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Research

Validated Rp-Hplc Method For Simultaneous Estimation Of Torsemide And Spironolactone In Bulk And Tablet Dosage Form.

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Abstract

Background: A simple, accurate and precise HPLC method for simultaneous determination of Spironolactone and Torsemide in pure and tablet dosage form has been developed. Aim: To develop and validate analytical method for simultaneous estimation of Spironolactone and Torsemide in pharmaceutical formulation by RP-HPLC.
Materials and Methods: HPLC of Waters (Model: Alliance 2695) with Phenomenex Luna C18 (4.6 mm I.D. × 250 mm, 5 µm) column was used for chromatographic separation. It contains waters injector and PDA Detector (Deuterium). Mobile phase consists of Methanol:Water (65:35% v/v) and flow rate adjusted was 1ml/min. Wavelength selected for detection was 220nm and injection volume was 10 µl.
Results and discussion: By using the developed method, retention time of Spironolactone and Torsemide was found to be 3.2min and 5.4min respectively. The method has been validated for linearity, accuracy and precision. Linearity of Spironolactone and Torsemide were in the range of 75–375µg/ml and 15–75µg/ml respectively. The percentage recoveries obtained for Spironolactone and Torsemide were found to be in range of 99.3 – 99.6%. LOD and LOQ were found to be 12.5µg/ml and 38.1µg/ml for Spironolactone 3.7and 11.4µg/ml for Torsemide.
Conclusion: The developed HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied for the analysis of pure drug and pharmaceutical dosage forms. From the present study it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2R1.

Keywords: Spironolactone, Torsemide, Simultaneous Estimation, RP- HPLC

INTRODUCTION

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity.[1]

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.[2,3]

The basic components of HPLC are

1. Pumping System[4]
2. Sample Introduction Device[5]
3. Chromatographic Column[5]
4. Detector[7]
5. Data handling Device[8]

Pumping System

The HPLC pump is very important component of the system. It delivers the constant flow of the mobile phase or phases so that the separation of the components of the mixture occur in a reasonable time. Its performance directly affects retention time, reproducibility and detector sensitivity. Three main types of pumps are used in HPLC to propel the liquid mobile phase through the system are as under;

a. Displacement pump: It produces a flow that tends to independent of viscosity and backpressure and also output is pulse free. But it possesses limited capacity (250 ml).

b. Reciprocating pump: It has small internal volume (35 to 400 µl). It has high output pressure (up to 10,000 psi) and constant flow rates. But it produces a pulsed flow.

c. Pneumatic or constant pressure pump: They are pulse free, suffer from limited capacity as well as a dependence of flow rate on solvent viscosity and column back pressure. They are limited to pressure less than 2000 psi.

There are two type of elution process, i.e. isocratic and gradient

Isocratic: In this system, the things are kept constant throughout the run. In the case of pumping of mobile phase, the mobile phase composition is kept constant throughout the run. The nominal flow rate accuracy required is ±1% of the set flow

Gradient: There is some change purposely incorporated during the particular sample run to achieve a better or/and faster separation. In case of pumping mobile phase, the composition of mobile phase is continuously varied during the particular run. The gradient accuracy of ±1% of the step gradient composition is typical.

Sample Introducing Device

It is not possible to use direct syringe injection on column like GC, as the inlet pressure in LC is too high. Insertion of the sample onto the pressurized column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself should have no dead (void) volume. There are three important ways of introducing the sample into injection port.

a. Loop injection: In which, a fixed amount of volume is introduced by making use of fixed volume loop injector.

b. Valve injection: In which, a variable volume is introduced by making use of an injection valve.

c. On column injection: In which, a variable volume is introduced by means of a syringe through a septum.

