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Research

Development And Validation Of Analytical Method For Simultaneous Estimation Of Econazole And Triamcinolone By Rp- Hplc

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Abstract

A Rapid and Precise Гeverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Econozole & Triamcinolone, in its pure form as well as in tablet dosage form. Chromatography was carried out on X-Terra C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 212 nm. The retention time of the Econozole and Triamcinolone was 2.090, 5.289 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Econozole and 45-225mg/ml of Triamcinolone. 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: Econozole, Triamcinolone, ГР-HPLC, validation.

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.
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 [12-14].

Requirements for good method development
Choosing the appropriate HPLC column

C18 columns are the commonly used columns in HPLC method analysis. C8 or Octyl bonded phases are also used occasionally. Like C18, they are non-polar, but not as hydrophobic. Therefore, retention times for hydrophobic compounds are typically shorter. Also, they may show somewhat different selectivity than C18 due to increased base silica exposure unique selectivity results in proton interaction of the bonded phase with electron deficient functional groups of solute molecules.

Retention is a mixed mechanism, resulting from both hydrophobic interactions and dipole interactions of the bonded phase C□N group with solute amino groups or p - p interactions with sites of unsaturation. It is the best for polar organic compounds and is versatile enough for use in both normal and reversed phase modes.
Each bonded phase has unique selectivity for certain sample types. For example: to separate toluene and ethyl benzene (differ by only one -CH2- unit), we would choose a C18 bonded phase. Further, we would want to narrow the decision to a particular packing material that shows good or excellent retention of such hydrophobic compounds (i.e. high % carbon load) to be able to maximize the particular separation. The effects of surface area and carbon load are discussed in the next section. The stationary phase must be able to "hold on" to the two compounds long enough to resolve them by differential migration.

Separation of many samples can be enhanced by selecting the right column temperature. Higher column temperature reduces system backpressure by decreasing mobile phase viscosity, which in turn allows use of longer columns with higher separation efficiency. However, an overall loss of resolution between mixture components in many samples occurs by increasing column temperature. The optimum temperature is dependent upon the nature of the mixture components. The overall separation can be improved by the simultaneous changes in column temperature and mobile phase composition [15-17].

Recently, normal phase HPLC is back popular with the birth of hydrophilic interaction liquid chromatography (HILIC) technology that proved to improve reproducibility in separating polar and hydrophilic compounds such as peptides, carbohydrates, vitamins, polar drugs and metabolites. In order to develop a HPLC method effectively, most of the effort should be spent in method development and optimization as this will improve the final method performance.

MATERIALS AND METHODS

Econazole /Triamcinolone-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Potassium Dihydrogen Phosphate-Finar Chemicals.

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 Buffer in 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 PDA Detector 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 ultra sonication.
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	:	Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20)
Column	:	X-Terra C18 (4.6×150mm, 5.0 µm)
Flow rate	:	1 ml/min
Wavelength	:	212 nm
Column temp	:	Ambient
Injection Volume	:	10 µl
Run time	:	10 minutes

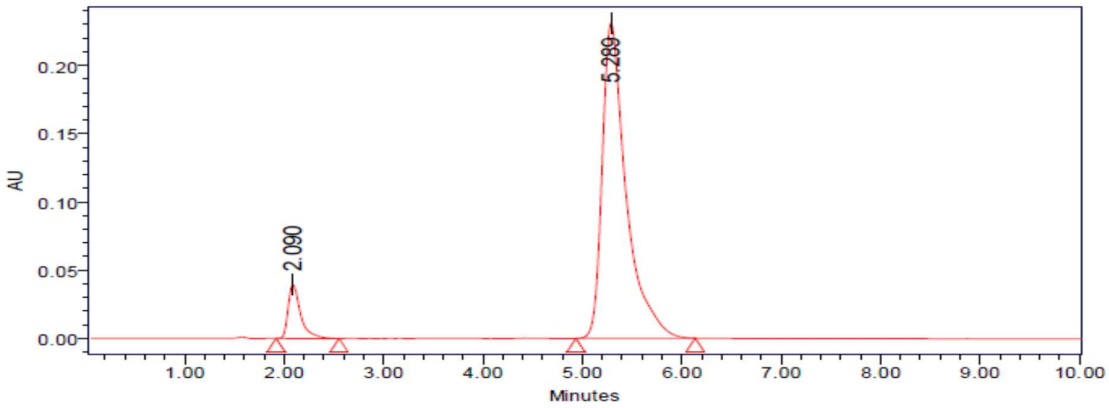

Fig 1: Optimized Chromatogram

Table 1: peak Results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Econozole	2.090	372127	39691		1.71	5588
2	Triamcinolone	5.289	3864999	231195	9.81	1.78	5699

