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REVERSE PHASE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TRITYL CANDESARTAN IN BULK DRUG AND DOSAGE FORMS.

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ABSTRACT

Trityl Candesartan is an angiotensin II receptor antagonist (more commonly called an "ARB", or angiotensin receptor blocker), Angiotensin-II is a substance produced in the body which causes blood vessels to tighten. It blocks the action of angiotensin-II and therefore relaxes blood vessels. This helps lower blood pressure. A simple, precise and reversed phase liquid chromatographic (RP-LC) method was developed and validated for estimation of Trityl Candesartan. The separation was achieved on C-18 1.7µ, (2.1 X 100) mm, Make: Waters, analytical column with mobile phase consisted of buffer TFA in water: Acetonitrile with UV detection wavelength was at 255 nm and 5µl of sample volume was injected. The retention time of Trityl Candesartan was found 1.4 minute. The method was successfully validated in accordance to ICH guidelines and usp pharmacopeia for accuracy, precision, specificity, linearity. The linear regression analysis data for calibration plots showed good linear relationship correlation factor 0.999 in the concentration range 50-150µg/mL for Trityl Candesartan. The % Recovery/Accuracy was within the range. The percentage RSD for precision method was found less than 2%. Therefore, the proposed method is simple, precise, and fast, so method should be successfully applied for routine analysis of Trityl Candesartan in bulk drug.

KEYWORDS: Accuracy, ICH, Isocratic, Linearity, Precision, RP-LC, validation, USP pharmacopeia.

INTRODUCTION

Trityl Candesartan is an angiotensin II receptor antagonist (more commonly called an "ARB", or angiotensin receptor blocker), Angiotensin-II is a substance produced in the body which causes blood vessels to tighten. It blocks the action of angiotensin-II and therefore relaxes blood vessels. This helps lower blood pressure. It is used alone or in combination with other antihypertensive agents. Trityl Candesartan is chemically described as a 2-ethoxy-1-[[(2-(1-triphenylmethyl-1Htetrazol-5-yl) biphenyl-4-yl-). It is a white to off-white crystalline powder with a molecular weight of 682.77g/mol., is slightly soluble in alcohol and methylene chloride and practically insoluble in water. Its empirical formula is C43H34N6O3, and the structural formula is shown in **figure-I**.[1-4]

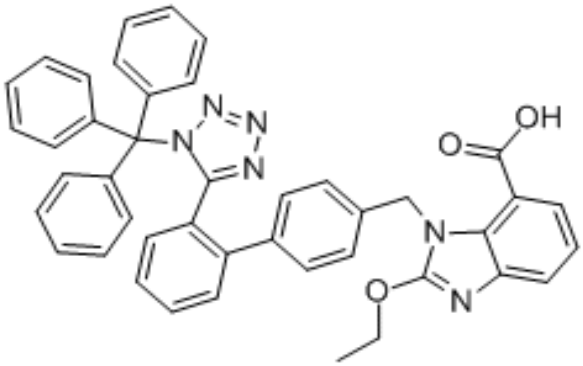


Figure-I Trityl Candesartan Structure.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The intended use of analytical methods is to assess product quality and validation is the process of generating experimental data that provides evidence that the performance of an analytical method is adequate for reliably assessing product quality.[5-8] The validation procedure has been performed by using fast liquid chromatography. The method has been validated for linearity, precision (system repeatability, method repeatability, and method reproducibility), accuracy, range, specificity, and solution stability.[9-11] Literature survey indicates that there is no RP-LC short run time method available for assay determination of Trityl Candesartan.[12-15] thus we aimed to develop it. Fast reverse phase Liquid chromatography is a modern separation technique. Chromatography depends on the distribution of the mixture between two phases, one of them is called Stationary phase and other is moile phase. The mixture is dissolved in the moving phase and passed over a stationary phase. When a mixture of components is introduced in to a LC column, they travel according to their relative affinities towards the stationary phase. The component which has

more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.[15-19] several drugs are used for treatment of Hypertension, cost of medicines are too much high. Here an attempt has been made to reduce the cost of medicine trityl candesartan (anti-hypertensive drug) by reducing the analysis cost and develop such type of analytical method in which

1) There is Minimum solvent consumption
2) Reduced analysis time
3) Chemicals and reagents which are used in the method are cheap and easily available.

