Research Article

Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Torsemide and Eplerenone in Bulk Drugs and Combined Dosage Form

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ABSTRACT

A simple, precise and economical UV spectrophotometric method has been developed for the simultaneous estimation of Torsemide and Eplerenone in their combined dosage form. Method is Absorbance ratio (Q- analysis method) which is based on measurement of absorption at wavelength of 239.80 nm (λ max of Eplerenone) and 260 nm (Isoabsorptive point). Linearity was observed in the concentration range of 4-20 µg/ml for Torsemide and 5-25 µg/ml for Eplerenone. The accuracy of method was assessed by recovery studies and was found to be within range of 99-101% for both Torsemide and Eplerenone. The developed method was validated with respect to linearity, accuracy (recovery) and precision. The results were validated statistically as per ICH guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of Torsemide and Eplerenone in the mixture.

Keywords: Torsemide, Eplerenone, Q-analysis, UV spectrophotometry.

INTRODUCTION

Torsemide (TOR) [1-3] is chemically 3-Pyridinesulfonamide, N-[[(1-methylethyl) amino] carbonyl]-4-[(3-methylphenyl)amino]-1-Isopropyl-3-[(4-m-toluidino-3-pyridyl) sulfonyl]urea. It acts by inhibits the Na+/K+/2Cl--carrier system in the lumen of the thick ascending portion of the loop of Henle, resulting in a decrease in reabsorption of sodium and chloride. This results in an increase in the rate of delivery of tubular fluid.

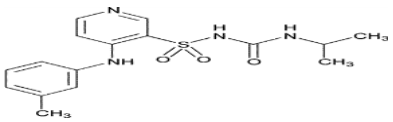

Figure 1: Structure of Torsemide.

Eplerenone[1-3,18] is designated Chemically as methyl (1'R,2R,2'S,9'R,10'R,11'S,15'S,17'R)- 2',15'-dimethyl-5,5'-dioxo-18'-oxaspiro[oxolane-2,14' pentacyclo[8.8.0.0^{1,17}.0^{2,7}.0^{11,15}]octadecan]- 6'-ene-9'-carboxylate is a compound of class Steroid Lactones, and use to treat Edema associated with CHF. It acts by Eplerenone binds to the mineralocorticoid receptor and thereby blocks the binding of aldosterone. Aldosterone synthesis, which occurs primarily in the adrenal gland, is modulated by multiple factors, including angiotensin II and non-RAAS mediators such as adrenocorticotropic hormone and potassium. Aldosterone binds to mineralocorticoid receptors in both epithelial and nonepithelial tissues and increases blood pressure through induction of sodium reabsorption and possibly other mechanisms. Various analytical methods have been reported for the estimation of Eplerenone as alone. They include stability indicating RP-HPLC[19-21], UV method[22-24], HP-TLC[25-26]. Eplerenone is Slightly Soluble in water, Sparingly Soluble in Methanol. Eplereonone is official in IP 2014[27] and analysed by Liquid chromatography.

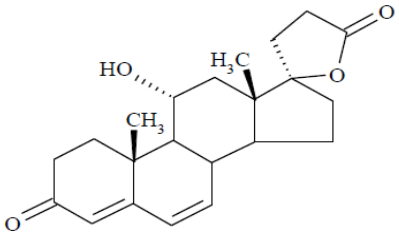

Figure 2: Structure of Eplerenone

MATERIALS AND METHODS

Instruments

UV double beam spectrophotometer of Simadzu-1800 with spectral band width of 1nm and wavelength accuracy of ±0.3 nm was used for analytical work along with matched quartz cell of length 1cm. The analysis was carried by using UV Solutions 2.42 software. All the weighing was carried out on the Reptech electronic weighing balance, Sonication of samples was carried out by Metrex sonicator.

Materials and reagents

Torsemide was obtained as gift sample from Sun Pharmaceuticals. Eplerenone was supplied as gift sample by Lupin Laboratories. The analytical grade methanol was purchased from Rankem Pvt. Ltd. (India). Tablets (Planep-T) were purchased from local pharmacy. The distilled water was used for analytical work and rinsing of clean glass wares.

Preparation of stock solution and selection of wavelength for analysis

Standard stock solutions of Torsemide and Eplerenone were prepared separately by adding 100 mg of drug to methanol taken in 100ml volumetric flasks and then sonicated for five minutes and the volume was made up with methanol. The resulting solutions contain 1mg/ml of the drug. The stock solutions of TOR and EPL were further diluted with methanol to obtain the concentration of 4 µg/ml and 10 µg/ml respectively. The resulting solutions were then scanned in UV spectrophotometer from 400 to 200nm. From the resulting spectra λmax for TOR and EPL were calculated separately (Fig. 3, 4). The overlay spectra of TOR and EPL were also recorded. From the overlay spectra iso- absorptive point of TOR and EPL was calculated (Fig. 5).