Chromatographic Column

Column is a heart of chromatography. The column is usually made up of heavy glass or stainless steel tubing to withstand high pressure. The columns are usually 10-30 cm long and 4-10 mm inside diameter containing stationary phase at particle diameter of 25 µm or less. Columns with an internal diameter of 5 mm give good results because of compromise between efficiency, sample capacity, and the amount of packing and solvent required.

Column packing:

The packing used in modern HPLC consists of small, rigid particles having a narrow particle size distribution. There are three main types of column packing in HPLC.

a. Porous, polymeric beds: Porous, polymeric beds based on styrene divinyl benzene co-polymers used for ion exchange and size exclusion chromatography. For analytical purpose these have now been replaced by silica based, packing which are more efficient and more stable.

b. Porous layer beds: Consisting of a thin shell (1-3 µm) of silica or modified silica on an spherical inert core (e.g. Glass). After the development of totally porous micro particulate packings, these have not been used in HPLC.

c. Totally Porous silica particles (dia. < 10 µm): These packing have widely been used for analytical HPLC in recent years. Particles of diameter > 20 µm are usually dry packed. While particles of diameter < 20 µm are slurry packed in which particles are suspended on a suitable solvent and the slurry so obtained is driven into the column under pressure.

Detector

The function of the detector in HPLC is to monitor the mobile phase as it merges from the column. There are several detectors available in the market. However UV Visible detector, photo diode array detector, fluorescence detector, conductometric and coulometric detector are more commonly used. The new ELSD detector is proving to be important detector, while the MS detector is outstanding. Detectors are usually of two types:

a. Bulk property detectors: It compares overall changes in a physical property of the mobile phase with and without an eluting solute e.g. refractive index, dielectric constant or density.

b. Solute property detectors: It responds to a physical property of the solute, which is not exhibited by the pure mobile phase e.g. UV absorbance, fluorescence or diffusion current.

Data handling Device

Computer-based system that controls all components of HPLC instrument (eluent composition (mixing of different solvents); temperature, injection sequence, etc.) and acquires data from the detector and monitors system performance (continuous monitoring of the mobile phase composition, temperature, back pressure, etc.)

MATERIALS AND METHODS

Spironolactone(Pure) & Torsemide(Pure) Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Spironolactone and Torsemide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 2.25ml of the above Spironolactone and 0.45ml of the Torsemide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and water in proportion 65:35 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 650ml (65%) of HPLC Methanol and 350ml of Water (35%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Column : Phenomenex Luna C18 (4.6×250mm) 5µ
Column temperature : 35˚C
Wavelength : 220nm
Mobile phase ratio : Methanol:Water(65:35 v/v)
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10minutes

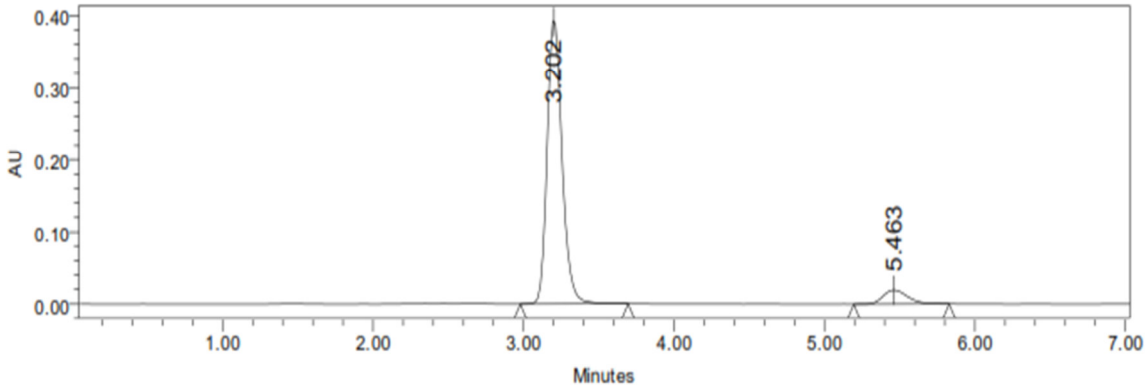


Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Spironolactone	3.202	2391746	39726	1.2	9028	
2	Torsemide	5.463	194627	8497	1.1	7398	7.4

