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Development and validation of analytical method for simultaneous estimation of econazole and triamcinolone by rp- hplc

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ABSTRACT

A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Econazole and Triamcinolone in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Phosphate Buffer (pH-4.2) (37:63% v/v)as the mobile phase at a flow rate of 1.0ml/min, thedetection was carried out at 275nm. The retention time of the Econazole and Triamcinolonewas found to be was 2.133, 3.692±0.02min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Econazole and 10-30mg/ml of Triamcinolone.The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Econazole and Triamcinolone, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

Method development

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use" [1-2]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these show unique selectivity. What is needed is a more informed

decision making process for column selection that may beusedbefore the chromatographer enters the laboratory. The method of column selection presentedhere involves a minimal investment in time initially, with the potential of saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [3, 4].

The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization[5]. The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [6-8].

The degraded drug samples obtained are subjected to preliminary chromatographic separation to study the number and types of degradation products formed under various conditions [9]. Scouting experiments are run and then conditions are chosen for further optimization [10]. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development [11]. Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds. Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time. However, by operating at pH extremes (2-4 or 8-10), not only is there a 10-30 fold difference in retention time that can be exploited in method development but also the method can be made more robust which is a desirable outcome with validation in minutes [12,13].

MATERIALS AND METHODS

Econazole from Sura labs, Triamcinolone from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK).Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

Hplc method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Econozole & Triamcinolone 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 0.1ml of the above Econozole and 0.3ml of the Triamcinolone 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 and Water: Acetonitrile and Methanol:Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Bufferin proportion 45:55 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDADetector 996 model.
Temperature : 35ºC
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.
pH : 4.6
Mobile phase :Acetonitrile: Phosphate Buffer(45:55 v/v)
Flow rate : 1ml/min
Wavelength : 245 nm
Injection volume : 10 µl
Run time : 7 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of mobile phase

Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital

ultra sonicator for 15 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

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)
Column : Phenomenex Luna C18 (4.6mm×250 mm) 5µm particle size
Column temperature : 35ºC
Wavelength : 275nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

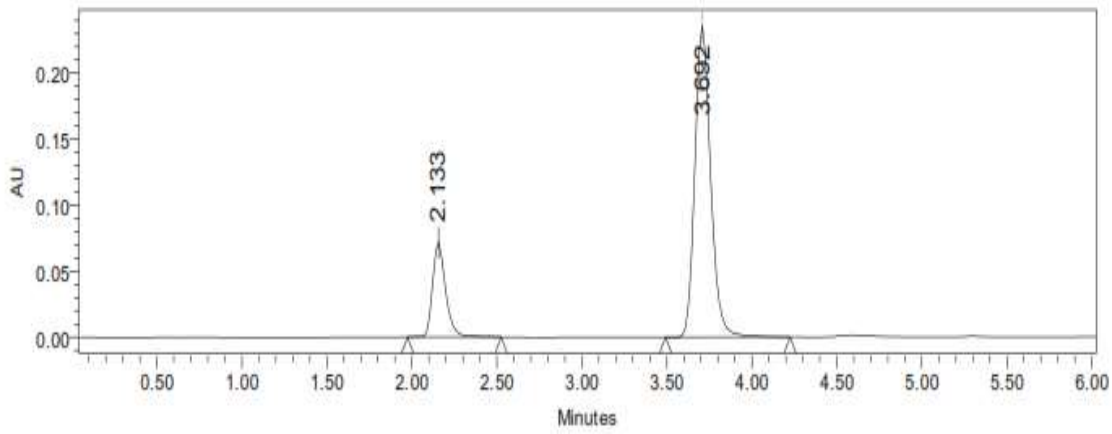

Fig 1: Optimized Chromatogram (Standard)

Table 1:Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate	Resolution
1	Triamcinolone	2.133	526389	86756	1.56	5679	
2	Econazole	3.692	1687285	367532	1.79	8685	9.8

Optimized Chromatogram (Sample)

