International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH |Publisher: Pharma Research Library
Journal Home Page: http://www.pharmaresearchlibrary.com/ijcps
DOI: https://doi.org/10.30904/j.ijcps.2025.4842
Int. J. of Chem. Pharm. Sci., 13(1), 2025: 43-51

Development and Validation of analytical method for simultaneous estimation of Econazole and Triamcinolone by RP- HPLC

Ch. Suresh*[1], Abdul Sameer[2], Madeha Nazneen[3], Nashara Buthul[2], Ramarthi Harthika[2]
[1]Professor and Principal, Department of Pharmaceutical Chemistry, Vijay College of Pharmacy, Nizamabad–503003, Telangana, India
[2]B.Pharm student, Vijay College of Pharmacy, Nizamabad–503003, Telangana, India

Abstract
A Rapid and Precise Reverse 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 (4.6x150mm, 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, RP-HPLC, validation

A R T I C L E I N F O

Corresponding Author:
Ch. Suresh
Professor and Principal
Department of Pharmaceutical Chemistry
Vijay College of Pharmacy, Nizamabad–503003, India

Article History:
Received : 29 April 2025
Revised : 24 May 2025
Accepted : 19 June 2025
Published : 14 July 2025

Citation: Ch. Suresh, *et al* (2025) Development and Validation of analytical method for simultaneous estimation of Econazole and Triamcinolone by RP- HPLC. Int. J. of Chem. and Pharm. Sci., 13(1), 43-51.

Contents:

1. 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.

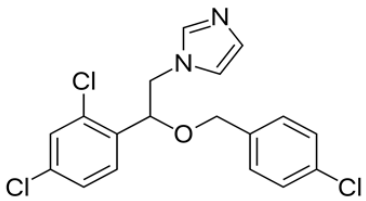

Fig.1: Econazole

Drug category: 14-alpha Demethylase Inhibitors Anti-Infective Agents Antifungal Agents Antifungal Agents (Vaginal) Antifungals for Dermatological Use Antifungals for Topical Use Azole Antifungals
Chemical name/ Nomenclature / IUPAC Name : 1-{2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl}-1H-imidazole
Molecular Formula: $C_{18}H_{15}Cl_3N_2O$
Molecular Weight : Average:381.684, Monoisotopic: 380.024996233

Physicochemical properties:
Description (Physical State): Solid
Dosage: Capsule
Melting point: 162 ºC
PKa: 6.48
Log P: 5.35

Pharmacokinetic properties: After topical application to the skin of normal subjects, systemic absorption of econazole nitrate is extremely low. Although most of the

applied drug remains on the skin surface, drug concentrations were found in the stratum corneum which, by far, exceeded the minimum inhibitory concentration for dermatophytes.

Metabolism: Hepatic.

Adverse effects: Improve treatment outcomes and reduce medical errors with our comprehensive and structured data on drug adverse effects. MeDRA and ICD10 IDs are provided for adverse effect conditions and symptoms.

Pharmacodynamics: Econazole is an antifungal medication related to fluconazole (Diflucan), ketoconazole (Nizoral), itraconazole (Sporanox), and clotrimazole (Lotrimin, Mycelex). Econazole prevents fungal organisms from producing vital substances required for growth and function. This medication is effective only for infections caused by fungal organisms. It will not work for bacterial or viral infections.

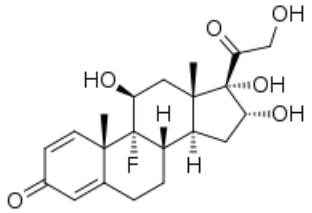

Fig.2: Triamcinolone

Drug category:Adrenal Cortex Hormones, Adrenals, Agents Causing Muscle Toxicity, Agents to Treat Airway Disease, Alimentary Tract and Metabolism, Anti-Inflammatory Agents, Corticosteroid Hormone Receptor Agonists, Corticosteroids

IUPAC Name : (1S,2R,3aS,3bS,9aS,9bR,10S,11aS)-9b-fluoro-1,2,10-trihydroxy-1-(2-hydroxyacetyl)-9a,11a-dimethyl1H,2H,3H,3aH,3bH,4H,5H,7H,9aH,9bH,10H,11H,11aH-cyclopenta[a]phenanthren-7-one

Molecular Formula : $C_{21}H_{27}FO_6$

Molecular Weight : Average: 394.4339,

Monoisotopic: 394.179166801

Physicochemical properties:

Melting point: 292° and 294°C.