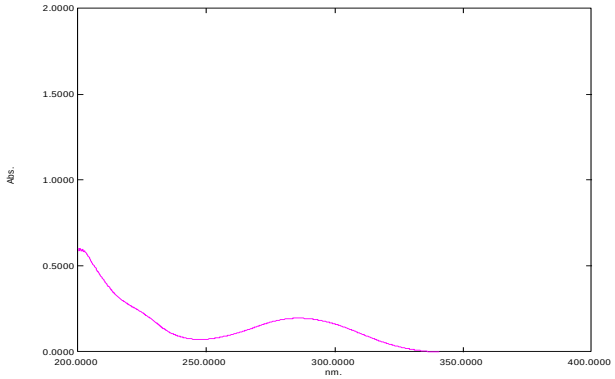

Figure 3: UV spectra of Torsemide.

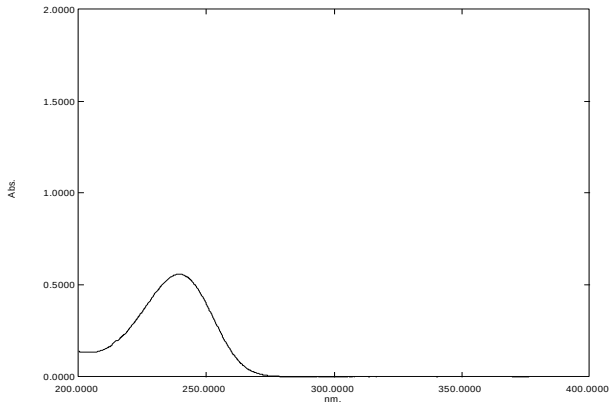

Figure 4: UV spectra of Eplerenone

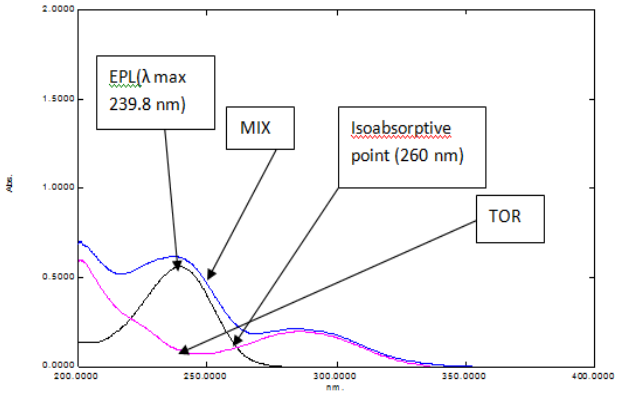

Figure 5: Overlay spectra of Torsemide and Eplerenone

Method: Absorbance Ratio Method (Q-Analysis Method)[28]

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length e.g. two dilutions of the same substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in mixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the λmax of one of the components (λ2) and the other being a wavelength of equal absorptivity of the two components (λ1), i.e., an iso- absorptive point. A series of standard solutions of TOR and EPL in the concentration range of 4-20 µg/ml and 5-25 µg/ml respectively were prepared in methanol and the absorbance of these solutions were measured at 260 nm (iso- absorptive point) and 293.80 nm (λmax of EPL) (Fig. 5). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

The concentration of two drugs in mixture was calculated by using the following equations:

$Cx = (Qm - Qy / Qx - Qy) \times (A1 / ax1)$

$Cy = (Qm - Qx / Qy - Qx) \times A1 / ay1)$

Where,

ax1 = A (1%, 1cm) of CIL at 239.80 nm

ay1 = A (1%, 1cm) of METO at 239.80 nm

ax2 = A (1%, 1cm) of CIL at 260 nm

ay2 = A (1%, 1cm) of METO at 260 nm

A1 and A2 are the absorbances of mixture at 239.80 nm and 260 nm. Cx and Cy are the concentrations of TOR and EPL.

