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STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF TRICLABENDAZOLE AS API AND ESTIMATION IN TABLET DOSAGE FORM

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Abstract:
A novel, specific, accurate, rugged, precise reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Triclabendazole in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using Phenomenex Luna C18 (4.6mm x 150mm, 5µm) column with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH-2.8 with ortho phosphoric acid in the ratio of 25:75%v/v. The flow rate was 1.0 ml/min and effluent were monitored at 249 nm and a peak eluted at 3.174 min and column oven temperature was maintained ambient. Calibration curve was plotted with a range from 10- 30 µg/ml. The LOD and LOQ values of Triclabendazole were found to be 1.3µg/ml and 3.9 µg/ml respectively. The percentage recovery of the Triclabendazole was found to be within the limits. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Triclabendazole in bulk drug and in its pharmaceutical dosage form. The proposed method was applied for the analysis of tablet formulations, to improve QC and assure therapeutic efficacy.

Keywords: *Triclabendazole, RP-HPLC, Accuracy, Validation, ICH Guidelines.*

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INTRODUCTION:

Aanalytical Chemistry [1]:

Chemistry is a science that deals with the composition, structure and properties of substance and with the transformation that they undergo.

Analytical chemistry is a branch of chemistry involved with the analysis of chemical composition of natural and artificial materials. It is the measurement of science consisting of a set of powerful ideas and method that are useful in all fields of science and medicine.

Pharmaceutical analysis is a specialized branch of analytical chemistry which is involved in separating, identifying and determining the relative amounts of components in a sample of matter. Pharmaceutical analysis plays a very important role in quality assurance and quality control of bulk drugs and their formulations. Method of analysis is routinely developed improved validated collaboratively studied and applied. The discipline of analytical chemistry consists of qualitative and quantitative analysis.

Qualitative analysis Refers to analysis in which substances are identified or classified on the basis of their chemical or physical properties, such as chemical reactivity, solubility, molecular weight, melting point, radioactive properties (emission, absorption), mass spectra, nuclear half-life, etc.

Quantitative analysis in which the amount or concentration of an analyte may be determined (estimated) and expressed as a numerical value in appropriate units.

Instrumental method of chemical analysis

Instrumental method of chemical analysis interacts with all the areas of chemistry and other areas of pure and applied science.

Analytical techniques play an important role in

- Production and evaluation of new drugs in bulk and formulation and also estimation from biological fluids.
- Detection and quantification of impurities and metabolites
- Accelerated stability studies
- Invitro dissolution studies
- Pharmacokinetic studies and drug metabolism studies
- Determination of bioavailability of two or more formulation.

Method of estimation of drugs are divided into

A. Physical methods.
B. Chemical methods.
C. Physicochemical methods.

Physical method:

Physical method of analysis involves the studying of physical properties of a substance. They include determination of the solubility transparency or degree of turbidity, density or specific gravity moisture content, melting, freezing and boiling points.

Chemical methods:

The chemical methods include the gravimetric and volumetric procedures which are based on complex formation acid base precipitation and redox reactions.

Types of chemical analysis:

Proximate analysis: The amount of each element in a sample is determined with no concern as to the actual compounds present.

Partial analysis deals with the determination of selected constituent in the sample

Trace constituent analysis Concerned with the determination of specified compounds present in minute quantity

Complete analysis Proportion of each component of the sample is determined.

Physicochemical methods Physicochemical methods include

Spectrophotometric techniques:

- UV- visible spectrophotometric techniques
- Flouroscence and Phosphorescence techniques
- Atomic spectrophotometric
- Infrared spectrophotometry
- X- ray spectrophotometry
- Nuclear magnetic resonance spectroscopy
- Mass spectroscopy
- Electron spin resonance spectroscopy

Electrochemical techniques:

- Potentiometry
- Voltametry
- Electrogravimetry
- Conductometry
- Amperometry

Chromatographic techniques:

- High performance liquid chromatography
- Gas chromatography
- High performance thin layer chromatography
- Thin layer chromatography
- GC-MS
- LC-MS

Miscellaneous techniques:

- Thermal analysis
- Kinetic technique
- Electrophoresis.

