STABILITY INDICATING RP-HPLC (PDA) METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF TRIENTINE HYDROCHLORIDE AND CHARACTERISATION OF ITS DEGRADATION PRODUCT BY SPECTROSCOPIC TECHNIQUES

A Dissertation submitted to
THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI-600032

In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
A.VARALAKSHMI
Reg.No : 261715708

Under the guidance of
Dr.P.G.SUNITHA, M.Pharm.,Ph.D.,
Assistant Professor
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College of Pharmacy, Madras Medical College

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE
CHENNAI-600 003
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EXAMINERS

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Thank
you

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I owe it all to the "**Almighty**" for guiding me with the wisdom, strength and protection needed to complete my project work successfully.

I would like to express my sincere thanks to, **Dr. R. Jayanthi, M.D., F.R.C.P,** Dean, Madras Medical College, Chennai for providing all the facilities required during the period of my academic study.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATION	EXPANSION
%	Percentage
µg	Microgram
µL	Microlitre
µm	Micrometer
ACN	Acetonitrile
cm	Centimeter
HPLC	High Performance Liquid Chromatography
PDA	Photodiode Array
LOD	Limit of Detection
LOQ	Limit of Quantitation
mg	Milligram
ICH	International Conference on Harmonization
Hrs	Hours
LC-MS	Liquid Chromatography Mass Spectroscopy
IP	Indian Pharmacopoeia
nm	Nanometer
RSD	Relative standard deviation
r^2	Correlation coefficient
RP	Reversed phase
v/v	Volume by Volume
Σ	Sigma
λ	Lambda
min	Minute
SD	Standard deviation
m	Slope
c	Intercept
DL	Detection limit
QL	Quantitation limit

R_t	Retention time
k'	Capacity factor
µg/ mL	Microgram per millilitre
API	Active pharmaceutical ingredient
°C	Degree Celsius
g/ mol	Gram per mole
avg	Average
AR grade	Analytical reagent grade
G	Gram
TNT	Trientine hydrochloride

DEDICATED TO THE ALMIGHTY GOD


Introduction

CHAPTER 1

INTRODUCTION

1.1 MOTIVATION AND PROBLEM STATEMENT

The quality of a drug plays an important role in ensuring the safety and efficacy of the dosage forms. Quality assurance and control of pharmaceuticals and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data. Hence, development of rugged and robust analytical methods are very important for statutory certification of drugs and their formulations with the regulatory authorities.[1-3]

1.2 CONCEPT OF ANALYTICAL CHEMISTRY

Analytical chemistry studies and uses instruments and methods to separate, identify and quantify the matter.[4] It also focusses on improvements in experimental design, chemometrics and the creation of new measurement tools having broad applications in forensic, medicine, science and engineering.

Analytical chemistry consists of classical, wet chemical methods and modern instrumental methods.[5]

- Classical qualitative methods use separations such as precipitation, extraction and distillation.
- Classical quantitative methods use mass or volume changes to quantify amount.
- Instrumental methods separate samples using chromatography, electrophoresis or field flow fractionation.

The development of pharmaceuticals brought a revolution in human health. These pharmaceuticals would serve their purpose only if they are free from impurities and administered in an appropriate amount. They may develop impurities at various stages of their development, transportation and storage which makes them risky to be administered, thus they must be detected and quantified.

In the field of pharmaceutical research, the analytical investigation of bulk drug materials, intermediates, drug formulations, impurities and degradation products and biological samples containing the drugs and their metabolites is very important. From the commencement of official pharmaceutical analysis, analytical assay methods were included in the compendial monographs with the aim to characterize the quality of bulk drug materials by setting limits of their active ingredient content. The assay methods in the monographs include titrimetry, spectrometry, chromatography, capillary electrophoresis and electroanalytical methods.

From the stages of drug development to marketing, analytical techniques play a great role in understanding the physical and chemical stability of the drug, selection and design of the dosage form, assessing the stability of the drug molecules, quantitation of the impurities and identification of these impurities, to evaluate the toxicity profiles to distinguish from the API, while assessing the content of drug in the marketed products.

1.3 IMPORTANCE OF NEWER ANALYTICAL METHODS

Newer analytical methods are developed for drugs or drug combination for the following reasons:

- ✓ Official compendia/ pharmacopoeia may not reveal an analytical procedure for the drugs or its combination.
- ✓ The literature search may not reveal an analytical procedure for the drug or its combination.
- ✓ Analytical methods may not be available for the drug combination due to interference caused by excipients.
- ✓ Analytical methods for the quantification of drug or drug combination from biological fluids may not be available.

On the other hand, existing procedure may

- Require expensive instrument, reagent or solvents.
- Involve any extraction or separation steps which may be time consuming.
- Not rapid, reliable or sensitive.

The newer analytical methods developed find importance in various fields:

1. Research
2. Quality control department in industries
3. Approved testing laboratories
4. Biopharmaceutics and bioequivalence studies and clinical pharmacokinetics.

1.4 TYPES OF ANALYTICAL METHODS

- **Spectral methods**

The spectral techniques measure electromagnetic radiation, which is either absorbed or emitted by sample. For example, UV-Visible spectroscopy, IR spectroscopy, NMR spectroscopy, ESR spectroscopy, Flame photometry, Flourimetry etc.

- **Electroanalytical methods**

Electroanalytical methods involve the measurement of current, voltage or resistance. For example, Potentiometry, Conductometry, Amperometry etc.

- **Chromatographic methods**

There are various advanced chromatographic techniques, which are most reliable and widely used for estimation of multi component drugs in their formulations.

- GLC
- HPLC
- HPTLC

1.5 METHOD DEVELOPMENT

Chromatography is probably the most powerful analytical technique available to the modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in a mixture by a single analytical procedure.[6,7]

High-performance liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture.[8]

1.5.1 Reversed-phase high-performance liquid chromatography (RP-HPLC)

Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character such as proteins, peptides and nucleic acids, can be separated by reversed phase chromatography with excellent recovery and resolution.[9] Nowadays reversed-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% of all HPLC separations are carried out in the reversed-phase mode. The reasons for this include the simplicity, versatility and scope of the reversed-phase method, as it is able to handle compounds of diverse polarity and molecular mass.[10-12]

1.5.2 Principle of RP-HPLC

RP-HPLC involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent. The solute mixture is initially applied to the sorbent in the presence of aqueous buffers and the solutes are eluted by the addition of organic solvent to the mobile phase. Elution can proceed either by isocratic conditions where the concentration of organic solvent is constant, or by gradient elution whereby the amount of organic solvent is increased over a period of time. The solutes are, therefore eluted in order of increasing molecular hydrophobicity.

The advantages of HPLC are :

- Excellent resolution achieved under a wide range of chromatographic conditions for very closely related molecules as well as structurally distinct molecules
- Experimental ease with which chromatographic selectivity can be manipulated through changes in mobile phase characteristics
- Generally high recoveries and high productivity
- Excellent reproducibility of repetitive separations.[13,14]

1.5.3 Instrumentation of HPLC

The instrumentation of HPLC consists of pump, injector, column, detector, integrator and display system as shown in **Figure 1.**

- **Solvent Reservoir:** The contents of mobile phase are present in solvent reservoir. In HPLC the mobile phase or solvent is a mixture of polar and non-polar liquid components. Depending on the composition of sample, the polar and non-polar solvents will be varied.
- **Pump:** The Pump suctions the mobile phase from solvent reservoir and forces it to column and then passes to detector. The operating pressure depends on column dimensions, particle size, flow rate and composition of mobile phase.
- **Sample Injector:** The injector can be a solitary infusion or a computerized infusion framework. An injector for a HPLC framework should give infusion of the fluid specimen inside the scope of 0.1 mL to 100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).
- **Columns:** Columns are typically made of cleaned stainless steel around 50mm and 300mm long and have an inward distance across of around 2 and 5mm. They are generally loaded with a stationary phase with a molecular size of 3µm to 10µm. Columns with inner diameters of <2 mm are regularly alluded to as microbore segments. Preferably the temperature of the mobile phase and the column should be kept consistent during investigation.
- **Detector:** The HPLC detector, situated toward the end of the column distinguishes the analytes as they elute from the chromatographic column. Regularly utilized detectors are UV-spectroscopy, fluorescence, mass spectrometric and electrochemical identifiers.
- **Data Collection Devices or Integrator:** Signals from the detector might be gathered on graph recorders or electronic integrators that fluctuate in many-sided quality and in their capacity to process, store and reprocess chromatographic information. The system coordinates the reaction of the integrator to every part and places it into a chromatograph. [15-22]

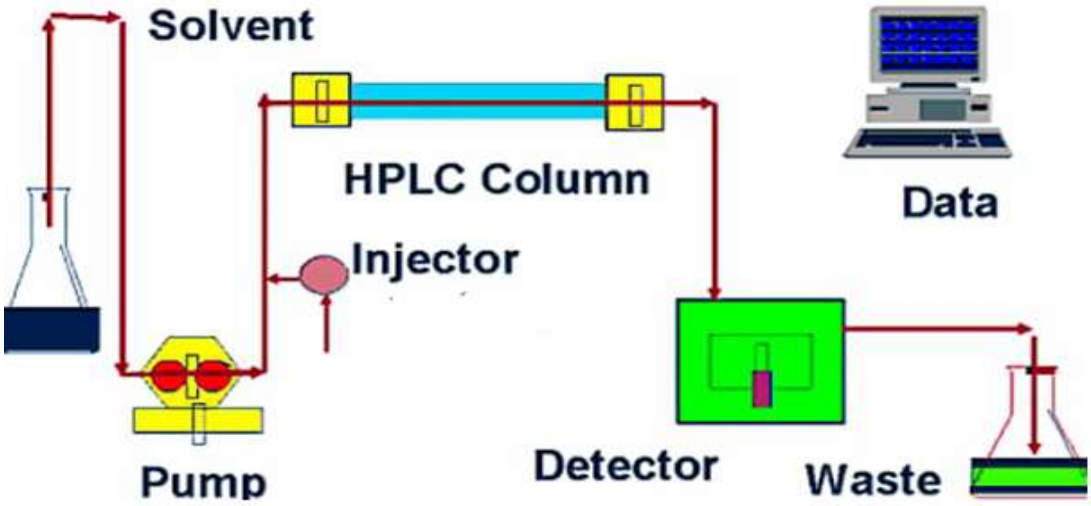


Figure 1. Diagramatic representation of HPLC Instrumentation

1.5.4 Applications of HPLC

The HPLC has several applications in the fields of pharmacy, forensic, environment and clinical. It also helps in the separation and purification of compounds.[23-49]

- Pharmaceutical applications -controlling drug stability, dissolution studies and quality control.
- Environmental applications-monitoring of pollutants and detecting components of drinking water.
- Forensic applications - analysis of textile dyes, quantification of drugs and steroids in biological samples.
- Food and Flavour applications - sugar analysis in fruit juices, detecting polycyclic compounds in vegetables, analysis of preservatives.
- Clinical applications - Detecting endogeneous neuropeptides, analysis of biological samples like blood and urine.

