Isocratic reverse phase HPLC estimation method of Torsemide and Spironolactone in pharmaceutical combined dosage form

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Validated high performance liquid chromatographic (HPLC) method for estimation of Torsemide (TOR) and Spironolactone (SPI) in tablet dosage form. Isocratic RP-HPLC separation was achieved on Licrosphere C_{18} column (250 x 4.6mm) using Methanol: Acetonitrile: Phosphate buffer, pH 6.5 (60:20:20 v/v) at flow rate of 1.5 ml/min at 30 ^{o}C temperature. Quantitation was achieved by UV detection at 252 nm over the conc. range 0-25 μg/ml for both the drugs with mean recoveries of 100.01% $\pm$ 0.12 and 100.64% $\pm$ 0.20 for TOR and SPI respectively. This method is simple, precise and sensitive and applicable for the simultaneous estimation of TOR and SPI in tablet pharmaceutical combined dosage form.

1. 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 7α-Acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone .It acts as potassium-spiring 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. Because of the absence of an official pharmacopoeial method for the simultaneous estimation of TOR and SPI in tablet dosage form, efforts were made to develop an analytical method for the estimation of TOR and SPI in tablet dosage form using Isocratic-Reverse Phase HPLC method.

2. Experimental

2.1 Apparatus

The HPLC method was performed on a Shimadzu HPLC system equipped with LC-10 TOR and SPI pump UV detector, and Rheodyne injector system fitted with 20μl loop. The HPLC analysis was performed on reversed phase high-performance liquid chromatographic system with isocratic elution mode using a mobile phase of water: Acetonitrile: buffer, pH 6.5 (65:35 v/v) at flow rate of 1.5 ml/min at 30 ^{o}C temperature.

2.2 Reagent and material

TOR and SPI pure powder were procured as gifts sample from Lupin Labs, Bhopal. Torlactone tablets (Sun Pharmaceuticals Ltd) were procured from local market. Label claim of Torlactone tablet for TOR and SPI were 5 mg and 25 mg respectively. Methanol HPLC grade, Acetonitrile HPLC grade were purchased from E.Merck (Mumbai, India), Potassium Dihydrogen Phosphate and o-phosphoric acid were purchased from SD fine chemical Ltd (Ahmedabad, India) and were of analytical grade Water of HPLC grade was used.

2.3 Chromatographic condition of method

The Licrosphere C_{18} column was used 25°C temperature. The mobile phase considered water: acetonitrile buffer (65:35 v/v) pH adjusted to 6.5 $\pm$ 0.1 with o-phosphoric acid. It was pumped at flow rate of 1.5 ml/min. the mobile phase was passed through nylon 0.45 μm membrane filters and degassed before use. The elution was monitored at 252 nm and the injection volume was 20 μl.

2.4 Preparation of standard stock solution

The equivalent of 10 mg each of TOR and SPI were accurately weighed in 100 ml volumetric flasks separately

and dissolve in 25 ml of methanol. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 μg/ml of TOR and SPI.

2.5 Preparation of sample solution

Ten tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 5 mg of TOR was taken in 25 ml volumetric flask and dissolved in 75 ml of methanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 50ml of volumetric flask through a.whatman no 41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100 ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent.

2.6 Method validation

2.6.1 Calibration graph (linearity)

Calibration graphs were constructed by plotting peak area Vs concentration of TOR and SPI and the regression

equation were calculated. The calibration graphs were plotted over 5 different concentrations in the range of 5-25μg/ml for both drugs. Accurately measured mixed standard solution aliquots of TOR and SPI (0.5, 1.0, 1.5, 2.0, 2.5 ml) were transferred to series of 10 ml volumetric flasks and diluted to mark with methanol. A liquots (20μl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

2.7 Accuracy

The accuracy of the method was established using recovery technique i.e. external standard addition method. The known amount of standard was added at three different levels to preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in Table 2.

Table 2. Recovery studies.

	TOR				SPI		
Label claimed	%Amount added	Found in(μg/ml)	%recovery	Label claimed	%Amount added	Found in(μg/ml)	%recovery
5	80	499	9998	25	80	2505	10010
	100	512	10001		100	2499	9996
	120	504	1000		120	2616	9995

2.7.1 Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) mixed standard solution of TOR and SPI.

2.7.2 Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of TOR and SPI at concentration 5μg/ml and 25μg/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation.

2.7.3 Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD with signal to noise (S/N) ratio of 6:2 and LOQ with (S/N) ratio of 10:1 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines [11] Where σ = the standard deviation (SD) of the response and S = the SD of the y-intercept of the regression line.

2.8 Stability of standard and sample solution

The standard solution of TOR and SPI (100 μg/ml for HPLC method) and sample solution of TOR and SPI (100 μg/ml for HPLC method) were prepared and analyzed after 24 hrs by storing the Solutions at room temperature.