From the above chromatogram it was observed that the Econozole & Triamcinolone peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogeram (Sample)

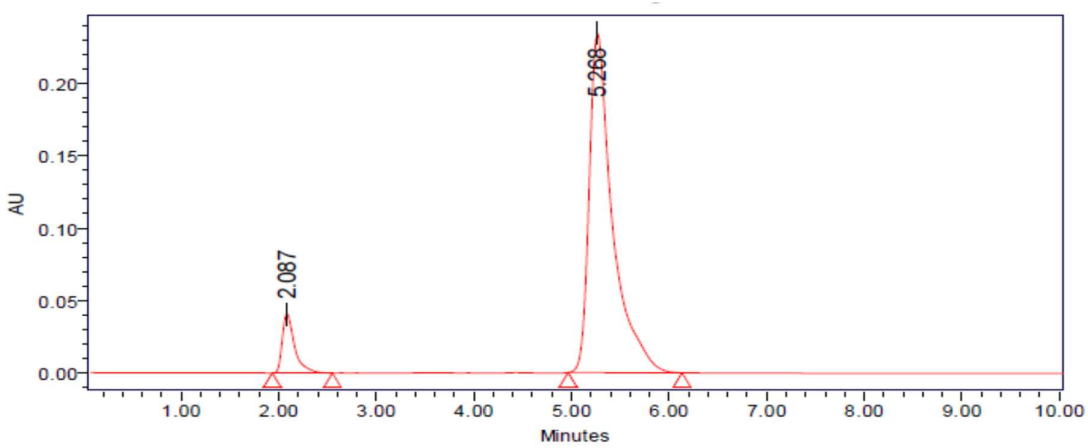

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Econozole	2.087	356548	41156		1.73	5556
2	Triamcinolone	5.268	3896494	234962	9.83	1.92	5805

- *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 wer within the limit.*

System suitability

Table 3: Results of system suitability for Econozole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Econozole	2.090	342127	39691	5464	1.42
2	Econozole	2.090	342425	39692	5577	1.42
3	Econozole	2.089	342563	39991	5099	1.44
4	Econozole	2.089	347977	40397	5144	1.43
5	Econozole	2.085	352915	40964	5675	1.47
Mean			345601.4			
Std. Dev			4757.233			
% RSD			1.376509			

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

Table 4: Results of system suitability for Econozole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Triamcinolone	5.289	3864999	231195	5787	1.46	9.80

2	Triamcinolone	5.289	3864997	232183	5909	1.47	9.81
3	Triamcinolone	5.338	3881444	231045	5488	1.48	9.81
4	Triamcinolone	5.327	3896953	231968	5033	1.40	9.83
5	Triamcinolone	5.262	3900104	233542	5388	1.43	9.82
Mean			3881699				
Std.Dev			16802.83				
% RSD			0.432873				

- *%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.*

Assay (Standard)

Table 5: Peak results for assay standard

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Econozole	2.090	348127	39691		1.70	5588	1
2	Triamcinolone	5.289	3864999	231195	9.81	1.77	5629	1
3	Econozole	2.089	352565	39991		1.66	5572	2
4	Triamcinolone	5.338	3881444	231045	9.92	1.83	5689	2
5	Econozole	2.089	357977	40397		1.68	5531	3
6	Triamcinolone	5.327	3896953	231968	9.91	1.86	5713	3

Assay (Sample)

Table 6: Peak Results for Assay sample

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Econozole	2.088	352291	40268		1.69	5517	1
2	Triamcinolone	5.276	3883795	231355	9.75	1.89	5678	1
3	Econozole	2.087	356548	41158		1.72	5556	2
4	Triamcinolone	5.268	3896494	234962	9.82	1.91	5805	2
5	Econozole	2.085	358915	40964		1.75	5488	3
6	Triamcinolone	5.262	3900104	233542	9.78	1.95	5791	3

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

The % purity of Econozole and Triamcinolone in pharmaceutical dosage form was found to be100.5%.