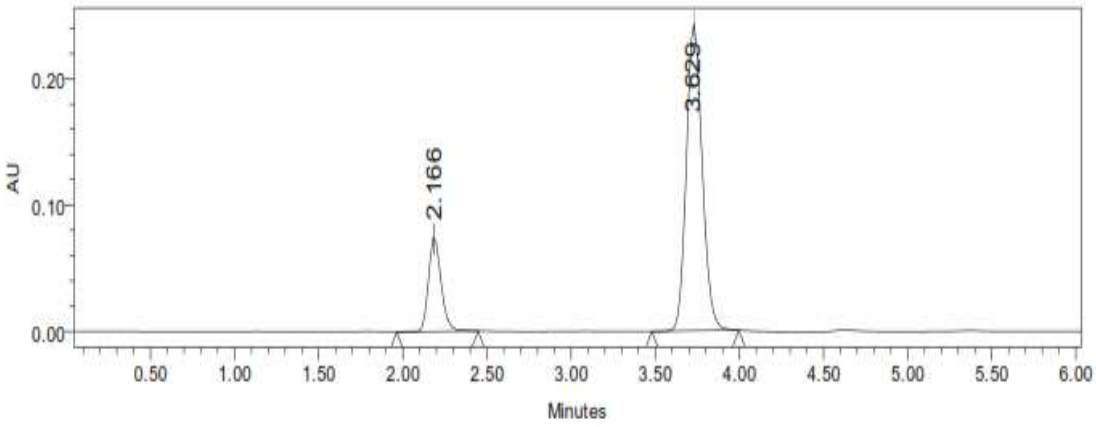

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate	Resolution
1	Triamcinolone	2.166	536587	77464	1.57	5789	
2	Econazole	3.629	1695846	378564	1.80	8795	10.01

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Sample)

Table 3: Peak results for Assay sample of Triamcinolone

S.No	Name	RT	Area	Height	USP	USP Plate	Injection
1	Triamcinolone	2.152	536859	87584	1.58	5789	1
2	Triamcinolone	2.150	532654	87965	1.59	5784	2
3	Triamcinolone	2.187	532685	87465	1.58	5769	3

Table 4: Peak results for Assay sample of Econazole

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Econazole	3.646	1698568	378562	1.81	8759	1
2	Econazole	3.651	1698574	375847	1.80	8795	2
3	Econazole	3.601	1698547	376584	1.81	8745	3

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

= 99.89%

The % purity of Econazole & Triamcinolone in pharmaceutical dosage form was found to be 99.89%