Qm= A2 / A1, Qx = ax2 / ax1 and Qy= ay2 /ay1

Assay of Tablets by Method

Tablet Planep-T contains 10mg of Torsemide and 25mg of Eplerenone equivalent to Metoprolol tartarate. It is manufactured by Lupin Ltd. Twenty tablets were weighed and triturated in a mortar pestle and the tablet powder equivalent to 100 mg of TOR and 250 mg of EPL was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 0.4 ml of this solution was diluted to 10 ml with methanol and 1.0 ml of this solution was further diluted to 10 ml with methanol. Absorbance of the resulting solution was measured at 239.80 nm and 260 nm against methanol. The concentration of TOR and EPL can be obtained by using following equations,

$Cx = (Qm - Qy / Qx - Qy) \times (A1 / ax1)$

$Cy = (Qm - Qx / Qy - Qx) \times A1 / ay1)$

METHOD VALIDATION[29]

The UV spectrophotometric method was validated as per ICH guidelines for method validation. The performance parameters like linearity, precision and accuracy were evaluated.

Linearity

Linearity was studied by diluting standard stock solution of TOR 4-20 µg/ml and EPL 5-25 µg/ml concentrations (n=3). Calibration curves with concentration verses absorbance were plotted at their respective wavelengths and the obtained data was subjected to regression analysis using the least square method. The standard curves for TOR and EPL are shown in Fig. 6,7,8 and 9 and data is presented in Table 1.

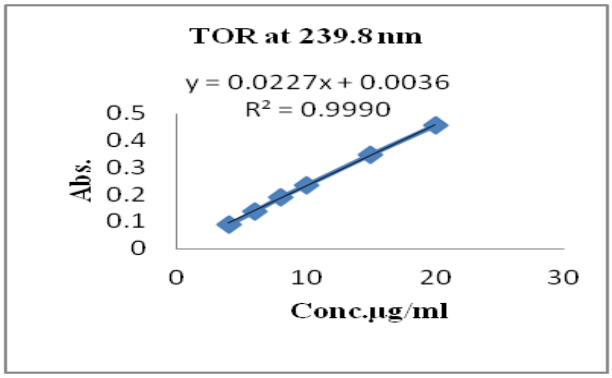

Figure 6: Calibration curve for TOR at 239.80 nm in methanol.

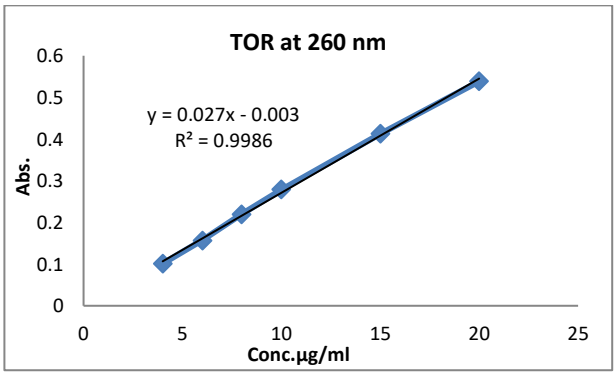

Figure 7: Calibration curve for TOR at 260 nm in methanol.

