

Simultaneous Spectrophotometric Estimation of Torsemide and Spironolactone in Tablet Dosage Form

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Abstract: Three simple, accurate, and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Torsemide (TOR) and spironolactone (SPI) in combined tablet dosage form. The methods employed were absorbance ratio, I; first order derivative spectroscopy method, II; and area under curve (AUC) method, III. Torsemide showed absorbance maxima at 288 nm and spironolactone showed at 238 nm in methanol as solvent. Beer's law was obeyed in concentration range of 0-25 mcg ml⁻¹ for both drugs for all proposed three methods. The first developed method makes use absorbance ratio method using 255 nm as isobestic point. The second method is based on first order derivative spectroscopy to overcome spectral interference from other drug, wavelengths 315 nm and 225 nm were selected for the determination of the TOR and SPI respectively. Third method is area under curve method, the sampling wavelengths range selected are 294-290 nm and 240-236 nm with linearity for TOR and SPI respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines.

Key words: Torsemide (TOR) and Spironolactone (SPI), Absorbance ratio method, first order derivative spectroscopy method, Area under curve method (AUC).

INTRODUCTION:

Torsemide (TOR) is sulfonylurea derivative and chemically known as 3-[4-[(3-methylphenyl) amino] pyridin-3-yl] sulfonyl-1-propan-2-ylurea. It acts as diuretic. Spironolactone (SPI) is steroidal derivative and chemically known as 7 -Acetylthio-3-oxo-17 -pregn-4-ene-21,17-carbolactone. It acts as potassium-sparing diuretics. Literature survey revealed that Spectrophotometric and HPLC methods^[1-10] are available for estimation of TOR and SPI individually and in combination with other diuretics in different formulation. The combination of the both drugs is not official in any pharmacopoeia; hence, no official method is reported for simultaneous estimation of TOR and SPI in formulations. Aim of present work was to develop simple, economical, rapid, accurate, and precise spectrophotometric methods for determination of these drugs in fixed dose combination. The proposed methods were optimized and validated as per the International Conference on Harmonization (ICH) guidelines^[11].

MATERIALS AND METHODS:

INSTRUMENTATION

UV/Visible double beam spectrophotometer (Shimadzu-1601) was employed with fixed slit width 2 nm using a pair of 1 cm matched quartz cells with automatic wavelength correction and wavelength repeatability for all measurement. All weighing were performed on an electronic single pan balance (balance Shimadzu Uni Bioc) calibrated ASGI glassware was used in the study.

REAGENTS AND CHEMICALS

Analytical pure standard sample of TOR and SPI were supplied as gift sample by Lupin lab, Jammu and used without further purification. The pharmaceutical dosage form used in study was TORLACTONE tablet (label claim: TOR- 5 mg, SPI- 25 mg) manufactured by Sun pharmaceuticals ltd, India.

PREPARATION OF STANDARD STOCK SOLUTION

Standard stock solution (100 mcg ml⁻¹) of TOR and SPI were prepared by dissolving 10 mg of drug each in 100

ml methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with solvent.

METHODS:

METHOD I: ABSORBANCE RATIO METHOD

Absorbance ratio method^[12] of analysis was based on the absorbance's at two selected wavelengths, one of which is an isobestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig.1) 255 nm (Isobestic point) and 238 nm (of SPI) were selected for the formation of Q absorbance equation (Eqn. 1 and 2). The absorbance at 255 nm and 238 nm for TOR and SPI were measured. The absorptivity values of each drug at both wavelengths were determined which was the mean of six independent values. The absorbance and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

Equation 1.....

$$C_X = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{a_{x1}}$$

And

Equation 2.....

$$C_Y = \frac{Q_M - Q_Y}{Q_Y - Q_X} \times \frac{A_1}{A_{y1}}$$

Q_M , Q_X , and Q_Y were obtained as below:

$$Q_M = A_2/A_1, Q_X = ax_2/ax_1, Q_Y = ay_2/ay_1$$

Where; A_1 and A_2 were the absorbance of the sample at 255 nm and 238 nm respectively, ax_1 and ax_2 were the absorptivity of TOR at 255 nm and 238 nm and ay_1 and ay_2 were the absorptivity of SPI at 238 nm and 255 nm. Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components. Results of which are reported in Table.1.

METHOD II: FIRST ORDER DERIVATIVE SPECTROSCOPY METHOD

In this method^[13] the standard stock solution of TOR and SPI were scanned from 200 nm to 400 nm. The spectra obtained were derivatized in first order and then overlain spectra recorded (Fig.2). From the entire derivative spectra obtained, the wave lengths were selected in a manner such that TOR had zero crossing point at 225 nm and SPI showed a measurable $dA/d\lambda$ where as the zero crossing point of SPI at 315 nm, TOR showed appreciable $dA/d\lambda$. Hence wavelengths 315 nm and 225 nm were selected as analytical wavelength for determination of TOR & SPI respectively. The mixed standards were scanned in the spectrum mode, derivatized in first order with derivative interval of 5 nm and absorbance were measured at the selected wavelengths. Calibration curve for TOR & SPI were plotted as dA/d verses concentration. By extrapolating the value of absorbance, the conc. of corresponding drugs in the sample was determined. Results of analysis of mixed standard are reported in Table.1.