1.6 STABILITY INDICATING METHOD

- Today a majority of the drugs used are of synthetic origin. These are produced in bulk and used for their therapeutic effects in pharmaceutical formulations. These biologically active chemical substances are generally formulated into convenient dosage forms such as tablets, capsules, dry syrups, liquid orals, creams or ointments, parenterals, lotions, dusting powders, aerosols, metered dose inhalers and dry powder inhalers etc. These formulations deliver the drug substances in a stable, non-toxic and acceptable form, ensuring its bioavailability and therapeutic activity.

- In view of the wide variety of excipients used in formulating drugs for administration to patients, drug substances can undergo transformation by interacting with one or more components of the formulation. Formulated drugs can degrade due to acidic or basic environments created by the formulation matrix. Drugs can degrade due to exposure to temperature, humidity and light during manufacturing, transportation and storage during its shelf life. Due to this, it is essential to know the degradation pathways of the drugs in acidic, basic, neutral, oxidation conditions and their susceptibility to temperature and humidity and to formulate them in a manner in which they are stabilized and retain its quality throughout their shelf life.[50-54]

- As most drugs contain functional groups which can participate in reactions in some way or the other, it is essential that the analytical methods developed for estimation of the purity and impurities, are capable enough to separate all the desired and undesired components and devoid of any interferences from the formulation matrix. When analytical methods are able to precisely and accurately quantify without missing any impurities, without under estimation or over estimation and detect all possible impurities and degradants which can form during stability studies, with adequate sensitivity and exactly reflect the quality of drug substances and drug products, those methods are called stability indicating methods.

- In recent times, the development of stability indicating assays has increased enormously, using the approach of stress testing as outlined in the International Conference on Harmonization (ICH) guideline $Q1AR_2$ and even this approach is being extended to drug combinations. This ICH guideline requires that stress testing on API and drug products should be carried out to establish their inherent stability characteristics, which should include the effect of temperature, humidity, light, oxidizing agents as well as susceptibility across a wide range of pH.

- The knowledge gained from stress testing can be useful for (i) The development of stable formulation and appropriate packaging design (ii) Controlling manufacturing and processing parameters (iii) Identification and isolation of toxic degradants during API synthesis (iv) Recommendation of appropriate storage conditions and shelf-life determination and (v) Designing and interpreting environmental studies, as the degradation of the drug in the environment will often be similar to degradation observed during stress-testing studies.[55-57] It is also recommended that analysis of stability samples should be done through the use of a validated stability-indicating testing method.

1.6.1 Forced Degradation Study

The ICH guideline Q1A on Stability Testing of New Drug Substances and Products gives indications for the testing of parameters which may be susceptible to change during long storage and are likely to affect quality, safety and efficacy by validated stability indicating testing methods. It is mentioned that forced degradation studies[58-60] or stress testing at temperatures in 10°C increments above the accelerated temperatures, extreme pH and under oxidative and photolytic conditions have to be carried out on the drug substance so as to set up the stability characteristics and degradation pathways to back up the suitability of the proposed analytical procedures.

1.6.2 Objectives of forced degradation study

Forced degradation study provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Lack of drug substance or drug product stability may affect the purity, potency and safety of the drug product.

- ✓ To establish the degradation pathways of drug substances and drug products
- ✓ To determine the intrinsic stability of a drug substance in formulation
- ✓ To provide information on drug substance / product characteristics
- ✓ Identification of potential degradants
- ✓ Establish a re-test period for the drug substance or shelf life of the drug product and recommended storage conditions
- ✓ To generate more stable formulations
- ✓ To differentiate degradation products that are related to drug products from those that are generated from non-drug products in a formulation
- ✓ Process development, design and optimization of manufacturing process
- ✓ To elucidate the structure of degradation products
- ✓ To understand the chemical properties of drug molecule
- ✓ Design of formulation (including selection of excipient for formulation)
- ✓ To generate a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions
- ✓ Packaging development
- ✓ To solve the stability related problems
- ✓ Provide data to support clinical trials, registration submission or commercialization.

Forced degradation studies are used to generate product related variants and develop analytical methods to determine the degradation products formed during accelerated and long term stability studies. The degradation products obtained reveal the degradation pathwayof the drug and facilitate the development of stability indicating methods. The degradation products should be evaluated for potential hazard and hence arises the need for characterisation and quantification.

Forced degradation of drug substances are usually conducted in solution and in solid state at temperature exceeding accelerated stability conditions (>40ºC). The degradation pathways include hydrolysis, oxidation, thermal, photolysis and polymerisation. Hydrolysis is investigated in solution over a broad pH range and in the solid state by exposure of the drug to high relative humidity. Oxidation in solution can be investigated through control of exposure to molecular oxygen or by addition of oxidising agents such as peroxides. The effects of thermolysis are usually assessed in

solid state by applying heat. Photolysis is investigated in solution or the solid state by irradiating samples with light of wavelengths in the range of 300-800nm. Polymerisation can be investigated by measuring the rate of degradation as a function of different initial drug concentrations in solution.

A degradation level of 10-15% is considered for validation of a chromatographic purity assay. The forced degradation conditions utilized for drug substances and finished dosage form is tabulated in **Table 1**.

Table 1. Conditions for Forced degradation study

Degradation type	Experimental conditions	Storage conditions	Sampling time
Hydrolysis	0.1N HCl	40ºC, 60ºC	1,3,5 days
	0.1 N NaOH	40ºC, 60ºC	1,3,5 days
	pH: 2,4,6,8	40ºC, 60ºC	1,3,5 days
Oxidative	3% H_2O_2	25ºC, 60ºC	1,3,5 days
	Peroxide control	25ºC, 60ºC	1,3,5 days
	Azobisisobutyronitrile (AIBN)	40ºC, 60ºC	1,3,5 days
Photolytic	Light, 1 X ICH	NA	1,3,5 days
	Light, 3X ICH	NA	1,3,5 days
	Light control	NA	1,3,5 days
Thermal	Heat environment	60ºC	1,3,5 days
	Heat environment	60ºC / 75%RH	1,3,5 days
	Heat environment	80ºC	1,3,5 days
	Heat environment	80ºC / 75%RH	1,3,5 days
	Heat control	Room temperature	1,3,5 days

1.7 METHOD VALIDATION

Analytical method validation is the process of demonstrating that an analytical method is reliable and adequate for its intended purpose. Reliable data for the release of clinical supplies, stability and setting shelf life can only be generated with appropriate validated methods.[61-67]

1.7.1 Validation Parameters

Accuracy

Accuracy is the measure of how close the experimental value is to the true value. The RSD of the replicates will provide the analysis variation or how precise the test method is. The mean of the replicates, expressed as % label claim, indicates how accurate the test method is.

Detection Limit (LOD) and Quantitation Limit (LOQ)

Detection limit is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Precision

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility.

(a) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

(b) Intermediate precision

Intermediate precision expresses within laboratory variations like different days, different analysts, different equipments, etc. Intermediate precision was previously known as part of ruggedness. The attribute evaluates the reliability of the method in a different environment other than that used during development of the method. The objective is to ensure that the method will provide the same results when similar samples are analyzed once the method development phase is over. Depending on time and resources, the method can be tested on multiple days, analysts, instruments, etc.

(c) Reproducibility

As defined by ICH, reproducibility expresses the precision between laboratories. Multiple laboratories are desirable but not always attainable because of the size of the firm.

Robustness

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. Robustness can be partly assured by good system suitability specifications. Testing by varying some or all conditions, e.g., age of columns, column type, column temperature, pH of buffer in mobile phase, reagents, is normally performed.

Sample Solution Stability

Solution stability of the drug substance or drug product after preparation according to the test method should be evaluated according to the test method. This is of concern especially for drugs that can undergo degradation by hydrolysis, photolysis or adhesion to glassware.

Specificity/Selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. A representative chromatogram or profile should be generated and submitted to show that the extraneous peaks either by addition of known compounds or samples from stress testing are baseline resolved from the parent analyte.

System Suitability Specifications and Tests

The accuracy and precision of data collected begin with a well behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose.

- **Capacity factor (**k')

The capacity factor is a measure of where the peak of interest is located with respect to the void volume. The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 2.

- **Relative retention**

Relative retention is a measure of the relative location of two peaks. This is not an essential parameter as long as the resolution (R) is stated.

- **Resolution (R)**

R is a measure of how well the two peaks are separated. For reliable quantitation, well-separated peaks are essential. R of >2 is desirable.