PKa: 0.84

Log P: 5.35

Pharmacokinetic properties: A 16mg oral dose of triamcinolone reaches a C_{max} of 5.23±0.84ng/mL with a T_{max} of 2.24±0.78h, an AUC of 36.0±6.2ng h/mL. A 2mg intravenous dose of triamcinolone acetonide has an AUC of 57.7ng h/mL. The bioavailability of 800µg of inhaled triamcinolone acetonide is 25%, with 10.4% coming from pulmonary absorption and the rest being accounted for by deposition on the oral mucosa and other underlying factors. An inhaled dose of triamcinolone acetonide reaches a C_{max} of 0.92ng/mL with a T_{max} of 1.74h and an AUC of 5.12ng*h/mL

Adverse effects: Side effects of triamcinolone are similar to other corticoids. In short-term treatment up to ten days, it has adverse effects; however, sometimes gastrointestinal bleeding is seen, as well as acute infections (mainly viral) and impaired glucose tolerance

Pharmacodynamics: Triamcinolone is a corticosteroid with anti-inflammatory properties.8 These properties are used to treat inflammation in conditions that affect various organs and tissues.15 Triamcinolone should not be administered as an epidural injection**.**

2. Materials and Methods

Table.1: Instruments used

S.N	Instruments	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA Detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table.2: Chemicals used

S.N	Chemical	Brand names
1	Econazole /Triamcinolone	Sandoz
2	Water and Methanol for HPLC	Lichrosolv (Merck)
3	Acetonitrile for HPLC	Merck
4	Potassium Dihydrogen Phosphate	Finar Chemicals

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

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.

Validation parameters

System suitability: Accurately weigh and transfer 10 mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). 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 Diluent.

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.

Specificity study of drug:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** 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 Diluent.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Econozole and Triamcinolone sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. 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 Diluent.

Preparation of drug solutions for linearity:

Accurately weigh and transfer 10 mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (6 ppm of Econozole & 18ppm of Triamcinolone): Pipette out 0.06ml of Econozole and 0.18ml of Triamcinolone stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (8 ppm of Econozole& 24ppm of Triamcinolone): Pipette out 0.08ml of Econozole and 0.24ml of Triamcinolone stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (10 ppm of Econozole& 30ppm of Triamcinolone): Pipette out 0.1 ml of Econozole and 0.3ml of Triamcinolone stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (12 ppm of Econozole& 36ppm of Triamcinolone): Pipette out 0.12 ml of Econozole and 0.36ml of Triamcinolone stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (14 ppm of Econozole& 42ppm of Triamcinolone): Pipette out 0.14ml of Econozole and 0.42ml of Triamcinolone stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

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

Repeatability

Preparation of Econozole and Triamcinolone Product Solution for Precision: Accurately weigh and transfer 10 mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). 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 Diluent. 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.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

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.