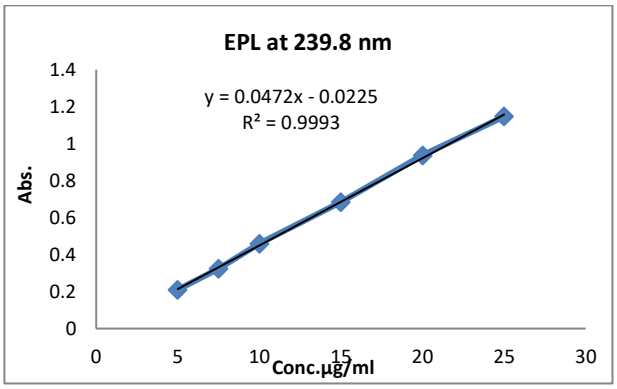

Figure 8: Calibration curve for EPL at 239.860 nm in methanol

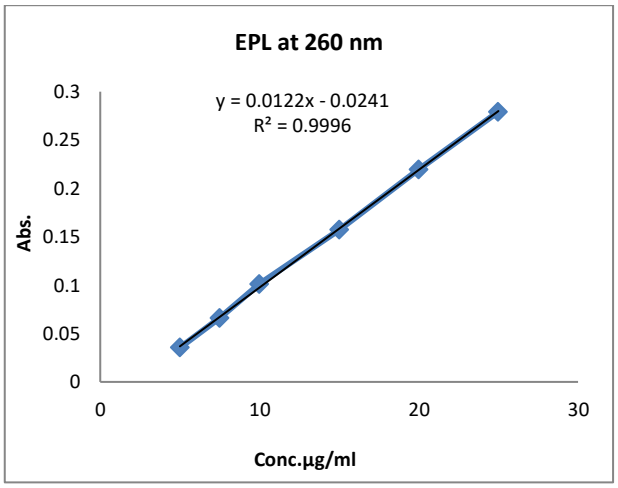

Figure 9: Calibration curve for EPL at 260 nm in methanol

Precision

Repeatability: 0.8 ml of working standard solution of TOR (100µg/ml) was transferred to 10 ml volumetric flask. 2 ml of working standard solution of EPL (100 µg/ml) was transferred to another 10 ml volumetric flask. The volume was adjusted up to mark with methanol in both the flask to get 8 µg/ml solution of TOR and 20µg/ml solution of EPL. The absorbances of solutions were measured spectrophotometry six times and % RSD was calculated. The data is represented in Table 2.

Intermediate precision

Intermediate precision is studied in terms of intraday and inter-day precision. Three concentrations of TOR and EPL were selected in a mixture and analyzed by method (n=3). For intraday, the analysis was carried out at different intervals on the same day and for inter day, the analysis was carried on different days. The results for intraday and inter-day studies respectively are given in Table 3.

Accuracy

To check the accuracy of the developed methods and to study interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug was added to tablet samples at three different concentrations level (80,100 and 120%). At each level, samples were prepared in triplicate and the mean percentage recoveries and % RSD value were calculated. Table 4. Shows the result for accuracy of the method.

Table 1: Regression analysis of calibration curves and summary of validation parameters

Sr. No.	Parameter	TOR		EPL	
		239.80 nm	260 nm	239.80 nm	260 nm
1	Linearity range (µg/ml)	4 -20	4 -20	5 – 25	5 – 25
2	Slope	0.0227	0.0274	0.0472	0.0122
3	Intercept	0.0036	0.003	0.0225	0.00241
4	Limit of Detection (µg/ml)	0.1642	0.0634	0.0982	0.1075
5	Limit of Quatificatiion (µg/ml)	0.4978	0.1921	0.2976	0.3258

Table 2: Repeatability data of TOR and EPL

Drugs	%R.S.D.	
TOR	239.80 nm	260 nm
	0.3296	0.3132
EPL	0.6622	0.3547

Table 3: Precision data

Drug	Intraday Precision(%RSD)		Interday Precision (%RSD)	
	239.80 nm	260 nm	239.80 nm	260 nm
TOR	0.4637-0.6044	0.5782-0.7496	0.5594-0.6592	0.6421-0.8193
EPL	0.3851-0.5831	0.3975-0.4389	0.4255-0.6448	0.5079-0.9205

Table 4: Recovery study data for TOR and EPL (n=3)

Drug	Pre-analyzed conc. (µg/ml)	Drug added (µg/ml)	239.80 nm		260 nm	
			Conc. recovered	% Recovery	Conc. recovered	% Recovery
TOR	4	0	3.97	99.41	4.01	100.25
		3.2	3.16	99.44	3.19	99.90
		4	4.01	100.16	3.95	99.37
		4.8	4.79	99.89	4.84	100.53
EPL	10	0	9.9	99.06	10.01	100.10
		8	7.96	99.80	7.98	99.93
		10	9.97	99.85	9.96	99.82
		12	12.03	100.14	11.98	99.91

Table 5: Analysis of marketed formulation (n=3)

Tablet	Actual concentration mg/tablet		Amount obtained mg/tablet		% Conc. Of Label claim	
Planep-T	TOR	EPL	TOR	EPL	% TOR ± S.D. (n=3)	% EPL ± S.D. (n=3)
	10	25	9.94	24.89	99.42± 0.07328	99.56 ± 0.12774

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient, precise and accurate way for simultaneous analysis of Torsemide and Eplerenone in its bulk and pharmaceutical dosage form. Absorbance maxima of EPL at 239.80 nm and isoabsorptive point 260 nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 4-20µg/ml for TOR and 5-25µg/ml for EPL with respective correlation coefficients of 0.9990 and 0.9993 respectively. The % RSD for repeatability (n=6), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery for TOR and EPL were found within the range of 99% and 101%. Values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of this method. The assay for TOR and EPL was found to be 99.42 ± 0.07328 and 99.56 ± 0.12774 respectively. The % RSD value for both TOR and EPL were found to be less than2%. In this study simultaneous estimation of Torsemide and Eplerenone were carried out by absorbance ratio method satisfactorily.

CONCLUSION

Based on the results obtained, it is found that the developed UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, sensitive and economical. These can become effective analytical tool for routine quality control of Cilnidine and Metoprolol succinate bulk drug combination and its combined pharmaceutical dosage form without any prior separation of components.

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