- **Tailing factor (T)**

The accuracy of quantitation decreases with increase in peak tailing. T of < 2 is desirable.

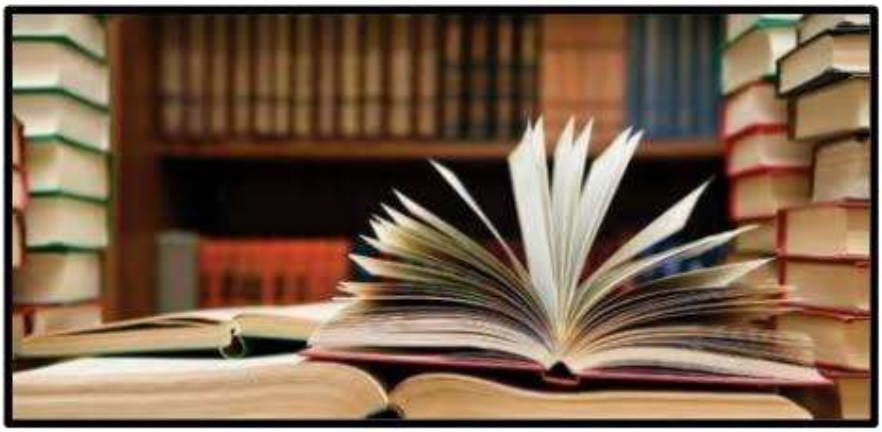
- **Theoretical plate number (N) and HETP**

Theoretical plate number is a measure of column efficiency, i.e., how many peaks can be located per unit run-time of the chromatogram. HETP, the height equivalent to theoretical plate, measures the column efficiency per unit length (L) of the column. The theoretical plate number depends on elution time but in general should be > 2000.

1.8 IN-SILICO TOXICITY PREDICTION

The OSIRIS property explorer calculates on-the-fly various drug related properties whenever a structure is valid. Prediction results are valued and colour coded. Properties with high risks of undesired effects like mutagenicity or poor intestinal absorption are shown in red whereas a green colour indicates drug-conform behaviour.

Literature Review



CHAPTER 2

REVIEW OF LITERATURE

- Eugene B.Hansen et al. (1985) reported the determination of Trientine hydrochloride in aqueous solution by reversed phase ion pairing HPLC and conductivity detection.[68] The mobile phase used in the study was ACN 20% : Water 80% and the detection wavelength used was 599nm.The conductivity detector response was linear over the concentration range of 10 to 100µg/mL.

- Katsumi Miyazaki et al. (1990) reported the determination of TNT in plasma of patients by HPLC using fluorimetric method.[69] Mobile phase used was 73% salt solution and 27% ACN, pH adjusted to 6.0 with 2M NaOH. The flow rate was 0.5ml/min and the pressure was approximately 70kg/cm^2. Fluorescence measurement was made with the excitation set at 380nm and emission at 485nm.

- Yukitaka Nakavo et al. (2002) studied the liquid chromatographic technique for TNT in human and rabbit sera based on intramolecular excimer forming fluorescence derivatization.[70] TNT and 1,6 hexanediamine used as internal standard were converted to excimer forming derivatives using pyrene reagent. The derivatives were separated within 20 min on a reversed phase column using isocratic elution and detected spectrofluorometrically at 480nm with excitation at 345nm.

- Asma Othman et al. (2007) developed and validated a rapid HPLC method for the determination of TNT and its two major metabolites in human serum.[71] Mobile phase employed was ACN : Ammonium acetate. 9-fluorenylmethylchloroformate (FMOC) was used for labelling the analytes and then separated using a reversed phase C_{18} column by gradient elution and detected spectrofluormetrically at 317nm with excitation at 263nm.

- Jun lu et al. (2007) carried out the determination of TNT and its metabolites in human plasma and urine by liquid chromatography – mass spectrometry (LC-MS).[72] Separation was achieved on a cyano column using 15% ACN and 85% water and 0.1% hepatafluorobutyric acid as mobile phase. MS detection was performed at $[M+H]^+$ values of 147 using N_1-acetylspermine as internal standard.

Based on the literature survey it is evident that no RP-HPLC method has been developed for the quantification of TNT in its pharmaceutical dosage form. Hence an attempt has been made to develop and validate a stability indicating RP-HPLC method for the quantification of TNT in its pharmaceutical dosage form.


AIM
&
OBJECTIVE

CHAPTER 3

AIM AND OBJECTIVE

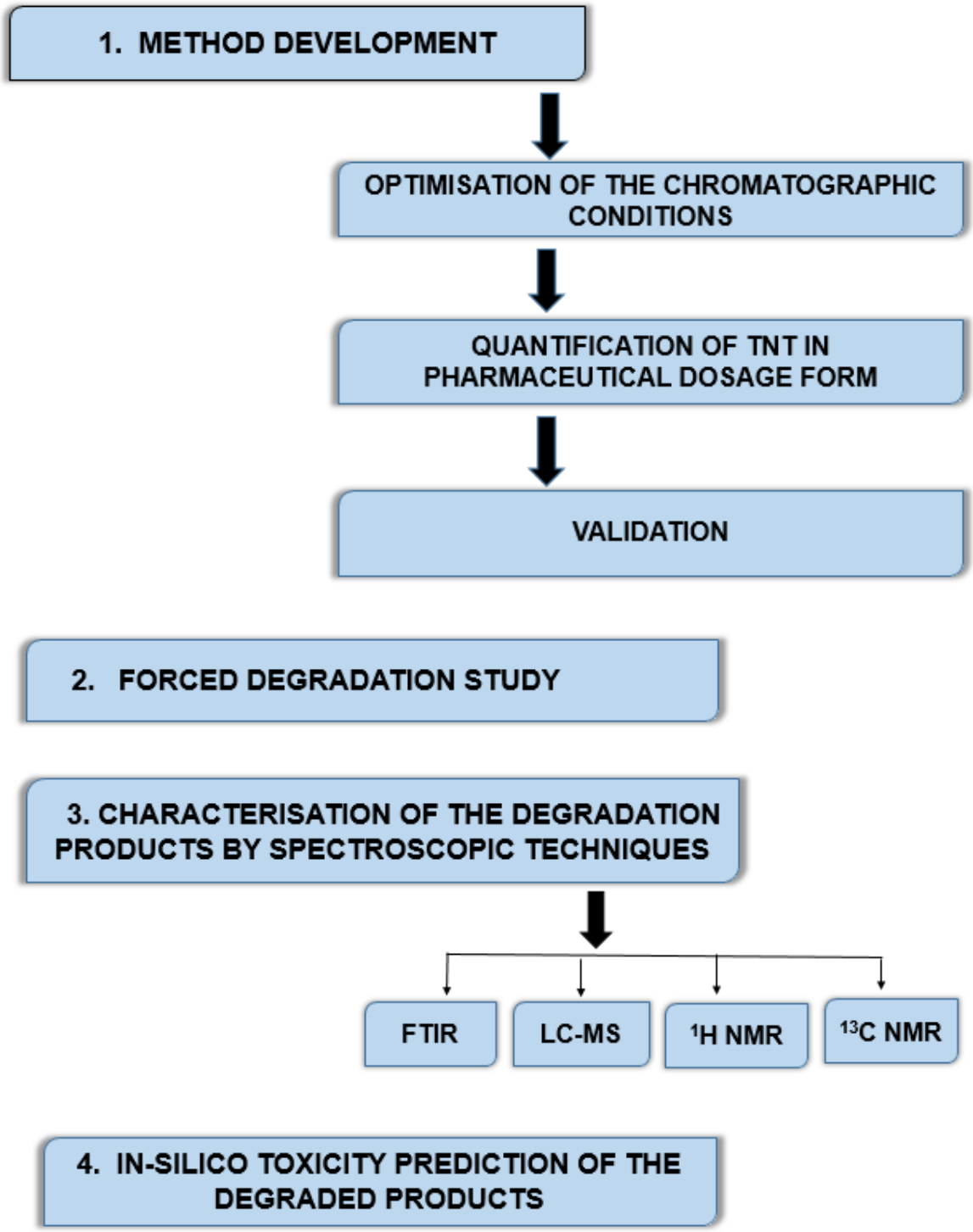
3.1 NEED FOR METHOD DEVELOPMENT

Wilson's disease is an autosomal recessive disorder of copper metabolism with a prevalence of 1 in 30,000 in general population. Penicillamine is a chelating agent used to decrease copper stores in Wilson's disease and also used in rheumatoid diseases. Penicillamine use is assosciated with numerous side effects, some are severe (hypersensitivity and liver damage) and require drug discontinuation. Trientine appears to have a more favourable side effect profile than penicillamine, also as an orphan drug, research is lacking with this moiety.The literature survey shows that no analytical method has been developed for quantification of TNT in pharmaceutical formulation. Hence an attempt has been made to develop a Stability indicating RP-HPLC (PDA) method for the quantification of TNT. The objectives of the study include the following :

- To develop Stability indicating RP-HPLC(PDA) method for the quantification of TNT in bulk and pharmaceutical formulation
- To validate the developed method
- To prove the Stability indicating nature of the developed method
- Characterisation of the degradation product of TNT
- In-Silico Toxicity prediction of the degradation product of TNT


PLAN

3.2 PLAN OF WORK



DRUG PROFILE

CHAPTER 4

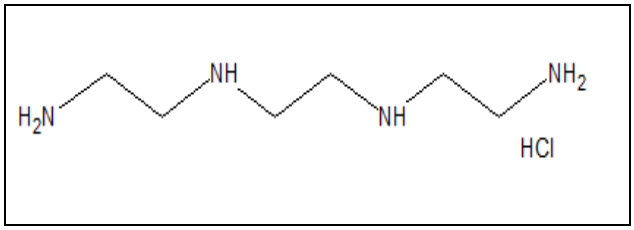
DRUG PROFILE

TRIENTINE HYDROCHLORIDE

Molecular Formula : $C_6H_{19}ClN_4$

Chemical Name : N'-(2-(2-aminoethylamino)ethyl)ethane-1,2-diamine hydrochloride.