Thus purpose of my research work to develop the analytical method for anti-hypertensive pharmaceutical drugs by liquid chromatography.

MATERIALS AND METHODS

Chemicals and Reagents

samples of Trityl Candesartan were obtained as gift. LC grade Acetonitrile and Trifluoroacetic acid were purchased from Merck Chemicals. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

LC instrumentation and chromatographic conditions

The analysis was carried out on a Waters Acquity UPLC (Ultra performance liquid chromatography). Binary Gradient System using 10µL injection loop column with auto injector. Column compartment having temperature control and for detection Ultraviolet Detector was employed throughout the analysis. Acquity BEH C-18 1.7µ, (2.1 X 100) mm, Make: Waters, analytical column was used for separation. Mobile phase consisted of buffer (0.1% TFA in water): Acetonitrile (5:95 v/v). Mix well and filter through 0.22µm filter. The mobile phase was prepared freshly and degassed by sonicating for 5min before use. Acetonitrile was used as diluents. The analysis was done on isocratic flow of 0.40ml/min with UV detection wavelength was performed at 255 nm at column temperature 40°C using 5.0 µL injection volumes with auto injector. Analysis run time is 3.0 minutes.

Standard solution Preparation

Accurately weigh and transfer about 20mg of Trityl Candesartan standard into a 20mL volumetric flask, add about 15mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same. Further pipette out 5mL of the above stock solution

into a 50mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.22µm filter. Obtain solution concentration was 100µg/ml.

Trityl Candesartan sample solution preparation

Accurately weigh and transfer equivalent to 20mg of Trityl Candesartan sample into a 20mL volumetric flask, add about 15mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same. Further pipette out 5mL of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.22µm filter. Obtain solution concentration was 100µg/ml.

These solutions were injected into LC system. To determine and measure the Peak area of Trityl Candesartan and calculate % Trityl Candesartan by following formulae.

Calculation

$$\text{TRITYL CANDESARTAN (\%)} = \frac{A_1 \times C_2}{A_2 \times C_1} \times P$$

Where,

A_1 = Area of Trityl Candesartan in sample

A_2 = Area of Trityl Candesartan in standard

C_1 = Concentration of Trityl Candesartan in sample (mg/ml)

C_2 = Concentration of Trityl Candesartan in Standard (mg/ml)

P = Potency of Standard

RESULTS AND DISCUSSION

Method validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (system repeatability, method repeatability and method reproducibility), accuracy, specificity, stability and system suitability.

Linearity

The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. It should be established initially by visual examination of a plot

of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods (e.g., by calculation of a regression line by the method of least squares). In some cases, to obtain linearity between the response of an analyte and its concentration, the test data may have to be subjected to a mathematical transformation. Data from the regression line itself may be helpful for providing mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted.

Five standard solutions of Trityl Candesartan were prepared from three stocks in the range of 50% to 150% of the nominal concentration and injected once. Linearity regression analysis demonstrated the acceptability of the method for quantitative determination of Trityl Candesartan over the concentration range of about 50ppm to 150ppm of the nominal concentration. Linearity graph was shown in **figure-2.**

