Development and Validation of a RP-HPLC Method for the Analysis of Trimetazidine Hydrochloride in Bulk Drug and Pharmaceutical Dosage Forms

A thesis presented for the partial fulfillment of the requirement for the degree of Master of Pharmacy

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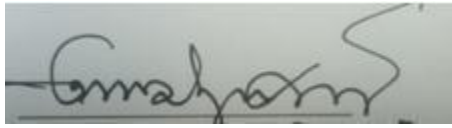BCSIR Laboratories
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DEDICATED TO … … … … …

MY BELOVED PARENTS

DECLARATION

I hereby certify to declare that, this thesis report is done by me under the supervision of Professor **Dr. Ahmad Ismail Mustafa,** Dean, Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University and **Suriya Sharmin**, Scientific Officer, PSRD, Bangladesh Council of Scientific and Industrial Research (BCSIR), impartial fulfillment of the requirement for the degree of Master of Pharmacy. I am declaring that this research is my original work. I am also declaring that thesis nor has any part there of been submitted elsewhere for the award of Master or any degree.

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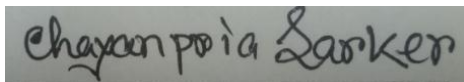Faculty of Allied Health Science
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ABSTRACT

This study presents the development, optimization and validation of a simple HPLC method for the determination of Trimetazidine Dihydrochloride in bulk drug and modified release dosage tablets as Trimetazidine Dihydrochloride modified release tablets is not included in any of the official monographs. Method development was carried out by using different columns to get satisfactory result. Mobile phase composition giving symmetrical peak shape was selected. It was also found that C18 column gives symmetric peaks with high theoretical plates and low tailing factor. Simple, fast, economical, accurate, precise and reproducible HPLC method was developed for the determination of Trimetazidine which works as a myocardial metabolism modifier antianginal agent. A simple and reproducible method was developed for Trimetazidine by Reverse phase high performance liquid chromatography (RP-HPLC). The separation was performed by C18 column at ambient temperature with mobile phase of 10 mM Trietylamine, pH 4.0 adjusted with acetic acid buffer and Acetonitrile at a ratio of 85:15 and flow rate of 1 mL/min. The wavelength for maximum absorbance was selected as 232 nm by spectral scan of the Trimetazidine standard solution in UV-VIS Spectrophotometer. The detection was performed by PDA (Photodiode array detection) detector at 232 nm. . The method which was developed was also validated in complete compliance with the current regulatory guidelines by using well developed analytical method validation techniques and tools which comprises with the analytical method validation parameters like linearity, accuracy, method precision, specificity, system suitability and robustness. The proposed method's results were found to be satisfactory and are suitable for determination of Trimetazidine for routine quality control of drugs in bulk drug and formulation wavelength. The method was found to be linear with regression coefficient value of 0.999 at concentration of 1.859 – 55.763 μg/mL of Trimetazidine. The range was determined at concentration of 39.67 – 65.25 μg/mL with acceptable accuracy (% Recovery: 93.6 – 101.2) and precision study (% RSD: 0.67 – 2.25) in three replicate analysis at three different concentration level of Trimetazidine sample solution. The method showed good precision with %RSD value of 0.16 – 0.64 for repeatability or intraday precision study and %RSD value of 0.20 – 2.02 for intermediate precision or interday precision study. Accuracy was checked for replicate analysis of three concentration level by standard addition method. % recovery value obtained was 97.40 –

99.42 % with % RSD of 0.08 – 0.78. LOD (Limit of Detection) and LOQ (Limit of Quantification) were found to be 0.307 μg/mL and 1.021 μg/mL respectively. Specificity was checked by comparing the peaks of standard and sample with excipients for presence of any interference. The developed method was found to be robust with pH variation of 3.95 – 4.05 and column temperature variation of 20 °C – 30 °C. System suitability parameters were checked for acceptance limit in terms of asymmetry, theoretical plates, capacity factor and relative standard deviation (%) of the six replicate injection in 10 µL value of standard solution at specific concentration. which is useful for the routine determination of Trimetazidine in bulk drug and in its pharmaceutical dosage form. Further study on degradation pathway of the compound will provide idea about the stability indicating nature of the method to discriminate the active constituent, Trimetazidine from its related impurities and degradants.

Keywords: Trimetazidine Dihydrochloride, Myocardial metabolism modifier, HPLC, Photodiode array detection, Method validation.

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CHAPTER : ONE

INTRODUCTION

INTRODUCTION

Pharmaceutical Analysis:

Pharmaceutical Analysis determines the quality of drug products via analytical chemistry. The aim of pharmaceutical analysis is to build and ensure quality in the finished drug product. In the field of pharmaceutical research, the analytical investigation of bulk drug materials, intermediates, drug products, drug formulations, impurities and degradation products, and biological samples containing the drugs and their metabolites is necessary to ensure the quality and safety of the drug products. Briefly it is the process which identifies, determines, quantifies, purifies and separates the active compound from the mixture (Garshell et. al. 2003).

Pharmaceutical Analysis: Role

Pharmaceutical analysis plays a major role in assuring the identity, safety efficacy and quality of a drug product. Safety and efficacy studies required that the new drug entity considered for drug substance and drug product should have the following critical requirements;

• Established Identity/purity

•Established dissolution/bioavailability

•Identity and purity requirements

• Regulatory compliance

Pharmaceutical Analysis: Major activities

Modern pharmaceutical analysis entails following major activities from discovery of chemical entity to final inclusion as a drug product:

• Discovery of new chemical entity and high throughput screening

• Solid-state analysis of the drug substance

• Degradation and impurity analysis of the drug substances

• Preformulation analysis

• Analysis of solid oral dosage forms

• Analysis of injectable dosage forms

• Development of new dosage forms

• Compendial testing

• Method development

• Setting specification

• Method validation

• Stability studies

• Analytical methodology transfer

• Documentation and inspections

• Innovative analytical platforms

Pharmaceutical Quality Management System

Quality management system (QMS) is the application of quality policies and objectives systematically to the organizational structure, procedures, processes and resources involved throughout the products lifecycle with a provision of continuous improvement of the policies to allow delivery of products, with quality attributes appropriate to meet the needs of patients, health care professionals, regulatory authorities (including compliance with approved regulatory filings) and other internal and external customers. The product lifecycle includes the following technical activities for new and existing products (Avdeef A et al, 2002).

Quality Assurance

"Quality assurance" is a wide-ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangements made with the object of ensuring that pharmaceutical products are of the quality required for their intended use. Quality assurance therefore incorporates GMP and other factors, including product design and development.

Good practices in Quality Control

Quality control is the part of GMP concerned with sampling, specifications and testing, organization, documentation and release procedures which ensure that the necessary and relevant tests are actually carried out and that materials are not released for use, nor products released for sale or supply, until their quality has been judged to be satisfactory. Quality control is not confined to laboratory operations but must be involved in all decisions concerning the quality of the product (Anu. Linna. et al. 2008).

Standards of the Quality

These compendial monographs are considered as standards for quality service to the people. Pharmaceutical products can usually be qualified by various Pharmacopoeias. Current existing pronounced standards include:

British Pharmacopoeia (BP)

European Pharmacopoeia (EP)).

Japanese Pharmacopoeia (JP).

The International Pharmacopoeia (IP).

United States Pharmacopoeia (USP).

Analytical Method Development: Choice of Instrumentation

The choice of analytical instrumentation and methodology should be based on the intended purpose and scope of the analytical method. So, analytical method development is the process of selecting an accurate assay procedure to determine the composition of a formulation.

The prerequisite for method development are follows:

1. Qualified and calibrated instruments

2. Documented methods

3. Reliable reference standards

4. Qualified analysis

5. Sample selection and integrity

6. Change control

Purposes of analytical method development

Analytical method development and finalizing the methods consist of

1. Standardizing the working standard from reference standard.

2. Optimizing the chromatographic condition, concentration of standard and sample solution and extraction procedure of the sample.

3. Analytical method verification or mini validation to be done before analyzing (routine samples) tests like assay, dissolution and related substance in development laboratories.

4. Prior starting the validation the satisfactory result should be found in mini validation and formulation should be finalized (MC Dowall R.D et al,2009).

Method Validation

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use (Gaurav T et al, 2010). Validation refers to

establishing documented evidence that a process or system, when operated within established parameters, can perform effectively and reproducibly to produce a medicinal product meeting its pre-determined specifications and quality attributes. The international standard ISO/TEC requires validation of nonstandard methods, laboratory designed or developed methods, standard methods used outside their intended scope, and amplifications and modifications of standard methods to confirm that the methods are suitable for their intended use. Analytical methods need to be validated or revalidated before their introduction into routine use. Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and whenever the method is changed and the change is outside the original scope of the method. (J. Ermer et al. 2006)

According to ICH guidelines the validation parameters are ICH Q2B guideline:

Linearity

Range

Accuracy

Precision

 Repeatability,

 Intermediate Precision

Specificity

Detection Limit, & Quantitation Limit

Robustness

System Suitability

1. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should.

2. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which the analytical procedure has a suitable level of precision, accuracy and linearity.

- for the assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration;

- for content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified;

3. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy may be inferred once precision, linearity and specificity have been established.

Accuracy Determination for Drug Substance

a) Application of an analytical procedure to an analyte of known purity (e.g. reference material);

b) Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined.

Accuracy Determination for Drug Product

a) Application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analyzed have been added;

4. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

i) Repeatability

Repeatability should be assessed using a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or a minimum of 6 determinations at 100% of the test concentration.

ii) Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. Typical variations to be studied include days, analysts, equipment, etc.

iii) Reproducibility

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias.

Forced Degradation

Forced degradation is the degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods when no known impurities or degradants are present and also provides an insight into degradation pathways and degradation products of the drug substance that could form during storage, and helps in elucidation of the structure of the degradation products (G Ngwa et al. 2010).
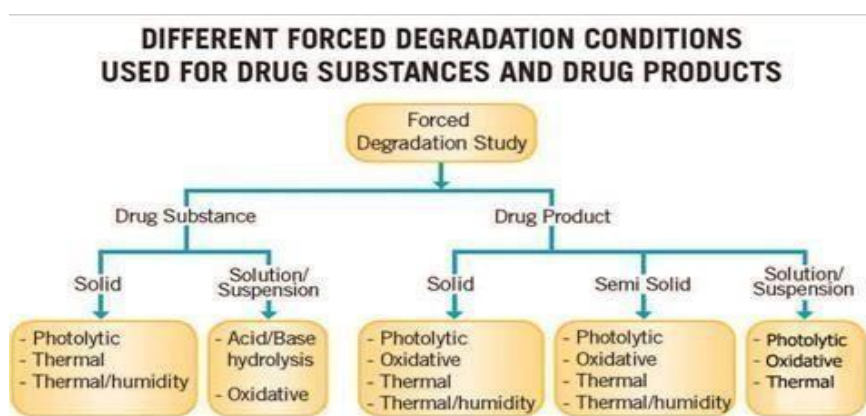

Figure 1: An illustrative flow diagram showing the different forced degradation conditions.

5. Limit of Detection and Limit of Quantitation

Limit of detection (LOD) and Limit of quantitation (LOQ) can be calculated by using the formula

1. By visual evaluation,

2. Based on S/N ratio for procedures exhibiting base line noise,

3. Based on Standard Deviation of response and slope, where

LOD = 3.3 x σ/s

LOQ = 10 x σ/s.

σ = Standard deviation of response can be obtained by Standard deviation of blank response, Residual standard deviation of the regression line, or Standard deviation of the y-intercept of the regression line;

S = Slope of regression equation.

7. Robustness

The robustness of an analytical procedure is the characteristic of its stability with respect to small variations of the system parameters possible under real conditions. This stability is usually evaluated in terms of the RSD of the results of analyses compared to the analogous data obtained using strictly observed conditions according to the validated analytical procedure.

8. System Suitability

System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. These tests are used to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. The parameters to be measured and their recommended limits for chromatographic procedures obtained from the analysis of the system suitability sample are shown below:

Parameters	Accepted Value
Theoretical Plate /Column efficiency (N)	Should be ≥ 2000
Capacity (Retention) factor	Should be ≥ 2
Tailing factor/Peak asymmetry	Should be ≤ 2
Relative retention time	The relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ± 2.5 %.
Resolution	Should be > 2 for main peaks and
	Should be > 1.5 between main peak and impurities
Relative Standard Deviation	Should < 1.

Titrimetric techniques
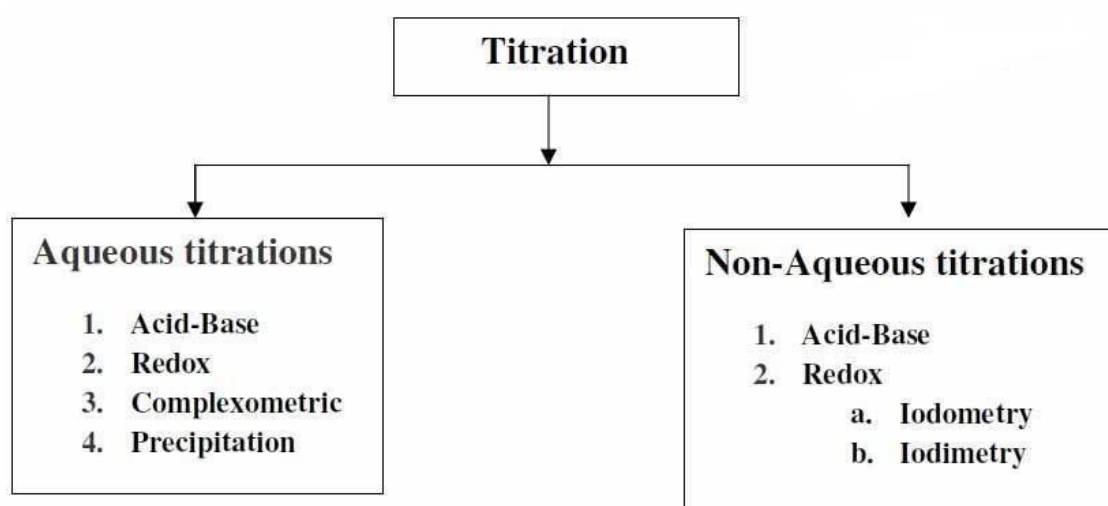
Chromatographic techniques

'Chromatography' is an analytical technique commonly used for separating a mixture of chemical substances into its individual components due to the differential affinities (strength of adhesion) of the various components of the analyte towards the stationary and mobile phase based on their polarity, charge, size etc.

- Thin layer chromatography (TLC)
- High performance thin layer chromatography (HPTLC)
- High performance liquid chromatography (HPLC)
- Gas chromatography (GC)

Spectroscopic techniques

Spectroscopy also known as absorption spectroscopy is of fundamental importance in pharmaceutical analysis. Many chemical substances absorb electromagnetic radiation. Through proper instruments and techniques the absorbed radiation can be measured. These will determine the amount and nature of drug substance, impurities and other substances in dosage form, reaction vessel or in biological systems. Most commonly used spectroscopic techniques used in pharmaceutical analysis are:

- UV-Visible spectrophotometry,
- Near infrared spectroscopy (NIRS),
- Atomic absorption spectroscopy,
- Mass Spectrometry,
- Raman Spectroscopy,
- Nuclear magnetic resonance (NMR) Spectroscopy,
- Fluorimetry and phosphorimetry,
- X-Ray diffraction, Emission and Absorption Spectrometry.

Hyphenated techniques

- GC-MS Technique,
- LC-MS Technique,
- CE-MS Technique,
- GC-MS Technique,
- LC-NMR Technique etc.

Technique Used in this Work

UV/VIS spectrophotometry

UV/VIS spectrophotometry involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution that results in the excitation of electrons, in both atoms and molecules, from lower to higher energy levels. After absorbing a particular amount of energy in the form of fixed wavelength of light one of these electronic transitions will occur resulting in the excitement of an electron is excited from a full (low energy, ground state) orbital into an empty (higher energy, excited state) anti-bonding orbital. For each wavelength the intensity of light passing through both a reference cell (Io) and the sample cell (I) is measured and detector converts the incoming light into a current. The absorbance (A) of the sample is related to I and Io according to the following equation:

$A = \log_{10}(Io/I)$

The fundamental law that governs the quantitative spectrophotometric analysis is the Beer-Lambert law.

Chromatographic Techniques:

In chromatographic tecnique, only separation is variation in the distribution of different compounds between two dissimilar phases-a stationary phase and a mobile phase based on their polarity, size, ionic charge etc.