Chemical Structure :



Molecular Weight : 182.696g/mol

Appearance : White or off-white powder

Solubility : Freely soluble in methanol, sparingly soluble in ethanol, insoluble in chloroform and ether.

Pharmacological action : Copper chelating agent [73]

Indications : Treatment of Wilson disease, also a potent Angio-genic.

Dosage : 500 – 750 mg /day for paediatric use
750 – 1250 mg/day for adults in divided doses.

Contraindications : Not indicated for the treatment of biliary cirrhosis.

Special precautions : Mineral supplements should not be given.

Brand name : Syprine.

Mechanism of action

Wilson's disease results from the defective ATP7B protein **(Figure 2)**.It is a copper transporting P-type ATP-ase which is encoded by the ATP7B gene. The Protein is located in the trans-golgi network of the liver and brain and balances the copper level in the body by excreting excess copper into bile and plasma. Wilson's disease is characterised by impaired copper metabolism which leads to chronic liver disease, neurological and pshychiatric disturbances. Trientine chelates copper, forming a stable complex with four constituent nitrogen atoms in a planar ring. In addition to increased urinary copper excretion, trientine also decreases intestinal copper absorption.

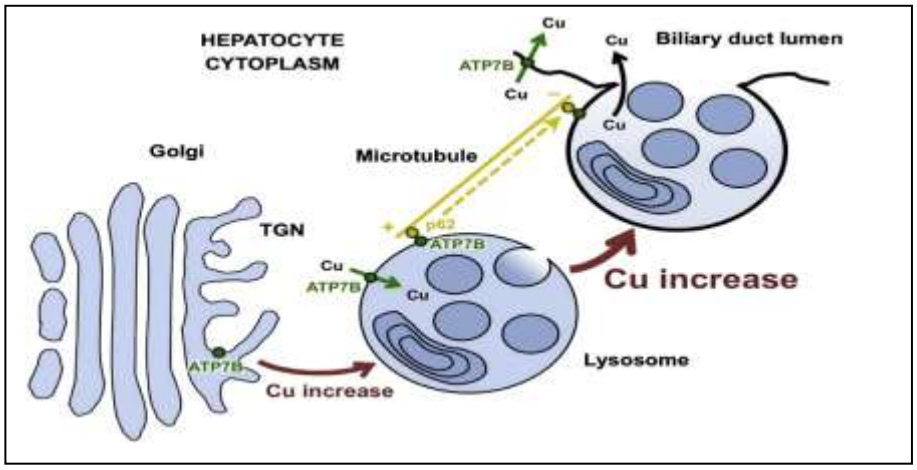


Figure 2. Mechanism of action of TNT

MATERIALS AND METHODS

CHAPTER 5

MATERIALS AND METHODS

5.1 REFERENCE STANDARD USED

Trientine hydrochloride standard was obtained as gift sample from Saimirra Innopharm Pvt Ltd, Chennai.

5.2 REAGENTS

All the solvents used for chromatographic study were of HPLC grade.

5.3 INSTRUMENTS

RP-HPLC

- Instrument - WATERS 2996 PDA detector
- Software - Empower 3
- Column - Waters Reliant C_8 (250 × 4.0mm, 4µ)
- Pump - LC - 20 ATVP Series
- Injector - Autosampler
- Detector –PDA Detector
- Syringe filter - PTFE, 0.45µm pore
- LC injection vials - 2mL with Teflon Coated Caps.

SPECTROSCOPIC STUDIES

- **IR Spectroscopy** - ABB MB3000-PH FT-IR Spectrometer
- **^{1}HNMR Spectroscopy** - 500MHz Bruker Topspin
- **^{13}CNMR Spectroscopy** - 100MHz Bruker Topspin
- **LC-MS Spectroscopy**-Agilent technologies, 6230B Time of Flight (TOF).

5.4 RP-HPLC METHOD DEVELOPMENT

5.4.1 Chromatographic conditions

Chromatographic separation was achieved on Waters Reliant C_8 column (250mm x 4.0mm ID, 4µm particle size) employing gradient elution using Acetonitrile and Ammonium formate buffer at pH 5.3 ± 0.05 (90:10) v/v as mobile phase. The mobile phase was filtered through membrane filter (0.45µm) and sonicated for 30min prior to use. Separation was performed at at a flow rate of 0.8mL/min and the run time was 35min. Detection was performed at a wavelength of 220nm.

5.4.2 Standard solution and construction of calibration curve

About 200mg of TNT standard was accurately weighed and transferred into a 100ml volumetric flask, dissolved and made upto volume with diluent. 10ml of standard stock solution was pipetted out into a 100ml volumetric flask, 40ml of diluent, 1ml of 1N sodium hydroxide and 1.5ml of ammonium hydroxide solution were added and shaken well. The flask was kept in an ice-bath to allow the solution to reach the temperature 2 to 8ºC and the same temperature was maintained for about 15min. 2ml of benzoyl chloride was added to the flask and kept in an ice-bath for 2min. The flask was kept at room temperature for about 30min and made upto volume with the diluent. Thus the standard stock solution of TNT containing 200µg/ml was prepared.

From the above stock solution, concentrations in the range of 40-120 µg/mL of TNT were obtained. The peak area for the different concentrations of TNT were recorded. The calibration curve was constructed between concentrations and respective peak area.

5.4.3 Analysis of formulation

For analysis of the capsule dosage form, twenty capsules (Saimirra Innopharm Pvt. Ltd) containing 250mg of TNT was weighed and the average weight was determined. The powder equivalent to the weight of 200mg of TNT was transferred to a 100ml volumetric flask, dissolved and made upto the volume with diluent. 10ml of the solution was pipetted out into a 100ml volumetric flask, 40ml of diluent, 1ml of

1N sodium hydroxide and 1.5ml of ammonium hydroxide solution were added and shaken well. The flask was kept in an ice-bath to allow the solution to reach the temperature 2 to 8ºC, the same temperature was maintained for about 15min. 2ml of benzoyl chloride was added to the flask and kept in an ice-bath for 2min. The flask was kept at room temperature for about 30min and made upto volume with diluent. 10ml of the above solution was pipetted out into a 25ml volumetric flask and made upto volume with the diluent to get the final concentration of 80µg/mL.20µL of solution was then injected for quantitative analysis. The identity of the compound was established by comparing the retention time of sample solution with that of standard solution and the amount of TNT was calculated.

5.5 VALIDATION OF THE PROPOSED METHOD

5.5.1 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 sample. Linearity test solution for the assay method was prepared by diluting the stock solution to the required concentrations. Five different concentration levels of the solutions were prepared in the range of 40 to 120µg/mL. Peak area under the curve (average peak area of five observations) was plotted against the respective concentration level. The calibration curve obtained from the regression analysis was used to calculate the corresponding predicted responses.

5.5.2 Accuracy

Accuracy of an analytical procedure expresses the closeness of agreement between a value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the assay method was evaluated in triplicate at three different concentration levels, 50%, 100%, and 150% i.e. 40, 80 and 120µg/mL in the bulk drug sample. Percentage recoveries were calculated.

5.5.3 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 (same day), intermediate precision (Interday-Three different day, Intraday- different time interval on the same day) and reproducibility (different labs). Precision was carried out by injecting six replicates at the 100% level. The RSD of the peak areas was calculated.

5.5.4 Limit of detection (LOD) and Limit of Quantification (LOQ)

The LOD is defined as the lowest amount of analyte in a sample which can be detected, but not necessarily quantifed as an exact value. LOQ is defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ for trientine was determined as S/N ratio of 3:1 and 10:1 respectively by injecting a series of dilute solutions with known concentration.

5.5.5 Robustness

The robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. To determine the robustness, deliberate changes in the following parameters were made: different columns, pH, wavelength and column temperature.

5.5.6 Selectivity

The selectivity was checked by injecting the solution of the drug into the HPLC system and recording the retention time.

5.5.7 Specificity

Specificity of the method was assessed by comparing the chromatogram obtained for the standard drug with the chromatogram obtained for formulation.

5.5.8 System suitability parameters

System suitability parameters were determined to ascertain the suitability of the selected chromatographic conditions.

5.5.9 Solution stability

Stability of sample solution was established by storage of the sample solution at 6°C for 48 hrs. Trientine hydrochloride was reanalysed after 24 and 48 hrs time intervals and the assay value was determined and compared against fresh sample.

5.6 FORCED DEGRADATION STUDY

Forced degradation study was carried out on Trientine hydrochloride capsules under various stress conditions like acid and base hydrolysis, oxidation, thermal and photolysis.

5.6.1 Acid hydrolysis

The study was performed by adding 10ml of 1M HCl to 50mg of TNT in a 50mL volumetric flask. The mixture was kept at 60°C for 1 hr and the mixture was neutralised with 10ml of 1M NaOH and the chromatogram was recorded.

5.6.2 Base hydrolysis

10ml of 1M NaOH was added to 50mg of TNT in a 50mL volumetric flask. The mixture was kept at 60°C for 1 hr and the mixture was neutralised with 10ml of 1M HCl and the chromatogram was recorded.

5.6.3 Oxidative degradation

Oxidative degradation was performed by adding 5ml of 3% H_2O_2 to 50mg of TNT in a 50mL volumetric flask and the mixture was kept at 60°C for 1 hr and the chromatogram was recorded.

5.6.4 Thermal degradation

Thermal degradation was done by treating 50mg of TNT at 105°C for 5 hrs in an oven, after which it was cooled to room temperature and the chromatogram was recorded.