DAY 2:

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.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.05ml of the above Econozole and 0.15ml of the Triamcinolone stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** 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 Diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.15ml of Econozole and 0.45ml of Triamcinolone from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%,100%,150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Econozole and Triamcinolone and calculate the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Econozole and 10mg of Triamcinolone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** 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 Diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (45:55), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

3. Results and Discussions

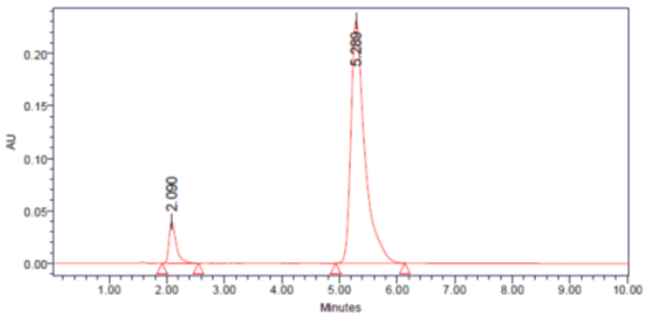

Fig.3: Optimized chromatogram (Standard)

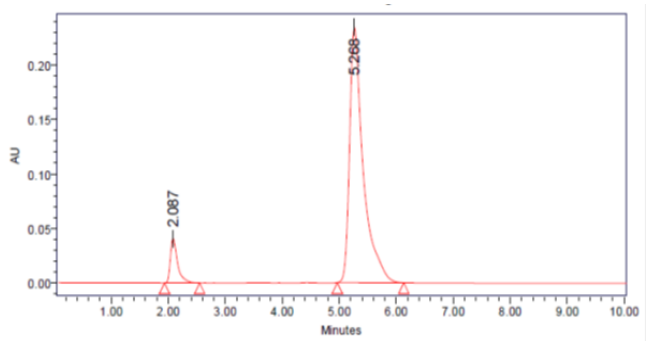

Fig.4: Optimized chromatogram (Sample)