High-performance liquid chromatography

There are different types of detectors as given in Table.

| Detector	Key Attributes	Limitations
UV/Vis/PDA	Most widely used and accepted; Near "universal" at low UV; Gradient compatible Qualitative and Quantitative; PDA peak purity/ homogeneity, spectral library searches/ID, contour maps and 3D spectral display; Nondestructive Cost; Very Reliable; Easy to use	Must have a chromophore; Solvents must be transparent; Widely varying response for different solutes
Light Scattering	Detects most non volatile analytes; Works well with gradient HPLC; Better sensitivity than RI detection	Requires the use of volatile buffers, optimization; Limited dynamic range; Reproducibility of methods
Corona discharge	Highest sensitivity of "universal" type detector; Wide dynamic range; Detects any non volatile or semi-volatile; Consistent response; Ease of use	Requires the use of volatile buffers
FL	Very selective and sensitive; Works well with gradients	Not all compounds fluoresce; Often requires derivative formation; Quenching; Cost for performance
Radioactivity	Gradient compatible; can determine distribution and mass balance for drug metabolite studies, wide response range	Large flow cell volumes increase peak broadening and decrease resolution
EC	Very selective and sensitive; Modern ECs are reliable and easy to use	Mobile phase must be conductive; susceptible to background noise and electrode fouling; only applicable to compounds that can be oxidized or reduced
Conductivity	Detector of choice for ion chromatography-inorganic ions and organic acids; Very selective; Low cost	Requires suppression of mobile phase background conductivity; Not all compounds are detected; Requires special HPLC systems and columns
RI	Original detector for HPLC in many methods; Excellent versatility/ Universal detection; Solvent compatibility; Nondestructive; Cost; reliable and easy to operate	Sensitivity; Gradient incompatible; Stability (Temperature and Flow)
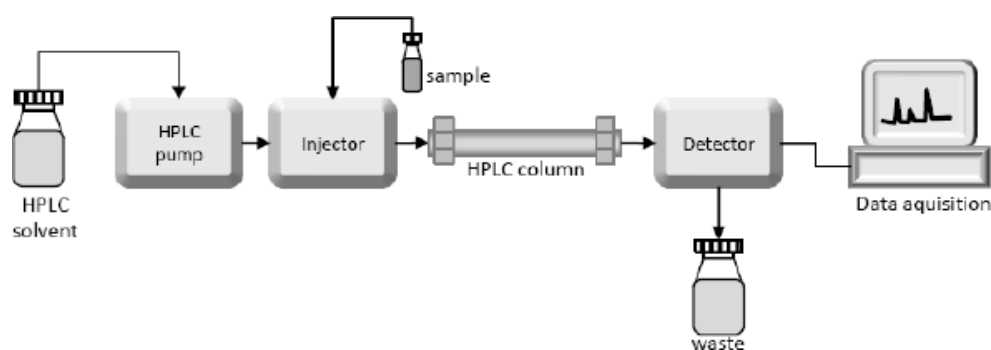
Figure 2: Block diagram of HPLC System

1) Mobile Phase in Solvent Reservoir: The main requirement for the mobile phase is that it has to dissolve the analytes up to the concentration suitable for the detection. In reverse phase applications water is usually the solvent. Other polar solvents such as Methanol, Acetonitrile or Tetrahydrofuran are added, pH is adjusted by buffers to modify separations of ionizable solutes. Ion- pairing reagents also enhance separation selectivity of charged analytes by increasing retention on hydrophobic bonding phases. Non polar solvents such as hexane, heptane iso-octane used in combination with slightly more polar solvents such as isopropanol, ethyl-acetate or chloroform in normal phase applications. Mobile phase degassing is important prior use to remove mobile phase entraped air from the atmosphere. Such bubbles can load to noise in detector, response or hinder flow of mobile phase through columns.

HPLC Method Development

The process is influenced by the nature of the analytes and generally follows the following steps:

- Step 1 - Initial studies
- Step 2 - Selection of initial conditions
- Step 3 - Selectivity optimization
- Step 4 - System optimization
- Step 5 - method validation

Aims and Objectives

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Analytical methods are continuously developed, improved, validated and finally included in the official monographs. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

Research on development of analytical methods can be done for drugs or drug products not included in pharmacopoeia or in cases of modification in the official methods. As HPLC is most efficient and reliable separation technique it is most widely used in pharmaceutical industry for qualitative and quantitative analytical method development and validation purposes. Development of stability-indicating analytical methods by

forced degradation study will ensure the capability of the analytical procedures to discriminate between the major active (intact) pharmaceutical ingredients (API) from any degradation (decomposition) product(s) formed under defined storage conditions.

Therefore, the aim of this research is to conduct method development and validation studies of the selected drug namely Trimetazidine by using high performance liquid chromatography as there is no standard compendial method present for analysis of this drug.

CHAPTER : TWO

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Literature survey is done on different analytical method development and validation documents available in order to recognize the work done till date, approach followed for development and validation. They were utilized as the basis for decisions relating to administering the drug to patients, play important role in new drug discovery, development, manufacture of pharmaceutical drugs and various other studies related to humans and animals. To analyze Trimetazidine Dihydrochloride (TMZ), a non-pharmacopoeial product novel method can be developed to reduce the cost besides time for better precision and ruggedness.

Gan Zhou et. al. (2010) Development and validation of a simple and sensitive liquid chromatography–tandem mass spectrometry method for quantifying trimetazidine in human plasma

Here, a simple and sensitive liquid chromatography–tandem mass spectrometry (LC-MS-MS) method was used. Sample preparation was based on deproteinating with acetonitrile. Chromatography was performed on a C18 analytical column (5 μm; 150 × 2.1, mm) and the retention times for trimetazidine and cetirizine (IS) were 1.8 and 3.0 min, respectively. The ionization was optimized using an electrospray ionization source. The calibration curve ranged from 0.1 to 200 ngmL-

A. Rajani et.al. (2014) RP-HPLC method development and validation for estimation of trimetazidine in tablet dosage forms.

In this method, a C-18 column (4.6 x 150mm, 5μ particle size) was used as stationary phase and Phosphate buffer:Acetonitrile (90:10, %v/v) was used as mobile phase at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 233 nm. The retention time for TMZ was 3.2 min.

B. Jeraldmaria antony et.al (2009) A new validated spectrophotometric method for determination of Trimetazidine in Formulation and comparison with UV method.

This method was based on the formation of a yellow ion pair complex between Trimetazidine and methyl orange using chloroform as solvent at pH 4 phosphate buffer. The lambda max and molar absorptivity of the chromogen were 427 nm and 5.0216 X 10^3 lit.mol.cm^{-1}, respectively. Chromogen obeys Beer's in the concentration range of 15-

50 µgmL^{-1} with linear regression of 0.9993, while the percentage recovery, LOD and LOQ were 99.65-99.98 %, 4.5 µgmL^{-1} and 10 µgmL^{-1} respectively.

C. Nagula et.al. (2014) Development and validation of a stability indicating RP-HPLC method for the determination of trimetazidine dihydrochloride.

In this method, the chromatographic conditions comprised of a reversed phase C18G column (250×4.6 mm, 5µm), with a mobile phase consisting of methanol:0.05% formic acid (90:10,%v/v) with flow rate of 1.0 mL/min. Detection was carried out at 232 nm. The retention time of TMZ was found to be 3.8 min. The linear regression analysis data for the calibration plots showed good linear relationship within the concentration range 10–80 µgmL^{-1}. The value of correlation coefficient wasfound to be 0.999. The recovery of trimetazidine hydrochloride was about 95–105%. TMZ is more sensitive to wards acidic degradation. The method was validated as per ICH guidelines.

# CHAPTER : THREE
DRUG INFORMATION

Coronary Artery Disease: Angina Pectoris

Angina also known as angina pectoris, is chest pain or pressure, usually due to absence of enough blood flow to the heart muscles to suppy the oxygen required by the heart.
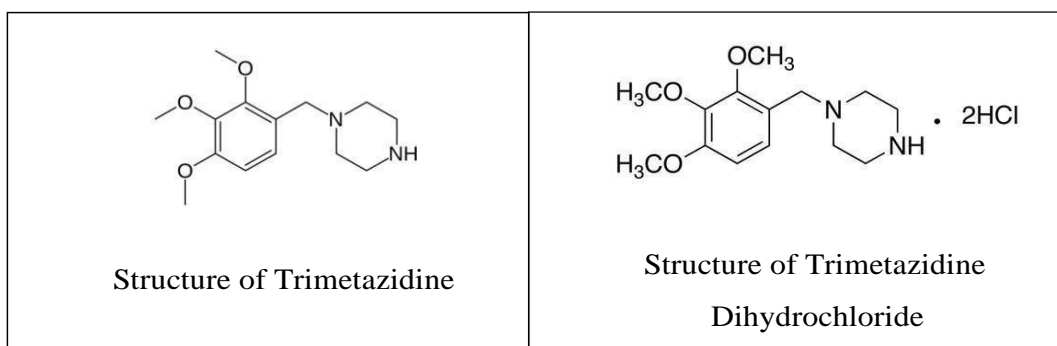
Angina occurs usually due to the obstruction or spasm of the coronary arteries. Other causes include anemia, abnormal heart rhythms and heart failure. The main mechanism of coronary artery obstruction is an atherosclerosis. The term derives from the Latin angere ("to strangle") and pectus ("chest"), and can therefore be translated as "a strangling feeling in the chest". There is a weak relationship between severity of pain and degree of oxygen deprivation in the heart muscle (i.e., there can be severe pain with little or no risk of a myocardial infarction (heart attack) and a heart attack can occur without pain (M Prinzmetal, et al. 1959).

Drug Choice: Trimetazidine (Antianginal agent)
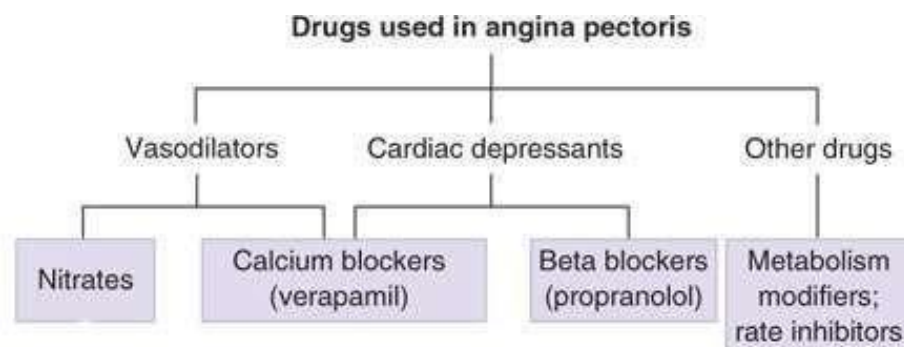
Trimetazidine: Physicochemical Properties

With evidence for efficacy in such diverse clinical settings such as stable coronary artery disease, reperfusion injury, and contrast-induced nephropathy, Trimetazidine (TMZ) is novel among cardiovascular agents. Although no single pharmacological mechanism has been hitherto universally accepted, TMZ is known to target deranged cellular energetics particularly in ischaemic myocardial tissue. Mechanistically, this separates the drug from conventional anti-anginal therapies, namely beta-adrenergic antagonists, calcium channel blockers, and nitrates.

| Structure of Trimetazidine | Structure of Trimetazidine Dihydrochloride |

Formula	C14H22N2O3	C14H24Cl2N2O3
Molar mass	266.336 g/mol	339.257 g/mol
Water Solubility	0.754 mg/mL	0.754 mg/mL
pKa (Strongest Basic)	9.21	9.21
Solubility (25°C)	Soluble in Methanol.	

CHAPTER : FOUR

MATERIALS AND METHODS

Materials and Methods

Materials:

All the materials, such as, active pharmaceutical ingredients, solvents, reagents, excipients etc. used were pharmaceutical grade.

Pharmaceutical ingredients:

a. Trimetazidine HCL standard (Beximco Pharmaceuticals Ltd., Bangladesh).

b. Trimetazidine HCL tablet sample (Square Pharmaceuticals Ltd., Bangladesh)

Solvents and reagents:

a. De-ionized water ,

b. Ammonium Acetate,

c. Ammonia solution (25%),

d. Trifluroacetic acid,

e. Triethylamine,

f. Acetic Acid,

g. Acetonitrile

h. Excipients   [Povidone,   Hydroxypropyl   Methylcellulose   (HPMC), Microcrystaline Cellulose, Purified Talc, Magnesium Stearate, Lactose).

Apparatus:

i. Volumetric flask (100 ml, 50 ml, 25ml, 10ml)

j. Conical flask

k. Measuring cylinder

l. Beaker

m. Spatula

n. Filter paper

o. Pipette

p. Micropipette

q. Mortar pestle

Instruments:

r. HPLC (Hitachi Chromaster) equipped with PDA detector.

s. Digital pH meter (SENSION, Spain).

t. Elcctronic balance (COPLEY, England)

u. UV- spectrophotometer (ANALYTIKJENA, Spectord 250 plus)

v. Sonicator (Wisd, Germany)

w. Hot air oven/Drier

Method:

Wavelength Determination:

A suitable standard solution was scanned in the wavelength range of 200-800 nm in the UV-VIS spectrophotometer, the wavelength for maximum absorbance λmax was determined.

Mobile Phase Selection:

Mobile phase effects resolution, selectivity and efficiency. Mobile phase composition (or solvent strength) plays an important role in HPLC separation.

Most common solvents for RP-HPLC are:

Organic: Methanol, Acetonitrile, Tetrahydrofuran etc.

Aqueous: Water, Buffer

The drug substance being analyzed should be stable in mobile phase solution (diluent). Satisfactory peak shape of analyte should be obtained by the selected mobile phase.

Different solvent systems were prepared for the selection of right solvent ratio as a mobile phase in which the peak of Trimetazidine HCL was stable and correct. Buffer solution checked are:

x.   Ammonium acetate buffer, pH 5.0

y.   Ammonium acetate buffer, pH 8.2

z.   0.1% Trifluroacetate buffer

aa. 10 mM Trietylamine buffer, pH 4.0

For this standard stock solution was prepared in the mobile phase (Buffer:Acetonitrile=50:50) and the standard solution was injected in the HPLC system for subsequent five days. The peak shape was observed.

Buffer preparation:

10 mM triethylamine of pH 4.0 adjusted with acetic acid was selected as buffer for the method. To prepare the buffer, 1.394 ml Triethylamine and approximately 0.572 ml acetic acid was well mixed with de-ionized water in a conical flask. Additional acetic acid was introduced to adjust the pH at 4. Then the mixture sample was kept into the sonicator for 3 minutes for proper mixing.

Assay of Trimetazidine Tablets:

Chromatographic condition used in assay is as follows:

Mode: LC

Detector: UV, 232 nm

Column: 4.6-mm x 25-cm; 5-úm packing (C18)

Mobile Phase: Buffer:Acetonitrile = 85:15

Diluent: Mobile phase

Flow Rate: 1 .0 mL/min

Injection Volume: 10 µL

Retention Time: Approximately 9 minutes

Diluent preparation:

In a 1000 mL conical flask, 850 mL Buffer and 150 mL Acetonitrile was heavily mixed with a stirrer by maintaining the ratio (Buffer:Acetonitrile = 85:15).

Standard solution preparation:

34.1 mg Trimetazidine HCL standard was mixed with diluent and the volume was increased up to 100 ml. After 5 minutes sonication the stock solution was then suitably diluted to obtain concentration of 21.693, 27.116, 32.540, 37.963 and 43.386 µg/mL. The standard solution was transferred into vial by passing through 0.2µm membrane filter.

Sample solution preparation:

10 tablets were weighed and finely powdered in a mortar pestle. Accurately weighed portions of the powder equivalent to 35 mg of Trimetazidine HCL was taken in a 50 mL volumetric flask and the volume was diluted up to 50 mL. The sample solution was filtered by the Whatman No. 1 filter paper. After discarding first few mL of the filtrate 2 mLof it was transferred into another volumetric flask and volume was increased up to 25 mL with diluent. The sample solution was transferred into vial by passing through 0.2µm membrane filter.

Concentration of the sample was calculated from the regression equation of the calibration graph.

Method validation of Trimetazidine using HPLC:

10 mM triethylamine of pH 4.0 adjusted with acetic acid buffer was prepared. The solvent system at ratio Buffer:Acetonitrile= 85:15 was used for development of analytical method and validation for Trimetazidine assay in bulk drug and tablet dosage form.

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution. A standard stock solution was prepared by dissolving 37.4 mg of Trimetazidine standard in 100 ml volumetric flask. Different amount of solution were taken into volumetric flask separately and increased up to 50 mL with diluents to obtain the concentration of 1.859, 3.718, 7.435, 18.588, 29.740, 33.458, 37.176, 40.893, 44.611 and 55.763 µg/mL.A standard curve was prepared with the peak response (area) against these concentrations and linear correlation was determined using least square method.

Range

Range was determined by analysis of the marketed sample at 80%, 100%, and 120% of the nominal test concentration for assay in triplicate. For this standard solution was prepared by dissolving 32.6 mg of Trimetazidine standard in 50 mL volumetric flask. The sample was then suitably diluted to obtain a fixed concentration of 51.951 µg/mL. The assay result was calculated for the samples tested. Linearity of the tested sample was evaluated by the visual inspection of the graph and reported by linear regression analysis of the curve. % RSD was determined at each concentration of tested sample for precision study and accuracy was determined by obtaining % recovery.

Precision

Repeatability

The precision was checked by repeated scanning and measurement of the response (peak area) of Trimetazidine standard solution at fixed concentration.

Intermediate precision

Precision of method was determined in terms of inter-day variations. Inter day precision was assessed from by analyzing three replicates of the nominal concentration of the standard drug solutions on consecutive three days in a week by maintaining proposed condition.

In both cases, % RSD of the peak area was calculated by the following equation:

% RSD = (Standard Deviation/Mean) x 100 %

Accuracy:

Percent recovery was determined by standard addition method. A known amount of previously assayed sample solution was added at 80%, 100% and 120% of the test concentration of the standard solution and the sample was analyzed. Accuracy is defined as the ratio of the observed result to the expected result expressed as a percentage. For this a standard stock solution was prepared by dissolving 34.1 mg of Trimetazidine HCL standard in 100 mL diluent. Then 3.2, 4.0 and 4.8 ml standard stock solution were transferred into 50 mL volumetric flask separately to obtain 80%, 100% and 120% of the nominal test concentration respectively. One previously tested marketed tablet was taken into a 50 mL volumetric flask and dissolved properly in diluent by sonication. After sonication and filtration 1 mL of the sample solution were transferred into each of the 50 ml volumetric flasks containing 80%, 100%, and 120% of the test concentration of standard separately. Accuracy study (% recovery level and % RSD) was carried out at similar operating condition.

Calculation of % Recovery of the test concentration sample:

Actual concentration of added sample (μg/mL):

= Concentration of standard solution + Concentration of added sample solution

% Recovery = (Sample Recovered/Sample Added)* 100

Sensitivity:

For this baseline noise value was calculated from software. Low concentration of Trimetazidine solution was prepared by suitable dilution of the standard stock solution and checked for any visible peak at the retention time of the analyte. The peak obtained at this low concentration was compared with blank as per ICH guideline. LOD and LOQ of the drug should be within the following limits:

LOD=3 x (S/N)

LOQ = 10 x (S/N)

Specificity

By Checking the Chromatogram

Specificity was evaluated by comparing the chromatogram of standard, sample and placebo with blank at LOQ level of drug substance. Chromatogram was checked and peak purity value was determined.