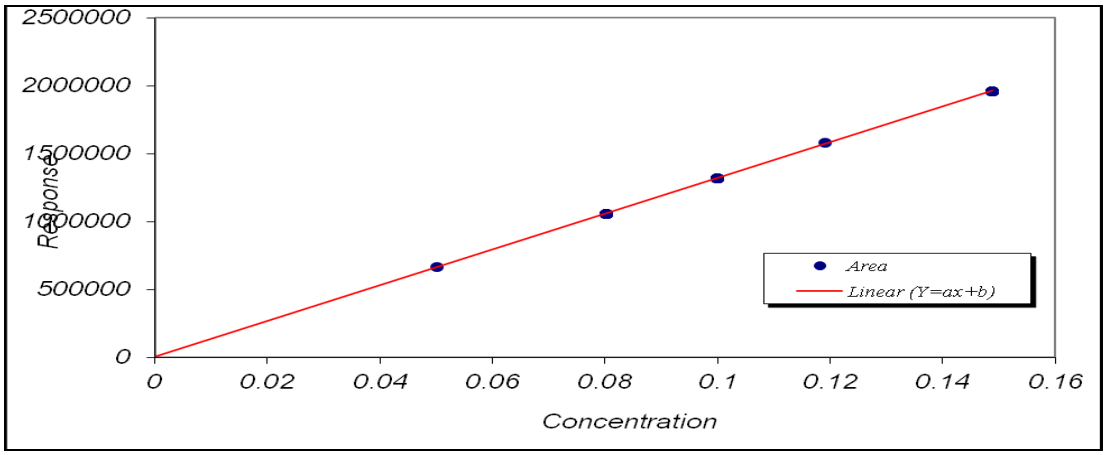


Fig-2 Linearity graph.

Table-1 Linearity Data And slope, intercept and correlation factor were shown in table-I.

Trityl Candesartan Concentration(ppm)	Trityl Candesartan Area
50.17ppm	733455
80.27ppm	1163404
100.04ppm	1469983
119.10ppm	1754906
148.87ppm	2202730
Slope	**14932076**
Intercept	**-20692**
Correlation factor	**0.9998**

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration, or a minimum of six determinations at 100% of the test concentration).

System Repeatability

Standard solution is prepared 100ppm were injected in six times and RSD of areas and retention times were calculated. The percentage RSD of areas was less than 2.0% and the % RSD of retention times was less than 5.0 %. Results are presented in **table-2.**

Table-2 System Repeatability data.

System Repeatability		
Concentration (ppm)	**Retention time (min)**	**Area**
100.04	1.389	1466157
100.04	1.389	1465118
100.04	1.389	1467130
100.04	1.389	1464561
100.04	1.388	1470990
100.04	1.386	1471268
Average	**1.388**	**1467537**
STDEV	**0.001**	**2919.6**
%RSD	**0.1**	**0.2**

Method repeatability

Six preparation of Trityl Candesartan sample was analyzed from sample preparation to final results by the same analyst and the percentage RSD of obtained results was less than 2% and obtained result were within given range 100 ± 2. Result are presented in **table-3**

Table-3 Method Repeatability data.

Method Repeatability			
Concentration (ppm)	**Retention time (min)**	**Area**	**% Trityl Candesartan**
99.84	1.387	1469983	100.2
99.45	1.386	1465370	99.8
100.29	1.384	1471599	99.9
100.54	1.386	1463535	99.1
100.88	1.385	1467996	99.0
99.74	1.383	1466832	100.1
Average	**1.385**		**99.7**
STDEV	**0.001**		**0.499**
%RSD	**0.1**		**0.5**

System Reproducibility

Three Trityl Candesartan sample are analysed by this method in duplicate preparation and obtain result are in **table-4.**

Table-4 Method Reproducibility data.

Method Reproducibility				
S No.	**Concentration (ppm)**	**Area**	**% Trityl Candesartan**	**% Trityl Candesartan Average**
Sample-I Pre-I	99.55	1468745	100.55	100.6
Sample-I Pre-II	99.35	1468859	100.61	
Sample-II Pre-I	99.65	1466896	100.18	100.2
Sample-II Pre-II	99.84	1470701	100.24	
Sample-III Pre-I	100.04	1469293	99.94	99.9
Sample-III Pre-II	100.14	1468686	99.80	

