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Conventional and Micellar Liquid Chromatography Method with Validation for *Torsemide and Spironolactone* in Tablet Combined Dosage Form

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Abstract

A new, simple, rapid and specific micellar liquid chromatographic (MLC) method was developed and validated for the determination of method for estimation of Torsemide (TOR) and Spironolactone (SPI) in tablet dosage form. Micellar liquid chromatographic (MLC) was achieved on Licrosphere C_{18} column (250 x 4.6mm) using Tween-20, n-Butanol Phosphate buffer (50:25:25 v/v) adjusted to pH 3.5 $\pm$ 0.01.Quantitation was achieved with UV detection at 261 nm based on peak area 254 nm and at flow rate of 1.5 ml/min at 30 ^{o}C temperature. Validation experiments were performed to demonstrate linear ranges, accuracy, precision, and robustness, limit of detection (LOD) and limit of quantification (LOQ). The method was applied to the determination of these Torsemide and Spironolactone in tablet dosage form in various pharmaceutical formulations.

Keywords: Torsemide, Spironolactone, Micellar liquid chromatography, Tween-20.

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. Micellar liquid chromatography has been reported as a suitable technique for pharmaceuticals and intermediate for drug and cosmetics interest[11]. Micellar solution can replace conventional aqueous organic mobile phase with good results. Micellar liquid chromatography (MLC) is a reversed phase liquid chromatographic (RPLC) mode with mobile phases containing a surfactant (Ionic or Non ionic) above its critical concentration (CMC)[12]. In these conditions the stationary phase is modified with a approximately constant amount of surfactants monomers, and solubilizing capability of mobile phase is altered by the presence of micelles, giving rise to diverse interactions (Hydrophobic, ionic and satiric) with major implications and selectivity.Literature survey revealed that no HPLC method has been reported for the estimation of in combined dosage form.Because of the absence of an official pharmacopoeial method for the Micellar liquid chromatography method of TOR and SPI in tablet dosage form; efforts were made to develop an analytical method for the estimation of ROS and EZE in tablet dosage form using HPLC method. Micellar mobile phases have been used with different bonded stationary phases (mostly C8, C18 and cyanopropyle). The most common surfactant are the anionic sodium dodecyl sulphate (SDS) cationic cetytrimethylammonium bromide (CTAB), and non-ionic Tween-20, several organic solvents have been used as modifiers, short/medium chain alcohols and acetonitrile being the most suitable. The presence of micellar contributes well above their solubility in water. 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 micellar liquid chromatographic method.

Materials and Methods

Experimental

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. Tween-20, n-Butanol and water were obtained from Merck. All reagents were of HPLC grade unless otherwise specified. 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.

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.

Chromatographic condition of method

The Licrosphere C_{18} column was used 25^{o}C temperature. The mobile phase considered 5% n-Butanol in 0.05 molL^{-1} Tween-20 pH adjusted to 3.5 $\pm$ 0.01 with o-phosphoric acid. It was pumped at flow rate of 1ml /min. the mobile phase was passed through nylon 0.45 µm membrane filters and degassed before use. The elution was moni TOR and SPI at 254 nm and the injection volume was 20 µl.

Preparation of standard stock solution

The equivalent of 5 mg and 25 mg each of TOR and SPI were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of n-Butanol. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 50 µg/ml of TOR and SPI. These standard stock solutions were observed to contain 100 µg/ml of TOR and SPI. The two main advantages of micellar procedure are the elimination of organic solvents and simplification of sample preparation step. The point's calibration graphs were constructed covering a concentration range. 0.5 to 10 mg/ml. linear relationship was obtained between the peak area ratio of TOR and SPI in the concentration range 10 ppm to 50 ppm. The correlation coefficient was found 0.9998. According to International Conference on Harmonization (ICH) guidelines the following expression is used to evaluate LOD and LOQ.

Preparation of sample solution

Twenty 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 50 ml of n-Butanol 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.

Method Validation

Calibration graph

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 tween 20 and n-butanol.A liquors (20µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

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.

Method precision (repeatability)

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

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.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD with signal to noise (S/N) ratio of 2:1 and LOQ with (S/N) ratio of 11:6 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines[13]
Where σ = the standard deviation (SD) of the response and S = the SD of the y-intercept of the regression line.

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.

Analysis of TOR and SPI in tablet dosage form
The response of sample solutions were measured at 254 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.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD with signal to noise (S/N) ratio of 2:1 and LOQ with (S/N) ratio of 11:6 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines[11]

$$LOD = 2.11 \times \sigma/S$$
$$LOQ = 4.5 \times \sigma/S$$

Where σ = the standard deviation (SD) of the response and S = the SD of the y-intercept of the regression line.

Analysis of ROS and EZE in tablet dosage form
The response of sample solutions were measured at 254 nm for quantitation of ROS and EZE by the method described above. The amount of ROS and EZE present in the sample solution were determined by applying values of peak area to regression equation of the calibration graph.

