Validated TLC Densitometric method for the quantification of Torsemide and Spironolactone in bulk drug and in tablet dosage form

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Abstract

Validated high performance thin liquid chromatographic (HPTLC) method for estimation of Torsemide (TOR) and Spironolactone (SPI) in tablet dosage form. A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the determination of Torsemide and Spironolactone in dosage form. The stationary phase used was precoated silica gel 50 F254. The mobile phase used was a mixture of ethyl acetate: aceton: acetic acid (10.5: 4: 1.5v/v/v). The detection of spot was carried out at 269.0 nm. Developed method was validated in terms of linearity, accuracy, precision, repeatability and specificity. Limit of detection and limit of quantification of Torsemide and Spironolactone were found to be 120 ng/spot and 178 ng/spot, respectively. The linearity range for Torsemide and Spironolactone was found to be 360-850 ng/spot with correlation coefficient of 0.998. The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of methods.

Keywords: Torsemide, Spironolactone, HPTLC, densitometric estimation.

Introduction

Torsemide (TOR) is sulfonylurea derivative and chemically known as 3-[4-[(3-methylphenyl) amino] pyridin-3-yl] sulfonyl-1-propan-2-yurea. It acts as diuretic. Spironolactone (SPI) is steroidal derivative and chemically known as 7α-Acetyltihio-3-oxo-17α-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. 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 HPTLC method.

**Result and Discussion**

The method was validated in terms of linearity, accuracy, inter-day and intra-day precision, specificity, repeatability of measurement of peak area as well as repeatability of sample application. The limit of detection and limit of quantification were also determined. The TLC plates were pre-washed with methanol, and activated by keeping at 95 °C for about 30 min. The stationary phase used was precoated silica gel 50 F254. The mobile phase used was a mixture of ethyl acetate: acetone: acetic acid (10.5: 4: 1.5 v/v/v). The detection of spot was carried out at 269.0 nm, chamber saturation time 25 min, distance 30 mm, wavelength scanning at 269 nm, band width 9 mm, slit dimension keeping the slit dimension at 5 ×0.45 mm scanning speed 15 nm/sec, and the source of radiation of deuterium lamp. On to a pre-washed and activated TLC plate, 5-10 ml of standard stock solution of TOR and SPI was spotted with Linomat V Semi applicator. The plates were developed and scanned. The peak areas of each standard were obtained from the system, and a calibration graph was plotted with concentration vs. peak area. The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day assay precision, repeatability of measurement, and repeatability of sample application. From the sample aliquot prepared, 2 and 6 ml solution was applied, and the plate was developed with the mobile phase. A triplicate of those was carried out, and the peak areas were noted. The mobile phase consisted of ethyl acetate: acetone: acetic acid (10.5: 4: 1.5 v/v/v) and Rf value of TOR and SPI were found to be 0.33 and 0.19 respectively. Detection was carried out at 269, 247 TOR and SPI respectively.

The proposed method has been validated for assay of TOR and SPI in bulk and tablet dosage forms using following parameters [11], [12]. The target analyte concentration of all the two drugs was fixed as 10 µg/ml. Linearity was observed in the concentration range of 400-1800 ng/spot and 600-2500 ng/spot correlation coefficients (r) being 0.9996 and 0.9998 with the slopes 3.843 and 2.0541 for TOR and SPI, respectively. The resolution (Rs) between TOR and SPI was 18.2. The efficiency of the method is studied by calculating number of theoretical plates and was found as 11,375. Peak area ratios of standard TOR and SPI to that of internal standard were measured.

A representative calibration graph of peak area ratio versus TOR and SPI concentration (100 to 650 ng/spot) resulted in regression equation y=0.3481 x+0.7821 (r = 0.9996). The intra- and inter-day precision were carried out at three different concentration levels, i.e., 200,400,600 ng/spot; 200, 500, 700 ng/spot for the determinations of TOR and SPI, respectively. The low values of percentage relative standard deviation (% RSD) for intra-and inter-day variation as shown in [Table-3] reveal that the proposed method is precise. Recovery studies of the drugs
were carried out for the accuracy parameters. These studies were carried out at three levels i.e. multiple level recovery studies. Sample stock solution from tablet formulation of 100 µg/ml of was prepared. To the above prepared solutions, 80%, 100% and 120% of the standard drug solutions were added. Dilutions were made and recovery studies were performed. Percentage recovery was found to be within the limits as listed in [Table 2]. The assay value for the marketed formulation was found to be within the limits as listed in [Table 2]. The low RSD value indicated the suitability of the method for routine analysis of TOR and SPI in pharmaceutical dosage forms. The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of TOR and SPI in bulk drug and tablet formulations.

