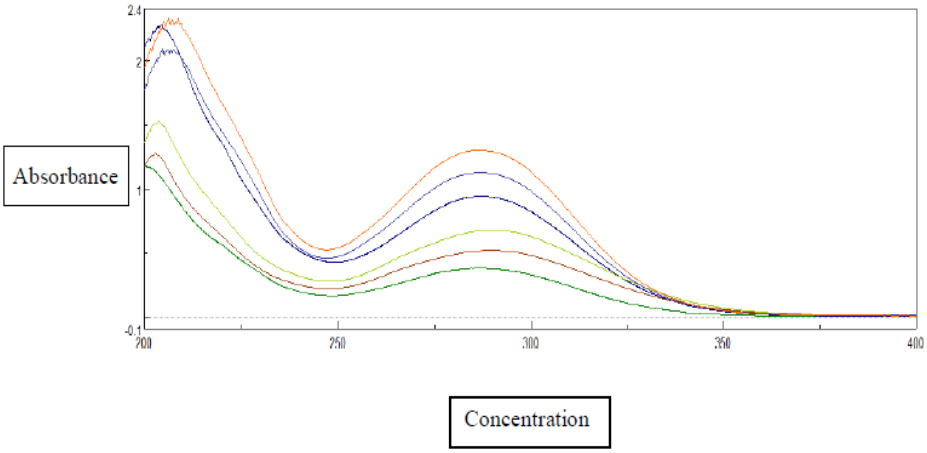

Fig. 9: Overlay spectra of torsemide (10-35 µg/m).

Table 1: Recovery study (accuracy) for torsemide tablet formulation.

The accuracy was determined by % recovery method. All the recovery values of torsemide and eplerenone were within ±1.5% by using standard addition method, results were obtained within accepted criteria as RSD of all the samples < 2, showing proposed method was accurate (Table 1 and Table 2).

	Level%	Tablet Taken (µg/ml)	Standard Added (µg/ml)	%Recovery	
TOR				Tablet	Added
	80	10	8	100.5	100.62
	100	10	10	101.21	101.45
	120	10	12	100.95	100.81
Mean				100.88	100.96
SD				0.359	0.434
%RSD				0.356	0.430

Table 2: Recovery study (Accuracy) for eplerenone tablet formulation.

	Level%	Tablet Taken (µg/ml)	Standard Added (µg/ml)	%Recovery	
EPT				Tablet	Added
	80	10	8	101.20	101.51
	100	10	10	101.00	101.20
	120	10	12	100.59	100.68
Mean				100.93	101.13
SD				0.310	0.419
%RSD				0.308	0.414

Table 3: Analysis of pharmaceutical dosage form.

Tablet formulation	Planeo-t 10			
	Torsemide		Eplerenone	
	244	262	244	262
Label claim (mg)	10	10	25	25
Amount found (mg)	10.17	10.19	25.27	25.18
%Assay				
Sd	0.499	0.970	0.283	0.245
%Rsd	0.508	0.989	0.287	0.247

The results of analysis of marketed formulation Torsemide and Eplerenone were obtained as shown in Table 3. The RSD of both the drugs were found to be < 2%.

Table 4: Optical regression Characteristics and Validation parameters.

Mixture	Torsemide & Eplerenone			
Method Parameters	TOR		EPL	
Wave length (nm)	244	262	244	262
Linearity range (µg/ml) (n=6)	10-35	10-35	10-35	10-35
Slope	0.016	0.0218	0.0396	0.0172
Intercept	0.0024	0.01	0.0455	0.0048
Correlation Coefficient (r)	0.9984	0.9997	0.9971	0.9994
Precision (±%RSD)				
Repeatability	101.39± 0.908	101.02± 0.558	101.05 ±1.00	101.66 ±0.226
Interprecision	101.08±0.574	101.38 ± 0.294	101.48 ± 0.375	101.15 ± 0.574
Intraprecision	101.06±0.236	101.22±0.578	101.58 ±0.292	101.60±0.205
%Assay	100.40±0.803	100.17±0.422	99.80±0.484	100.73±0.247

All the tested parameters are within recommended limits. The method was found to be linear throughout the range , as the correlation coefficient (r^2) was 0.99 (n=6). Repeatability and intermediate precision are shown in table 4. Inter-day and intra-day precision were assessed by % RSD. The RSD of all the samples were < 2%, which confirmed that proposed method was precise.(Table 4)

8. CONCLUSION

After investigating, the results obtained shows that the proposed spectrophotometric method was found to be simple, accurate and precise for determination of Torsemide and Eplerenone in combined dosage form. The method utilizes easily available and cheap solvent for analysis of Torsemide and Eplerenone in combined dosage form. The common excipients and additives that are usually present in the combined dosage form do not interfere in the analysis of Torsemide and Eplerenone in the method, hence it can be conveniently adopted for routine quality control of the drug in mixture or combined pharmaceutical formulation.

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