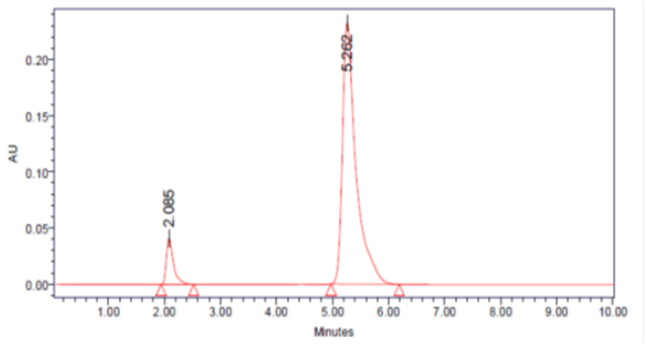

Fig.5: System suitability

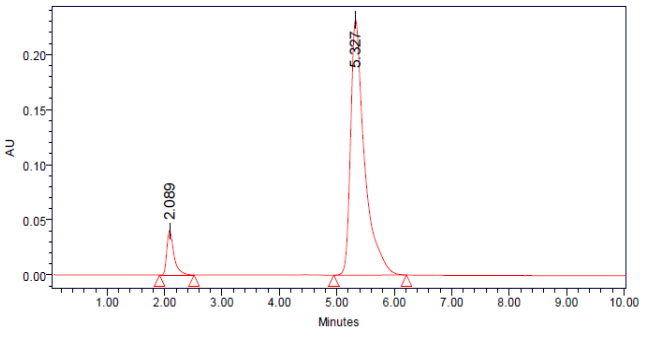

Fig.6: Chromatogram for assay Standard

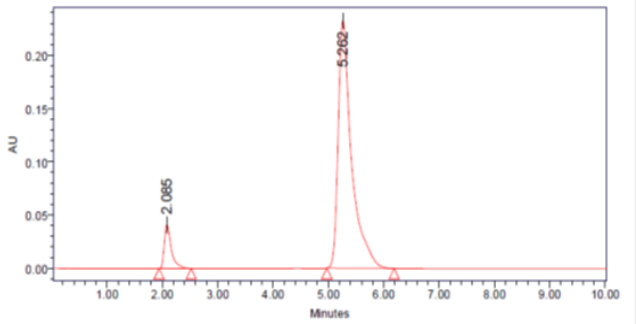

Fig.7: Chromatogram for assay Sample

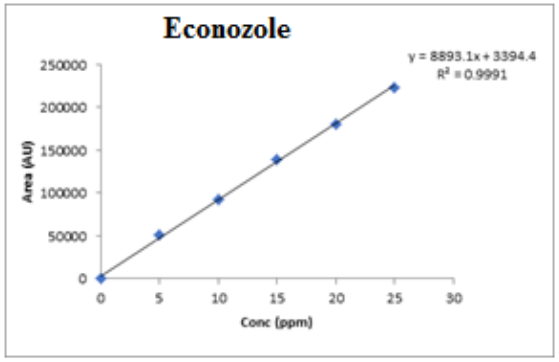

Fig.8: Calibration graph for Econozole

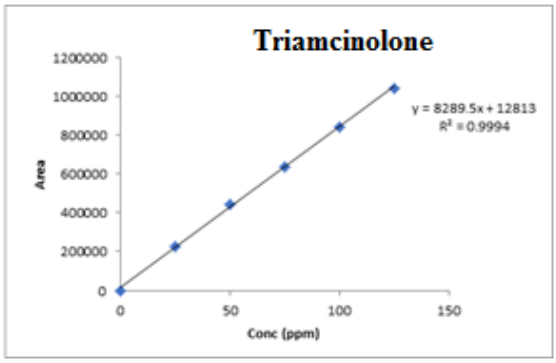

Fig.9: Calibration graph for Triamcinolone

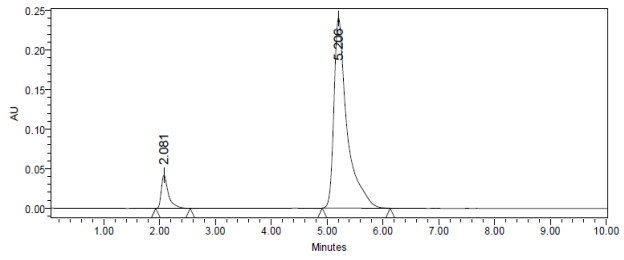

Fig.10: Calibration graph for precision

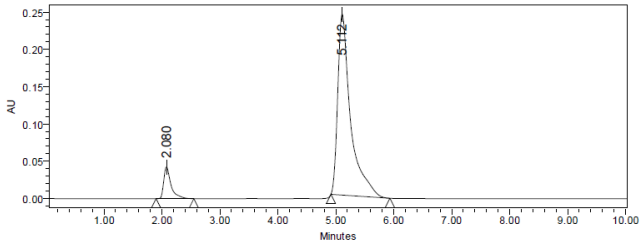

Fig.11: Calibration graph for Intermediate precision

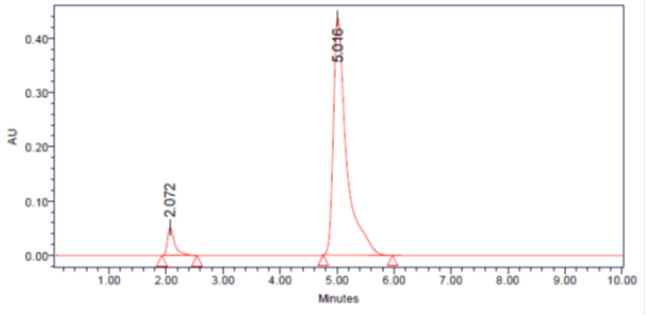

Fig.12: Accuracy-50%

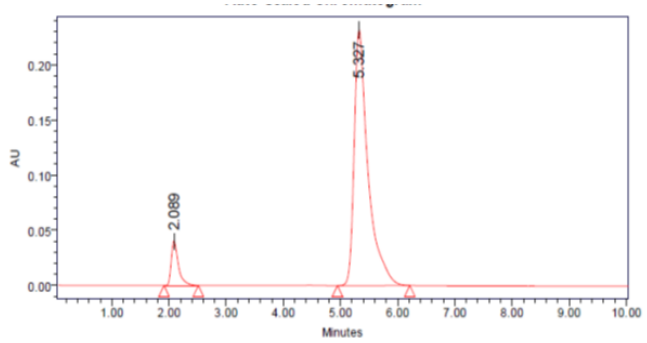

Fig.13: Accuracy-100%

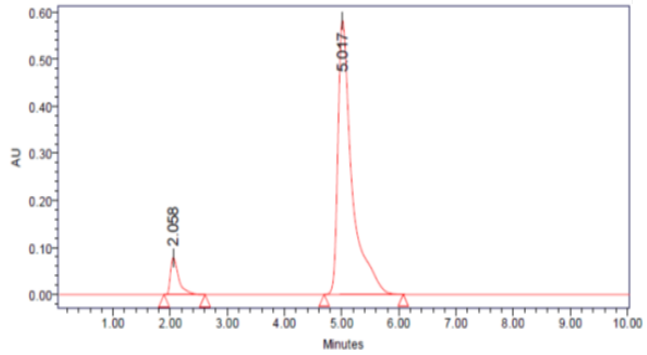

Fig.14: Accuracy-150%

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Econozole:

=3.3 × 5088/24679

=0.6µg/ml

Triamcinolone:

=3.3 × 84406/28674

=9.7µg/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result:

Econozole:

=10×5088/24679
= 2.0µg/ml

Triamcinolone:

=10 × 84406/28674
= 29.4µg/ml

Robustness:

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Econozole and Triamcinolone. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Econozole and Triamcinolone were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

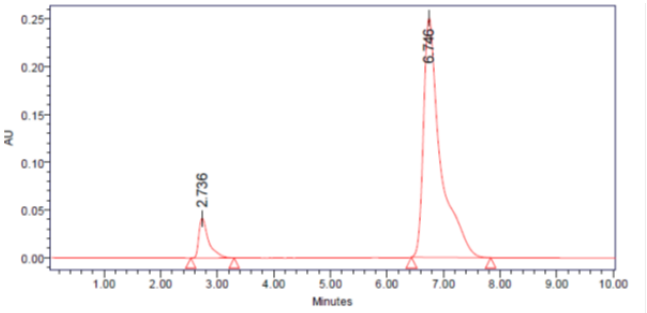

Fig.15: Chromatogram shows less flow of 0.9ml/min

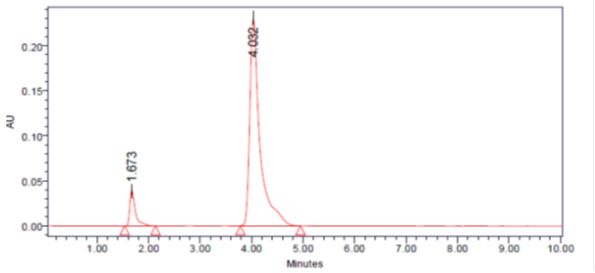

Fig.16: Chromatogram shows more flow of 1.1 ml/min

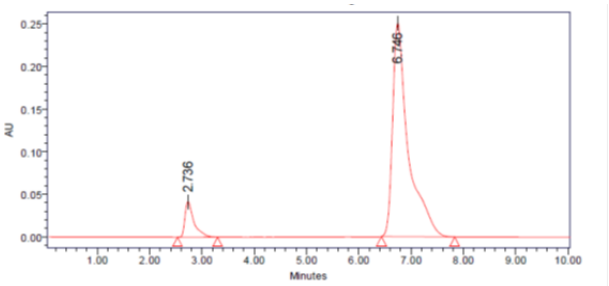

Fig.17: Showing less organic composition

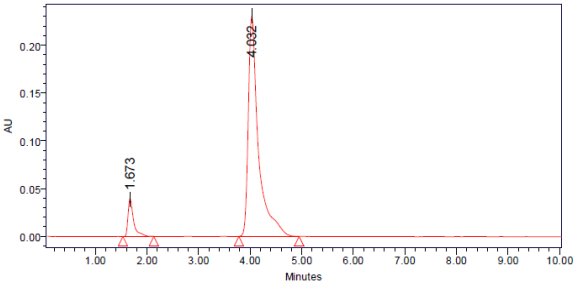

Fig.18: Showing more organic composition

Table.3: Peak Results for Assay sample

S.n	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

Table.4: 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			

Table.5: 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				

Table.6: 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			

Table.7: 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				

Table.8: 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	98.7%
100%	374223	16	13.8	98.67	
150%	555892.3	21.5	22.4	99.2	