Accuracy

It is defined as the closeness of test results obtained by the method to the true value. It may often be expressed as percent recovery by the assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method. The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration). The three different concentrations of Trityl Candesartan standard solutions were determined from three replicate injections, using the linear regression lines (linearity section). The deviations of the obtained results (expressed as percentage accuracy) were calculated from the true values were presented in **table-5.**

Table-5 Accuracy test data of Trityl Candesartan

Injection No	Level	Concentration(ppm)	Area	Calculated concentration (ppm)	Accuracy (%)
1	80 %	80.27	1163404	79.500	99.04
2			1163947	79.536	99.08
3			1166830	79.729	99.33
Average			**1164727**		**99.1**
1	100 %	100.04	1469983	100.03	99.99
2			1465370	99.722	99.68
3			1471599	100.14	100.10
Average			**1468984**		**99.9**
1	120 %	119.10	1754906	119.11	100.01
2			1755618	119.16	100.05
3			1755004	119.12	100.02
Average			**1755176**		**100.0**

The average deviations from true value are less than 2.0 %.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials. The specificity of the method was verified by testing the blank, standard and sample (un-spiked and spiked), determined the resolution factors between analyte peak (Trityl Candesartan) and the nearest peak. Sample of Trityl Candesartan sample spiked with other known impurity.

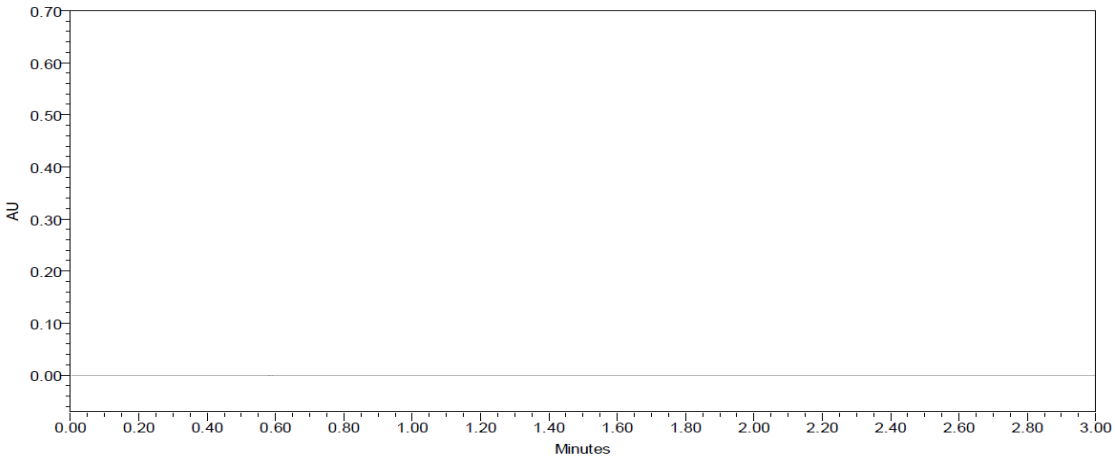
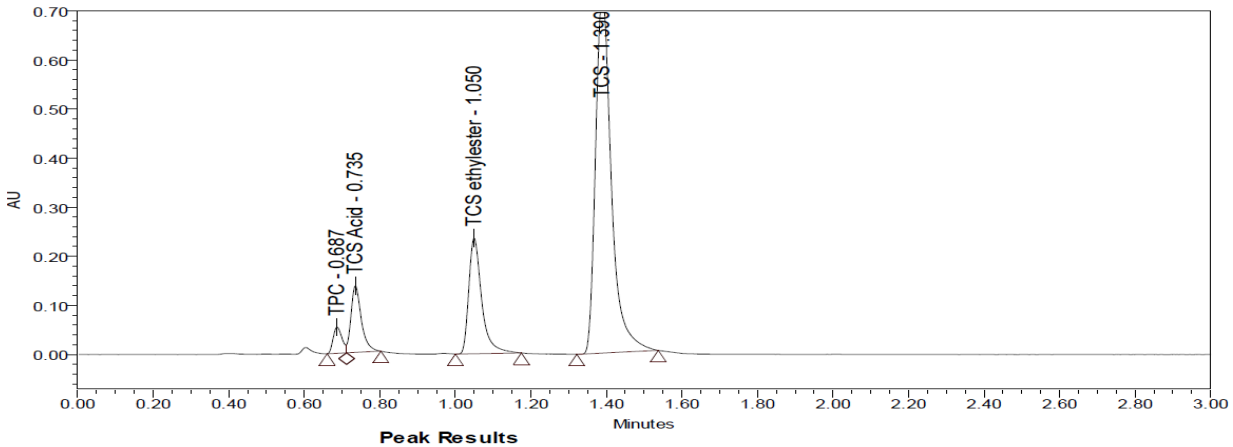