Table.1: Results of analysis of mixed standards for method I, method II, and method III

Sr. No.	Conc. Present (mcg ml ⁻¹)		(% Conc. found)					
			Method I		Method II		Method III	
	TOR	SPI	TOR	SPI	TOR	SPI	TOR	SPI
01	5	25	102.0	98.8	98.0	101.6	98.0	99.2
02	10	20	99.0	99.0	102.0	100.5	102.0	99.5
03	15	15	100.66	98.66	100.6	98.6	100.6	98.6
04	20	10	99.5	99.0	99.5	100.0	99.5	99.0
05	25	5	99.2	98.0	99.2	100.0	98.8	100.0

METHOD III: AREA UNDER CURVE METHOD (AUC)

Area under curve method ^[14], 294-290 nm and 240-236 nm were selected as the two sampling wavelength intervals for TOR and SPI respectively. Fig.1 represents the overlain spectra of TOR and SPI with AUC ranges. TOR and SPI exhibited linearity in the concentration range of 0-25 mcg ml⁻¹ at their respective selected wavelength intervals. Coefficients of correlation were found to be 0.9951 and 0.9999 for TOR and SPI respectively. For the simultaneous estimation, mixed standards containing TOR and SPI in the 1:5 were prepared by appropriate dilution of the standard stock solutions. The AUC of mixed standard solutions were measured at selected wavelength intervals. Results of analysis of mixed standards are reported in Table.1. A set of two simultaneous equations were established using the mean absorptivity coefficients of TOR and SPI at the selected wavelength intervals.

$$A_1 = 0.1612 C_{\text{TOR}} + 0.0016 C_{\text{SPI}} \dots$$

.....eqn.3 at 294(λ₁)-290 nm (λ₂)

$$A_2 = 0.0906 C_{\text{TOR}} + 0.2172 C_{\text{SPI}} \dots$$

.....eqn.4 at 240(λ₃)-236 nm (λ₄)

Where; 0.1612 and 0.0906 are mean absorptivity value of TOR at 294(λ₁)-290 nm (λ₂) and 240(λ₃)-236 nm (λ₄) respectively.

0.0016 And 0.2172 are mean absorptivity value of SPI at 294(λ₁)-290 nm (λ₂) and 240(λ₃)-236 nm (λ₄) respectively.

A₁ and A₂ are the AUC of mixed standard of sample

solution at 294(λ₁)-290 nm (λ₂) and 240(λ₃)-236 nm (λ₄) respectively.

C_{TOR} and C_{SPI} are concentration on gl⁻¹.

The concentration of TOR and SPI in mixed standard and tablet formulation can be obtained by solving equation.3 and equation.4.

ASSAY OF TABLET FORMULATION

Twenty tablets were accurately weighed and a quantity of tablet powder equivalent to 5 mg of TOR and 25 mg of SPI was weighed and dissolved in 100 ml methanol with the aid of ultrasonication for 15 min. The solution was then filtered through whatmann filter paper no.41 and diluted further to obtain final concentration of 5 mcg ml⁻¹ of TOR and 25 mcg ml⁻¹ of SPI. The sample solutions were analyzed as per the procedure for mixed standards. The concentration of each drug in sample solutions were calculated using eqn.1 and eqn.2 for the absorbance ratio method, eqn.3 and eqn.4 for the area under curve method and using calibration curve for the first order derivative spectroscopy method. Analysis was repeated six times to study the precision of the methods. The results of the analysis and statistically validation data of the tablet formulation are given in Table.2.

RECOVERY STUDIES

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80%, 100%, and 120%) within range of linearity for both the drugs.

