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Simultaneous Estimation of Torsemide and Spironolactone in Combined Dosage Form Using Reverse Phase Liquid Chromatography

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ABSTRACT

Simple, accurate, precise, and sensitive reversed-phase high-performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Spironolactone (SPR) and Torsemide (TOR) in combined tablet dosage form has been developed and validated. Beer's law was obeyed in the concentration range of 5-25 µg/mL for both drugs in methanol. The RP-HPLC method uses a Shimadzu LC 10 AT_{VP} system with a Luna C_{18} column and methanol: acetonitrile: phosphate buffer, pH 3.5(60:20:20 %v/v) as the mobile phase. The detection was carried out using a diode array detector set at 238 nm. The recoveries were found to be in the range of 99.64 ± 0.04 to 100.75 ± 0.15 and 99.56 ± 0.35 to 100.33 ± 0.56 for TOR and SPR, respectively. Developed method was found to be simple, precise, sensitive and may be used for routine analysis of TOR and SPR in a pharmaceutical formulation. Results of analysis were validated statistically per ICH guidelines.

Keywords: absorptivity, robustness, simultaneous equation, validation
Abbreviations: RP-HPLC, reverse phase high performance liquid chromatography; **SPR**, Spironolactone; **TOR**, Torsemide

INTRODUCTION

Torsemide (TOR) is sulfonylurea derivative and chemically known as 3-[4-[(3methylphenyl) amino] pyridin-3-yl] sulfonyl-l-propan-2-ylurea. It acts as diuretic. Spironolactone (SPI) is steroidal derivative and chemically known as 7a-Acetylthio-3oxo-l7a-pregn-4-ene-21, 17-carbolactone (**Fig. 1**). It acts as potassium-sparing diuretics (Jankowski *et al.* 1996). Literature survey revealed that spectrophotometric and HPLC methods are available for estimation of TOR and SPI individually and in combination with other diuretics in different formulation (Begona *et al.* 1996; Gupta and Ghanekar 2006; Sandall *et al.* 2006). The combination of the both drugs is not official in any pharmacopoeia, and therefore an attempt was made to develop an RP-HPLC method for the estimation of TOR and SPI in combined tablet dosage form. This paper describes simple, accurate, precise, and sensitive reversed-phase (RP)-HPLC methods for simultaneous determination of TOR and SPR in a combined tablet dosage form (Parimoo *et al.* 1995; Luis *et al.* 1999). The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines (Anonymous 1996; Dubey *et al.* 2011; Mandhanya *et al.* 2011).

MATERIALS AND METHODS

Drugs and chemicals

Acetonitrile, methanol and THF (Tetra Hydro Furon) were purchased from Merck (Mumbai, India). All other reagents used were of HPLC grade. Standard bulk drug samples of TOR (99.81% pure) and SPR (99.76% pure) were provided by Lupin Labs Ltd., India as gratis samples. Torlactone tablet (Sun Pharmaceuticals Ltd., India) containing TOR (5 mg) and SPI (25 mg) of three different batches were purchased from a local pharmacy.

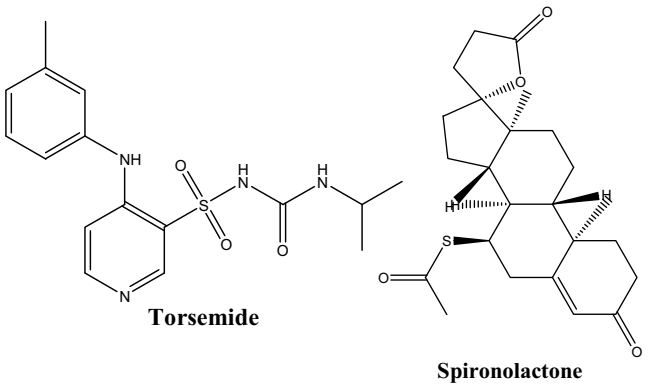

Fig. 1 Chemical structure of TOR and SPR.

Instruments

An HPLC system consisting of LC 10 AT $_{VP}$ pump equipped with diode array detector (Shimadzu, Japan) and Luna C_{18} (4.6 mm id) column and Class-M10A software was used. A Rheodyne (Rohnert Park, CA) injector with 20 µL loop was used for injecting the sample.

In the RP-HPLC method, separation and analysis of TOR and SPR were carried out on a Luna C_{18} column (4.6 mm i.d.) with the photo diode array detector set at 238 nm. Mobile phase consisting of Methanol: acetonitrile: phosphate buffer with pH 3.5 ± 0.1 (60: 20: 20, v/v filtered through a 0.2 µm membrane filter, degassed and sonicated) was used with a flow rate of 1 mL/min.

1. Standard stock solutions

Standard stock solutions containing 100 µg/mL TOR or 100 µg/mL SPR were prepared by dissolving the pure drugs separately in the mobile phase.

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2. Preparation of the calibration curves

Aliquots sufficient to produce 5, 10, 15, 20, and 25 µg/mL were transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with the mobile phase for TOR and SPR. Each solution was injected, and chromatogram was recorded. The peak area of TOR and SPR were noted, and respective calibration curves were plotted as peak area against concentration of each drug.

3. Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 5 mg TOR and 25 mg SPR weighed and transferred to a 50-mL volumetric flask containing about 70 mL mobile phase, ultrasonicated for 10 min, and the volume was made up to the mark with the mobile phase. The solution was filtered through Whatman (Florham Park, NJ) No. 41 paper, 0.2 mL filtrate was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. The tablet sample solution was injected, the chromatogram was obtained and the peak areas were recorded. A representative chromatogram is given in **Fig. 3**. Form the peak area the both the drugs concentration of each drug/tablet was estimated from the respective calibration curves.

4. Robustness studies

The influence of small, deliberate variations of the analytical parameters on the retention time of the drugs was examined. The following factors were selected for change: the wavelength at which the drugs were recorded (238 ± 1 nm) and the flow rate of the mobile phase (1.0 ± 0.02 mL/min). One factor at the time was changed to estimate the effect (Croo *et al*. 1985). The solutions containing 20 µg/mL TOR and 20 µg/mL SPR were applied onto the column. Six replicate analyses (n = 6) were conducted at 3 levels of the factor (–, 0, +).

5. Recovery studies

To study the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100, and 120%.

6. Precision

Precision of the method was checked by 3 replicate readings at 3 concentration levels of within range expressed as RSD values.

Statistical analysis

Means, standard deviation (SD), Relative standard deviation (RSD), and linear regression analyses were calculated using Microsoft Excel 2003.

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for TOR and SPR. Different mobile phases containing methanol, acetonitrile, water, tetrahydrofuron and buffers with different pH were examined, and the mobile phase Methanol: acetonitrile: phosphate buffer with pH 3.5 ± 0.1 (60: 20: 20, v/v) was selected as optimal for obtaining well-defined and resolved peaks. The optimum wavelength for detection and quantitation was 238 nm, at which the best detector response for both the substances was obtained. Mean retention times for TOR and SPR were found to be 3.254 and 4.577min, respectively (**Fig. 2**). Straight line calibration curves were obtained for TOR and SPR in the spectrophotometric and RP-HPLC methods. **Table 1** summarizes the Beer's law limit, linear regression equation, correlation coefficient, standard deviations (SD), and limit of detection (LOD) and limit of quantitation (LOQ) values for both methods. System suitability parameters for the RP-HPLC method are listed in **Table 2**.

Table 1 Regression analysis of calibration curve.

Parameters	RP-HPLC Method	
	TOR	SPI
Linearity range µg/mL[a]	5-45	5-25
Correlation coefficient	0.9971	0.9988
Linear regression equation [b]		
Intercept	201.36	417.82
Slope	22859	57741
SD [c]	15.42	17.56
Detection limit, µg/mL	0.01	0.004
Quantitation limit, µg/mL	0.03	0.010

[a] Detection wavelength at 238 nm for HPLC method.
[b] y = mx + c, where y is the absorbance and x is the concentration (µg/mL).
[c] SD = standard deviation of the slope.

Table 2 System suitability parameters for RP-HPLC method.

Parameters	TOR	SPI
Calibration curve range, µg/mL	5-25	5-25
Retention time	3.245	4.577
Theoretical plate number	1905	2372
HETP [a]	0.0120	0.0072
Tailing factor	1.36	1.30
Capacity factor (k')	1.70	1.20
Resolution	1.25	1.23

[a] HETP = Height equivalent to theoretical plate, cm

Table 3 Results of analysis of commercial formulation.

RP-HPLC	Label claim, mg/tablet		% claim, estimated [a]	
	TOR	SPI	TOR	SPI
	5	25	99.87 ± 0.32	98.23 ± 1.12

[a] Mean ± Relative standard deviation, n = 6.

Table 4 Recovery studies of Torsemide and Spironolactone

Drug	Concentration taken, µg/ml for methods	Concentration added, µg/ml for methods	% Recovery[a]
TOR	5	4	99.64 ± 0.04
	5	4.92	100.14 ± 0.29
	5	5.97	100.75 ± 0.15
SPI	25	19.93	99.62 ± 0.19
	25	24.96	100.33 ± 0.56
	25	29.96	99.56 ± 0.35

[a] mean ± relative standard deviation (n = 3).

Robustness studies of the HPLC method, carried out after deliberate alterations of the analytical wavelength and flow rate of mobile phase, showed that small changes of these operational parameters did not lead to changes of retention times for the peaks of interest. The effect of a single factor at two levels indicated that the selected factors remained unaffected by small variations of these parameters. Therefore, this method is suitable for use in routine analysis (**Table 3**).

System suitability studies were conducted indicating well resolved peaks at retention time of 3.2 and 4.5 min for TOR and SPI, respectively (**Table 2**). The proposed methods were also evaluated in the assay of commercially available tablets containing TOR and SPR (**Fig. 3**). Six replicate determinations were performed on the accurately weighed amounts of tablets. Tablets were found to contain 99.87 ± 0.32% and 98.23 ± 1.12% (mean ± RSD, n = 6) for TOR and SPI respectively (**Table 3**). The recoveries were found to be in the ranges of 99.64 ± 0.04% to 100.75 ± 0.15% and 99.56 ± 0.35% to 100.33 ± 0.56% for TOR and SPI, respectively (**Table 4**).