By Checking the Response of Placebo

Placebo preparation:

Excipient stock powder was prepared using some common excipients used for modified release formulation within their acceptable usage range and taken into vial maintaining the ambient temperature for further use.

Placebo Constituents		
Lactose	:	390 mg
HPMC	:	400 mg
Microcrystalline Cellulose	:	400 mg
Povidone	:	300 mg
Talc	:	80 mg
Magnesium Stearate	:	80 mg

Standard Stock Preparation:

mg of standard was dissolved in 100 ml diluent to prepare standard stock solution.

Placebo Stock Preparation:

mg of the placebo mixture was dissolved in 100 mL of the diluent in a volumetric flask.

Working solutions preparation with fixed excipient stock solution and different standard solution:

The standard stock solution was taken at specified amount to five (05) 50 mL volumetric flasks to obtain a concentration of 80, 90, 100, 110 and 120 % of the nominal concentration of Trimetazidine present in the final assay sample. Fixed amount of the excipient stock solution was added to each of the previously 05 standard solution after filtration with Whatman No. 1 filter paper to obtain a concentration present in the final assay sample. The solution was adjusted to 50 mL with diluent. The sample solution was transferred into vial by passing through 0.2 µm membrane filter.

Working solutions preparation with fixed standard stock solution and different excipients solution:

The standard stock solution was taken at specified amount to five (05) 50 mL volumetric flasks to obtain a concentration of 100 % of the nominal concentration of Trimetazidine present in the final assay sample. Fixed amount of the excipient stock solution was added to each of the previously 05 standard solution after filtration with Whatman No. 1 filter paper to obtain a concentration of 80, 90, 100, 110 and 120% of the nominal concentration of excipient present in the final assay sample. The solution was adjusted to 50 mL with diluent. The sample solution was transferred into vial by passing through 0.2 µm membrane filter.

Robustness

- Content of the organic solvent in the eluent($\pm$2%);

- pH of the buffer solution ($\pm$0.05);

- HPLC column temperature ($\pm$ 5 °C);

- Mobile phase flow rate; and

- Changing wavelength of analysis.

Robustness study was carried out by three (03) injection of the standard solution at the same chromatographic condition.

System suitability

32.6 mg standard was transferred into 50 mL volumetric flask and the volume was adjusted with diluents. 2 mL of this solution was diluted up to 25 mL in a volumetric flask. System suitability study was carried out by six (06) injection of this solution at the same chromatographic condition.

CHAPTER : FIVE

RESULT

Result:

Wavelength Determination

Maximum Absorbance Wavelength of Trimetazidine:
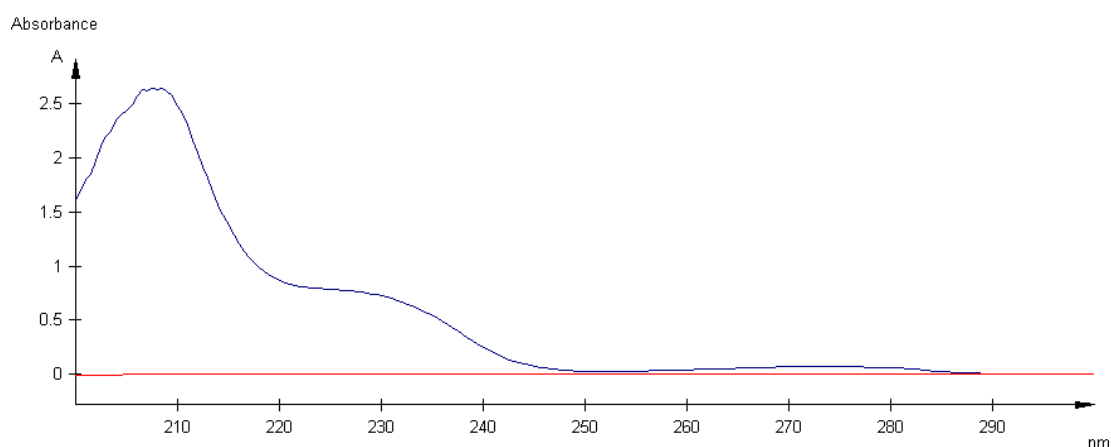
Figure 3: Maximum Absorbance Wavelength of Trimetazidine

Meas. values

Sample 1 - C 1
227 nm : 0.7757 A	227 nm : 0.7712 A	228 nm : 0.7660 A
228 nm : 0.7600 A	229 nm : 0.7531 A	229 nm : 0.7452 A
230 nm : 0.7359 A	230 nm : 0.7254 A	231 nm : 0.7133 A
231 nm : 0.6998 A	232 nm : 0.6848 A	232 nm : 0.6682 A
233 nm : 0.6501 A	233 nm : 0.6304 A	234 nm : 0.6093 A

The maximum absorption was observed at 232 nm.

Assay:

Assay result was calculated from calibration curve of the standard solution. Calibration standard concentration at each of the five concentration levels was determined from the following equation:

Sequence Summary Report

HPLC DATA

Sequence name: Assay1_9.8.18.rst

Description:

Acquisition date: 8/9/2018 12:44:48 PM

.::Standard statistics::.

Compound: Trimetazidine   Signal: DAD-CH1 232 nm

Injection Data File Name	Sample Name	RT [min]	Peak Asymmetry	Peak Purity	Peak Theoretical Plates USP	Area	Height	Concentration	Unit
Calibration_01.dat	Trimetazidine	8.62	1.35	0.9493	5921	1068532	62300	21.693	µg/ml
Calibration_02.dat	Trimetazidine	8.61	1.38	0.9729	5806	1354202	77989	27.116	µg/ml
Calibration_03.dat	Trimetazidine	8.59	1.44	1.0000	5461	1650885	92969	32.540	µg/ml
Calibration_04.dat	Trimetazidine	8.57	1.53	0.9929	5318	1905343	106504	37.963	µg/ml
Calibration_05.dat	Trimetazidine	8.56	1.54	0.9975	5215	2209707	122659	43.386	µg/ml
	Standard Devi	0.03	0.08461						
	RSD	0.30	5.84096						
	Average	8.59	1.44859						

.::Sample statistics::.

Compound: Trimetazidine   Signal: DAD-CH1 232 nm

Injection Data File Name	Sample Name	RT [min]	Peak Asymmetry	Peak Purity	Peak Theoretical Plates USP	Area	Height	Compound Concentration	Unit
Sample_01.dat	Trimetazidine	8.59	1.445	0.9778	5567	1436606	81443	28.69	µg/ml
Sample_02.dat	Trimetazidine	8.56	1.535	0.9867	5110	1433691	79531	28.64	µg/ml
	Mean	8.577							
	StdDev	0.024							
	RSD	0.275							

HPLC Chromatograph
Assay

Sample Name: Calibration Standard_01
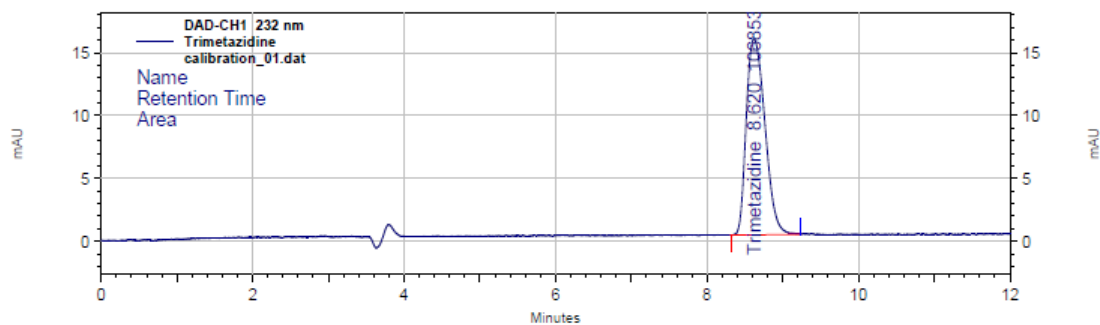
Sample Name: Calibration Standard_02
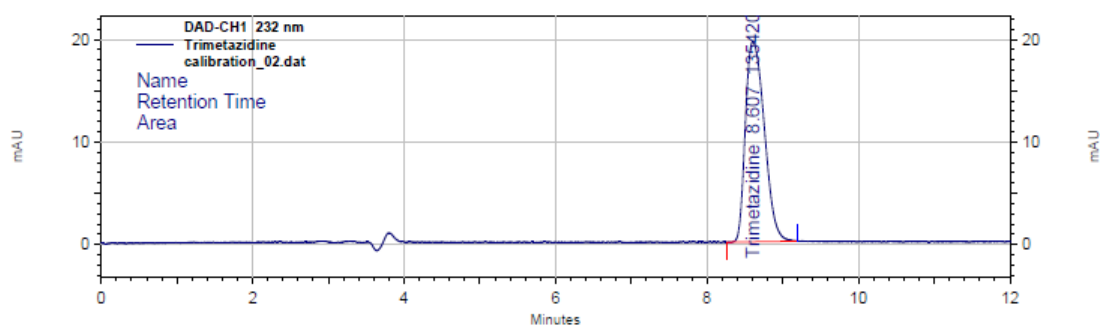
Sample Name: Calibration Standard_03
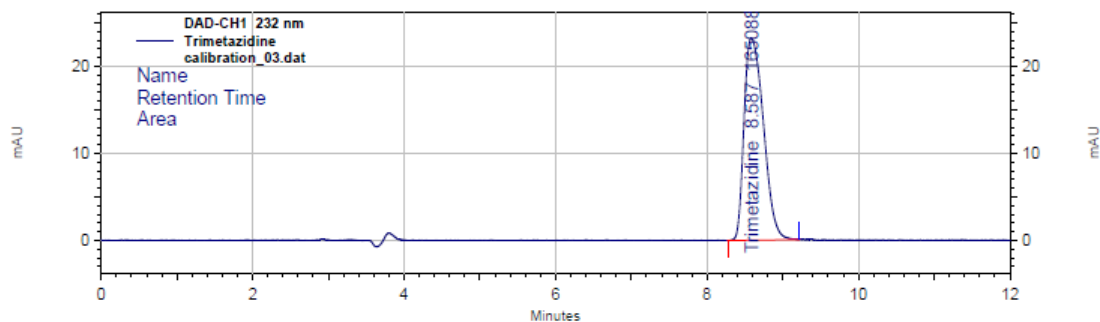
Sample Name: Calibration Standard_04
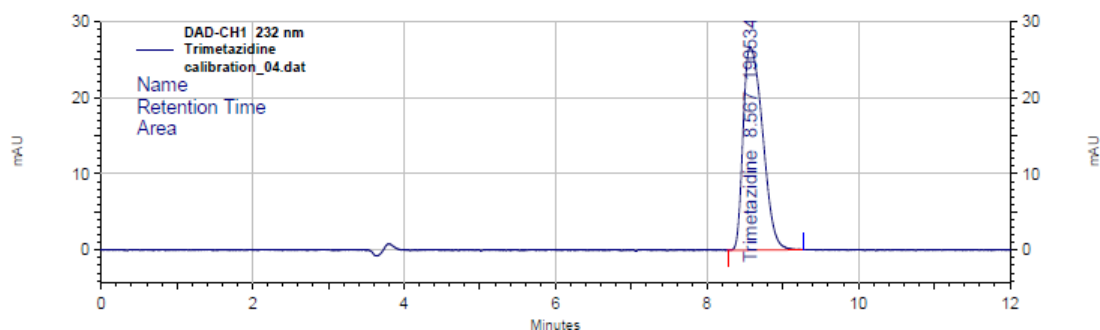
Sample Name: Calibration Standard_05
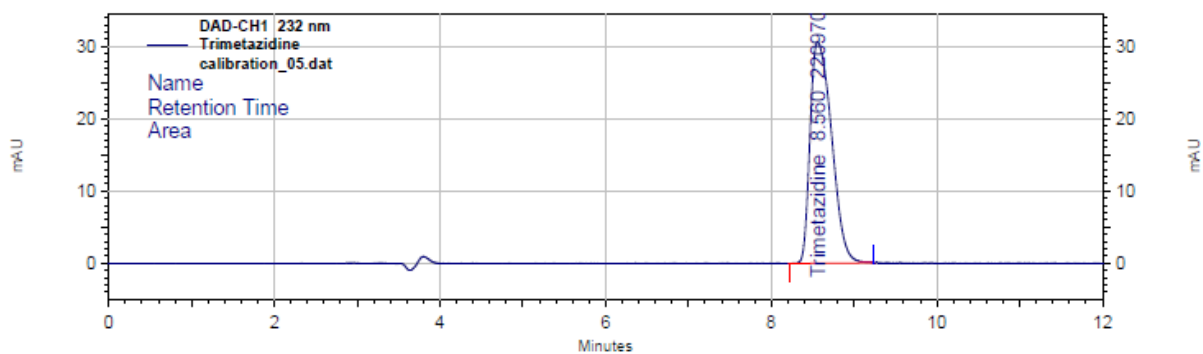
Sample Name: Sample_01
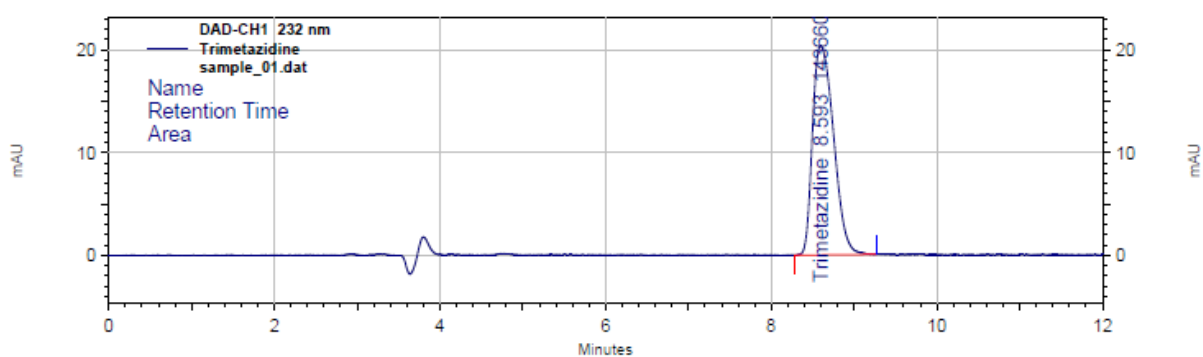
Sample Name: Sample_02
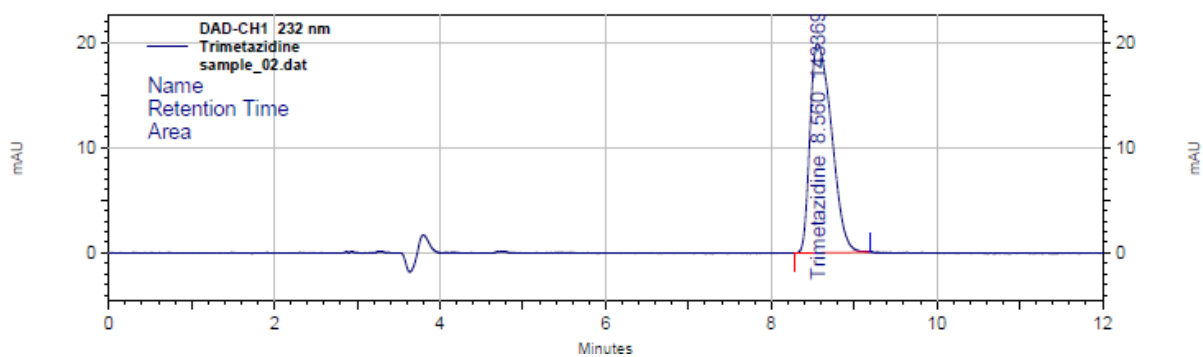
Sample concentration was calculated by the regression equation obtained from the calibration graph:

y = 50431.4x

Table 1: Assay study of Trimetazidine

Sample	Weight Taken (mg)	Peak Area	Amount (mg/Tablet)
Sample_01	208.1 mg	1436606	34.64
Sample_02	207.4 mg	1433691	34.69

Linearity

Concentration of the standard solution was prepared from the following equation:

Table 2: Linearity Study of Trimetazidine

Nominal Concentration	Concentration (µg/mL)	Peak Area
37.176	1.859	89602
	3.718	192326
	7.435	373365
	18.588	929157
	29.740	1475110
	33.458	1656044
	37.176	1812736
	40.893	2058421
	44.611	2260168
	55.763	2823560
Correlation coefficient (r^2): 0.999		
y-intercept: 0.00		
Slope of the regression line: 50328.8		
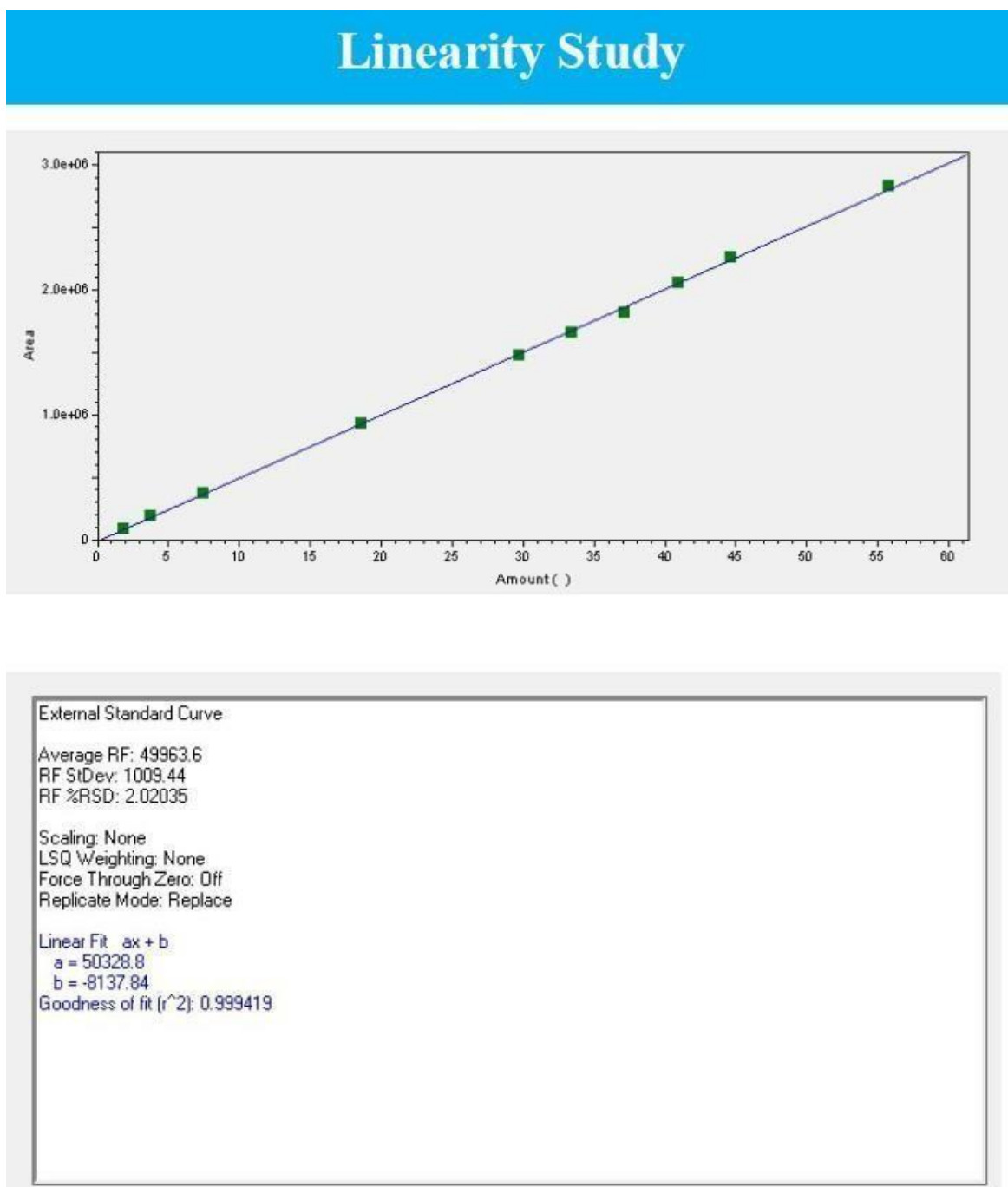
Figure 4: Linearity study of Trimetazidine

HPLC Chromatograph
Linearity Study

Sample Name: Linearity 5.0%
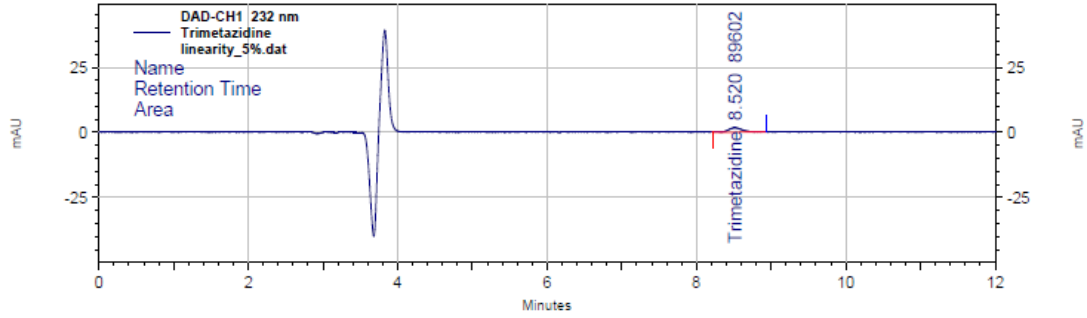
Sample Name: Linearity 10.0%
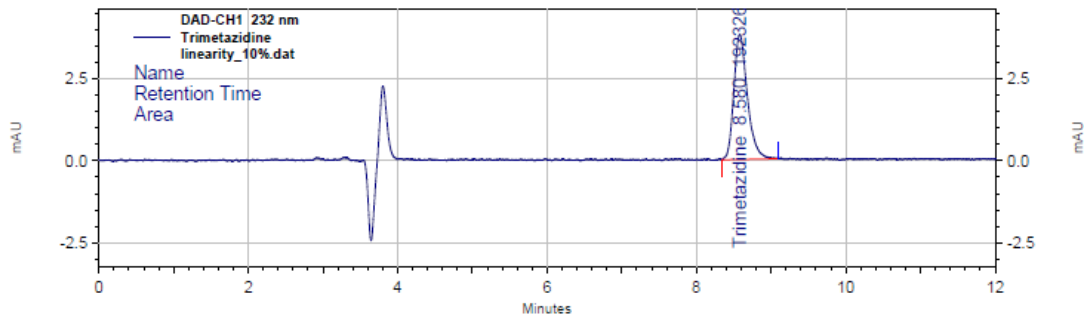
Sample Name: Linearity 20.0%
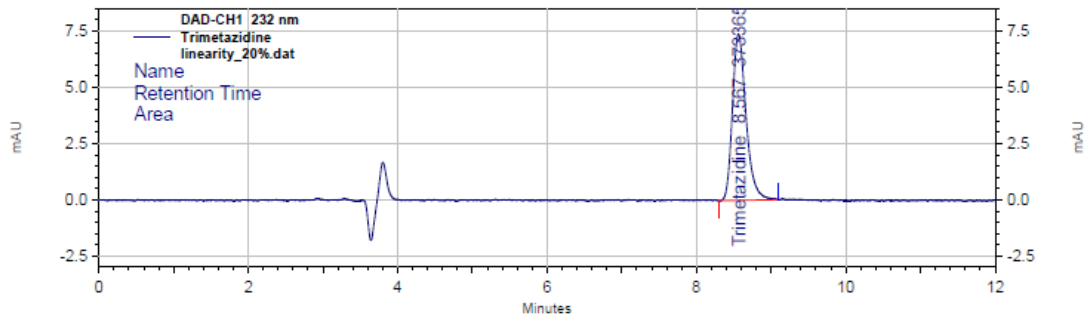
Sample Name: Linearity 50.0%
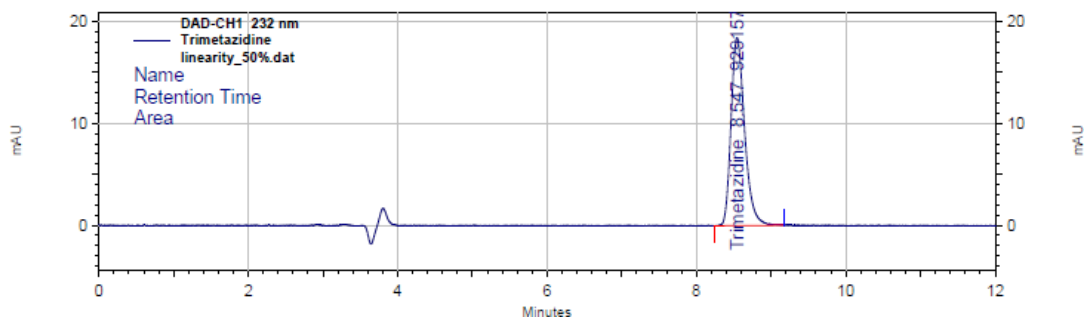
Sample Name: Linearity 80.0%
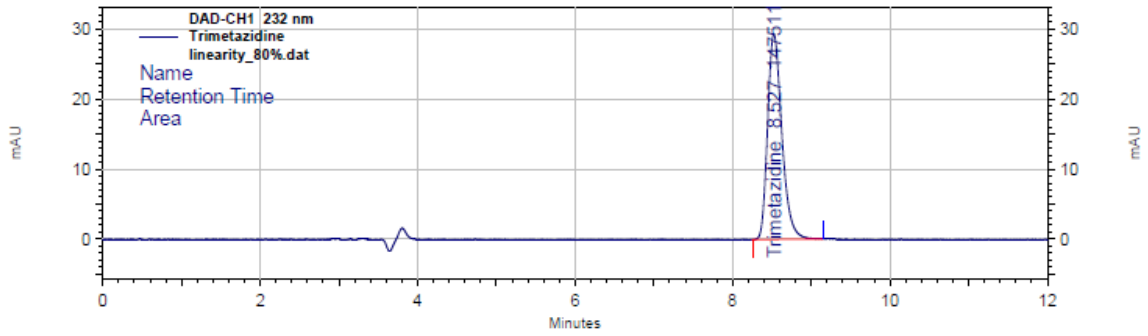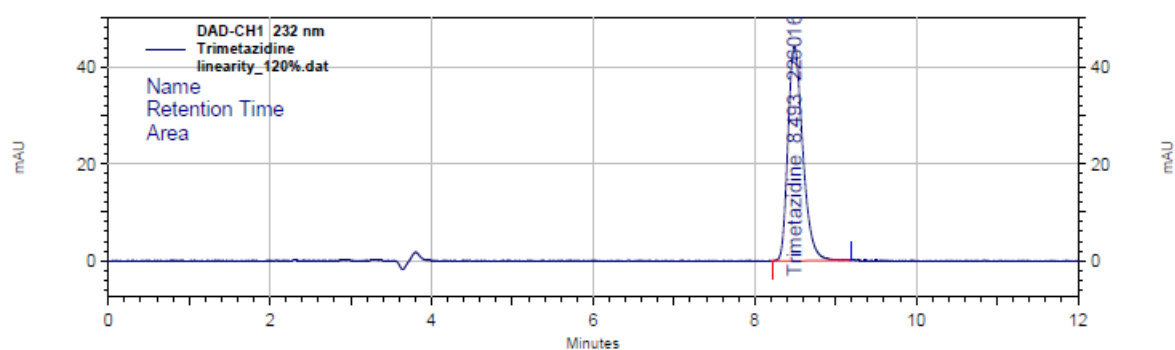

Sample Name: Linearity 90.0%
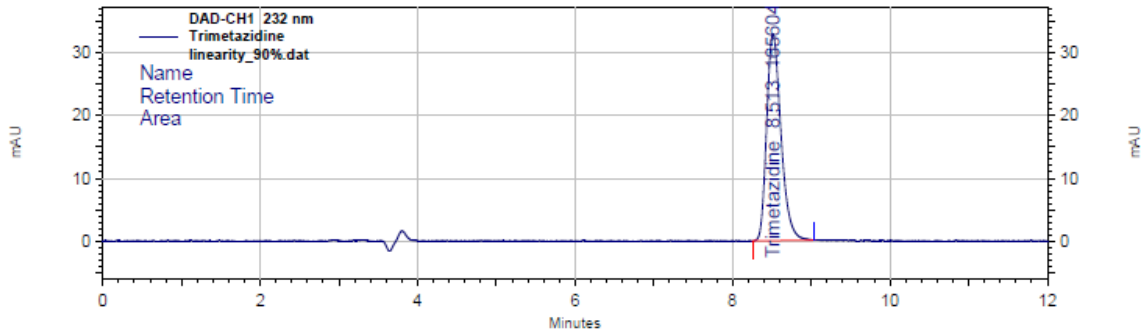
Sample Name: Linearity 100.0%
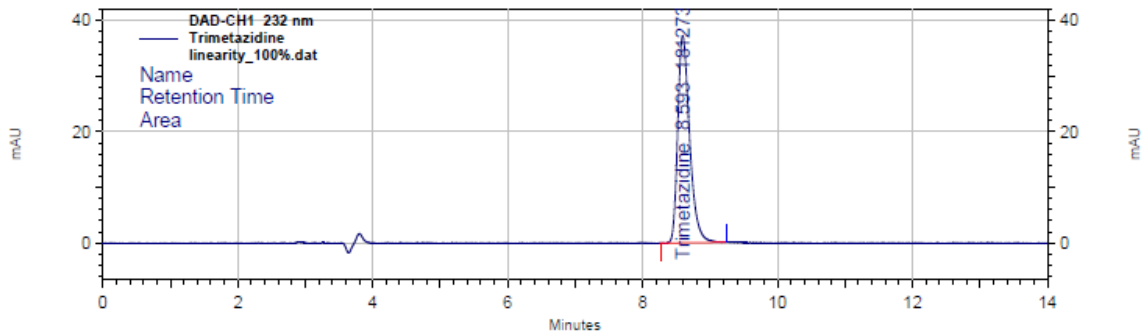
Sample Name: Linearity 110.0%
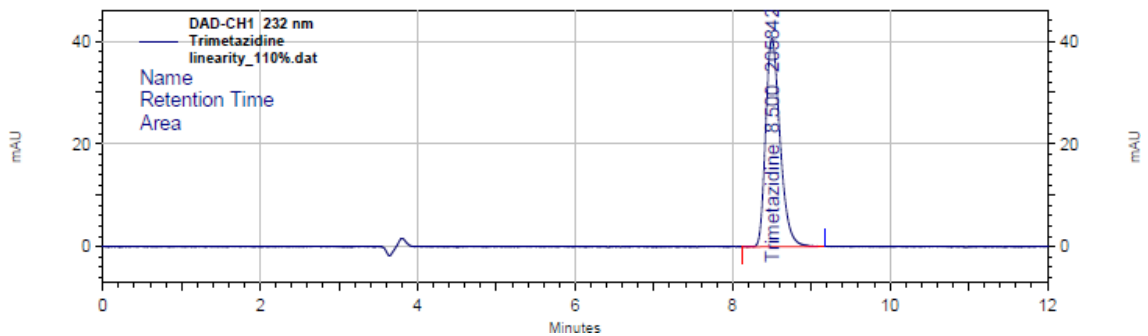
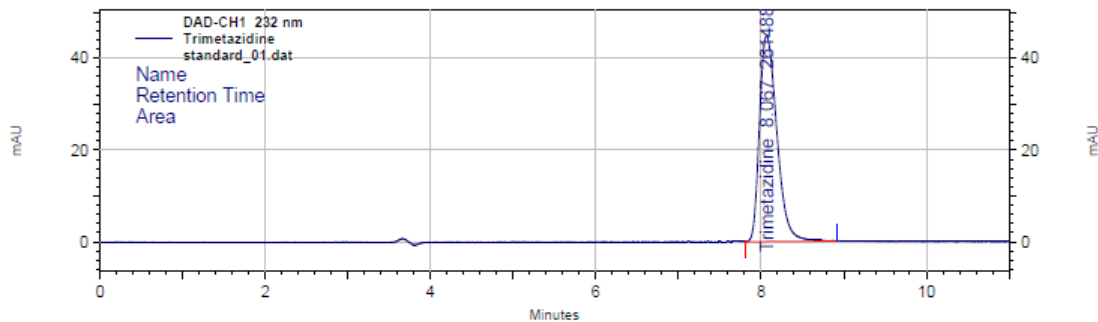Sample Name: Linearity 120.0%
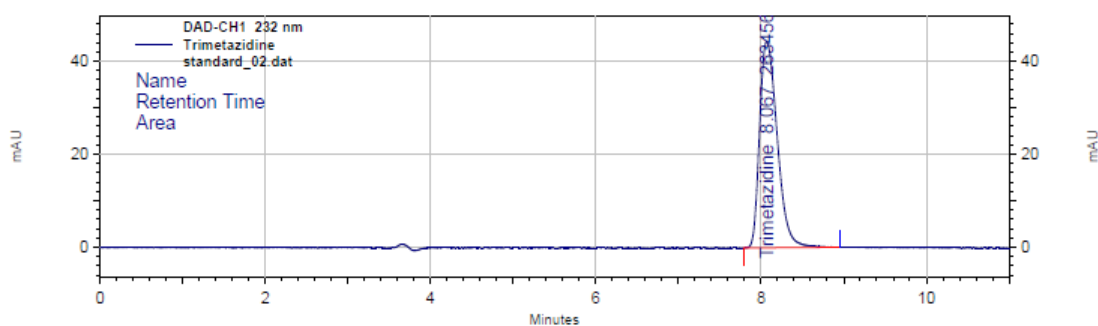