Linearity

Table 5: Chromatographic Data for Linearity Study of Triamcinolone

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

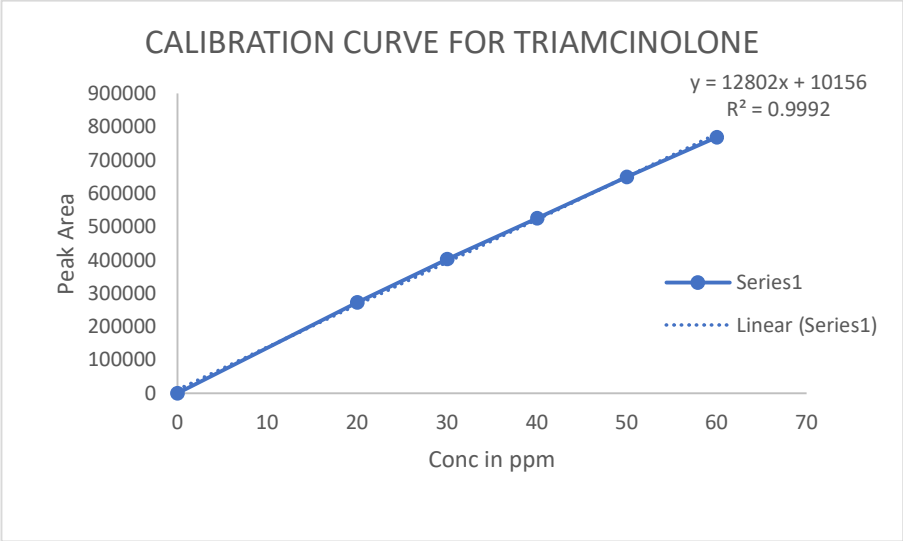

Fig 3: Calibration Curve of Triamcinolone

Chromatographic data for linearity study of econazole

Table 6: Chromatographic Data for Linearity Study of Econazole

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

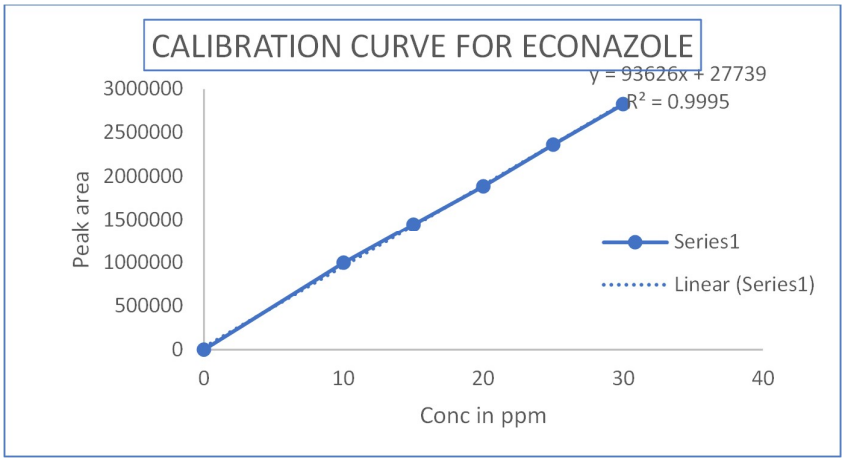

Fig 4: Calibration Curve of Econazole

Repeatability

Table 7: Results of repeatability for Triamcinolone

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Triamcinolone	2.157	526358	86598	5689	1.56
2	Triamcinolone	2.159	524856	86542	5687	1.57
3	Triamcinolone	2.186	526985	86578	5684	1.56
4	Triamcinolone	2.160	528654	86354	5689	1.56

5	Triamcinolone	2.170	528457	86958	5639	1.56
Mean			527062			
Std.dev			1569.114			
%RSD			0.297709			

- *%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 Econazole

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Econazole	3.603	1687589	367859	8659	1.79
2	Econazole	3.608	1685987	368547	8679	1.80
3	Econazole	3.600	1685987	367985	8645	1.80
4	Econazole	3.696	1685754	365874	8695	1.79
5	Econazole	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std.Dev			749.493			
%RSD			0.044447			

Intermediate precision

Table 9: Results of Intermediate precision day 1 for Triamcinolone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Triamcinolone	2.198	546585	87589	5898	1.58	100%
2	Triamcinolone	2.196	548758	87985	5879	1.59	100%
3	Triamcinolone	2.160	549854	87452	5868	1.58	100%
4	Triamcinolone	2.160	548798	87421	5847	1.59	100%
5	Triamcinolone	2.160	542659	87963	5896	1.58	100%
6	Triamcinolone	2.186	548754	87254	5874	1.59	100%
Mean			547568				
Std.			2631.576				
% RSD			0.480593				

- *%RSD of five different sample solutions should not more than 2*

Table 10: Results of Intermediate precision day 2 for Econazole

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Econazole	3.623	1698587	385482	8789	1.81	9.8	98%
2	Econazole	3.611	1698574	385698	8759	1.80	9.8	98.2%
3	Econazole	3.696	1698532	385748	8754	1.81	9.9	98.7%
4	Econazole	3.696	1698574	386958	8754	1.81	10.01	99.7%
5	Econazole	3.696	1698532	385755	5798	1.80	9.98	98.5%
6	Econazole	3.642	1698547	386558	8762	1.80	10.02	98.2%
Mean			1698558					
Std. Dev.			23.77113					
% RSD			0.001399					

- *%RSD of five different sample solutions should not more than 2*

Table 11: Results of Intermediate precision Day 2 for Triamcinolone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Triamcinolone	2.198	536854	8758	5789	1.58

2	Triamcinolone	2.196	536985	8795	5726	1.59
3	Triamcinolone	2.178	536587	8746	5742	1.58
4	Triamcinolone	2.142	532546	8754	5746	1.59
5	Triamcinolone	2.177	534587	8725	5798	1.58
6	Triamcinolone	2.177	538598	8726	5785	1.59
Mean			536026.2			
Std. Dev.			2131.492			
% RSD			0.397647			

- *%RSD of five different sample solutions should not more than 2.*

Table 12: Results of Intermediate precision Day 2 for Econazole

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Econazole	3.611	1678598	356875	8875	1.82	9.9
2	Econazole	3.623	1678985	358985	8856	1.83	10.01
3	Econazole	3.684	1678984	358754	8862	1.82	9.9
4	Econazole	3.697	1678985	352412	8849	1.83	10.01
5	Econazole	3.684	1678549	358987	8873	1.82	9.9
6	Econazole	3.684	1678984	358986	8842	1.83	10.01
Mean			1678848				
Std. Dev.			212.8048				
% RSD			0.012676				

- *%RSD of five different sample solutions should not more than 2.*

Accuracy

Table 13: The accuracy results for Triamcinolone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

The percentage recovery was found to be within the limit (98-102%).

Table 14: The accuracy results for Econazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 15: The robustness results for TRIAMCINOLONE

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52
Less organic phase	516854	2.200	5163	1.57

More Organic phase	506898	2.172	5098	1.51

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

Table 16: The robustness results for ECONAZOLE

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

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

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Econazole and Triamcinolonein bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Econazole was found to be freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane.Triamcinolone (hydrochloride) was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide,soluble in water.

Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Econazole and Triamcinolonein bulk drug and in Pharmaceutical dosage forms.

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