The objective of the method development was to resolve chromatographic peaks for active drug ingredients (TOR and SPI) with less asymmetric factor. Various mixtures containing aqueous buffer-methanol, acetonitrile were tried as mobile phases.

The mobile phase consisting of Methanol: acetonitrile: phosphate buffer with pH 3.5 ± 0.1 (60: 20: 20, v/v) was selected which gave sharp, well resolved peaks for TOR

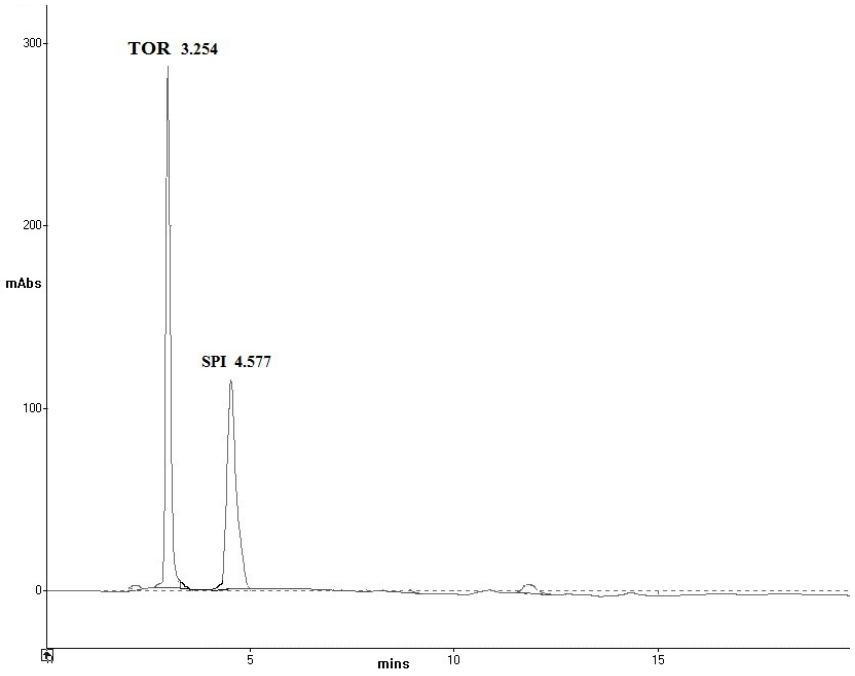

Fig. 2 Chromatogram of standard TOR and SPR solution.

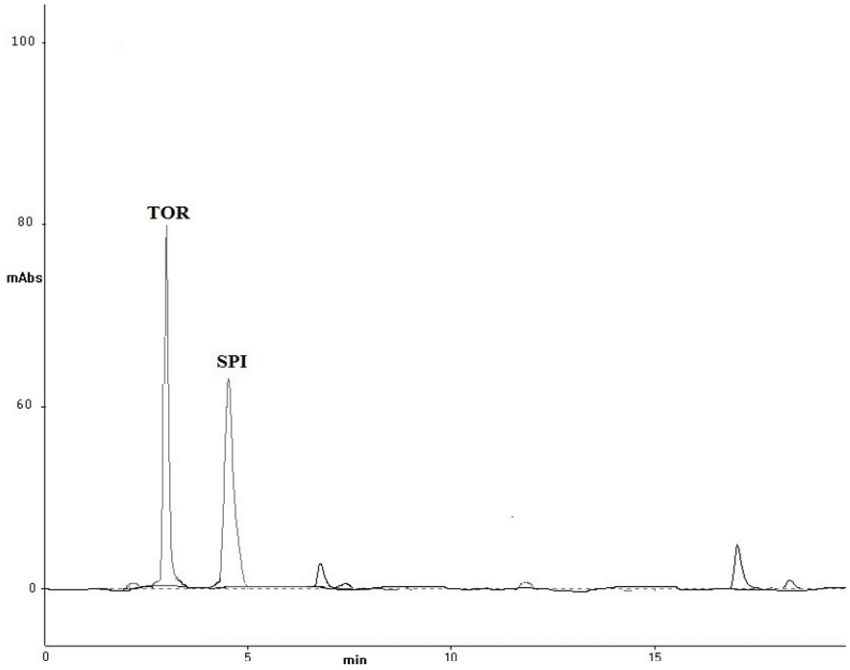

Fig. 3 Chromatogram of TOR and SPR in tablet dosage form.

and SPI. The flow rate was maintained at 1.0 ml/ min. The retention times for TOR and SPI were 3.2 and 4.5 min respectively with the resolution of 4.8.

CONCLUSIONS

The proposed RP-HPLC method was found to be simple, fast, accurate, precise, and sensitive. Thus, it may be used for routine analysis of TOR and SPR in combined tablet dosage form.

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