Table-1: Classification of chromatographic methods

MOBILE PHASE	STATIONARY PHASE
GAS Gas Chromatography (GC)	LIQUID Gas-Liquid Chromatography (GLC)
	SOLID Gas-Solid Chromatography (GSC)
LIQUID Liquid Chromatography (LC)	LIQUID Liquid-Liquid Chromatography (LLC)
	SOLID Liquid-Solid Chromatography (LSC)

Chromatographic methods [2]:

Chromatography is the powerful techniques in which differential migration of components take place between two phases, one is stable which is known as stationary phase and another is movable which is known as a mobile phase. Species in the sample undergo repeated interactions (partitions) between the mobile phase and stationary phase. The stationary phase may be solid or a liquid (supported on a solid or a gel), and packed in a column, spread as a layer or film. The mobile phase may be gaseous or liquid. Those solutes, distributed preferentially in the mobile phase, will move rapidly through the system than those distributed preferentially in the stationary phase. This forms the basis of separation of component present in a sample. The distribution of a solute between two phases results from the balance of forces between solute molecules and the molecule of each phase. It reflects the relative attraction or repulsion that molecule or ions of the competing phase shown for the solute and for them. These forces can be polar in nature arising from permanent on induced and dipole moment. In ion exchange chromatography, the forces on the solute molecules are substantially ionic in nature but include polar and non-polar forces as well.

Chromatographic method must having essentially:

- Stationary phase
- Mobile phase
- Sample injection system
- Solvent delivery system
- Column (support for stationary phase)
- Detection by detecting agent.

All chromatographic methods involve modifications in these basic components. Chromatographic techniques are predominantly used in the pharmaceutical industry for a large variety of samples.

MATERIALS AND METHODS:

Atazanavir from Sura labs, Ritonavir 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 Triclabendazole 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 2ml of the above Triclabendazole 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.

Optimized chromatographic conditions:
Instrument used : Waters HPLC with auto sampler and PDA 996 detector model.
Temperature : Ambient
Column : Phenomenex Luna C18 (4.6mm x 150mm, 5µm)
Mobile phase : Acetonitrile: Phosphate Buffer (pH-2.8) (25:75% v/v)
Flow rate : 1.0mL/min
Wavelength : 220 nm
Injection volume : 10 µl
Run time : 8 minutes

Validation:

Preparation of mobile phase:
Preparation of mobile phase:
Accurately measured 250 ml of Acetonitrile (25%) and 750 ml (75%) Phosphate Buffer (pH-2.8) were mixed and degassed in a digital ultra sonicater 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)
Column : Phenomenex Luna C18 (4.6mm x 150mm, 5µm)
Column temperature : Ambient
Wavelength : 249 nm
Mobile phase ratio : Acetonitrile: Phosphate Buffer (Ph-2.8) (25:75% v/v)
Flow rate : 1.0mL/min
Injection volume : 10 µl
Run time : 8 minutes

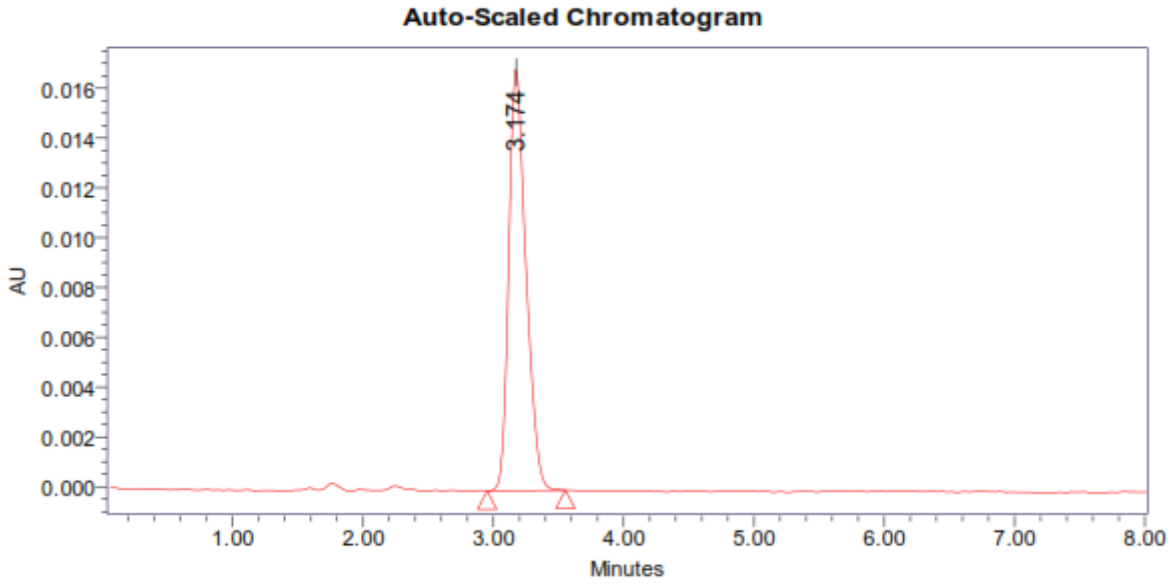

Figure-1: Optimized Chromatogram (Standard)

