Journal of Pharmaceutical and Biomedical Analysis Letters

Journal Home Page: www.pharmaresearchlibrary.com/jpbmal

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

B. Swathi, V. Hari Baskar*, Ramesh Dhani, M. Bharathi, M. Pavani

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur, Muthukur, SPSR Nellore– 524 346

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 are 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, RP-HPLC

A R T I C L E I N F O

CONTENTS

1. Introduction 49
2. Materials and Methods 49
3. Results and discussion 49
4. Conclusion 52
5. References. 53

Article History: Received 18 March 2017, **Accepted** 21 June 2017, **Available Online** 18 July 2017

***Corresponding Author**
V. Hari Baskar
Department of Pharmaceutical Analysis,
Ratnam Institute of Pharmacy,
Pidathapolur, Nellore, A.P, India
Manuscript ID: JPBMAL3433

PAPER-QR CODE

Citation: V. Hari Baskar, *et al.* Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Torsemide and Spiranolactone in Bulk Drug and Pharmaceutical Dosage Form. *J. Pharm, Biomed. A. Lett*., 2017, 5(2): 48-53.

1. Introduction

Analytical methods: 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 [1,2]. 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

Description of the Various Analytical Methods

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].

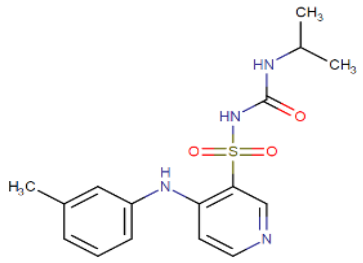

Figure 1: Torsemide

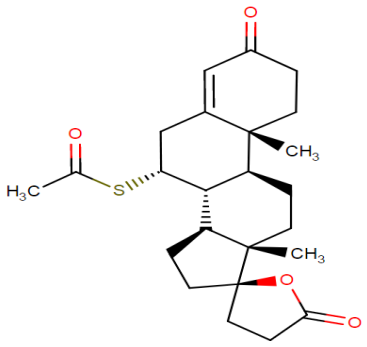

Figure 2: Spiranolactone

2. Materials and Methods

Apparatus:

WATERS, software: Empower, 2695 separation module UV detector. UV/VIS spectrophotometer, Digital weighing balance, P^H meter [10].

Reagents and Materials:

Ortho phosphoric acid, HCl, H_2O_2, NaOH, Acetonitrile, Methanol and Water

Selection of detection 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 trials for Simultaneous Estimation of Torsemide and Spiranolactone by RP-HPLC.

Optimization chromatographic conditions

Instrument used	:	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:

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Torsemide and 25 mg of Spiranolactone working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 10 mg of Torsemide and 25 mg of Spiranolactone sample into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: 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.

3. Results and Discussion

Method Validation Parameters

Linearity:

Preparation of stock solution: Accurately weigh and transfer 10 mg of Torsemide and 25 mg of Spiranolactone working standard into a 10 ml clean 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)