Table.9: 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	99.7%
100%	3927798	136	134.9	99.8	
150%	5858666	203.5	202.2	99.8	

Table.10: Robustness Results for 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

Table.11: 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	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

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-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.

5. References

[1] Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.

[2] A.BraithWait and F.J.Smith, Chromatographic Me thods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.

[3] Andrea Weston and Phyllisr.Brown, HPLC Princip le and Practice,1st edition, Academic press, (1997), PP 24-37.

[4] Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.

[5] Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.

[6] D. H. Shewiy, E. Kaale, P. G. Risha, B. Dejaegher, J. S. Verbeke, Y. V. Heyden, Journal Pharmaceut. Biomed. Anal, 66, 2012, 11-23.

[7] M. D. Rockville, General Tests, Chapter 621 – Chromatography System Suitability, United States Pharmacopeial Convention (USP), USP 31, 2009.

[8] FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Centre for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER), 2000.

[9] Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modelling in HPLC method development. J Adv Res, 3, 2012, 53-63.

[10] Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. Lc Gc N. Am, 75, 2002, 49-50.

[11] Snyder LR, Kirkland JJ, Glajach JL. X. In Practical HPLC Methods Development. John Wiley, New York, 295, 1997, 643-712.

[12] Swartz M, Murphy MB. New Fronties in Chromatography. Am Lab, 37, 2005, 22-27.

[13] Dolan JW. Peak tailing and resolution. Lc Gc N. Am, 20, 2002, 430-436.

[14] Chan CC, Leo YC, Lam H. Analytical method validation and Instrument Performance Validation. Vol-I, Wiley Interscince, 2004.

[15] Doserge, Wilson and Gisvold's text book of organic medicinal and pharmaceutical chemistry, 8thed, Lippincott Company, 1982, P.183-197.

[16] Michael e. S., Ira s. K., "analytical method development and validation", Marcel Dekker, inc., New York, 1997; 25-29.

[17] Becket and stenlake, practical pharmaceutical chemistry, part 24th edition CBS publications and distributors, 2005, 157-168.

[18] Practical HPLC method development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, second edition, 1, 420-430,686-704.

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

[20] Connors K.A, "a text book of pharmaceutical analysis", Wiley-inter science, Singapore, 1999; 175.

[21] Willard-H.H., Lynnel. M. Jr., john a., dean F.A.," instrumental methods of analysis", 7th edn. CBS publishers and distributors, New Delhi; 1-12, 580-610, 614-652.

[22] Davidson A.G, "basis of Spectrophotometry", 4th ed., part-2, CBS publishers, New Delhi, 2002; 264-74.

[23] Fronka. S., Handbook of instrumental techniques for analytical chemistry, 1st Edn., Pearson education, 2004 ; 7.

[24] Kalsi P.S., "Spectroscopy of organic compounds", 5thed, new age international publishers New Delhi, 2002; 7.

[25] Braun R.d.,"Introduction to instrument analysis", Pharma book syndicate, Hyderabad, 2005; 261.

[26] Beckett, A.H., Stenlakej. B., "Practical Pharmaceutical Chemistry", (1997), 4th edition, part 2, CBS publishers and distributors; 275-337.

[27] Sethip. D., "High Performance Liquid Chromatography: Quantitative Analysis of Pharmaceutical Formulation", 2001; 1st edn; 5–11, 141.