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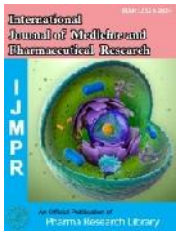

Research Article **Open Access**

Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Torsemide and Spiranolactone in Bulk Drug and Pharmaceutical Dosage Form

V. Haribaskar, Ramesh Dhani, B. Swathi*

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur, Nellore, A.P, India

A B S T R A C T

Simple precise and accurate method was developed for the estimation of Torsemide and Spiranolactone. The mobile phase consisting 20% Buffer: 80%ACN. The column was used: Inertsil ODS 4.6*210mm, 5µ with flow rate 1ml/min using PDA detection at 235 nm. The estimation of Torsemide and Spiranolactone was done by RP-HPLC. The assay of Torsemide and Spiranolactone was performed with tablets and the % assay was found to be 99.47 and 100.02 which shows that the method is useful for routine analysis. The linearity of Torsemide and Spiranolactone was found to be linear with a correlation coefficient of 0.998 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.1 and 0.7 for Torsemide and Spiranolactone which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.2 for Torsemide and Spiranolactone which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.74% and 99.40% for Torsemide and Spiranolactone. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Torsemide was found to be 2.98 and 10.00 and LOD and LOQ for Spiranolactone was found to be 3.00 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Keywords: Torsemide, Spiranolactone, Accuracy, Precision, RP-HPLC.

A R T I C L E I N F O

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***Corresponding Author**
B. Swathi
Department of Pharmaceutical Analysis,
Ratnam Institute of Pharmacy,
Pidathapolur, Nellore, A.P, India
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1. Introduction

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2]. Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials[3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements[5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6]. The advent of high-performance liquid chromatography (HPLC) in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution[8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC[9].

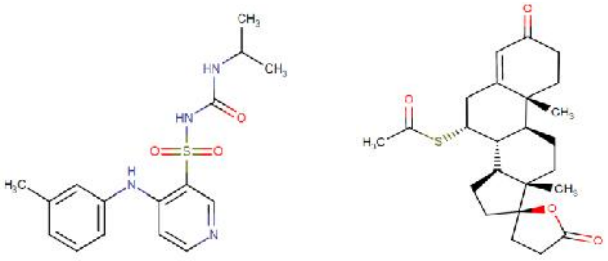

Figure 1: Structure of Torsemide, Spiranolactone

2. Materials and Methods

Materials

WATERS, software: Empower, 2695 separation module UV detector. UV/VIS spectrophotometer, Digital weighing balance, P^H meter [10]. Ortho phosphoric acid, HCl, H_2O_2, NaOH, Acetonitrile, Methanol and Water.

Detection of wavelength:

UV spectrum of 10µg/ml Torsemide and 10µg/ml Spiranolactone in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 235 nm. At this wavelength both the drugs show good absorbance.

Optimization chromatographic conditions:

Instrument	: Waters HPLC with auto sampler and UV detector.
Temperature	: Ambient (25° C)
Mode of separation	: Isocratic mode
Column	: Inertsil ODS 4.6*210mm, 5µ
Buffer	: 0.1% OPA
pH	: 3.0
Mobile phase	: 20% Buffer: 80%ACN
Flow rate	: 1 ml per min
Wavelength	: 235 nm
Injection volume	: 20 µl
Run time	: 10 min.

Assay:

Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for the Torsemide & Spiranolactone peaks and calculate the %Assay by using the formulae. Accurately weigh and transfer 10 mg of Torsemide and 25 mg of Spiranolactone working standard into a 10 mclean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. ***(Stock solution).***

Linearity:

Prepare Level – I,II,III,IV, V by changing the concentration of Torsemide & Spiranolactone. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Ruggedness:

The standard solution was injected for five times and measured the area for all Five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Accuracy:

Three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyze and chromatograms are recorded for the same.