5.6.5 Photolytic degradation

Photolytic degradation was done by subjecting TNT to 350nm UV-Visible light for 5 hrs and the chromatogram was recorded.

5.7 CHARACTERIZATION OF DEGRADATION PRODUCT OF TNT

The degradation product obtained in the forced degradation study was targeted for its structural characterization. The degradation product was analysed by LC-MS. The mass spectrometer was run in positive ionization mode and negative ionization mode with turbo ion spray interface and mass to charge (m/z) ratio was recorded. Further structure elucidation of the degradation product was done by ^{1}H NMR, ^{13}C NMR and IR spectroscopic studies.

5.8 TOXICITY PREDICTION

Toxicity Prediction was done by OSIRIS Property explorer, the online software of Thomas Sander Actelion Pharmaceuticals Ltd, Switzerland.

RESULTS AND DISCUSSION

CHAPTER 6

RESULTS AND DISCUSSION

The present work deals with the development of a new analytical method (RP-HPLC) for the quantification of Trientine hydrochloride. Only with proper validation, the data obtained can be reliable and trustworthy. Thus the developed method was validated as per ICH guidelines to prove the reliability of the method. The parameters which were validated are linearity, accuracy, precision, robustness and system suitability parameters. The validation acceptance criteria was met for the developed method.

The linearity of an analytical method is based on its ability to elicit test results, that are directly or by a well-defined mathematical transformation proportional to the concentration of the analyte present in the sample within the given range. The developed method was found to be linear in the concentration range reported. The regression analysis was carried out to check the correlation coefficient, intercept and slope of the regression line, which estimates the degree of linearity.

Analytical methods need to be robust, so that they can be used routinely without problems and can be easily transferred for use in another laboratory if necessary. Robustness was tested by making small deliberate changes in the chromatographic conditions such as flow rate, variation in mobile phase, column, mobile phase consumption, etc., and measuring the peak areas. The percentage RSD should not be greater than 2. The percentage RSD obtained in the proposed method was found to be below 2, which was within the acceptance criteria.

Accuracy of the proposed method was confirmed by recovery studies which indicate that, the co-formulated substances do not interfere in the determination. The low values of standard deviation in the recovery data indicate the reproducibility of the proposed method.

6.1 DETERMINATION OF TNT BY RP-HPLC METHOD

RP-HPLC method has not been reported for quantitative analysis of TNT in pharmaceutical dosage form, hence an attempt was made to develop a new stability indicating RP-HPLC method for the quantification of TNT. The RP-HPLC separation was conducted on Waters Reliant C_8 column as stationary phase using ammonium formate buffer : Acetonitrile in the ratio of 90:10v/v as the mobile phase. The detection of TNT was carried out at 220nm. The chromatogram in **Figure 3.** shows the retention time of Trientine hydrochloride as 12.497min. The calibration curve was constructed between concentrations and respective peak area. **Figure 4.** represents the linearity curve of TNT.

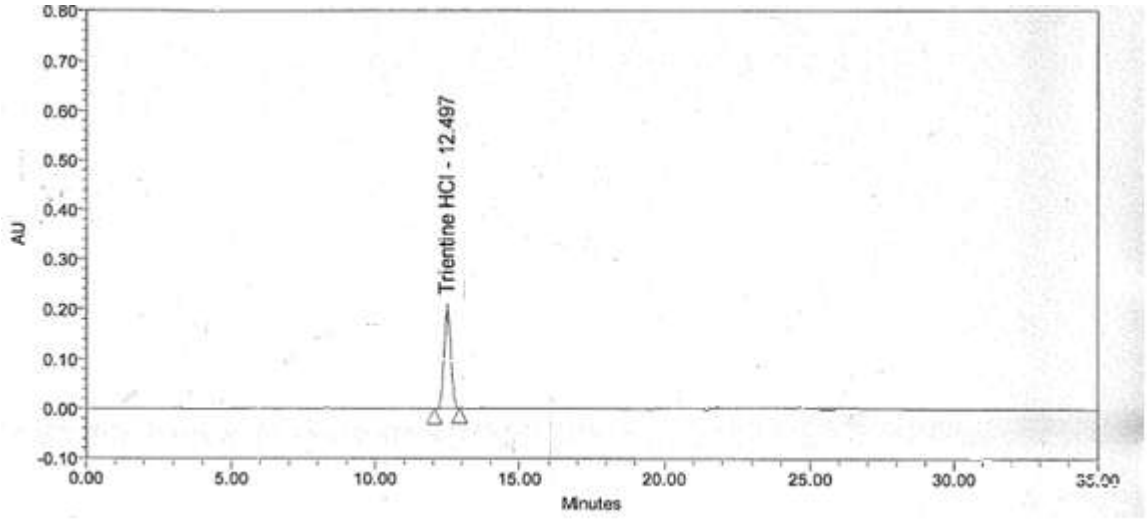


Figure 3. Chromatogram showing R_t of TNT

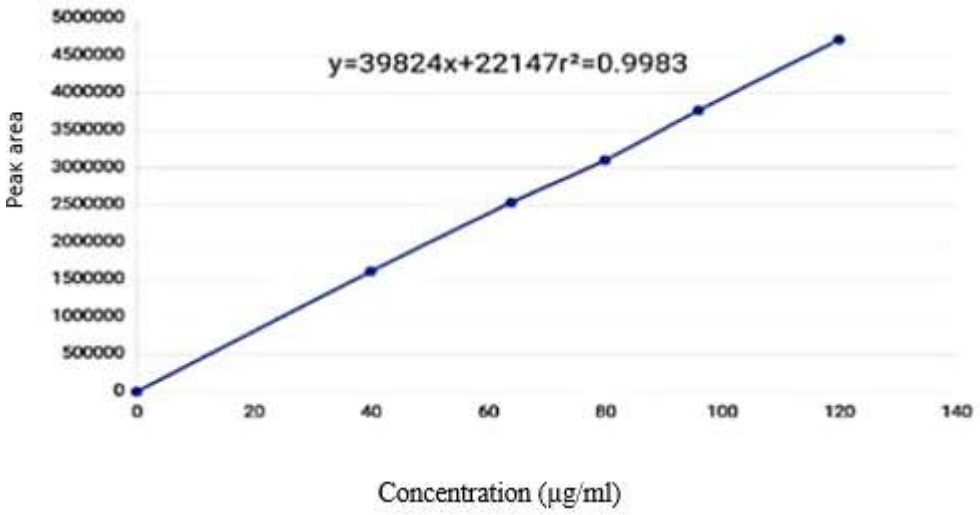


Figure 4. Linearity curve of TNT

6.1.1 Method Validation

The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain. The proposed method was validated in the light of ICH guidelines. The developed method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity as per ICH guidelines. All the validation parameters were checked by replicate injections of the sample and standard solutions.

Linearity

Linearity was obeyed in the concentration range of 40-120 µg/ml. From the data obtained correlation coefficient, y-intercept and slope were calculated to provide mathematical estimates of the degree of linearity as shown in **Table 2**.

Accuracy

Accuracy of the developed method was carried out by adding known amount of drug corresponding to three concentration levels 50%,100% and 150% of the label claim. The accuracy was expressed as the percentage of analyte recovered by the assay method. The results of percentage recovery are shown in **Table 3**.

Precision

Precision was studied by repeatability and intermediate precision studies. The results are reported in terms of relative percentage standard deviation (% RSD) as in **Table 4**.

Limit of detection (LOD) and limit of quantification (LOQ)

The lowest amount of the analyte in the sample which can be detected and the lowest amount of the analyte which can be quantitatively determined were studied and LOD and LOQ values are reported in **Table 5**.

Analysis of Formulation

The proposed method was applied for the quantitation of TNT in its pharmaceutical dosage form. The results of analysis were in good agreement with the label claim as shown in **Table 6.**

Robustness

The robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. It was observed that there was no marked change in the chromatogram which demonstrated that the method developed is robust as shown in **Table 7**.

Selectivity

The selectivity was checked by injecting the solution of the drug into the HPLC system. A sharp peak of TNT was obtained at the retention time of 12.497minutes. It was observed that the excipients did not interfere with the retention time of the drug so the method developed is said to be selective.

Specificity

Specificity of the method was assessed by comparing the chromatogram obtained for the standard drug with the chromatogram obtained for capsule solution. The chromatogram of the formulation is shown in **Figure 5.** The retention time of standard drug and the drug in the sample solution were same, so the method is specific.

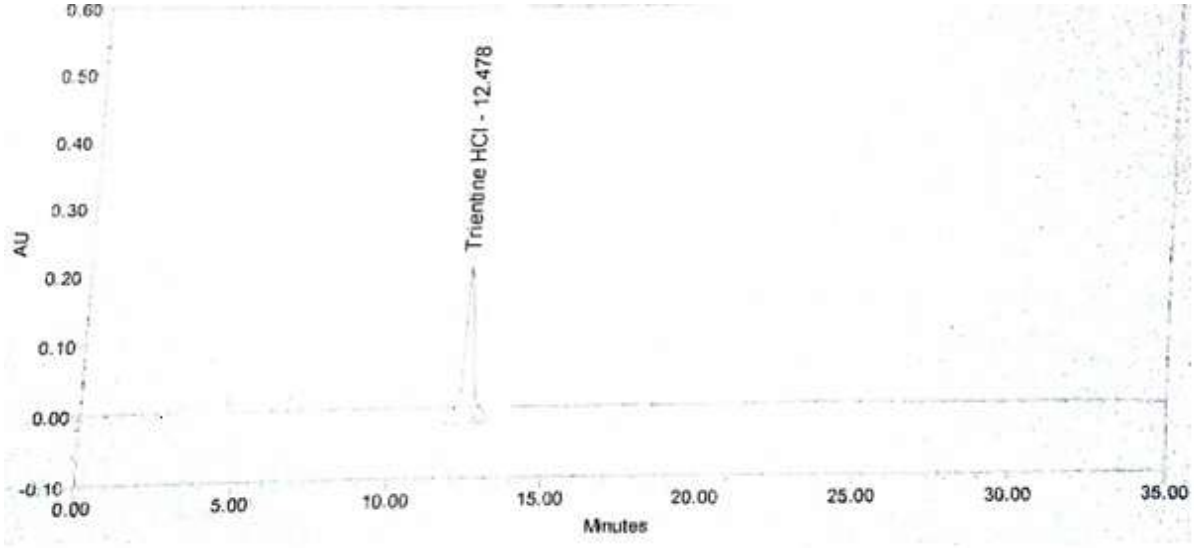


Figure 5. Chromatogram showing R_t of TNT Formulation

System suitability parameters

The results of the system suitability parameters for the developed method are shown in **Table 8**.