Optimized Chromatogram (Sample)

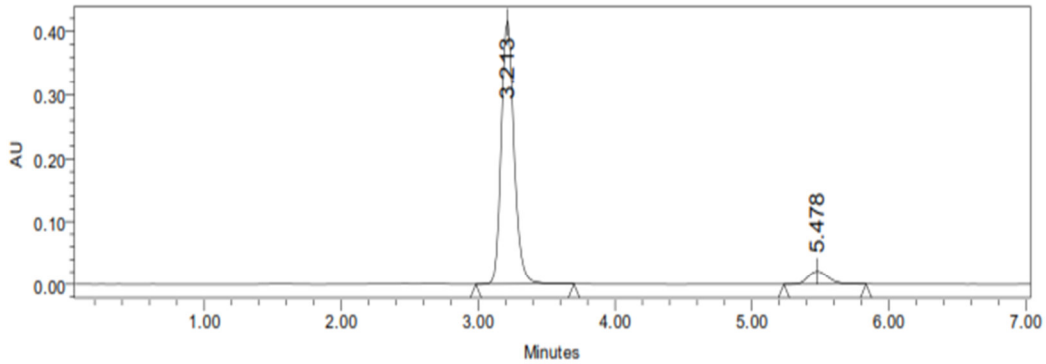


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Spironolactone	3.213	2381649	391846	1.2	9472	
2	Torsemide	5.478	191057	8104	1.1	8936	7.5

Assay (Standard)

Table 3: Peak results for assay standard

Spironolactone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Spironolactone	3.211	2397162	397161	1.2	9472
2	Spironolactone	3.222	2394721	389173	1.2	9745
3	Spironolactone	3.254	2389461	391723	1.2	8917

Torsemide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Torsemide	5.414	198462	7811	1.1	8492	7.49
2	Torsemide	5.453	198472	8193	1.1	8916	7.52
3	Torsemide	5.424	198735	7972	1.1	9372	7.44

Assay (Sample)

Table 4: Peak results for Assay sample

Spironolactone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Spironolactone	3.297	2391741	381612	1.2	9472
2	Spironolactone	3.294	2389166	391746	1.2	8927
3	Spironolactone	3.295	2361731	381634	1.2	9017

Torsemide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Torsemide	5.435	198641	8174	1.1	9284	7.18
2	Torsemide	5.417	196547	8942	1.1	8974	7.44
3	Torsemide	5.434	194027	7294	1.1	9017	7.38

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=2380879/2393781*10/225*225/0.3238*99.8/100*1.6194/50*100
=99.2%
The % purity of Spironolactone and Torsemide in pharmaceutical dosage form was found to be 99.2%.

System suitability

Table 5: Results of system suitability for Spironolactone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Spironolactone	3.200	2391746	394171	8952	1.2
2	Spironolactone	3.248	2391647	381946	9561	1.2
3	Spironolactone	3.299	2381647	391746	6572	1.2
4	Spironolactone	3.297	2385631	386562	6452	1.2
5	Spironolactone	3.297	2385635	389164	7452	1.2
Mean			2387261			
Std. Dev.			4363.771			
% RSD			0.182794			

%RSD of five different sample solutions should not more than 2
The %RSD obtained is within the limit, hence the method is suitable.