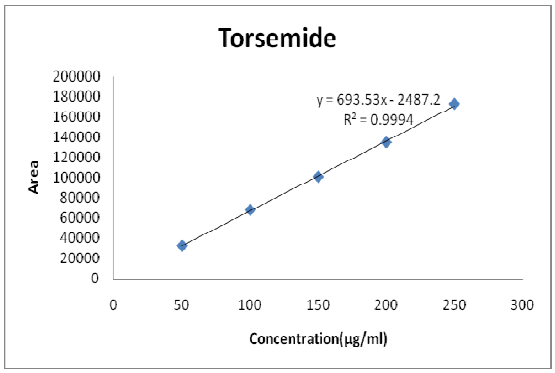

Figure 3: Calibration graph of Torsemide

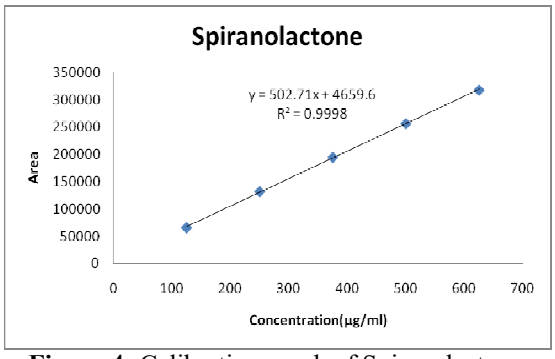

Figure 4: Calibration graph of Spiranolactone

Preparation of Level – I (50ppm & 125ppm of Torsemide & Spiranolactone):
0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.
Preparation of Level – II (100ppm & 250ppm of Torsemide & Spiranolactone):
1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.
Preparation of Level – III (150ppm & 375ppm of Torsemide & Spiranolactone):
1.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.
Preparation of Level – IV (200ppm & 500ppm of Torsemide & Spiranolactone):
2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.
Preparation of Level – V (250ppm & 625ppm of Torsemide & Spiranolactone):
2.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.
Procedure:
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:
Preparation of stock Solution:
Accurately weigh and transfer 10 mg of Torsemide and 25 mg of Spiranolactone working standard into a 10 ml clean 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)
Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents
Procedure: 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.
Intermediate Precision/Ruggedness:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.
Preparation of stock solution:
Accurately weigh and transfer 10 mg of Torsemide and 25 mg of Spiranolactone working standard into a 10 ml clean 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)
Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents
Procedure: 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
For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.
Preparation of Standard stock solution:
Accurately weigh and transfer 10 mg of Torsemide and 25mg of Spiranolactone working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents
Preparation Sample solutions:
For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 5 mg of Torsemide and 12.55 mg of Spiranolactone working standard into a 10 ml clean 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). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents
For preparation of 100% solution (With respect to target Assay concentration): Accurately weigh and transfer 10 mg of Torsemide and 25 mg of Spiranolactone working standard into a 10 ml clean 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). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.
For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 30 mg of Torsemide and 37.5 mg of Spiranolactone working standard into a 10 ml clean 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). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure: Inject the standard solution, Accuracy -10%, Accuracy -100% and Accuracy -110% solutions. Calculate the Amount found and Amount added for Torsemide & Spiranolactone and calculate the individual recovery and mean recovery values. **Limit of Detection:**

Preparation of Torsemide solution:

Preparation of 150µg/ml solution:

Accurately weigh and transfer10 mg of Torsemide working standard into a 10 ml clean 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)

Further 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:

Further 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: Accurately weigh and transfer 25 mg of Spiranolactone working standard into a 10 ml clean 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). Further 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:

Further 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: Accurately weigh and transfer 10 mg of Torsemide working standard into a 10 ml clean 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). Further 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:

Further 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:

Accurately weigh and transfer 25 mg of Spiranolactone working standard into a 10 ml clean 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)

Further 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:

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

Table 1: Area of different concentration of Torsemide and Spiranolactone

S. No	Torsemide		Spiranolactone	
	Concentration (µg/ml)	Area	Concentration (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

Results of Precision for Torsemide

Injection	Area for Torsemide
Injection-1	107339
Injection-2	107232
Injection-3	107131
Injection-4	107399
Injection-5	107018
Injection-6	107089
Average	107201.3
Standard Deviation	148.4
%RSD	0.1

Results of Precision for Spiranolactone

Injection	Area for Spiranolactone
Injection-1	191345
Injection-2	191232
Injection-3	191671
Injection-4	191999

Injection-5	192898
Injection-6	194679
Average	192304.0
Standard Deviation	1308.1
%RSD	0.7

Results of Intermediate precision for Torsemide

Injection	Area for Torsemide
Injection-1	104533
Injection-2	104232
Injection-3	104531
Injection-4	104399
Injection-5	104018
Injection-6	104689
Average	104400.3
Standard Deviation	241.9
%RSD	0.2

Results of Intermediate precision for Spiranolactone

Injection	Area for Spiranolactone
Injection-1	192345
Injection-2	192432
Injection-3	192971
Injection-4	192899
Injection-5	192898
Injection-6	192333
Average	192646.3
Standard Deviation	305.8
%RSD	0.2

Accuracy (recovery) data for Torsemide

% Concentration (at specification Level)	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	

Accuracy (recovery) data for Spiranolactone

%Concentration (at specification Level)	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	

Results of LOD

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

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.

5. References

[1] Becket and Stenlake, Practical pharmaceutical chemistry, part 24th edition CBS publications and distributors, 2005.

[2] P.D. Sethi, HPLC quantitative analysis of pharmaceutical formulations CBS publications and distributors, 1st Edition, 2001.

[3] B.K Sharma, instrumental method of chemical analysis, 23rd Edition, goal publishers 2004.

[4] Practical HPLC method development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, second edition.

[5] International conference on harmonization**:** ICH Q 2 (R1) Validation of Analytical Procedures: Text and Methodology 1995.

[6] Bhojani Maulik, Dadhania Ketan, Faldu Shital, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Furosemide and Spironolactone in their Combined Tablet Dosage Form, JPSBR, 2012, 2(3): 144-147.

[7] Hiresh K. Golher, Kavita Kapse and Sachin K. Singh, Simultaneous Spectrophotometric Estimation of Torsemide, Spironolactone in Tablet Dosage Form, International Journal of Pharm Tech Research, 2010, 2(4): 2246-2250.

[8] M. C. Sharma, Smita Sharmaa, D. V. Kohlib, A. D. Sharmac, Validated TLC Densitometric method for the quantification of Torsemide and Spironolactone in bulk drug and in tablet dosage form, Scholars Research Library, Der Pharma Chemica, 2010, 2(1): 121-126.

[9] Smit A Bhadja, Usmangani K Chhalotiya, Dimal A Shah, Falgun A Mehta, Kashyap K Bhatt, Simultaneous Estimation Of Torsemide And Amiloride Hydrochloride In Their Pharmaceutical Dosage Form By Dual Wavelength Uv Spectroscopic Method, Adv J Pharm Life sci Res, 2014 2;1:21-28.