Stability of analytical solution

Stability of sample solution was established by storage of the sample solution at 6°C for 48 hrs. Trientine hydrochloride was reanalysed after 24 and 48 hrs time intervals and assay value was determined and compared against fresh sample. Sample solution does not show any appreciable change in assay value when stored at 6°C upto 48 hrs. The percentage label claim of Trientine hydrochloride at 0, 24, 48 hrs were 99.9%, 100.2% and 100.1% respectively.

Table 2. Linear regression data for the Calibration curve*

Parameter	TNT
Linearity	40-80 µg/mL
r^2	0.9983
Slope	39824
Intercept	22147

*n=5

Table 3. Accuracy study for the determination of TNT*

Amount added (%)	Theoretical content(mg/caps)	Measured conc.±SD	Recovery (%)	%RSD
50	125.15	124.98	99.87	0.62
100	250.23	250.21	99.99	0.58
150	375.44	375.73	100.08	0.15

*n=9.

Table 4. Results of Precision study*

Drug conc. (µg/mL)	Repeatability Conc.± SD	% RSD	Intermediate precision Conc.± SD	% RSD
40	40.20±0.17	0.42%	39.90±0.04	0.09%
80	80.04±0.34	0.43%	80.39±0.43	0.53%
120	120.50±0.81	0.67%	120.15±0.56	0.47%

*n=9.

Table 5. Limit of detection (LOD) and Limit of quantitation(LOQ)

Parameter	TNT
LOD	1.785µg/mL
LOQ	5.409µg/mL

Table 6. Analysis of the marketed formulation*

Drug	Label claim	Drug content(%)±SD	% RSD
TNT	250mg	250.11mg ± 1.07	0.43

*n=6.

Table 7.Robustness evaluation of the proposed method*

Factor	Level	Retention time(R_t)	Asymmetry(T)
A. Columns from different manufactures			
Waters C_8 column		12.521	1.02
Phenomenex C_8 column		12.315	1.05
B. Column Temperature			
29°C	-1	12.502	1.04
30°C	0	12.499	1.02
31°C	+1	12.501	1.01
C. Wavelength			
218nm	-2	12.511	1.11
220nm	0	12.502	1.15
222nm	+2	12.503	1.09
D. Buffer pH			
5.2	-1	12.498	1.03
5.3	0	12.497	1.05
5.4	+1	12.502	1.07

* (n=6)

Table 8. System suitability parameters

Parameter	TNT	Reference values
Theoretical plates(N)	12765	NLT 2000
Tailing Factor	1.04	NMT 2.0

6.2 STABILITY INDICATING RP-HPLC METHOD FOR TRIENTINE HYDROCHLORIDE

Stress studies were performed to evaluate the stability indicating properties and the specificity of the method. During the conversion of drugs into formulations a number of excipients are employed. Drugs undergo transformation by interacting with these excipients. Formulated drugs may undergo degradation due to the acidic or basic environment of the formulation matrix. Drugs can degrade due to exposure to environmental factors like temperature, light, humidity, etc., during manufacturing, transport and storage. Due to this, it is essential to know the degradation pathway of the drug in various environmental conditions. ICH guidelines require that stress testing on active pharmaceutical ingredient (API) and the drug products should be carried out to establish their inherent stability characteristics.

Stability indicating study is essential for the following reasons:

- Development of stable formulation
- Identification of toxic degradants
- Recommendation of appropriate storage conditions
- Elucidation of the possible degradation pathways.

Stability indicating method helps in understanding the quality of the drug, that varies with passage of time, when it is exposed to different environmental factors. A stability indicating assay should be able to measure the active ingredient without interference from degradation products.

TNT was subjected to acid and alkali hydrolysis, oxidation, photo degradation and thermal stress conditions. Stress testing of TNT under different conditions using ammonium formate buffer: acetonitrile (90:10v/v) as the mobile phase solvent system suggested the degradation behaviour.

Initially 1M HCl was used at 60ºC for 1hr, no degradation was observed. The drug was found to be stable in 1M NaOH for 1hr. The drug was found to be labile in oxidative condition using 3% H_2O_2. The drug was found to be stable when treated