Limit of detection:

Preparation of Torsemide solution:

Preparation of 150µg/ml solution:

Pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 4.2 µg/ml solution:

Pipette 0.28 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Spiranolactone solution:

Preparation of 375 µg/ml solution:

Pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 2.66 µg/ml solution:

Pipette 0.071 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Limit of Quantification:

Preparation of Torsemide solution:

Preparation of 150 µg/ml solution:

Pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 14.04 µg/ml solution:

Pipette 0.936ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Spiranolactone solution:

Preparation of 375 µg/ml solution:

Pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 8.74 µg/ml solution:

Pipette 0.233ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

3. Results and Discussion

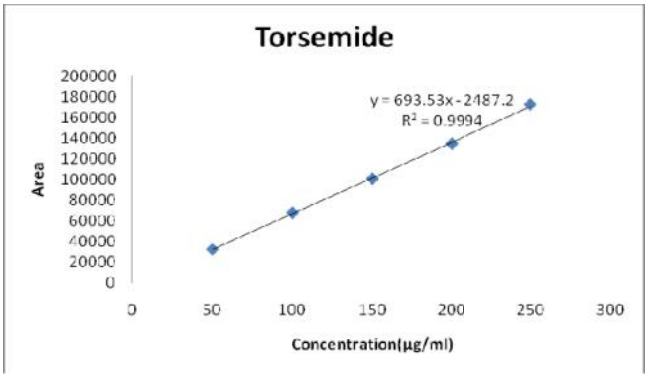

Figure 2: Calibration graph of Torsemide

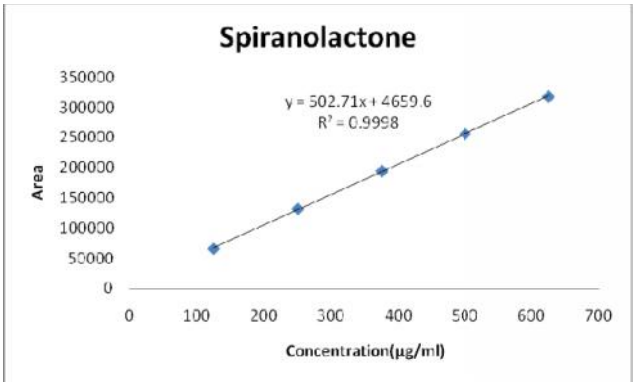

Figure 3: Calibration graph of Spiranolactone

Table 1: Area of different concentration of Torsemide and Spiranolactone

S. No	Torsemide		Spiranolactone	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
1	50	32441	125	65787
2	100	67728	250	131783
3	150	100630	375	194311
4	200	134448	500	256245
5	250	172463	625	317748

.

Table 2: Area of different concentration of Torsemide and Spiranolactone

Injection	Area for Torsemide	Area for Spiranolactone
Injection-1	107339	191345
Injection-2	107232	191232
Injection-3	107131	191671
Injection-4	107399	191999
Injection-5	107018	192898
Injection-6	107089	194679
Average	107201.3	192304.0
Standard Deviation	148.4	1308.1
%RSD	0.1	0.7

Table 3: Results of Ruggedness for Torsemide and Spiranolactone

Injection	Area for Torsemide	Area for Spiranolactone
Injection-1	104533	192345
Injection-2	104232	192432
Injection-3	104531	192971
Injection-4	104399	192899
Injection-5	104018	192898
Injection-6	104689	192333
Average	104400.3	192646.3
Standard Deviation	241.9	305.8
%RSD	0.2	0.2

Table 4: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Torsemide	58	173	2.98
Spiranolactone	58	174	3.00

Table 5: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Torsemide	58	580	10.00
Spiranolactone	58	579	9.98

4. Conclusion

The estimation of Torsemide and Spiranolactone was done by RP-HPLC. The assay of Torsemide and Spiranolactone was performed with tablets and the % assay was found to be 99.47 and 100.02 which shows that the method is useful for routine analysis. The linearity of Torsemide and Spiranolactone was found to be linear with a correlation coefficient of 0.998 and 0.999, which shows that the method is capable of producing good sensitivity. The

acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.1 and 0.7 for Torsemide and Spiranolactone which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.2 for Torsemide and Spiranolactone which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.74% and 99.40% for Torsemide and Spiranolactone. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Torsemide was found to be 2.98 and 10.00 and LOD and LOQ for Spiranolactone was found to be 3.00 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Table 6: Accuracy (recovery) data for Torsemide

% Concentration	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	53846	5	5.01	100.24	99.74
100%	107344	10	9.99	99.91	
150%	159676	15	14.86	99.08	

Table 7: Accuracy (recovery) data for Spiranolactone

% Concentration	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	95105	12.5	12.43	99.47	99.40
100%	191399	25	24.92	99.67	
150%	285309	37.5	37.14	99.05	

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