Sample Name: Linearity 150.0%
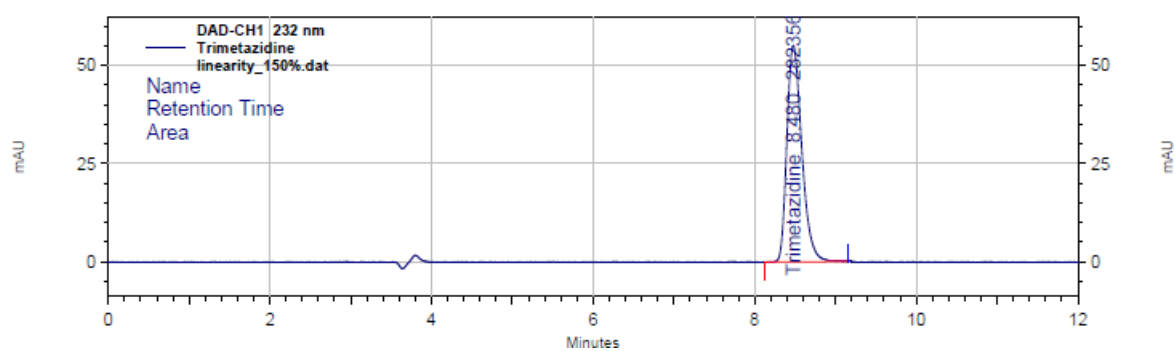
Range

Range was calculated by comparing the samples with known concentration of Trimetazidine standard.
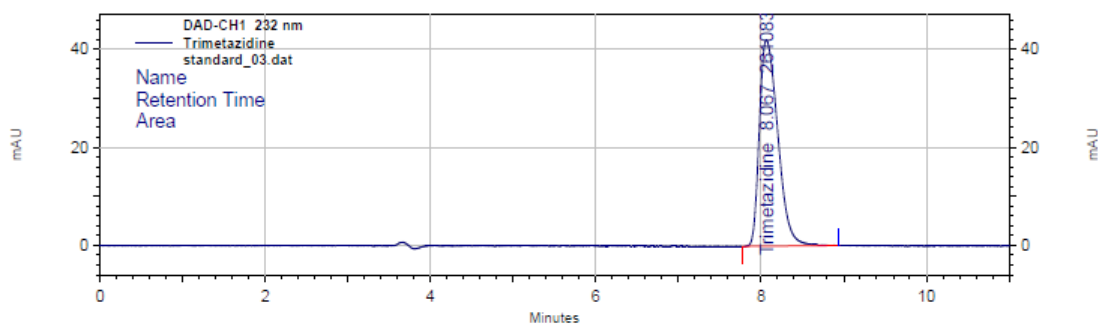
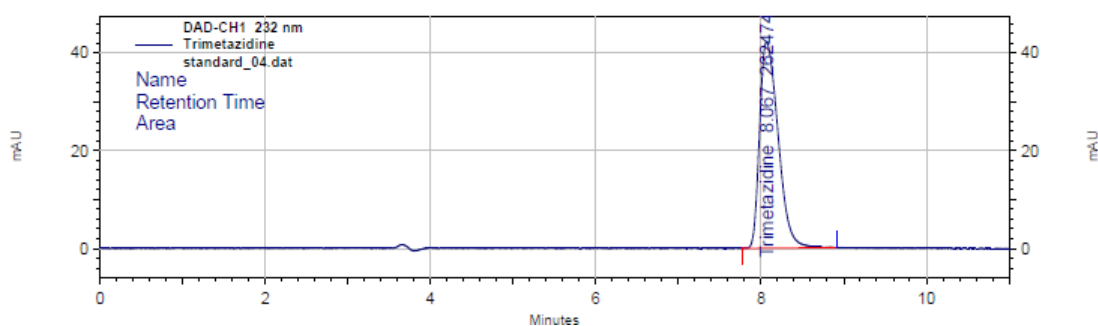# HPLC Chromatograph
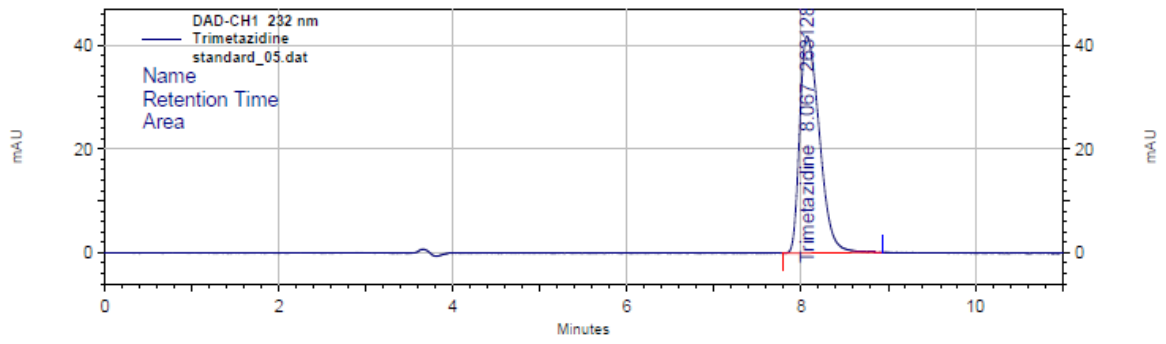# Range Study

Sample Name: Standard_01

Sample Name: Standard_02
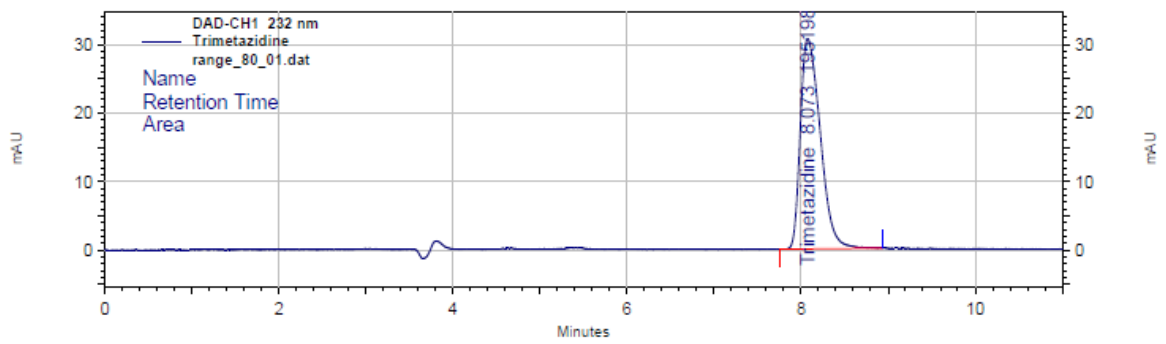

Sample Name: Standard_03
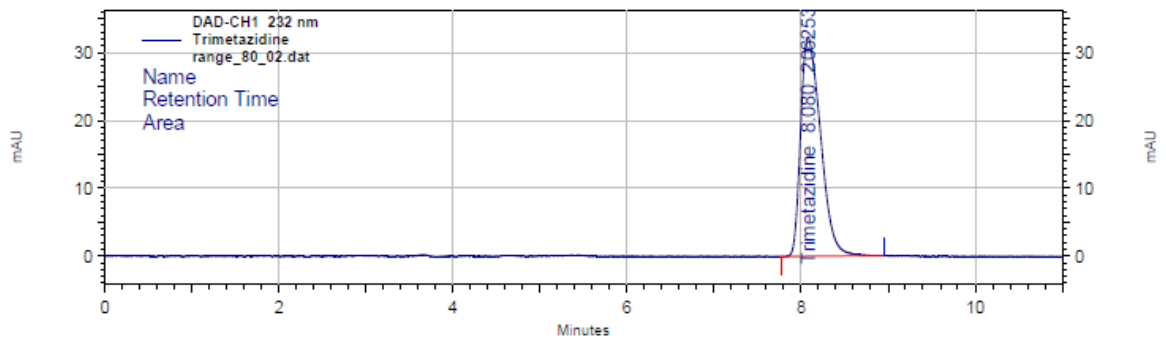

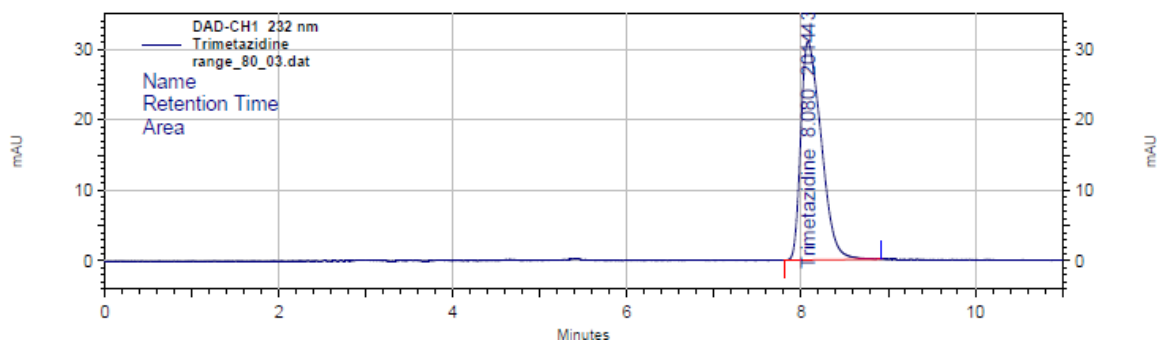## Sample Name: Standard_04

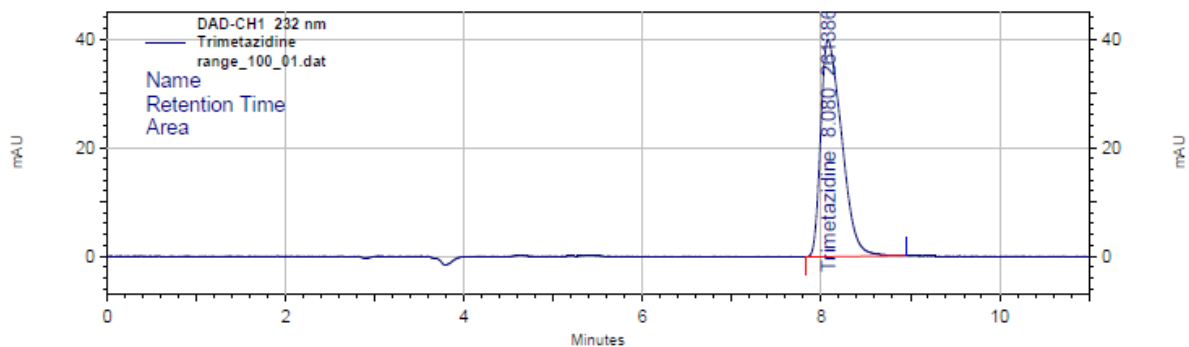## Sample Name: Standard_05

Sample Name: Range_80%_01

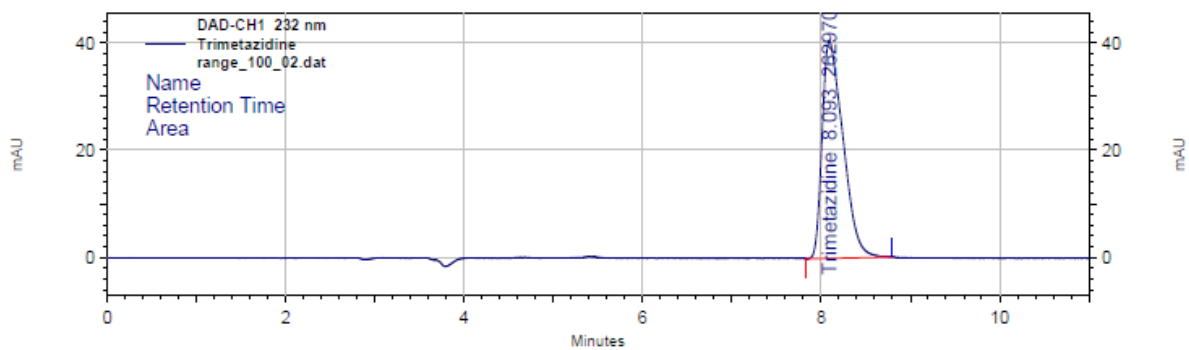## Sample Name: Range_80%_02

Sample Name: Range_80%_03

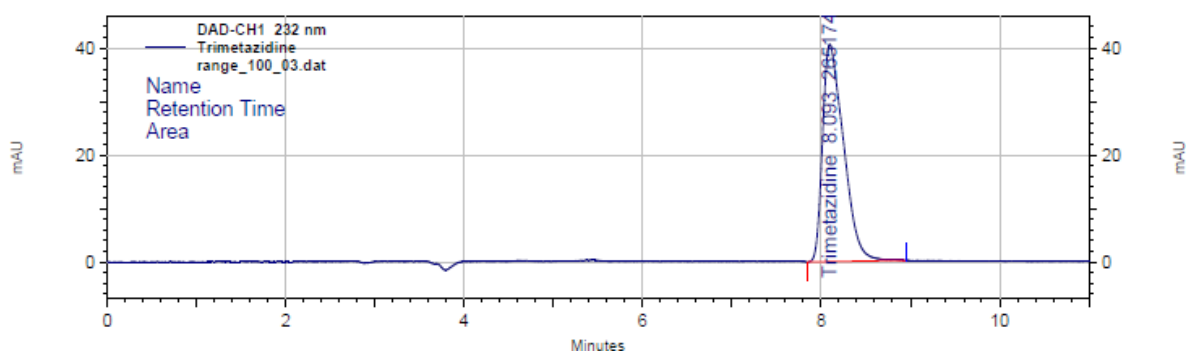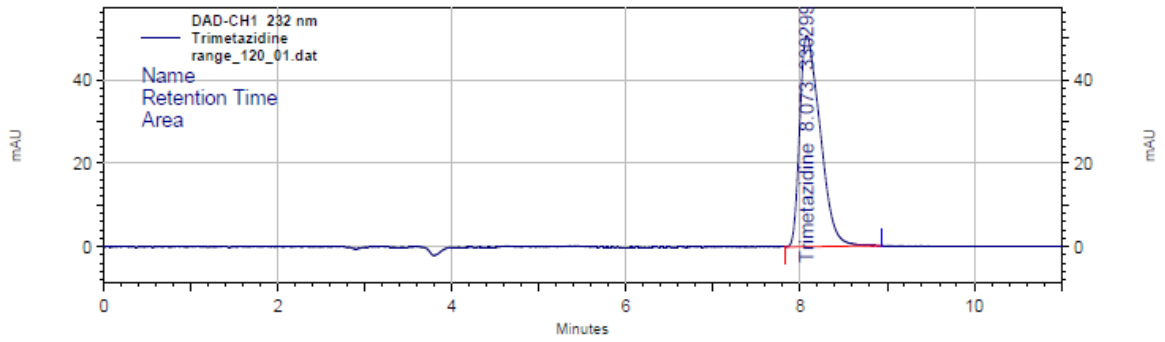## Sample Name: Range_100%_01

Sample Name: Range_100%_02

Sample Name: Range_100%_03

Sample Name: Range_120%_01

Sample Name: Range_120%_02
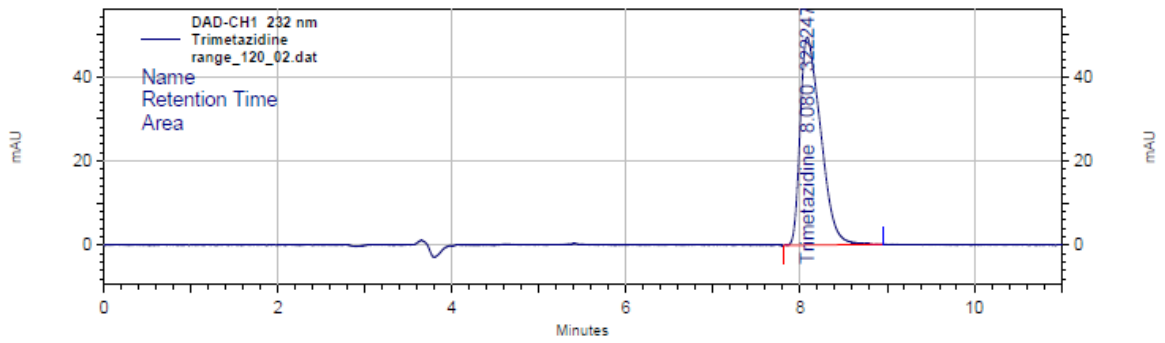
Sample Name: Range_120%_03
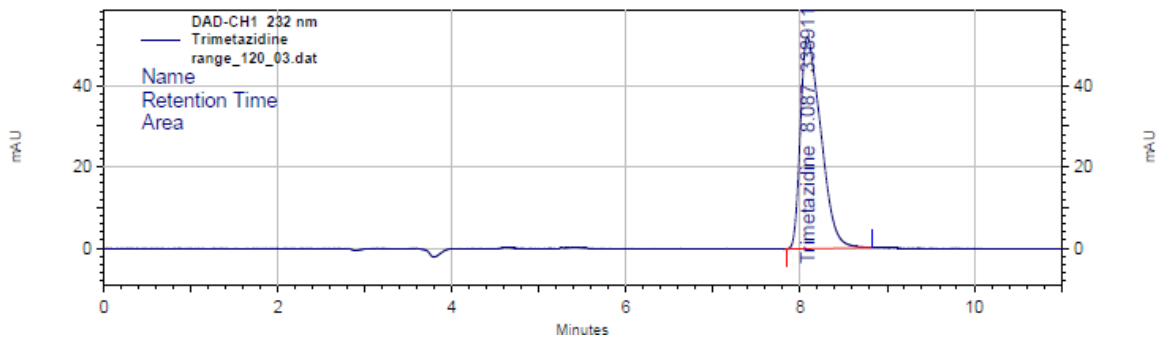
Table 3: Range Study

Nominal Concentration	Percent	Sample Weight (mg)	Concentration (µg/mL)	Peak Area	Amount (mg/Tablet)
51.951 µg/mL	80	191.4	38.54	1951986	32.56
		194.2	40.72	2062536	34.41
		193.6	39.77	2014438	33.22
	100	194.6	51.61	2613867	34.31
		194.2	51.92	2629707	34.59
		195.3	52.36	2651741	34.69
	120	196.9	65.21	3302994	34.67
		196.3	63.62	3222478	33.93
		197.6	66.91	3389110	35.45

Standard deviation and % relative standard deviation at each concentration was calculated.

Table 4: Range Study (%RSD Calculation)

Concentration Level	Average Concentration (µg/mL)	Average Peak Area	% RSD of Concentration
80%	39.68	2009653	2.25
100%	51.96	2631772	0.68
120%	65.25	3304861	2.07
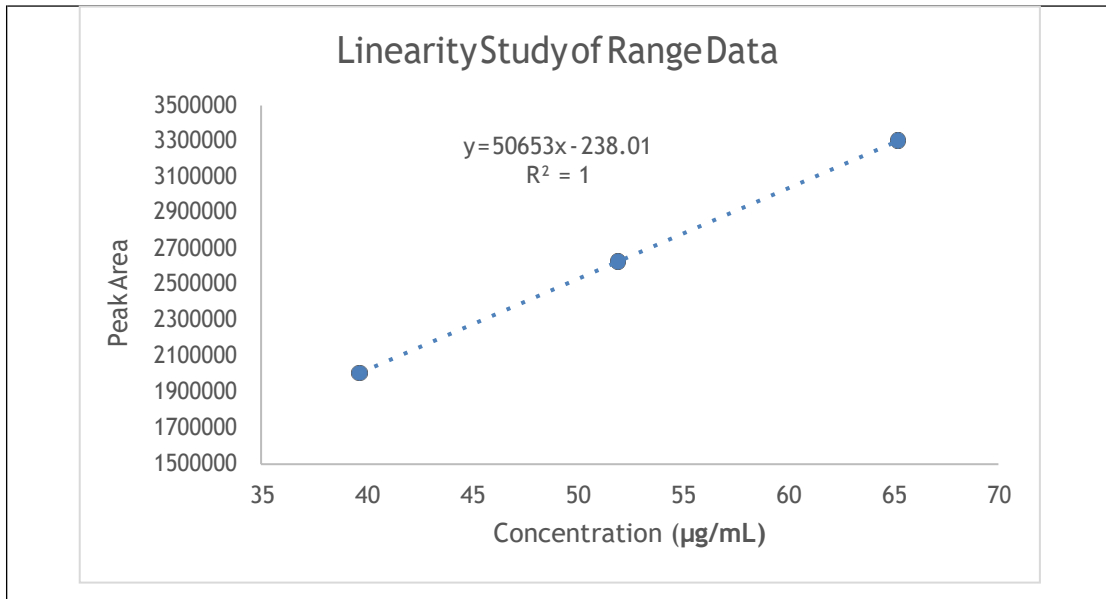
Precision:

Repeatability:

Repeatability or intraday precision was determined by replicate analyses of standard solution at 80%, 100% and 120% of the nominal test concentration in a single day at different time interval.