Table 6: Results of system suitability for Torsemide

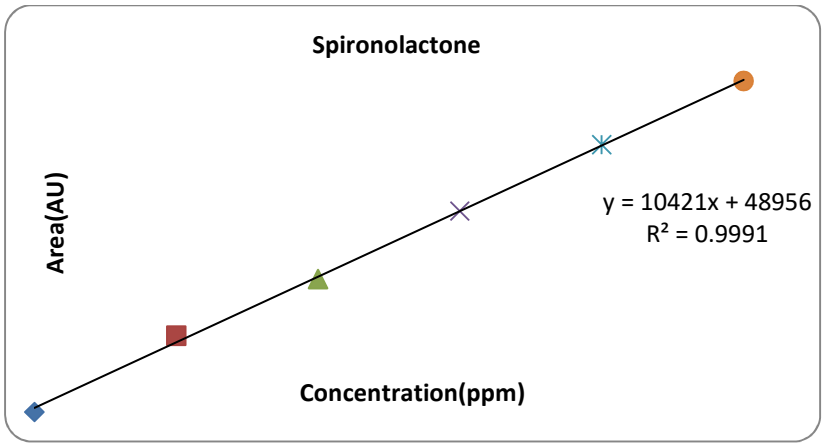
S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Torsemide	5.413	198362	7917	5272	1.1
2	Torsemide	5.484	197486	7486	6291	1.1
3	Torsemide	5.405	198354	7859	6184	1.1
4	Torsemide	5.405	197352	7926	7145	1.1
5	Torsemide	5.409	198453	7946	6946	1.1
Mean			198001.4			
Std. Dev.			535.1774			
% RSD			0.27029			

%RSD of five different sample solutions should not more than 2
The %RSD obtained is within the limit, hence the method is suitable.

LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

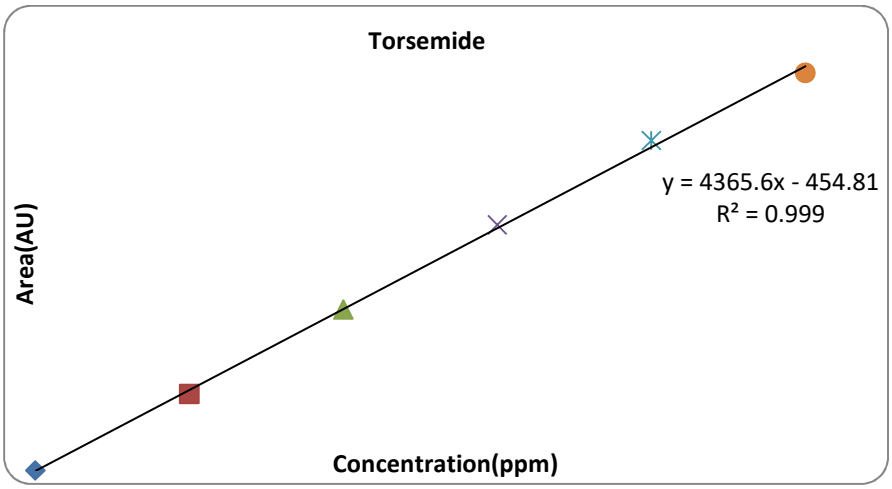
Spironolactone

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	75	909889
80	150	1583641
100	225	2395378
120	300	3185089
140	375	3943725



Torsemide

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	15	61953
80	30	130213
100	45	198697
120	60	267002
140	75	321658



Repeatability

Table 7: Results of repeatability for Spironolactone

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Spironolactone	3.213	2397164	381741	8155	1.2
2	Spironolactone	3.253	2391741	371742	9174	1.2
3	Spironolactone	3.297	2371846	391746	7154	1.2
4	Spironolactone	3.215	2361748	391847	9917	1.2
5	Spironolactone	3.254	2371649	384622	9247	1.2
Mean			2378830			

Std.dev	14958
%RSD	0.628797

%RSD for sample should be NMT 2,
The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 8: Results of repeatability for Torsemide

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Torsemide	5.441	198464	7291	6274	1.1
2	Torsemide	5.442	193643	7219	6592	1.1
3	Torsemide	5.409	196462	7194	6028	1.1
4	Torsemide	5.520	194644	8174	6927	1.1
5	Torsemide	5.424	198464	8653	5920	1.1
Mean			196335.4			
Std.dev			2190.191			
%RSD			1.115536			