Result and Discussion

Optimization of HPLC method
To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation of TOR and SPI with good peak symmetry and steady baseline was obtained with mobile phase Tween-20, n-Butanol Phosphate buffer (50:25:25 v/v) adjusted to pH 3.5 $\pm$ 0.01.Quantitation was achieved with UV detection at 261 nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained (Figure 1). The system suitability test parameters are shown in Table 1.

Validation of the proposed method

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 Table3.

Accuracy
The recovery experiments were performed by standard addition method. The recoveries obtained were 99.99 ± 0.23% and 100.09 ±0.02% for TOR and SPI respectively. (Table 4).

Method precision
The RSD values for TOR and SPI were found to be 0.231 % and 0.178 % respectively (Table 4).

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

LOD and LOQ
LOD values for TOR and SPI were found to be 0.03 and 0.08µg/ml respectively. LOQ values for TOR and SPI were found to be 0.002 and 0.004 µg/ml respectively. (Table 4)

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 1 System suitability test parameter for TOR and SPI

Property (n*=6)	TOR	SPI
Retention time(min)	4216	5601
Tailing factor	138	208
Capacity factor	354	561
Theoretical plates number	9086	8761
Resolution	166	153
Linearity range (mg/ml)	1 to 100	1 to 100
Peak asymmetry	251-275	189-198
Peak Width (min)	12.10	16.25
Regression equation	y = 67412 x + 32156	y = 98713 x + 16313

* n = Number of determination, TOR- Torsemide SPI—Spironolactone

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	5.03	100.01	25	80	24.99	99.98
	100	4.99	99.98		100	25.04	100.03
	120	5.08	100.12		120	25.12	100.11

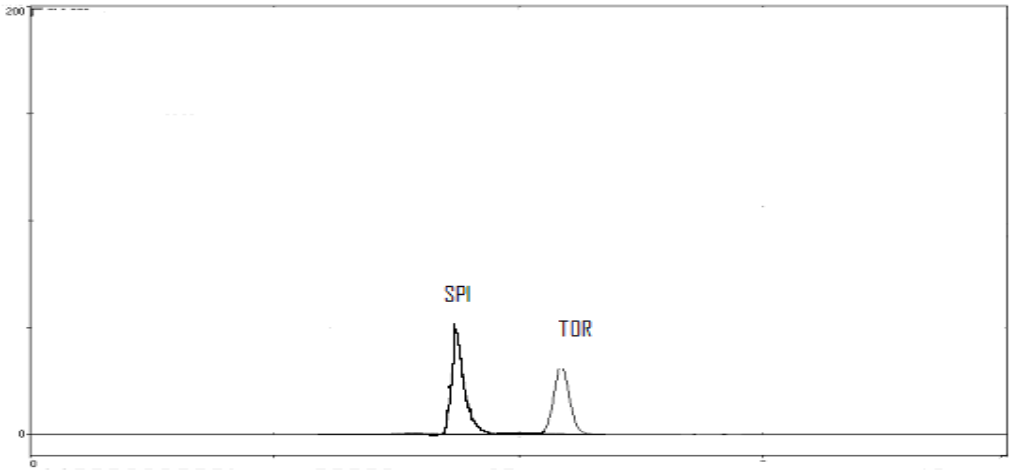
TOR- Torsemide SPI-- Spironolactone

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	11284	23197
SD[$] of the slope	1098	1784
Intercept	34932	43289
SD [a] of the intercept	12954	21309
Correlation coefficient	0.9999	0.9998

Table 4 Summary of validation parameter

Parameter	**TOR**	**SPI**
LOD[a]	0.03µg/ml	0.08µg/ml
LOQ[b]	0.002 µg/ml	0.004µg/ml
Accuracy, %	10001% ± 012	10064% ± 020
Repeatability(RSD[c], %, n =6)	0.231	0.178
Precision (RSD, %)		
Intra day(n =3)	0.022	0.045
Inter day(n = 3)	0.0014	0.026



Micellar Liquid Chromatography Method Development for *Torsemide and Spironolactone*

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)**
5	4.99	25	25.04
	4.97		24.98
	5.02		25.01
	5.11		24.98
	5.0		24.99
	4.99		25.01
Mean	5.03	Mean	25.11
±SD	0.278	±SD	0.341

Conclusion

The method is accurate, precise, rapid and selective for simultaneous estimation of TOR and SPI in tablet dosage form. Hence it can be conveniently adopted for routine analysis. The proposed miceller chromatographic method has been evaluated over the linearity, precision, accuracy, specificity and proved to be conveninant and effective for the quality control The proposed method has advantage of simplicity and convenience for the separation and quantitation of TOR Torsemide and SPI Spironolactone in the combination and cab be used for the assay of their dosage form.

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