Table 5: Intraday Precision Study:

Nominal Test Concentration	Percent	Concentration (µg/mL)	Peak area	Average	%RSD
36.102	28.881µg/mL, (80%)	Initial	1454968	1464851	0.52
			1466219		
			1473365		
		2nd Hour	1461644	1476090	0.77
			1477248		
			1489379		
		6th Hour	1460998	1462513	0.26
			1467772		
			1458770		
	36.102 µg/mL,	Initial	1826893	1839517	0.49
			1845856		

	(100%)		1845802		
		2nd Hour	1835527	1842992	0.31
			1844115		
			1849334		
		6th Hour	1840753	1835890	0.85
			1814856		
			1852060		
	43.322 µg/mL, (120%)	Initial	2222197	2215085	0.25
			2208864		
			2214194		
		2nd Hour	2214194	2201595	0.65
			2208864		
			2181728		
		6th Hour	2163309	2180701	0.61
			2182944		
			2195850		
Relative standard deviation at each concentration was calculated. Linearity data was also evaluated.					

Table 6: Intraday Precision Study (%RSD Calculation)

Concentration Level	Concentration (µg/mL)	Average Peak Area	% RSD of Concentration
80%	28.881	1467818	0.40
100%	36.102	1839466	0.16
120%	43.322	2199127	0.64

Linearity Study of Intraday Precision Data

$y = 50641x + 7239.3$
$R^2 = 0.9999$
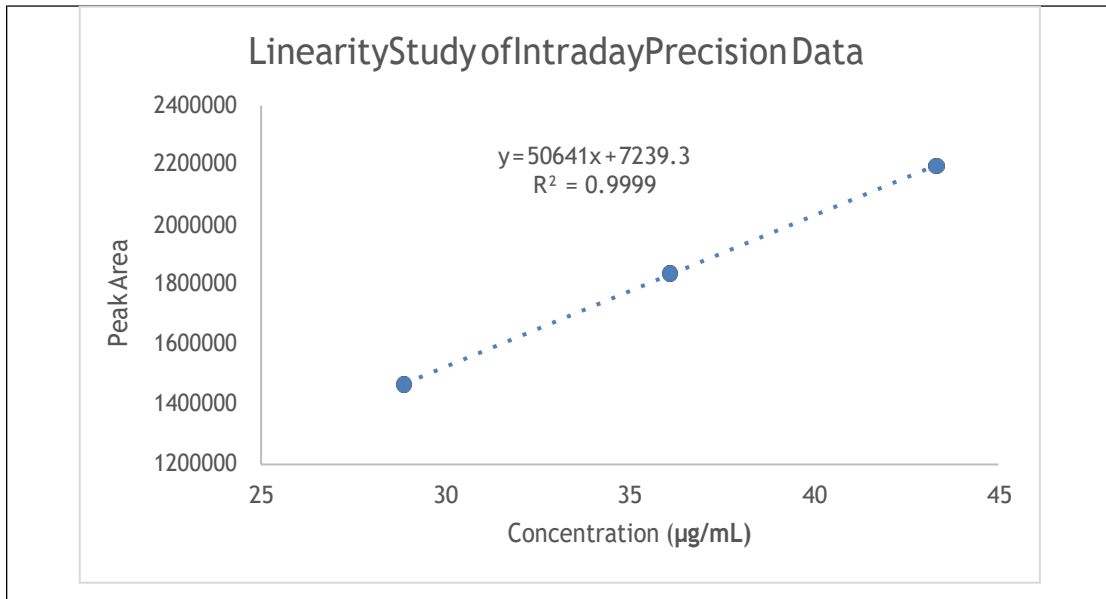
Intermediate precision:

Intermediate precision or Interday precision was determined by replicate analyses of standard solution at 80%, 100% and 120% of the nominal test concentration for three (03) consecutive days.

Table 7: Interday precision study:

Nominal Test Concentration	Percent	Concentration (µg/mL)	Peak area	Average	%RSD
36.102	28.881µg/mL, (80%)	1st Day	1464851	1467818	0.40
			1476090		
			1462513		
		2nd Day	1378030	1398759	2.00
			1380004		
			1438242		
		3rd Day	1445577	1447886	0.44
			1456616		
			1441464		
	36.102 µg/mL,	1st Day	1839517	1839466	0.16
			1842992		

	(100%)	1835890		
	2nd Day	1821338	1830495	0.52
		1843517		
		1826630		
	3rd Day	1820442	1834411	0.61
		1848062		
		1834730		
43.322 µg/mL, (120%)	1st Day	2215085	2199127	0.64
		2201595		
		2180701		
	2nd Day	2227354	2229566	1.29
		2265710		
		2195633		
	3rd Day	2190269	2178319	0.39
		2173787		
		2170901		
Relative standard deviation at each concentration was calculated. Linearity data was also evaluated.				

Table 8: Interday Precision Study (%RSD Calculation)

Concentration Level	Concentration (µg/mL)	Average Peak Area	% RSD of Concentration
80%	28.881	1438154	2.02
100%	36.102	1834791	0.20
120%	43.322	2202337	0.96
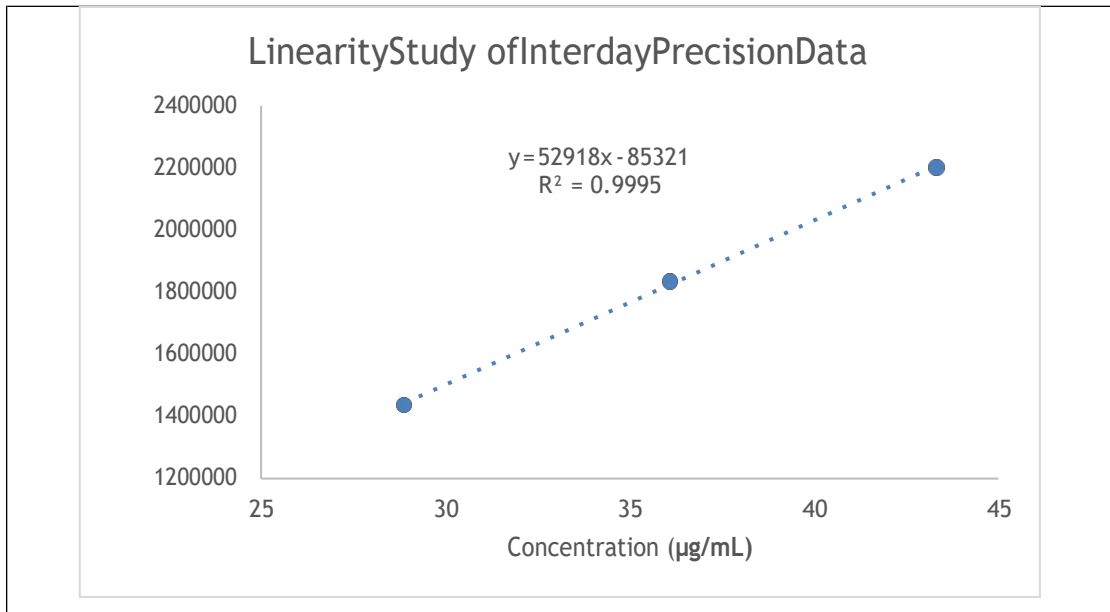
Accuracy:

% Recovery of the test sample was determined by the following formula:

= (Actual Sample Recovered/Theoretical Sample Added) X 100

Table 9: Accuracy Study (Theoretical Concentration)

Level	Standard Solution Concentration (µg/mL)	Sample Solution Added (µg/mL)	Theoretical Concentration(µg/mL)
80%	21.693		28.707
	21.693		28.707
	21.693		28.707
100%	27.116		34.13
	27.116	7.014	34.13
	27.116		34.13
120%	32.539		39.553
	32.539		39.553
	32.539		39.553

Actual Sample Recovered Concentration Calculation:

Sample recovered from the HPLC assay was determined by the Calibration curve of the standard solution. Calibration standard concentration at each level was determined by the following formula:

Sample concentration was calculated by the regression equation obtained from the calibration graph:
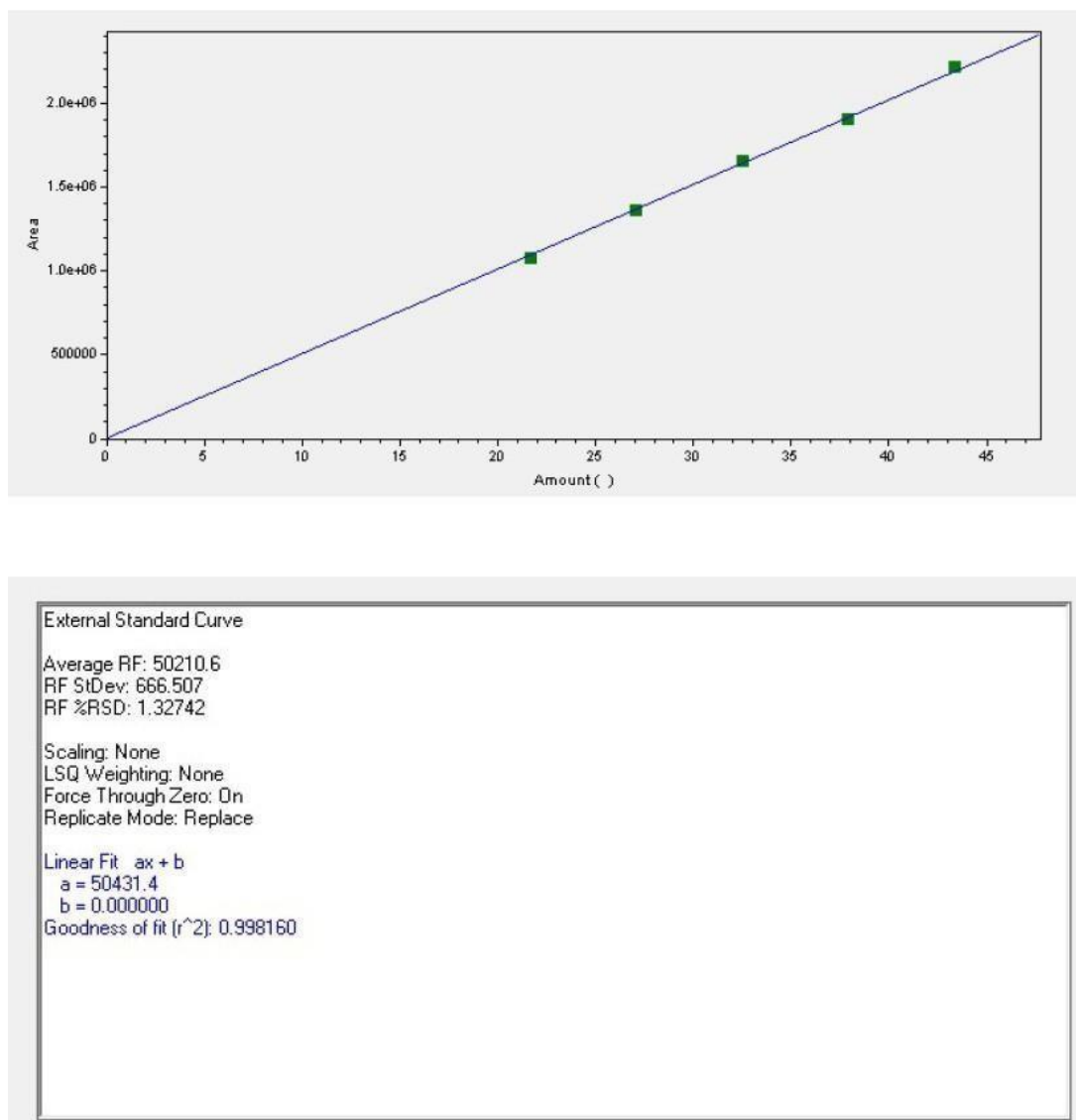
$y = 50431.4x$ _____

Figure 5: Accuracy study of Trimetazidine

Table 10: Accuracy Study

Level	Theoretical Concentration(µg/mL)	Actual Solution Concentration (µg/mL)	% Recovery	%RSD
80%	28.707	29.18	98.38	0.38
	28.707	28.94	99.19	
	28.707	29.17	98.41	
100%	34.13	34.79	98.10	0.78
	34.13	34.97	97.60	
	34.13	34.33	99.42	
120%	39.553	40.56	97.52	0.08
	39.553	40.61	97.40	
	39.553	40.53	97.59	

Sensitivity:

Sensitivity was determined using signal to noise ratio.

Figure 6: Noise Data for the method obtained from HPLC
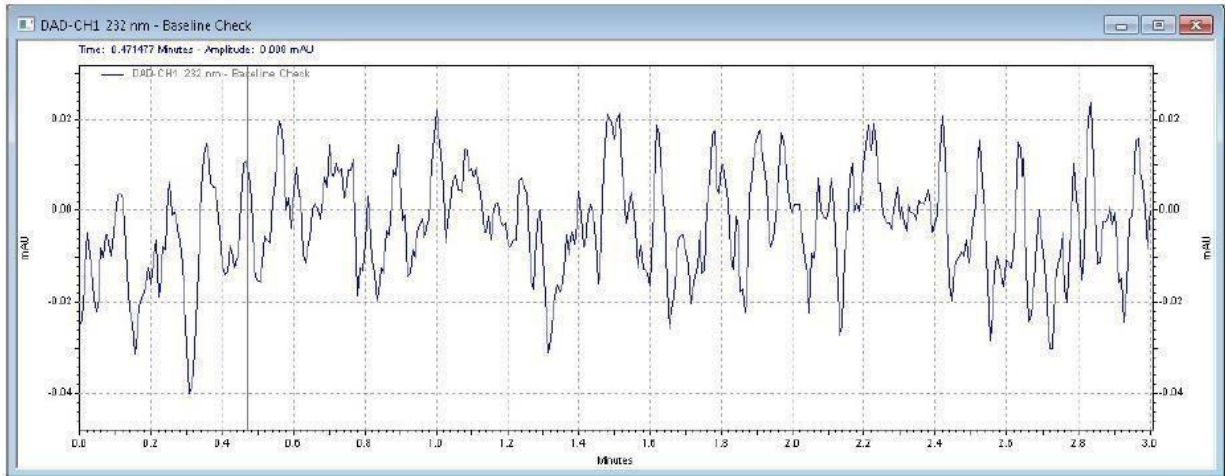
Figure 7: Noise Chromatogram for the method obtained from HPLC

Limit of Detection (LOD):

Concentration of LOD was determined by the following equation,

LOD = 0.307 µg/mL

Here, Height = 851

S/N = 851/199.8 = 4.23

Limit of Quantitation (LOQ):