Intermediate precision

Table 9: Results of Intermediate precision for Spironolactone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Spironolactone	3.211	2389572	395275	9375	1.2
2	Spironolactone	3.211	2391847	392175	9275	1.2
3	Spironolactone	3.210	2319472	312947	8265	1.2
4	Spironolactone	3.212	2306842	310585	6254	1.2
5	Spironolactone	3.211	2375972	310694	9028	1.2
6	Spironolactone	3.297	2396746	358373	8928	1.2
Mean			2363409			
Std. Dev.			39730.83			
% RSD			1.681082			

%RSD of six different sample solutions should not more than 2

Table 10: Results of Intermediate precision for Torsemide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Torsemide	5.411	197284	7194	8264	1.2
2	Torsemide	5.410	197849	7294	9174	1.2
3	Torsemide	5.420	196572	7147	9164	1.2
4	Torsemide	5.423	195028	7927	9733	1.2
5	Torsemide	5.419	199474	8238	9194	1.2
6	Torsemide	5.409	197482	7638	8973	1.2
Mean			197281.5			
Std. Dev.			1466.354			
% RSD			0.74328			

%RSD of six different sample solutions should not more than 2

Day 2

Table 11: Results of Intermediate precision Day 2 for Spironolactone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Spironolactone	3.211	2389562	391741	9264	1.2
2	Spironolactone	3.233	2381654	391047	9746	1.2
3	Spironolactone	3.244	2381946	391748	9816	1.2
4	Spironolactone	3.297	2391741	391746	9917	1.2
5	Spironolactone	3.297	2386452	381641	9742	1.2
6	Spironolactone	3.202	2374763	381645	9017	1.2
Mean			2384353			
Std. Dev.			6183.339			
% RSD			0.25933			

%RSD of six different sample solutions should not more than 2

Table 12: Results of Intermediate precision Day 2 for Torsemide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Torsemide	5.411	197486	7582	6272	1.1
2	Torsemide	5.410	197486	7184	6174	1.1
3	Torsemide	5.420	196746	7456	5184	1.1
4	Torsemide	5.405	195862	7814	6194	1.1
5	Torsemide	5.409	196582	7194	6292	1.1
6	Torsemide	5.463	198463	7745	6191	1.1
Mean			197104.2			
Std. Dev.			903.542			
% RSD			0.458408			

%RSD of six different sample solutions should not more than 2

ACCURACY

The accuracy results for Spironolactone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1217218	112.5	112.4	99.6	99.3
100%	2397141	225	225	100	
150%	3514547	337.5	332.5	98.5	

The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

The accuracy results for Torsemide

%Concentration (at specification Level)	Area	Amount dded (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	98598.67	22.5	22.4	99.9	99.6
100%	198359.7	45	45	100	
150%	291512.3	67.5	66.8	99	

The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness
Spironolactone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	2391746	3.202	9028	1.2
Less Flow rate of 0.9mL/min	2371831	3.639	7381	1.2
More Flow rate of 1.1mL/min	2218319	2.859	9311	1.1
Less organic phase (about 5 % decrease in organic phase)	2294821	3.460	7462	1.2
More organic phase (about 5 % Increase in organic phase)	2394811	3.022	6817	1.1

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 13: Results for Robustness

Torsemide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.1mL/min	194627	5.463	7398	1.1
Less Flow rate of 0.9mL/min	183738	6.250	6883	1.1
More Flow rate of 0.8mL/min	198373	4.863	9917	1.2
Less organic phase (about 5 % decrease in organic phase)	178471	6.196	8372	1.1
More organic phase (about 5 % Increase in organic phase)	189462	5.010	7716	1.2

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

The developed HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied for the analysis of pure drug and pharmaceutical dosage forms. From the present study it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2A/B.

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