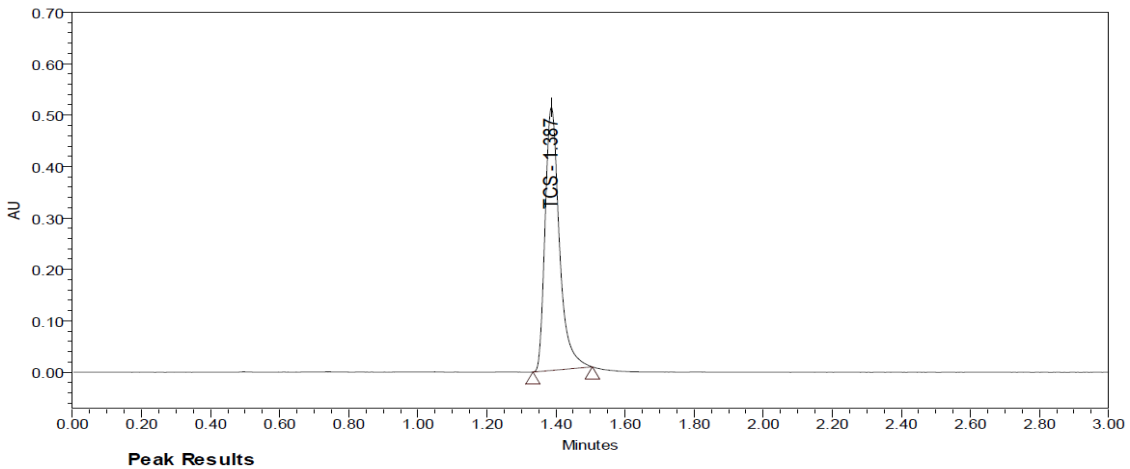
Figure-3 Typical Blank Chromatogram.



Peak Results

	Name	RT	Area	% Area	USP Tailing	USP Plate Count	USP Resolution
1	TPC	0.687	87742	2.89		3571	
2	TCS Acid	0.735	258806	8.54		3635	1.0
3	TCS ethylester	1.050	561747	18.53	1.5	5101	5.7
4	TCS	1.390	2123010	70.04	1.5	5974	5.1
Sum			3031305.6				

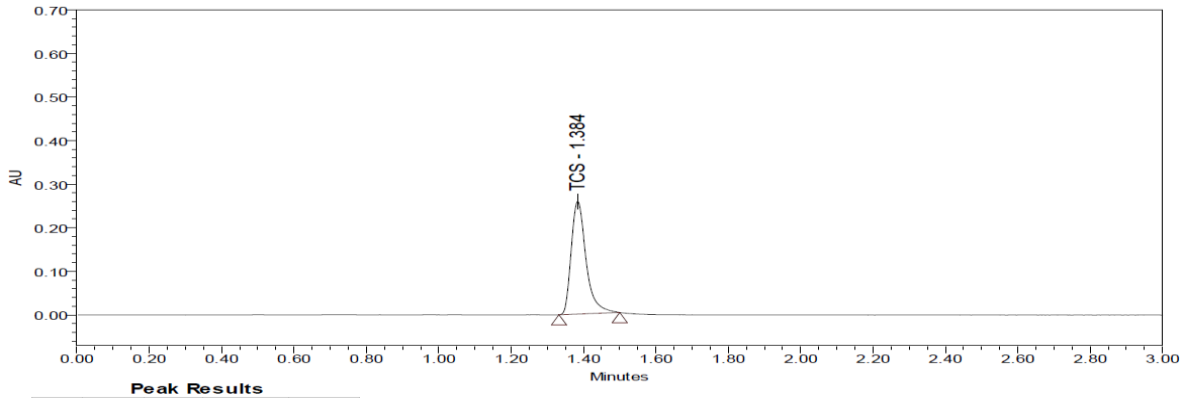
Figure-4 Typical System Suitability Chromatogram.