Concentration of LOD was determined by the following equation,

LOQ = 1.021 µg/mL

Here, Height = 2359

S/N = 2359/199.8 = 11.81

HPLC Chromatograph
Sensitivity Study

Sample Name: Limit of Detection (LOD)
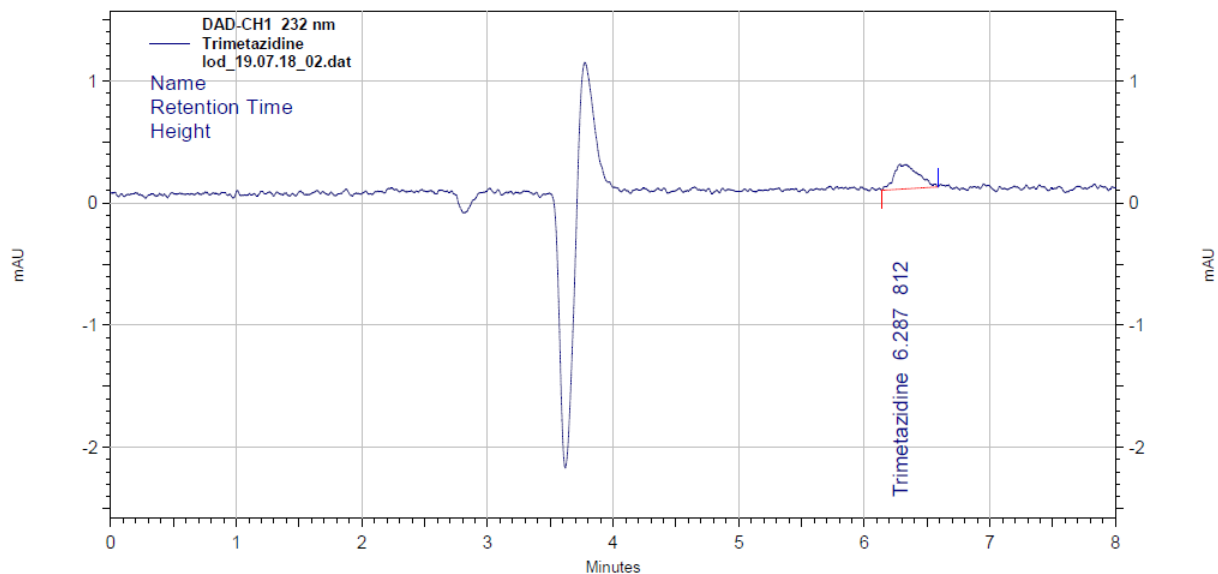
Sample Name: Limit of Quantiation (LOQ)
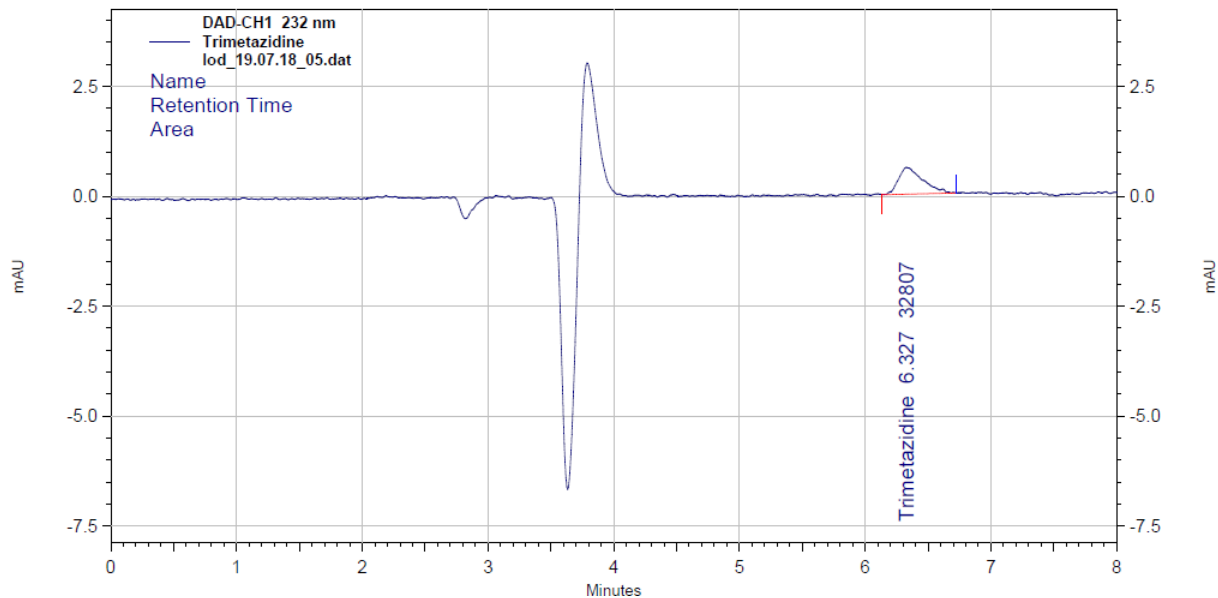
Specificity

Chromatogram Observation: Chromatogram of diluent (blank), standard, sample and excipient solution at LOQ level was observed for any interference.

HPLC Chromatograph
Specificity Study

Excipient
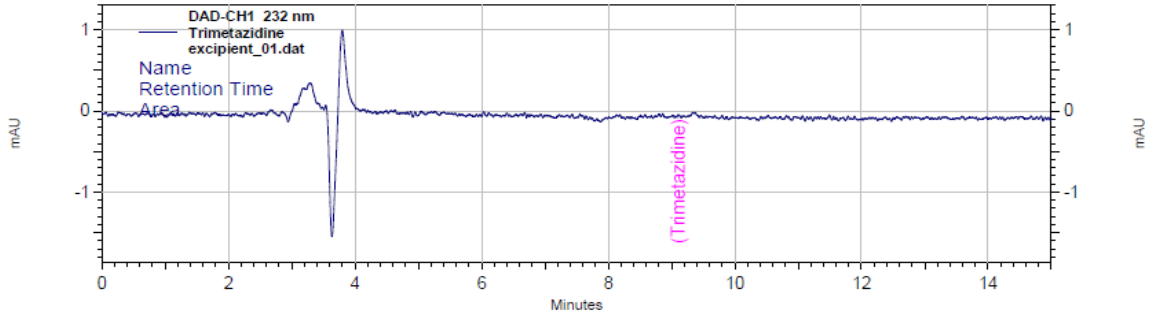
Standard
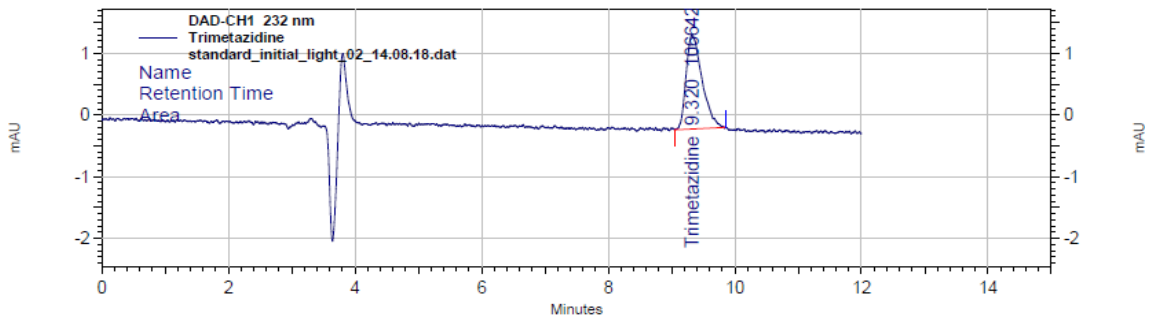
Sample
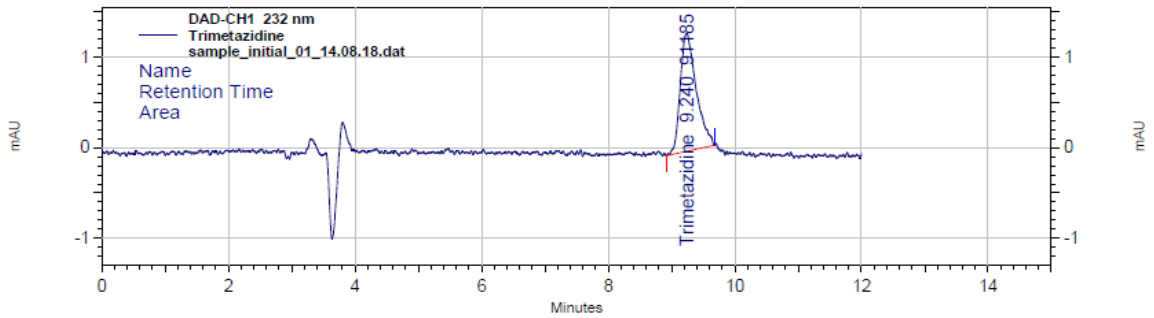
Blank
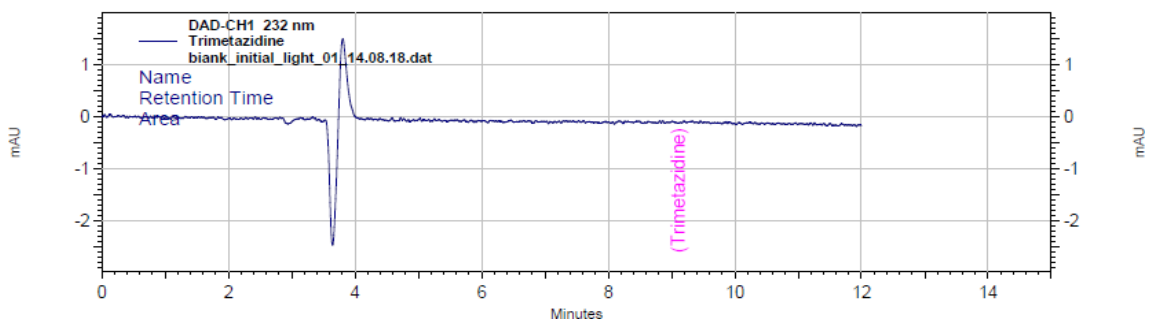
Table 11: Fixed excipient concentration with different standard concentration

Nominal Excipient Concentration (µg/mL)	Drug Substance Concentration (µg/ml)	Area	Peak Purity
216.00	25.62	1254016	0.9869
	28.83	1444884	0.9579
	32.03	1580370	0.9743
	35.23	1725559	0.9661
	38.44	1910064	0.9945

Specificity Study

$y = 49714x - 9353.8$

$R^2 = 0.9965$

Concentration (µg/mL)

Peak Area
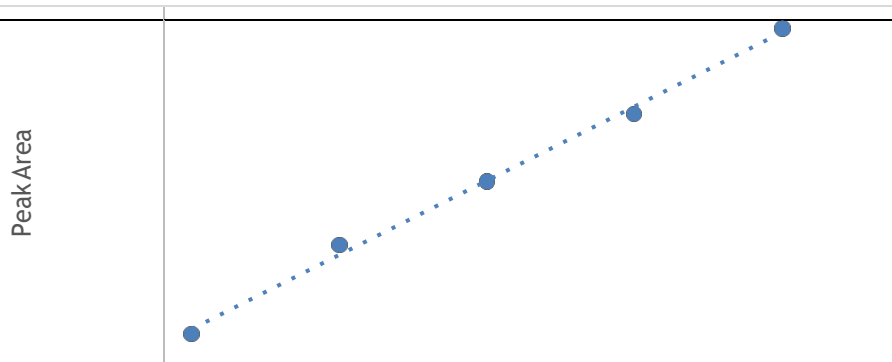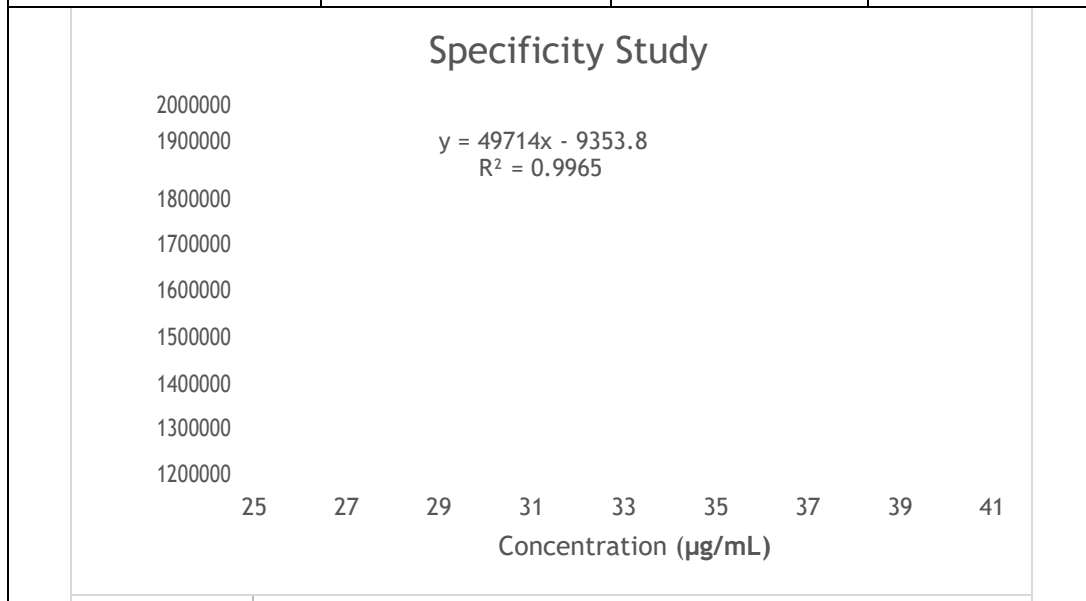
HPLC Chromatograph
Specificity Study

Sample Name: Excipient_100%_Standard_80%
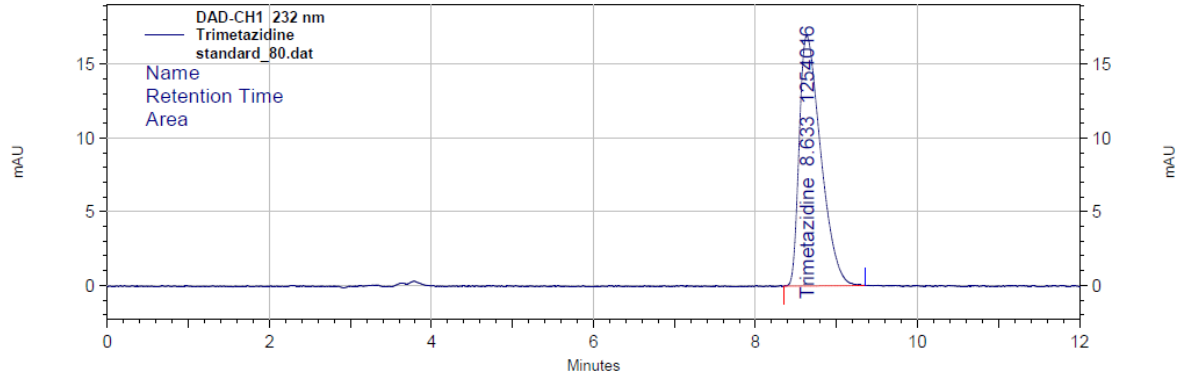
Sample Name: Excipient_100%_Standard_90%
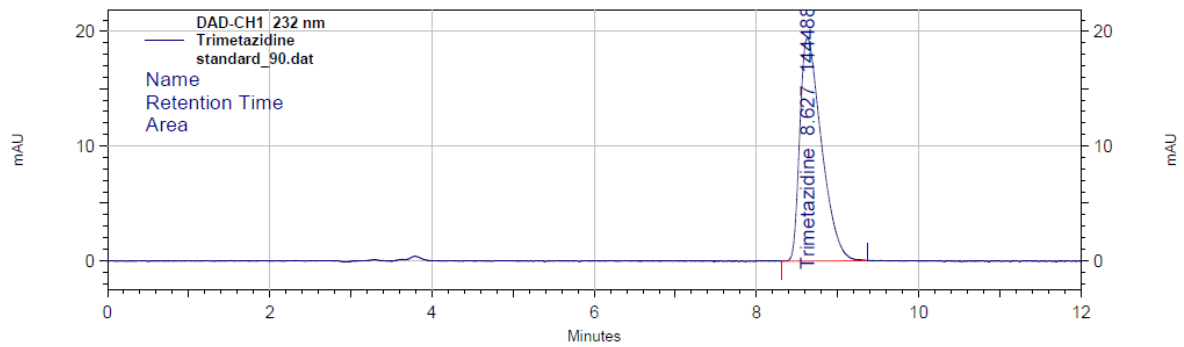
Sample Name: Excipient_100%_Standard_100%
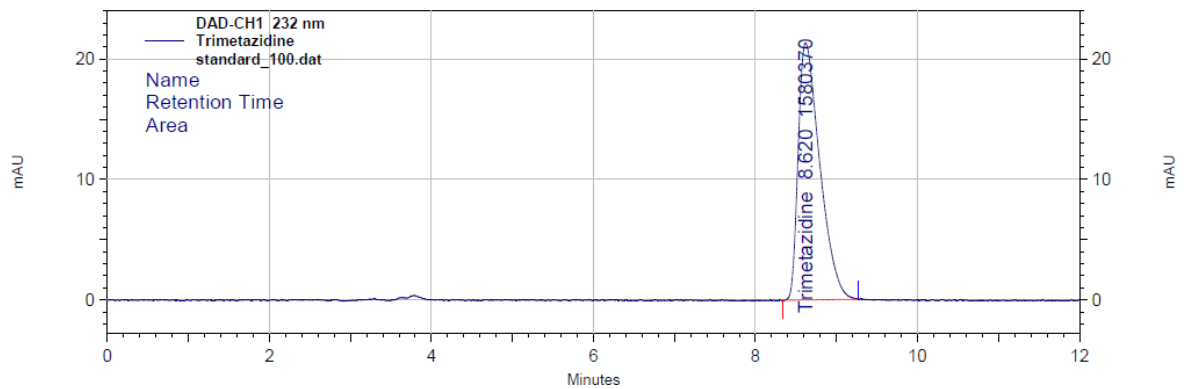
Sample Name: Excipient_100%_Standard_110%
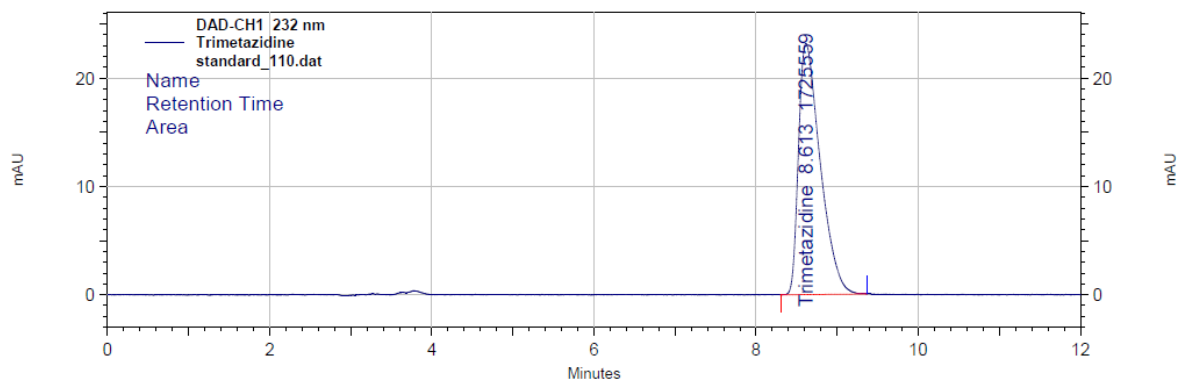
Sample Name: Excipient_100%_Standard_120%
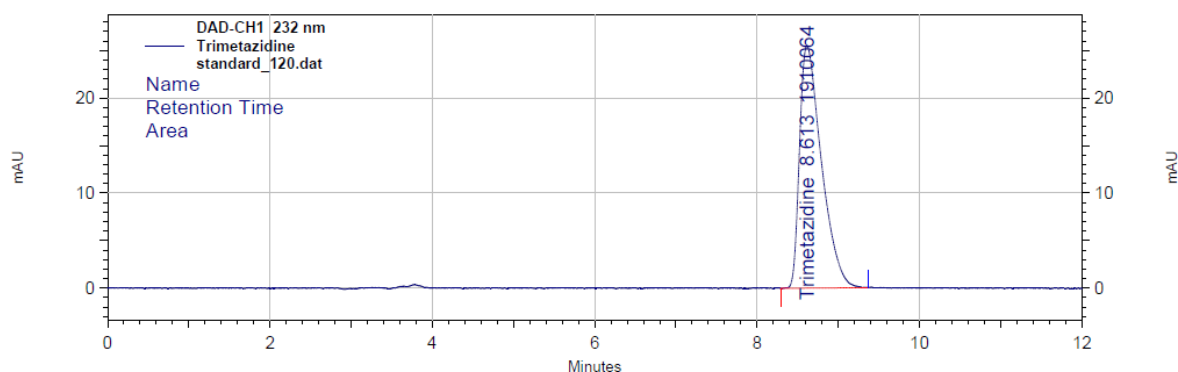
Table 12: Fixed standard concentration with different excipient concentration