Linearity

Chromatographic data for linearity study

Econozole

Concentration Level (%)	Average Peak Area
6	134437
8	245572
10	371549
12	499025
14	619831

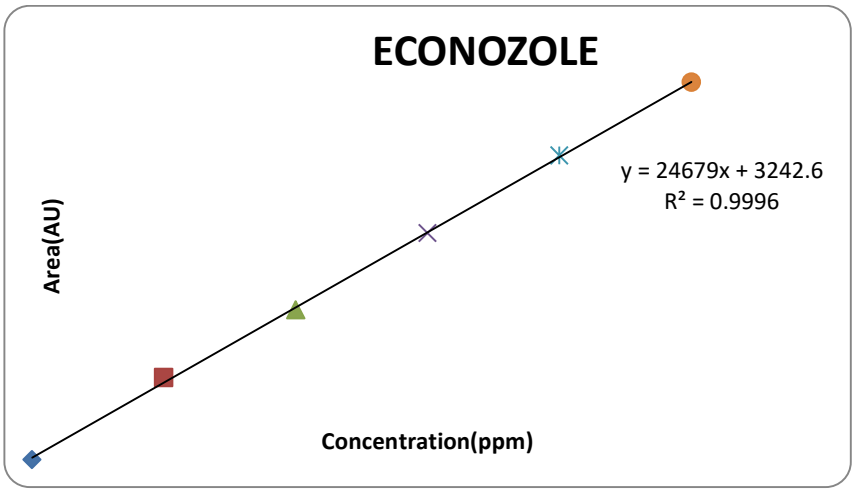

Fig 3: calibration graph for Econozole

Triamcinolone

Concentration Level (%)	Average Peak Area
18	1330055
24	2728975
30	3917064
36	5300023
42	6412696

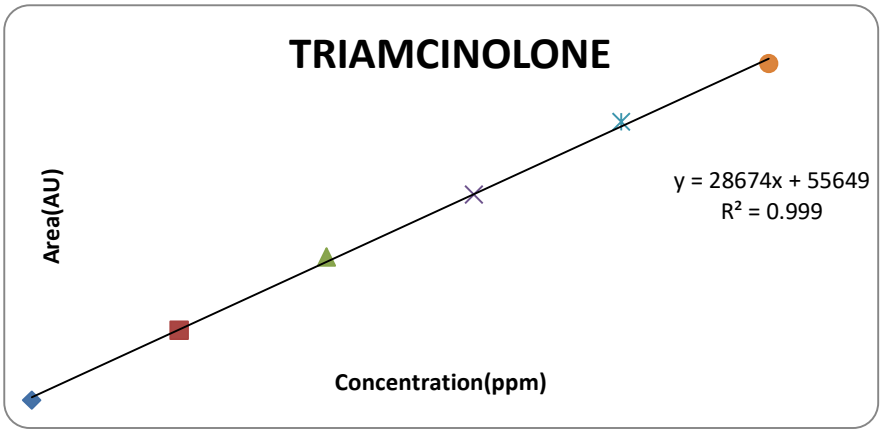

Fig 4: calibration graph for Triamcinolone

Precision
Repeatability

Table 7: Results of repeatability for Econozole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Econozole	2.086	362267	41698	5082.3	1.8
2	Econozole	2.083	364903	41403	5145.1	1.8
3	Econozole	2.083	366871	41541	5119.1	1.8
4	Econozole	2.081	367274	42257	5148.3	1.8
5	Econozole	2.081	368102	42144	5102.8	1.8
Mean			365883.4			

Std.Dev	2338.314
% RSD	0.639087

- *%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 method precession for Triamcinolone