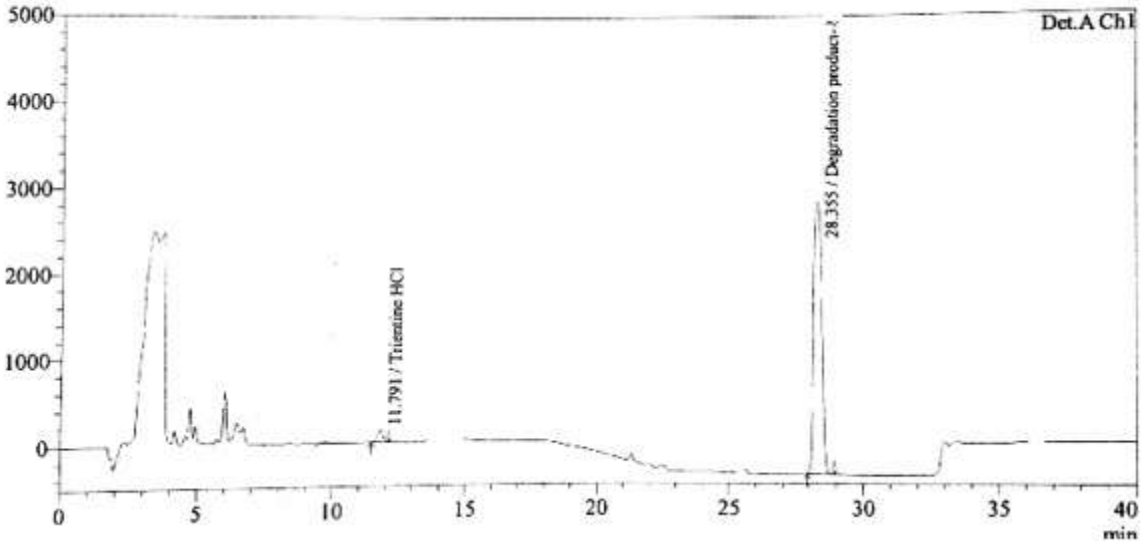


Figure 8. Oxidative degradation chromatogram of TNT

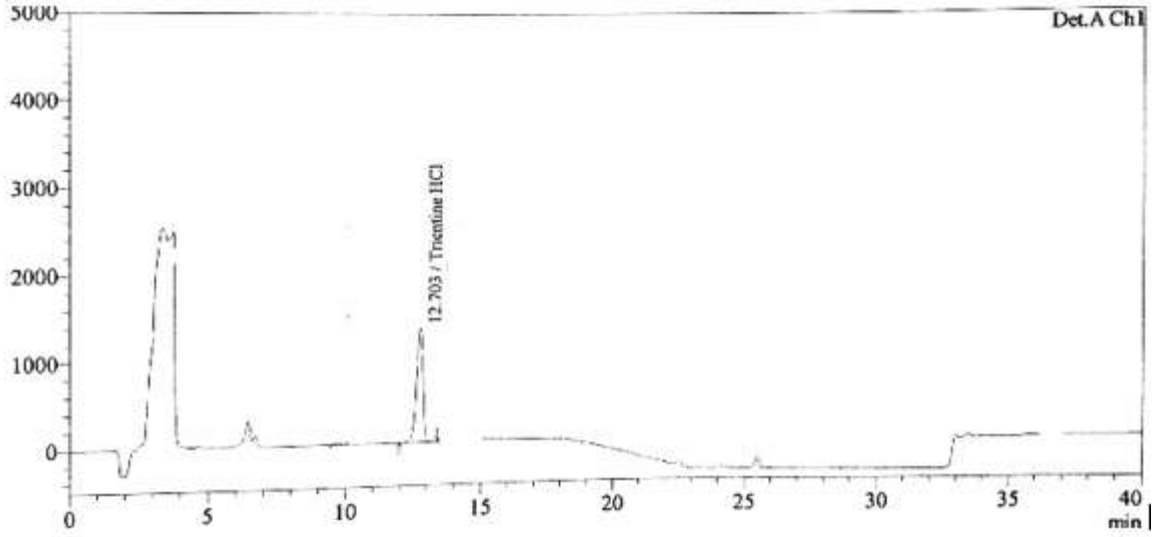


Figure 9. Photolytic degradation chromatogram of TNT

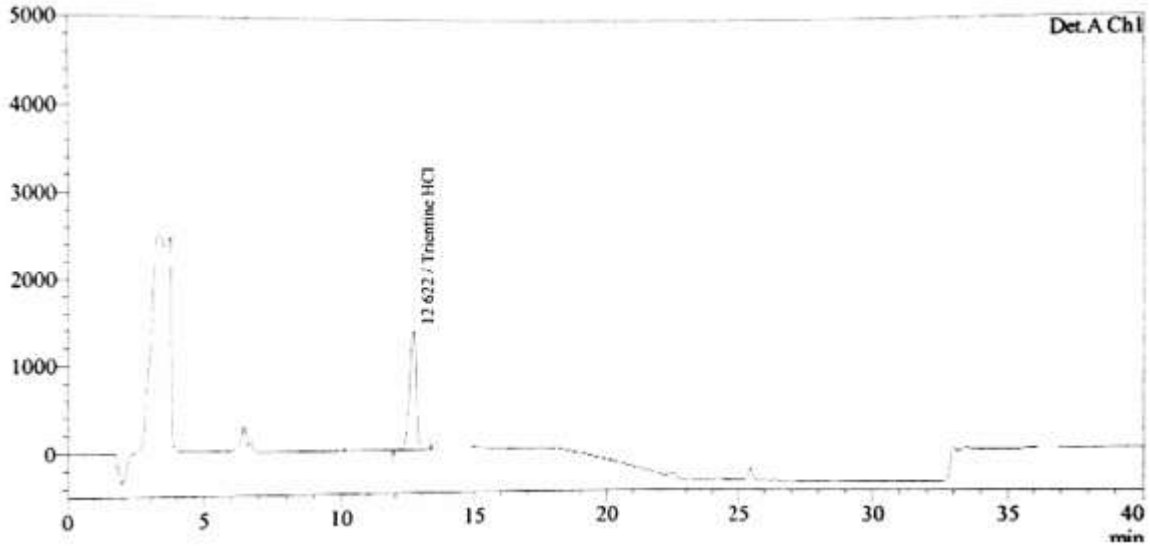


Figure 10. Thermal degradation chromatogram of TNT

6.2.1 Characterisation of Degradation Product

The degradation product obtained in oxidative stress condition was further subjected to LC-MS study for characterization and structural elucidation. LC-MS spectrum thus obtained is shown in **Figure 11**.

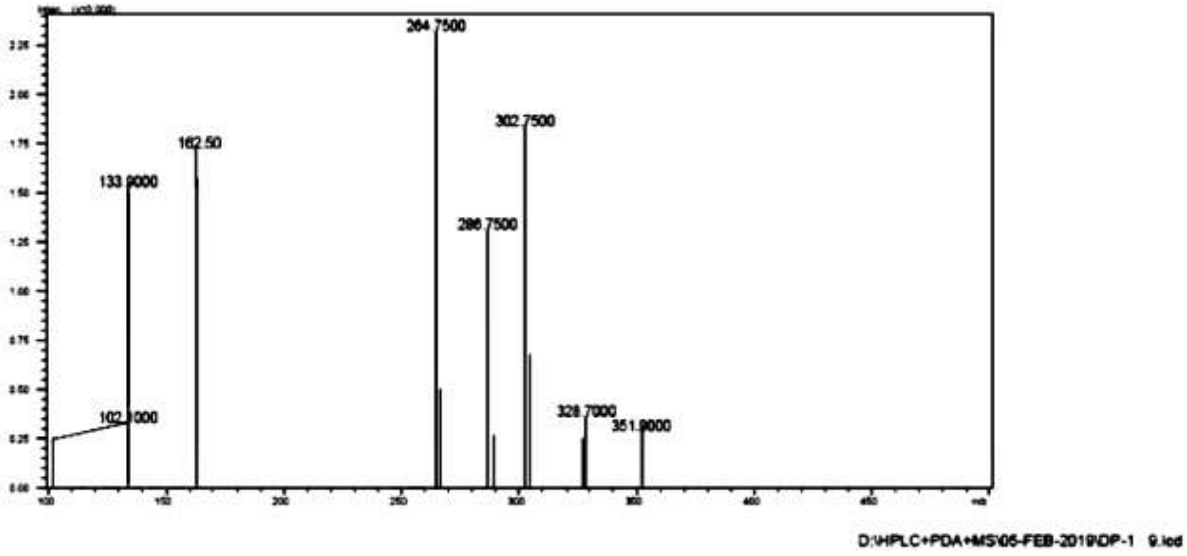


Figure 11. LC-MS Spectrum of degradation product

The m/z value of 162 in the spectrum is indicative of the formation of N-Oxide during oxidative degradation. The proposed fragmentation pathway is shown in **Figure 12**.

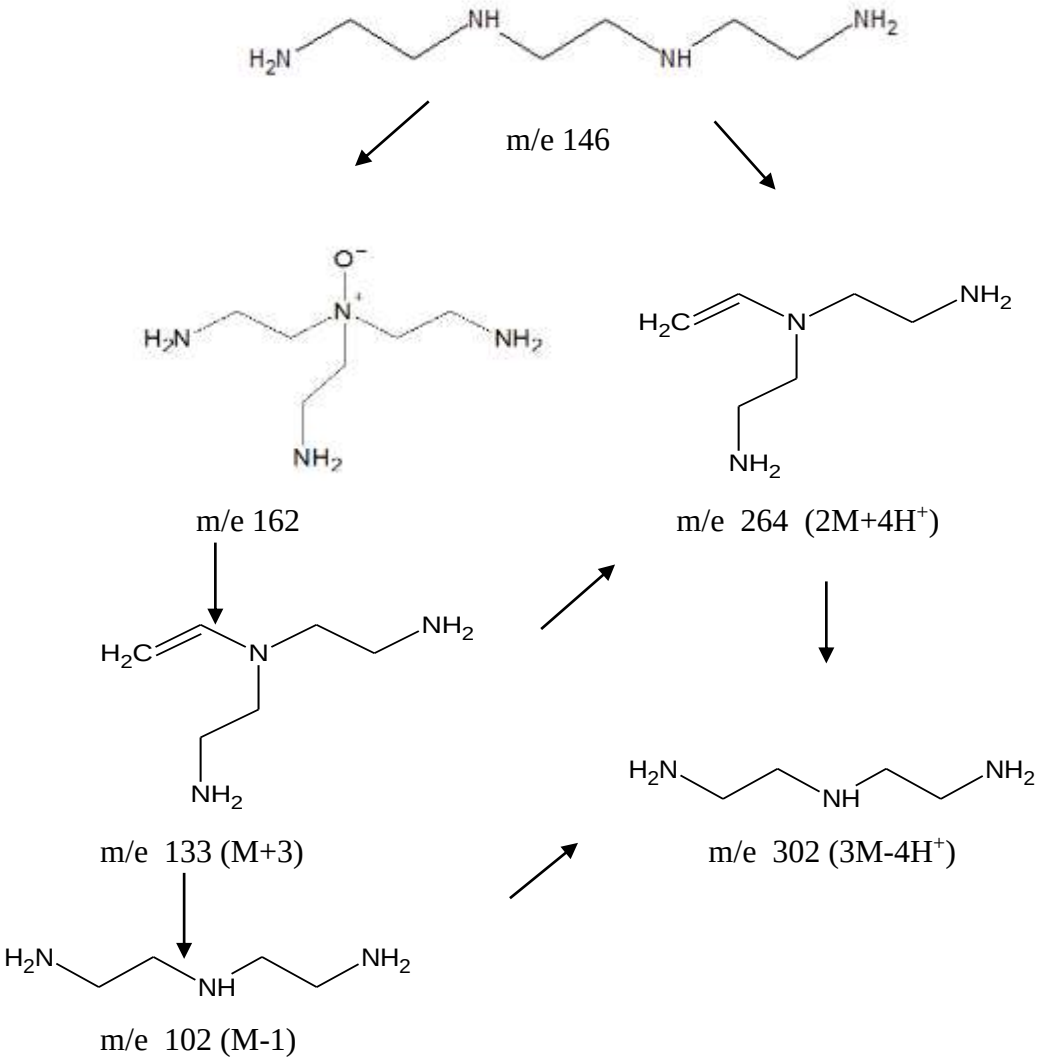


Figure 12. Proposed fragmentation pathway of TNT

^{1}H NMR and ^{13}C NMR.

^{1}H NMR and ^{13}C NMR spectra were recorded for the degradation product as shown in **Figure 13** and **Figure 14** respectively.

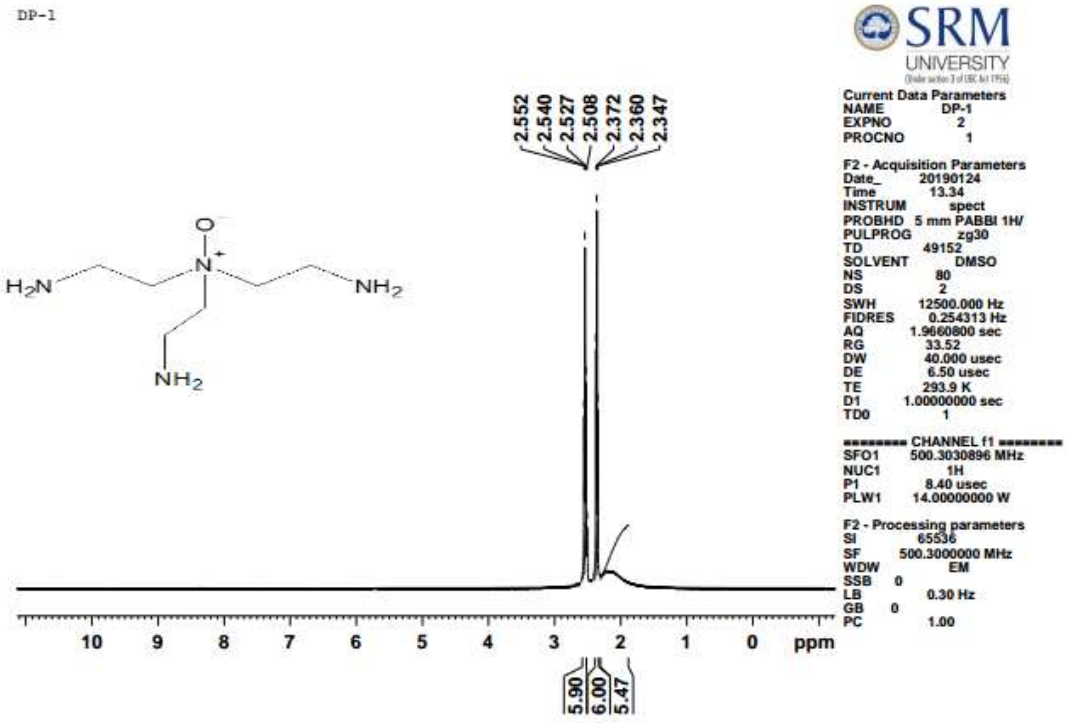


Figure 13.^{1}H NMR Spectrum of degraded product

^{1}H NMR (500MHz, DMSO, δ in ppm)