Nominal Standard Concentration (μg/mL)	Excipient Concentration (μg/ml)	Area	Peak Purity
	172.8	1570710	0.9975
	194.4	1574043	0.9670
32.03	216.00	1576056	0.9726
	237.6	1578608	0.9898
	259.2	1586137	0.9798
		% RSD = 0.39	

Specificity Study

$y = 163.98x + 2E+06$
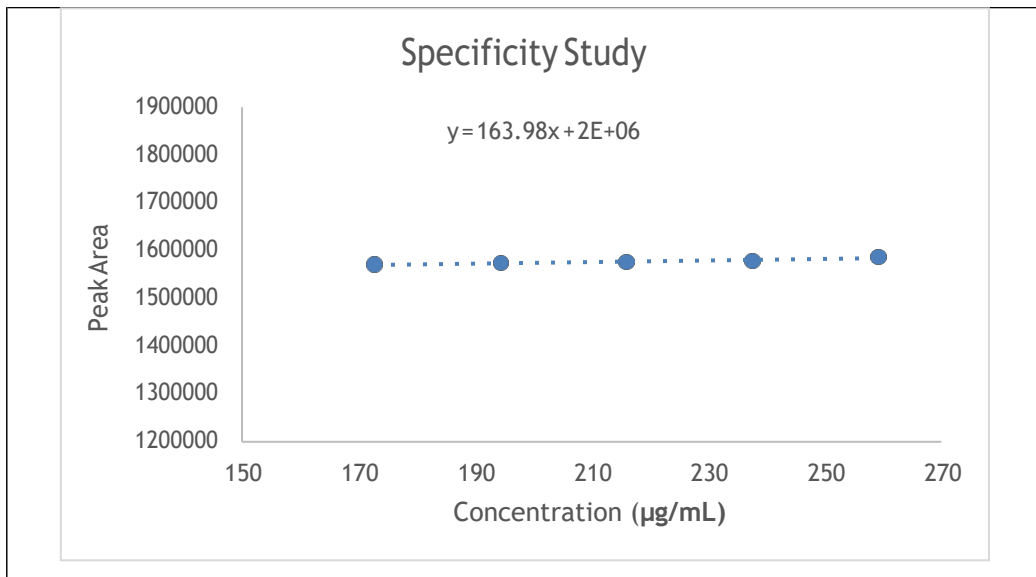
HPLC Chromatograph
Specificity Study

Sample Name: Excipient_80%_Standard_100%
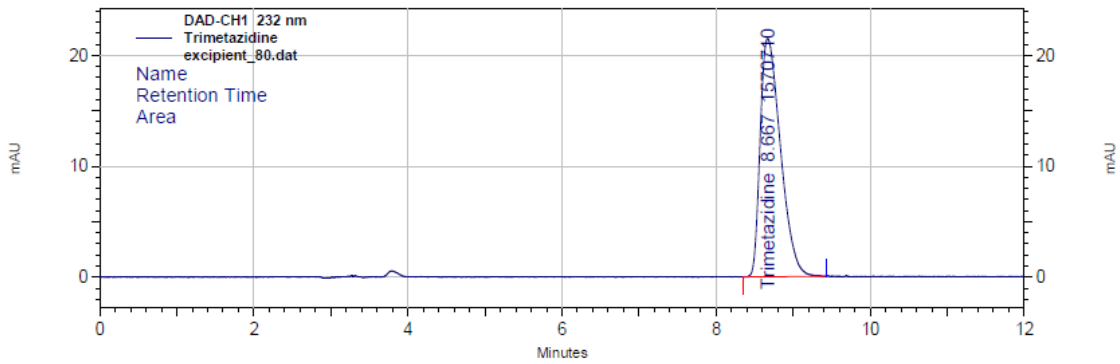
Sample Name: Excipient_90%_Standard_100%
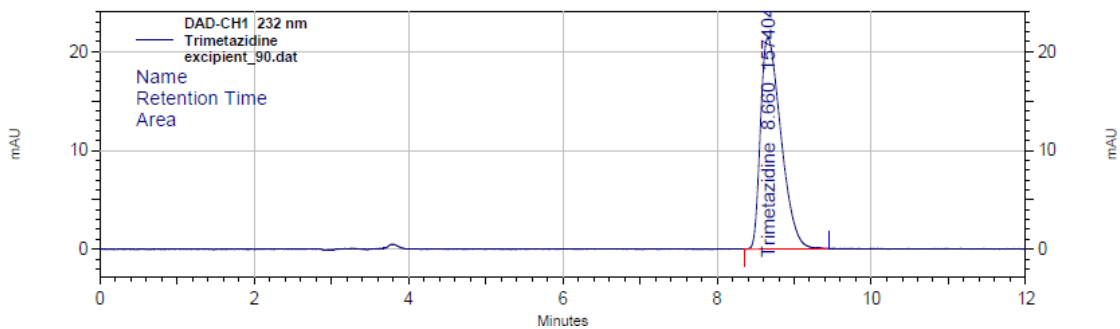
Sample Name: Excipient_100%_Standard_100%
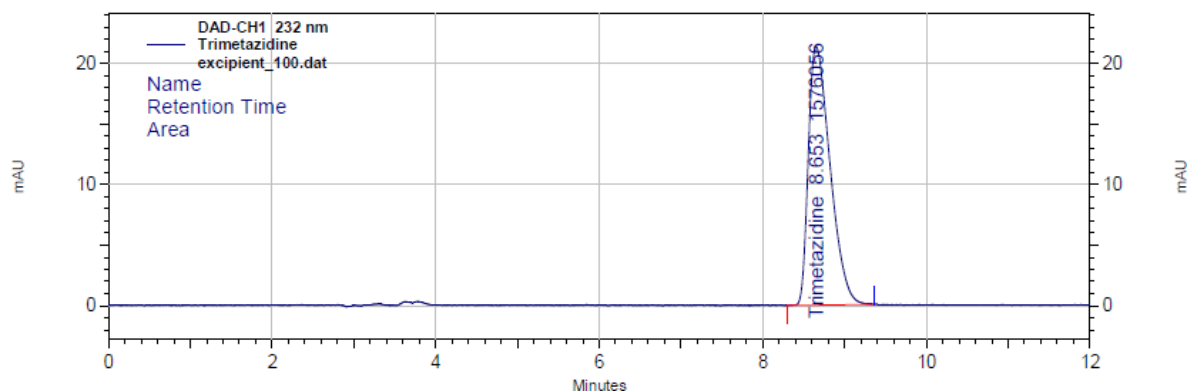
Sample Name: Excipient_110%_Standard_100%
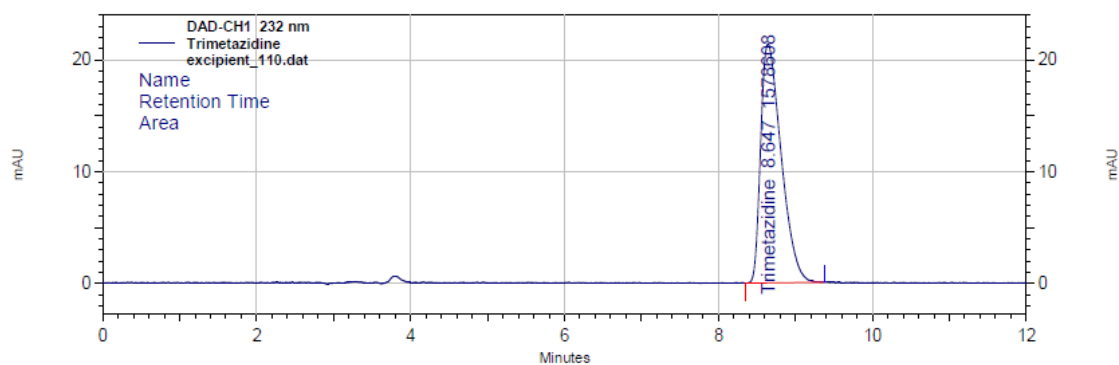
Sample Name: Excipient_120%_Standard_100%
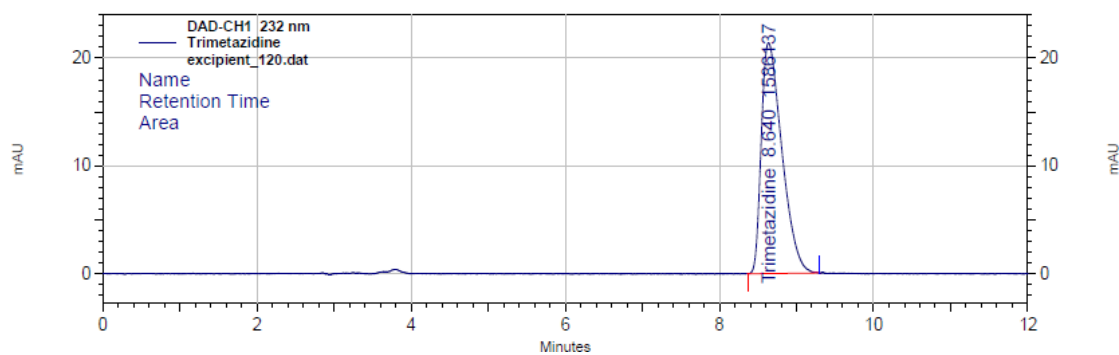
Robustness

Robustness was determined by 03 replicate injection of the standard solution by changing the different system parameters at approximately LOQ concentration level.

(i) Changing the organic solvent content in the eluent (±2%):

| Buffer:Methanol	RT (min)	Peak area±RSD
83:17	8.03	2111464±0.36
84:16	8.33	2114985±0.22
85:15	10.87	2293058±0.38
86:14	11.08	2116807±0.07
87:13	12.68	2119580±0.77

(ii) Changing pH of the buffer solution (±0.5):

Buffer pH	RT (min)	Peak area±RSD
3.95	7.75	2574578±0.15
4.00	7.39	2531470±0.81
4.05	7.89	2570966±0.40

(iii) Changing HPLC column temperature (±5 °C):

Column Temp. (°C)	RT (min)	Peak area±RSD
20	7.37	2356860±0.18
22	7.35	2354750±0.42
25	7.33	2359176±0.35
28	7.29	2371109±0.53
30	7.22	2345364±0.62

(iv) Changing mobile phase flow rate:

Flow rate (mL/min)	RT (min)	Peak area±RSD
0.8	8.29	3120490±0.44
0.9	7.35	2779178±0.44
1.0	6.67	2522700±0.15
1.1	6.03	2283218±0.03
1.2	5.53	2098107±0.81

(vi) Changing wavelength:

Wavelength (nm)	RT (min)	Peak area±RSD
228	6.38	3125146±0.24
230	6.38	2995947±0.29
232	6.35	2694533±0.46
234	6.38	2415024±0.29
236	6.38	2043068±0.20

System Suitability

System suitability result was obtained six replicate injection of the standard solution and following parameters were checked

Parameters	Obtained value
Theoretical Plate /Column efficiency (N)	6529
Capacity (Retention) factor	1.20
Tailing factor/Peak asymmetry	1.45
Relative Standard Deviation	1.43

Sequence Summary Report

HPLC DATA

Sequence name: System suitability.rst

Description:

Acquisition date: 9/5/2018 11:02:15 AM

Compound: Trimetazidine   **Signal:** DAD-CH1 232 nm

Injection Data File Name	Sample Name	RT [min]	Peak Asymmetry	Peak Capacity Factor	Peak Purity	Peak Theoretical Plates USP	Area	Height
standard_01.dat	Trimetazidine	8.07	1.39	1.20	1.00	7188	2603443	178744
standard_02.dat	Trimetazidine	8.07	1.42	1.20	0.99	7000	2621890	177211
standard_03.dat	Trimetazidine	8.07	1.45	1.20	0.98	6325	2603190	168162
standard_04.dat	Trimetazidine	8.07	1.48	1.20	1.00	6232	2615445	168475
standard_05.dat	Trimetazidine	8.07	1.51	1.20	0.99	6106	2620447	167147
standard_06.dat	Trimetazidine	8.07	1.45	1.20	0.98	6324	2604113	168170
	Mean	8.067	1.453	1.204	0.990	6529	2611421	171318
	StdDev	0.000	0.043	0.000	0.010	448.671	8855.122	5200.443
	RSD	0.000	2.942	0.000	0.991	6.872	0.339	3.036

CHAPTER : SIX

DISCUSSION

Discussions:

Wavelength Determination

Every compound absorb energy at a particular wavelength depending on its chromophore group constituent. The UV absorption spectrum of Trimetazidine in methanol showed two absorption peaks with maximaat approximately 232 nm and 278 nm, with greater intensity at theshorter wavelength, 232 nm which is λmax for Trimetazidine. To increase sensitivity and specificity of the method 232 nm was selected for this work.

Buffer Solution Selection

From different buffer solution checked 10mM triethylamine buffer, pH 4.0 adjusted with acetic acid was selected for solution stability and acceptable peak property such as peak asymmetry, peak purity etc. of Trimetazidine in the buffer solution.

Assay

Marketed sample of Trimetazidine dihydrochloride 35 mg MR tablets were checked for their content by the developed method. The average assay result was 34.67 which is 98.76% content of Trimetazidine dihydrochloride in sample.

Linearity

Linearity checked at a concentration of 1.859 – 55.763µg/mL of Trimetazidine with a regression coefficient value of 0.999.

Range

The marketed sample was assayed at three different concentration in three replication. The assay result was within 94.3% - 101.2% with %RSD value of 0.67 – 2.25 at these three concentrations.

Precision:

Repeatability or intraday precision study at three different concentration in three replication exhibited a %RSD value of 0.16 – 0.64 at these three concentrations with a regression coefficient value of 0.999.

Intermediate precision or interday precision study at three different concentration in three replication exhibited a %RSD value of 0.20 – 2.02 at these three concentrations with a regression coefficient value of 0.995.

According to FDA guidance within a day and day to day variations for Trimetazidine revealed that the proposed method is precise and repeatable.

Accuracy:

% recovery at three concentration level was 97.40 – 99.42 % with % RSD value of 0.08 – 0.78.

Sensitivity:

From the signal to noise ratio LOQ was found to be 0.307 µg/mL and 1.021 µg/mL respectively. S/N value was 4.23 for LOD and 11.81 for LOQ.

Specificity

No chromatographic interference was observed at the retention time of the standard solution for the excipients.

Addition of different amount of excipient solution with specific amount of standard solution produce response with a %RSD of 0.39.

The result of both experiments indicate the specificity of the method for analysis of Trimetazidine.

Robustness

Robustness study gives an idea about the changes may occur in the peak property with slight variation in the system parameters.

(i) Changing the organic solvent content in the eluent (±2%) exhibited a change in the retention time of ±2.8 value. No significant change in peak area was observed here.

(ii) The method was found to be robust within the pH of 3.95 to 4.05 of the buffer solution. No significant change in peak area was observed here.

(iii) Changing HPLC column temperature from 20°C to 30 °C (±5 °C) did not produce any significant change in the response.

(iv) Changing mobile phase flow rate from 0.8 to 1.2 mL/min changes the retention time from 8.29 to 5.53 minute with significant change in peak response.

(vi) Changing wavelength from 228 to 236 nm exhibited significant change in peak response.

System Suitability

System suitability was found to be acceptable in comparision with the standard value.

CHAPTER : SEVEN

CONCLUSION

CONCLUSION

Controlling and minimizing the side effects of drugs are the key issues in assuring the safety of drug therapy. At the same time drug analysts play a predominant role in assuring the quality of bulk drug materials and drug formulations and this is also closely related to the safety issue. The goal of an analytical method development is tocreate a method that is suitable for its intendedpurpose. The performance of the analyticalprocedure is established by conforming to the appropriate acceptance criteria of the validation parameters. Trimetazidine works as an antianginal agent by inhibiting fatty acid oxidation in the heart in response to to inhibition of 3-ketoacyl-CoA thiolase (3-KAT).High performance liquid chromatography at present is one of the most sophisticated tool of the analysis. The proposed RP-HPLC method for analysis of Trimetazidine was found to be simple and rapid. The method was found to be accurate, precise, linear, specific and robust. The sample recoveries in the formulation were in good agreement with their respective label claims suggested noninterference in the estimation. Hence, the methods can be easily and conveniently adopted for routine analysis of Trimetazidine bulk drug and tablet formulation. The simplicity ensures that the RP-HPLC method can be applied for estimation of Trimetazidinein different dosage forms.The method was found to be accurate, precise, linear, specific and robust. Mobile phases used in the method are simple to prepare and economical in terms of solvent consumption. The system suitability parameters were within limit, hence all the system as a whole were suitable to perform the assay.The methodcan be considered for routine quality control analysis of Trimetazidine in bulk drug and tablet formulation.

CHAPTER : EIGHT

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