Peak Results

	Name	RT	Area	% Area
1	TCS	1.387	1469983	100.00
Sum			1469982.8	

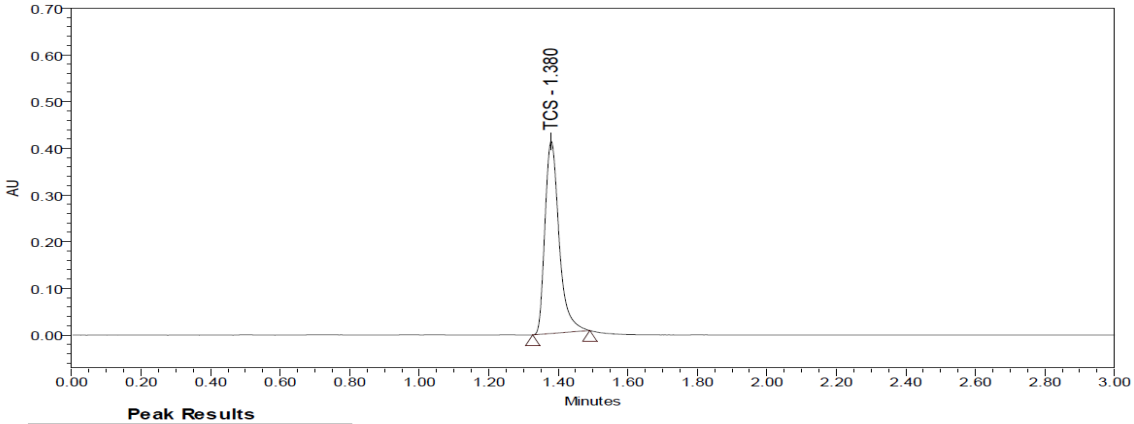
Figure-5 Typical Standard Chromatogram (100ppm).



Peak Results

	Name	RT	Area	% Area
1	TCS	1.384	733455	100.00
Sum			733455.1	

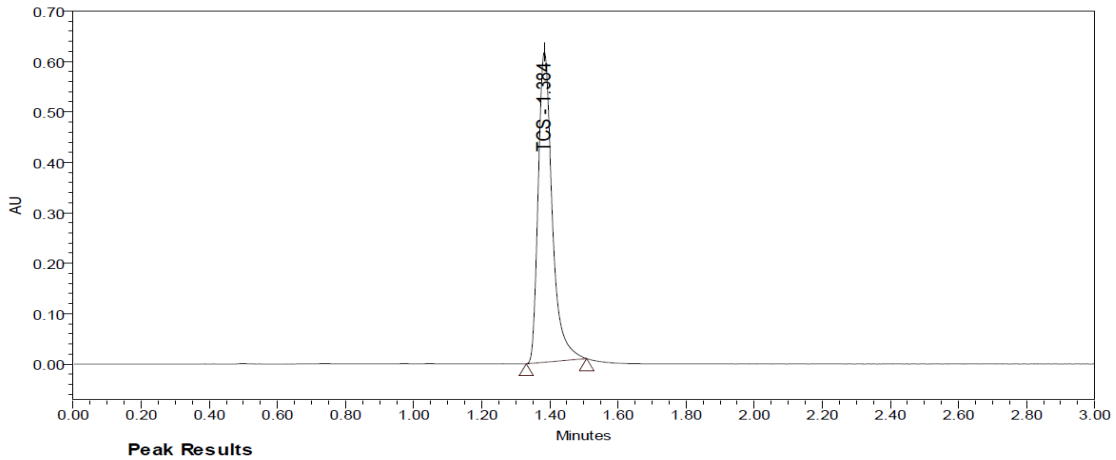
Figure-6 Typical Standard Chromatogram (50ppm).