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Triamcinolone	5.178	3903549	240180	5989.3	2.1	9.8
2	Triamcinolone	5.199	3905818	235524	5857.3	2.0	9.7
3	Triamcinolone	5.235	3916121	238579	5931.2	2.0	9.9
4	Triamcinolone	5.202	3916543	238815	5937.9	2.0	9.8
5	Triamcinolone	5.206	3920944	241007	5041.0	2.0	9.5
Mean			3912595				
Std.Dev			7508.046				
% RSD			0.191894				

- *%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.*

Intermediate precision
Day 1

Table 9: Results of Intermediate precision for Econozole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Econozole	2.083	369247	42278	5538.8	1.6
2	Econozole	2.083	370767	42709	5562.8	1.6
3	Econozole	2.089	370841	42066	5488.3	1.6
4	Econozole	2.083	370842	42067	5490.3	1.6
5	Econozole	2.082	371043	42569	5584.2	1.8
6	Econozole	2.080	371387	42212	5534.2	1.8
Mean			370687.5			
Std. Dev			740.7368			
% RSD			0.18			

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

Table 10: Results of Intermediate precision for Triamcinolone

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Triamcinolone	5.229	3743004	242956	5268.7	2.2	10.2
2	Triamcinolone	5.203	3845358	242254	5101.5	2.1	10.0
3	Triamcinolone	5.133	3885015	242853	5128.6	2.1	10.0
4	Triamcinolone	5.229	3743004	242957	5268.7	2.2	10.2
5	Triamcinolone	5.151	3722514	240345	5049.8	1.5	9.9
6	Triamcinolone	5.112	3728788	237639	5998.2	1.6	9.9
Mean			3777948				
Std. Dev			69193.4				
% RSD			1.9				

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

Day 2

Table 11: Results of Intermediate precision Day 2 foR Econozole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Econozole	2.078	370978	42979	3084.0	1.9
2	Econozole	2.082	371042	42569	3584.2	1.8
3	Econozole	2.080	371387	42212	3532.2	1.8

4	Econozole	2.089	369247	42278	1538.8	1.6
5	Econozole	2.083	370841	42066	1488.3	1.6
6	Econozole	2.089	369247	42278	1536.8	1.6
Mean			370457.4			
Std.Dev			954.6006			
% RSD			0.27			

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

Table 12: Results of Intermediate precision for Triamcinolone

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Triamcinolone	5.077	3841405	246819	5209.0	2.1	10.1
2	Triamcinolone	5.151	3885013	242855	5128.6	2.1	10.0
3	Triamcinolone	5.112	3743002	242956	5268.7	2.2	10.2
4	Triamcinolone	5.133	3743007	242954	5268.7	2.2	10.2
5	Triamcinolone	5.203	3885015	242853	5126.6	2.1	10.0
6	Triamcinolone	5.133	3743004	242956	5268.7	2.2	10.2
Mean			3806741				
Std.Dev			71613.48				
% RSD			1.9				

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

Accuracy

The accuracy results for Econozole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	192447.6	7.6	7.3	98.7	
100%	374223	16	13.8	98.67	98.7%
150%	555892.3	21.5	22.4	99.2	

The accuracy results for Triamcinolone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2001753	67.6	67.4	99.7	
100%	3927798	136	134.9	99.8	99.7%
150%	5858666	203.5	202.2	99.8	

- *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

Table 13: Results for robustness

Econozole

Parameter used for sample analysis	Peak Area	Retention Time	Theotetical plates	Tailing factor
Actual Flow tate of 1.0 mL/min	372127	2.090	5588	1.70
Less Flow rate of 0.9 mL/min	356766	2.736	5433	1.82
MoRe Flow rate of 1.1 mL/min	342357	1.673	5645	1.91
Less organic phase	312435	2.736	5099	1.82
More organic phase	305624	1.673	5124	1.91

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

Triamcinolone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3864999	5.289	5699	1.77

Less Flow rate of 0.9 mL/min	3546738	6.746	5547	1.88
MoRe Flow rate of 1.1 mL/min	3857217	4.032	5123	1.91
Less organic phase	3810346	6.746	5035	1.88
More organic phase	3875643	4.032	5613	1.91

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 ГР-HPLC method was developed for the quantitative estimation of Econozole & Triamcinolone in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Artemether and Lumefantrine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) 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 Econozole & Triamcinolone Drug and in Pharmaceutical dosage forms.

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