Table-2: Peak results for Optimized Chromatogram (Standard)

S.No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Triclabendazole	3.174	856985	69854	1.25	8547

Observation:
This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all system suitability parameters. So, it's optimized chromatogram.
Retention time of Dabrafenib–2.808min

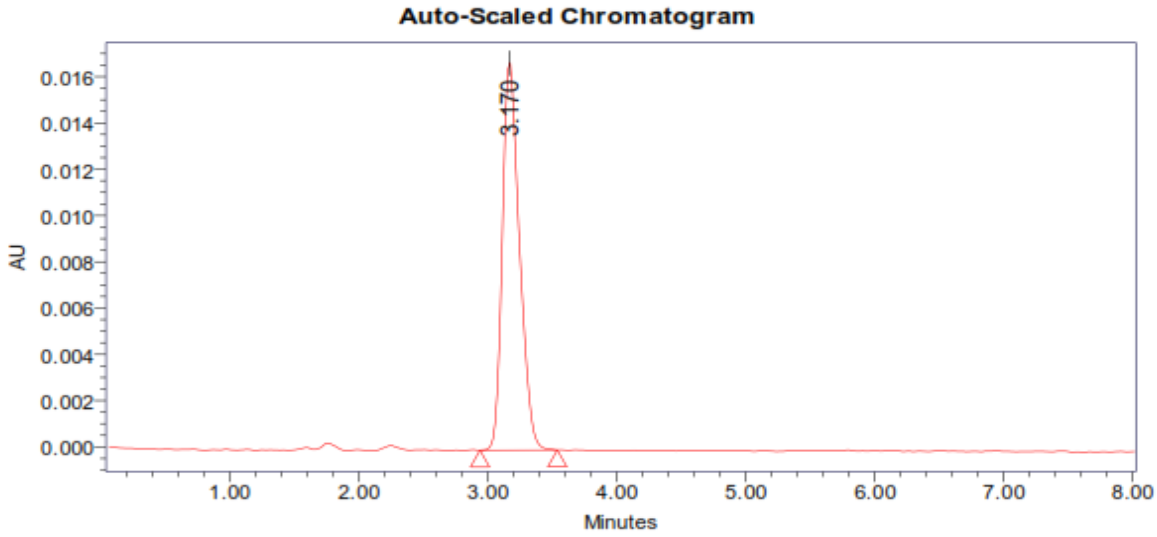

Figure-2: Optimized Chromatogram (Sample)

Table-3: Results of Optimized Chromatogram (Sample)

S.No.	Name	Retention time (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Triclabendazole	3.170	865845	69857	1.26	8659

Acceptance criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.

Assay (Standard):

Table-4: Results of Assay (Standard) for Triclabendazole

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Triclabendazole	3.170	866854	70152	8659	1.26
2	Triclabendazole	3.174	868478	69987	8657	1.27
3	Triclabendazole	3.170	865987	70154	8654	1.26
4	Triclabendazole	3.157	865896	69985	8659	1.27
5	Triclabendazole	3.153	859864	69587	8674	1.27
Mean			865415.8			
Std. Dev.			3272.034			
% RSD			0.378088			

Acceptance Criteria:

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

Assay (Sample):

Table-5: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Triclabendazole	3.155	875845	70025	1.28	8659	1
2	Triclabendazole	3.155	876584	70066	1.27	8696	2
3	Triclabendazole	3.155	874598	69989	1.28	8785	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 Triclabendazole in pharmaceutical dosage form was found to be 99.87%.