2.9 Analysis of TOR and SPI in tablet dosage form

The response of sample solutions were measured at 252 nm for quantization of TOR and SPI by the method described above. The amount of TOR and SPI present in the sample solution were determined by applying values of peak area to regression equation of the calibration graph.

3. Results and discussion

3.1 Optimization of HPLC method

Optimization of mobile phase was performed based on peak symmetry, peak width, and run time. The mobile phase of water and acetonitrile (65:35 v/v) was found to be satisfactory. The Fig. 1 shows typical chromatograms

obtained from the analysis of a standard and sample solutions of TOR, SPI using the proposed method. The retention time observed (5.12 min) permits a rapid determination of the drug, which is important for routine analysis. System suitability parameters for this method are reported in Table 1.The parameters were with in the acceptance limits. Complete resolution of the peaks with clear baseline separation was obtained (Fig. 1).

Table 1. System suitability test parameter for TOR and SPI.

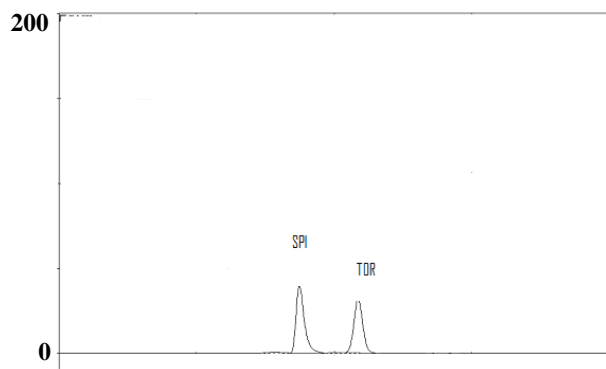| Property (n*=6)	TOR	SPI
Retention time(min)	5321	6321
Tailing factor	265	298
Capacity factor	122	143
Theoretical plates number	6364	8643
Resolution	125	123
Linearity range (mg/ml)	1 to 100	1 to 100
Peak asymmetry	121 – 154	176- 198
Peak Width (min)	007	009
Regression equation	y = 35823 x + 12766	y = 47516 x + 11091

* n = Number of determination

Fig. 1. Isocratic RP-HLPC chromatogram of TOR and SPI with detection at 252 nm.

3.2 Validation of the proposed method

3.2.1 Linearity

Linear correlation was obtained between peak areas and concentration of TOR and SPI in the range of 0 -

25μg/ml for both the drugs, respectively. Data of the regression analysis are summarized in Table 3.

Table 3. Regression analysis of calibration graph for TOR and SPI.

Parameter	TOR	SPI
Concentration range	0-25 μg/ml	0-25 μg/ml
Slope	16831	43209
SD$^\$$ of the slope	1189	2374
Intercept	65921	53891
SD[a] of the intercept	17311	32803
Correlation coefficient	09998	09999

$^\$$ SD = Standard Deviation

3.2.2 Accuracy

The recovery experiments were performed by standard addition method. The recoveries obtained were 100.17 $\pm$ 0.12 % and 105.02 $\pm$ 0.21% for TOR and SPI respectively. (Table 4).

3.2.3 Method precision

The RSD values for TOR and SPI were found to be 0.143 % and 0.165 % respectively (Table 4).

3.2.4 Intermediate precision

The RSD values were found to be < 1%, which indicates that the proposed method is reproducible. (Table 4)

Table 4. Summary of validation parameter.

Parameter	TOR	SPI
LOD[a]	001μg/ml	0009μg/ml
LOQ[b]	0010 μg/ml	0001μg/ml
Accuracy, %	10001% $\pm$ 012	10064% $\pm$ 020
Repeatability(RSD[c], %, n =6)	0149	0198
Precision (RSD, %)		
Intra day(n =3)	0012	0065
Inter day(n = 3)	0082	0099

3.2.5 LOD and LOQ

LOD values for TOR and SPI were found to be 0.05 and 0.010μg/ml respectively. LOQ values for TOR and SPI were found to be 0.01 and 0.001μg/ml respectively. (Table 4)

3.2.6 Assay of the tablet dosage form (TOR 5mg / tablet and SPI 25 mg / tablet)

The proposed validated method was successfully applied to determine TOR and SPI in tablet dosage form. The result obtained for TOR and SPI were comparable with corresponding labeled amounts. (Table 5)

Table 5. Result of assay of tablet formulation.

TOR		SPI	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
	511		250
	503		2509
5	498	25	2515
	508		2503
	50		2495
	497		2597
Mean	4983	Mean	25142
±SD	00432	±SD	0243

4. Conclusions

The proposed method has advantage of simplicity and convenience for the separation and quantitation of TOR and SPI in the combination and cab be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for simultaneous estimation of Torsemide and Spironolactone in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

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