### Table 1 Regression Analysis of Calibration Graph for TOR and SPI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TOR</th>
<th>SPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_F ) (SD)</td>
<td>0.24</td>
<td>0.33</td>
</tr>
<tr>
<td>Linearity and range (ng/spot)</td>
<td>400</td>
<td>600</td>
</tr>
<tr>
<td>Linearity detection (ng/spot)</td>
<td>132</td>
<td>160</td>
</tr>
<tr>
<td>Limit of quantification (ng/spot)</td>
<td>269</td>
<td>235</td>
</tr>
<tr>
<td>Repeatability of application (%RSD)</td>
<td>0.45</td>
<td>0.61</td>
</tr>
<tr>
<td>Repeatability of measurement (%RSD)</td>
<td>0.36</td>
<td>0.71</td>
</tr>
<tr>
<td>Intraday (%RSD)</td>
<td>0.12</td>
<td>0.29</td>
</tr>
<tr>
<td>Inter day (%RSD)</td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>LOD(^a)</td>
<td>0.459</td>
<td>0.761</td>
</tr>
<tr>
<td>LOQ(^b)</td>
<td>0.132</td>
<td>0.539</td>
</tr>
</tbody>
</table>

\(^a\) SD = Standard Deviation

![Figure: HPTLC-Chromatogram of Torsemide and Spironolactone](image)
Table 2 - Recovery Studies

<table>
<thead>
<tr>
<th>Label claimed</th>
<th>% Amount added</th>
<th>Found in (µg/ml)</th>
<th>% Recovery</th>
<th>Label claimed</th>
<th>% Amount added</th>
<th>Found in (µg/ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>80</td>
<td>10.12</td>
<td>100.12</td>
<td>10</td>
<td>80</td>
<td>9.98</td>
<td>99.98</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10.01</td>
<td>100.01</td>
<td></td>
<td>100</td>
<td>10.04</td>
<td>100.04</td>
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<tr>
<td></td>
<td>120</td>
<td>10.09</td>
<td>100.09</td>
<td></td>
<td>120</td>
<td>10.06</td>
<td>100.06</td>
</tr>
</tbody>
</table>

Method Validation

For ruggedness, study was carried out for two different parameters i.e., days and analyst. The results of estimation by proposed method are very much similar under variety of conditions. The assay results of TOR and SPI in bulk and tablet dosage forms were comparable with the value of labeled claim. The obtained results are given in [Table]. To study the accuracy of the proposed method recovery studies were carried out using standard addition method. The percent recovery was calculated by using the formula, % recovery = (T - A) / S × 100, where T is total amount of drug estimated, A is the amount of drug contributed by tablet powder and S is the amount of pure drug added.

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.

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

The limit of quantification (LOQ) represents the concentration of the analyte that would yield a signal-to-noise ratio of 10^3. The LOD and LOQ were found to be 240 and 420 ng/spot, respectively for TOR 24 and SPI 70 ng/spot.

Materials and Methods

TOR and SPI pure powder were procured as gifts sample from Lupin Labs, Jammu. 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. Ethyl acetate: acetone: acetic acid (10.5: 4: 1.5 v/v/v) were purchased from SD fine chemical Ltd (Ahmedabad, India) and were of analytical grade Water of HPLC grade was used.
HPTLC method and chromatographic conditions:
The chromatography estimation was performed using the following conditions: stationary phase was precoated silica gel 55 F_{254} aluminum sheets (10 x 10 cm, E. Merck) and the mobile phase used was ethyl acetate: acetone: acetic acid in the ratio of (10.5:4:1.5 v/v/v). The chamber saturation time employed was 15 min and the developing distance used was 4 cm. Scanning wavelengths for TOR and SPI was 269 nm with slit dimensions of 5.0 x 0.45 mm and scanning speed of 15mm/s were employed. Spotting parameters used were, 5 mm bandwidth, 15 mm space between two bands and spraying rate 20 s/µl.

Preparation of standard solution:
The equivalent of 10 mg each of TOR and SPI were accurately weighed in 25 ml volumetric flasks separately and dissolve in standard stock solution of 0.6 mg/ml. The aliquots (0.1 to 1.0 ml) of stock solution were transferred to 10 ml volumetric flasks and the volume of each was adjusted to 10 ml with methanol to obtain working standard solution containing 10, 30, 54, 74, 84 and 98 µg/ml.

Calibration curve for TOR and SPI- Standard solutions of TOR and SPI (10 µl) were applied in triplicate on TLC plate. The plate was developed in a solvent system of ethyl acetate: acetone: acetic acid in the ratio in the ratio of (10.5:4:1.5 v/v/v) up to distance of 4 cm. After development, the plates were dried in hot air and scanned at 269 nm. The peak areas were recorded. Calibration curve of TOR and SPI was obtained by plotting peak area vs concentration of TOR and SPI applied. Linearity was performed by applying six times the stock solution to give concentrations of 400-1800 ng/spot and 600-2500 ng/spot of TOR and SPI, respectively. Calibration curve was established by plotting peak area on ordinate and corresponding concentration on abscissa.

Assay of tablets:
Twenty tablets of TOR and SPI were crushed and ground to fine powder. A powder equivalent to 20 mg of drug was transferred to a conical flask and extracted with methanol (2 X 50 ml) by sonication. The extracts were filtered through Whatman No. 41 filter paper and the residue was washed with sufficient amount of methanol. The extract and its washings were pooled, transferred to a 100 ml volumetric flask and the final volume was made up to 100 ml with methanol to give a sample solution of 200 µg/ml. A fixed volume of 2 or 4 µl of working standard solutions (60 µg/ml) and 4 or 5 µl of sample solutions were spotted as sharp bands on the TLC plate and the plate was developed as mentioned above. The band of the drug was scanned at 269 nm. Precision of the method is expressed in terms of% RSD.

Conclusion

The proposed HPTLC method was found to be rapid, specific, precise and accurate. The proposed method has advantage of simplicity and convenience for the separation and quantitation of TOR and SPI in the combination and can be used for the assay of their dosage form. To confirm the specificity of the proposed method, Torsemide and Spironolactone was spotted on TLC plate, developed and scanned as described earlier. Simultaneous estimation of
Torsemide and Spironolactone in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

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Reference