LINEARITY:

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Data for Linearity of Triclabendazole

Concentration µg/ml	Average Peak Area
10	442986
15	652547
20	856985
25	1063654
30	1268475

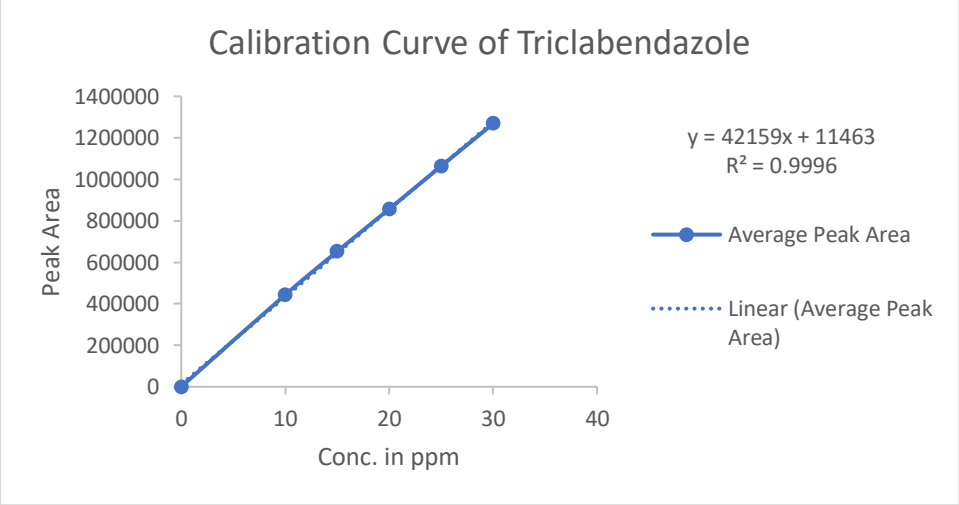

Fig3: Calibration Curve of Triclabendazole

REPEATABILITY:

Table-6: Results of method precision for Triclabendazole:

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Triclabendazole	3.165	856985	69856	8569	1.26
2	Triclabendazole	3.163	856898	69845	8597	1.25
3	Triclabendazole	3.158	856789	69865	8589	1.26
4	Triclabendazole	3.167	859854	69874	8569	1.25
5	Triclabendazole	3.171	854789	69798	8564	1.26
6	Triclabendazole	3.167	856978	69859	8599	1.25
Mean			857048.8			
Std.dev			1617.106			
%RSD			0.188683			

Acceptance criteria:

- %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:

Table-7: Results of ruggedness for Triclabendazole

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Triclabendazole	3.158	865845	70023	8659	1.27
2	Triclabendazole	3.163	864356	70015	8667	1.27
3	Triclabendazole	3.167	867584	69989	8654	1.28
4	Triclabendazole	3.165	865987	70114	8645	1.28
5	Triclabendazole	3.171	865975	69985	8635	1.27
6	Triclabendazole	3.171	865982	69998	8695	1.28
Mean			865954.8			
Std. Dev.			1022.223			
% RSD			0.118046			

Acceptance criteria:

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

Table-8: Results of Intermediate precision Analyst 2 for Triclabendazole

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Triclabendazole	3.173	878548	70254	8758	1.26
2	Triclabendazole	3.134	874598	70265	8798	1.27
3	Triclabendazole	3.161	874589	69989	8742	1.26
4	Triclabendazole	3.174	875984	70145	8759	1.26
5	Triclabendazole	3.199	875981	70158	8746	1.27
6	Triclabendazole	3.199	875984	69998	8796	1.27
Mean			875947.3			
Std. Dev.			1444.511			
% RSD			0.164908			

Acceptance criteria:

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

ACCURACY:

Table-9: The accuracy results for Triclabendazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	429549.7	10	9.916	99.16%	99.68%
100%	856189.3	20	20.036	100.18%	
150%	1272534	30	29.912	99.706%	

Acceptance Criteria:

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:

Table10: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	856985	3.174	8547	1.25
Less Flow rate of 0.9 mL/min	841542	3.488	8256	1.23
More Flow rate of 1.1 mL/min	812546	2.877	8146	1.20
Less organic phase	802654	4.705	8365	1.16
More organic phase	826549	2.090	8154	1.14

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

CONCLUSION:

Hence the proposed method was found to be rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and economical. The method shows non-interference of formulation excipients in the estimation. This method is also having an advantage that the retention time of the Triclabendazole is below 5 min and the drug can be assayed with the short time. Thus, the method is not time consuming and can be used in laboratories for the routine analysis of single and combination drugs.

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