Peak Results

	Name	RT	Area	% Area
1	TCS	1.380	1163404	100.00
Sum			1163404.4	

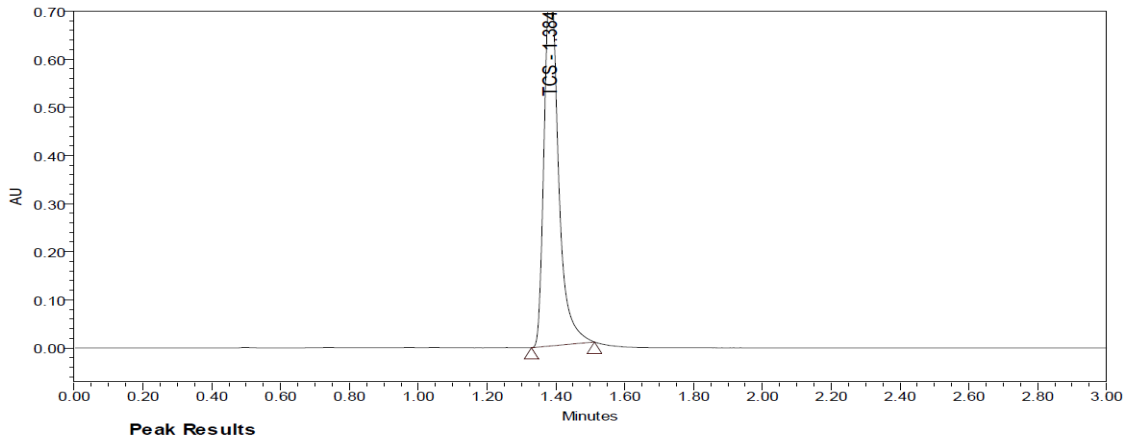
Figure-7 Typical Standard Chromatogram (80ppm).



Peak Results

	Name	RT	Area	% Area
1	TCS	1.384	1754906	100.00
Sum			1754906.1	

Figure-8 Typical Standard Chromatogram (120ppm).



Peak Results

	Name	RT	Area	% Area
1	TCS	1.384	2202730	100.00
Sum			2202730.3	

Figure-9 Typical Standard Chromatogram (150ppm).

No significant interfering peak appeared in the blank, System suitability and standard chromatogram at the retention times of the analyte peaks.

Range

The range obtained from Linearity, Precision and Accuracy is summarized - ibesartan-50ppm to 150ppm (50% to 150% of nominal sample concentration).

Solution Stability

The time period and storage conditions of testing the stability of standard and sample solutions will be according to accumulated knowledge. Stability shall be verified in the glassware specified for the particular solution in the method, e.g. transparent or amber glass. Solution stability was verified by retesting the solutions after 4 hours stored in transparent vials. The comprehensive results of this study are presented in the **table-6**.

Table-6 Solution stability.

Solution Stability after 4 hours		
S. No.	**Solution**	**(%) Trityl Candesartan**
1	Initial	99.73%
2	After 4 Hrs	99.17%
% Difference		**0.56%**

No new degradation peak was observed. The obtained results demonstrated a good stability of the sample solution stored at room temperature in vial for at least 4 hours.

CONCLUSION

The method validation was demonstrated-The Method "Estimation of Trityl Candesartan by reverse phase liquid Chromatography is selective, precise, linear and accurate for performing the determination over the required concentration ranges of 50 to150 % of Trityl Candesartan nominal sample concentration so it should be applied routine analysis of determination of candesartan in bulk drug and dosage forms.

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