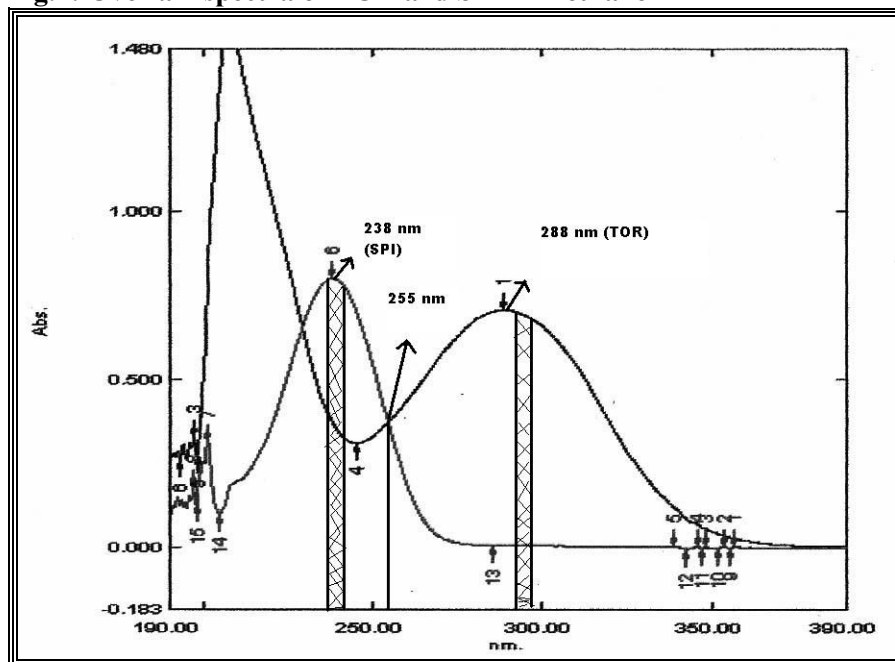
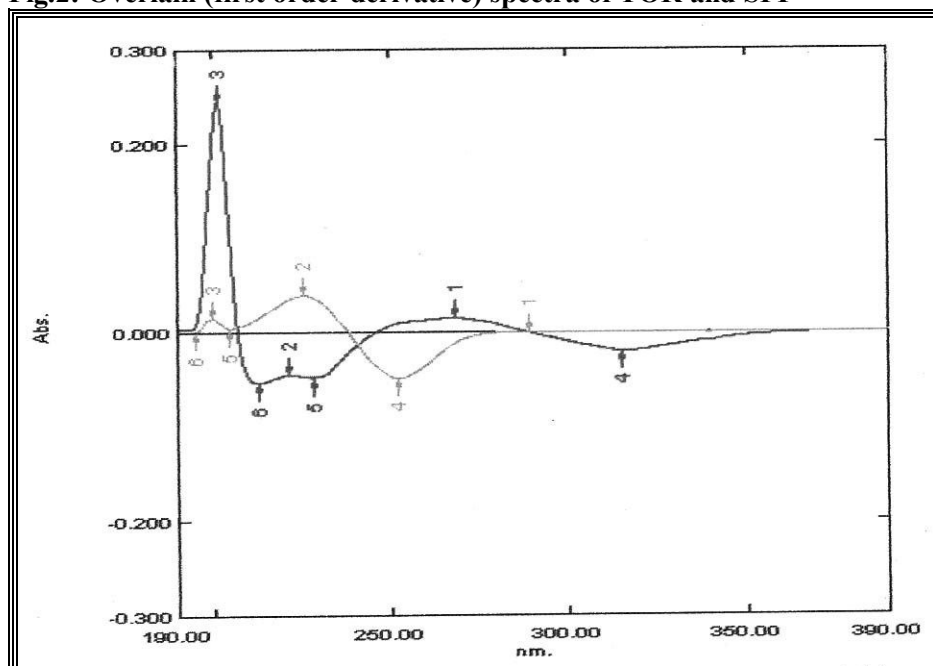
Table.2: Results of analysis of tablet formulation

Methods	Label claim (mg/tab)	% Label claim estimated* (Mean± SD)	% RSD
Absorbance ratio method	TOR- 5	99.200±1.789	1.80
	SPI - 25	100.0± 0.632	0.63
First order derivative method	TOR- 5	99.600± 1.673	1.68
	SPI - 25	99.800± 1.510	1.51
Area under curve method (AUC)	TOR- 5	99.200± 1.789	1.80
	SPI - 25	100.08± 1.25	1.24

*Mean of six determinations, RSD is relative standard deviation

Table.3: Results of recovery studies of TOR and SPI

Drug	% Recovery* (Mean ± SD)		
	Method I	Method II	Method III
TOR	99.77 ± 1.113	100.60 ± 0.667	99.25 ± 1.406
SPI	100.0 ± 1.219	100.11 ± 0.538	100.14 ± 1.233

Fig.1: Overlain spectra of TOR and SPI in methanol**Fig.2: Overlain (first order derivative) spectra of TOR and SPI****RESULT AND DISCUSSION:**

Under experimental condition described, calibration curve, assay of tablets, precision and recovery studies were performed. The drugs obey Beer's law in the concentration range of 0-25 mcg ml⁻¹ for both the drugs for all the three methods with good correlation coefficient > 0.998. The results of analysis of tablet formulation are presented in Table.2. Results of recovery studies are shown in Table.3. The accuracy is reproducibility is evident from the data as results are close to 100 % and low standard deviation. All three

developed methods are simple, economical, rapid, precise, and accurate. Hence these can be used for routine analysis of TOR and SPI in tablet formulation.

CONCLUSION:

The validated spectrophotometric methods employed here proved to be simple, economical, rapid, precise, and accurate. Thus these can be used for routine simultaneous estimation of TOR and SPI in tablet dosage form.

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