2.54 (t, 6H C$\underline{H}_2$)– 1,4,7) ; 2.35 (t, 6H C$\underline{H}_2$ – 2,5,8) ; 2.24 (t, 6H N$\underline{H}_2$ – 3,6,9)

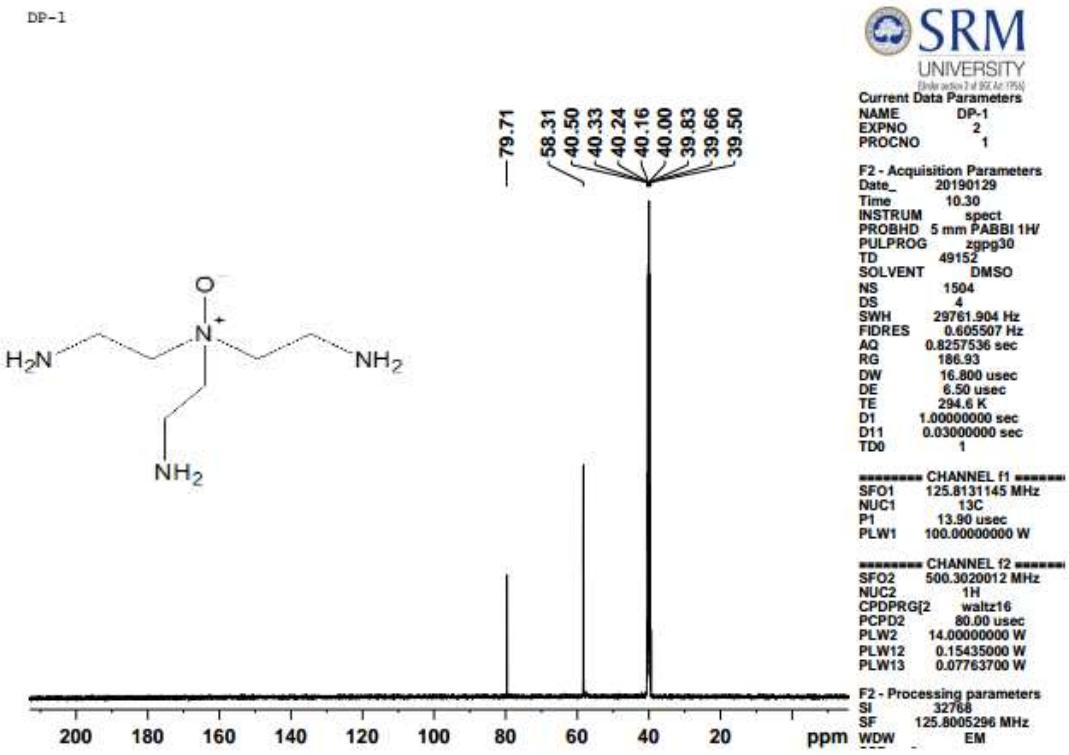


Figure 14. ^{13}C NMR Spectrum of degraded product

^{13}C NMR (100MHz, DMSO, δ in ppm)

C_1- 58.31 ; C_2 – 39.50 ; C_3 – 40.50; C_4 – 39.66 ; C_5 – 40.16 ; C_6 – 39.83.

Spectral characterisation of Oxidative degradation product

The IR spectrum was recorded for the oxidative degradation product and was compared with that of the parent drug as shown in the **Table 9.**

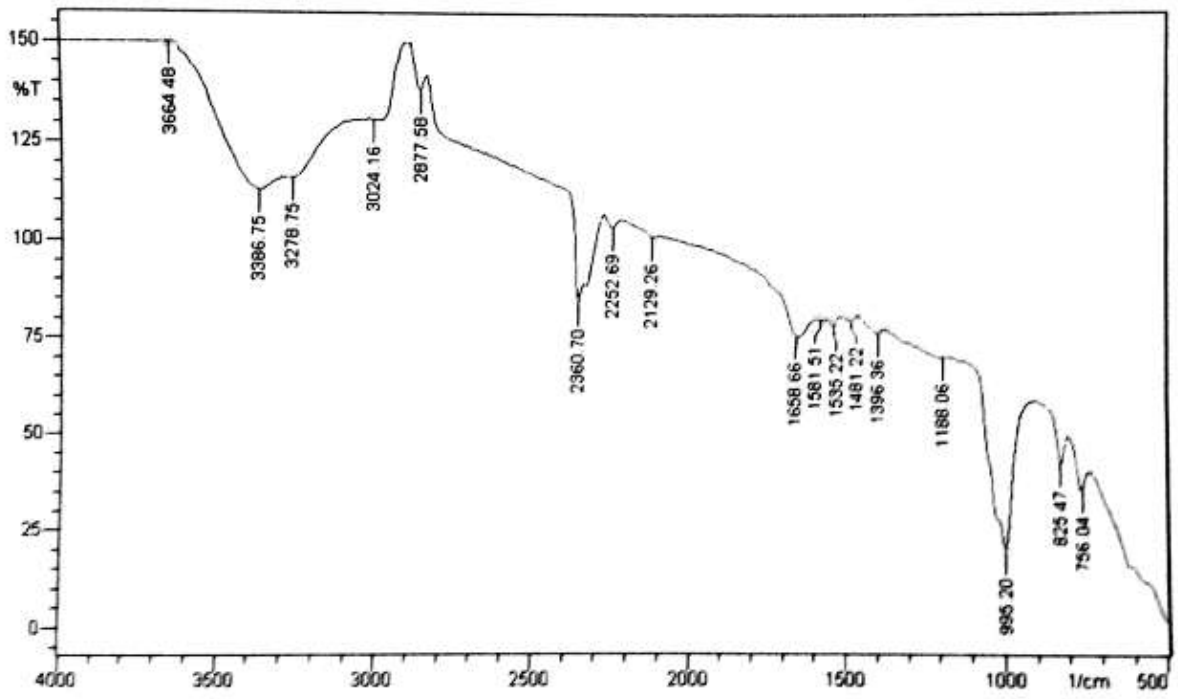


Figure 15. IR Spectrum of TNT Degradation product

Table 9. IR Interpretation of TNT degradation product

Wave number cm^{-1}	**Types of stretching**	**Remarks**
2877.58	C-H Stretching	Presence of alkane
3386.75	N-H Stretching	Presence of aliphatic primary amine
1581.51	N-H Bending	Presence of primary amine
995.20	N-O Stretching	Presence of amine oxide

The results of LC-MS and the spectroscopic studies indicate the formation of N-Oxide of TNT as the degradation product in oxidative degradation.

The degradation product has 16 amu more than Trientine showing the addition of one oxygen atom in Trientine moiety. Thus the structure of degradation product (N-Oxide) may be as shown in **Figure 16**.

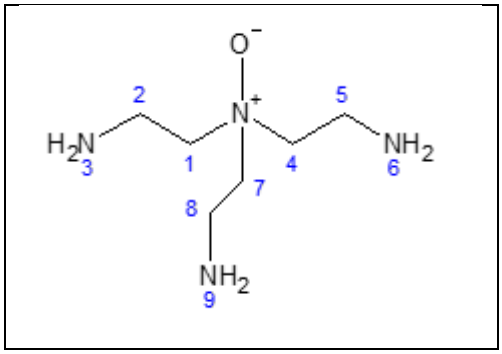
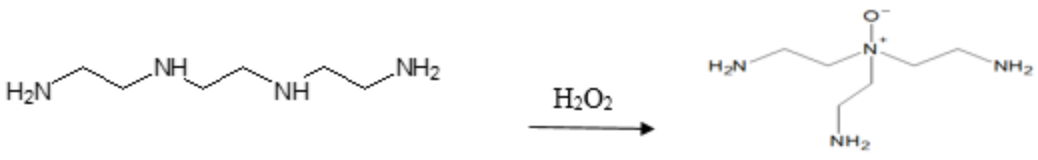


Figure 16. Proposed structure of TNT Degradation product

The developed method could be conveniently used to quantify the drug in presence of its degradation product. As the method could effectively separate the drug from its degradation product, it can be regarded as stability indicating. The probable degradation reaction of TNT is as shown in **Figure 17.**



TNT
Mol wt – 146

N-Oxide of TNT
Mol wt – 162

Figure 17. Probable degradation pathway of the drug

This work demonstrates the practical utility of IR, NMR and Mass spectroscopic studies in the effective structural elucidation of the degradation product. Based on the spectral data (^{1}H NMR, ^{13}C NMR, IR and Mass spectra) the degradation product was characterised. The pure drug was well resolved from the degradation product peak with significantly different R_t values. Statistical analysis proved that the method is repeatable and specific for the estimation of TNT. Application of the method for estimation of TNT from capsule dosage form and stressed samples showed that neither the degradation product nor the excipients interfered in the estimation of the drug. As the method could effectively separate the drug from the degradation product, it can be regarded as stability indicating.

The developed method was found to be precise, accurate, reproducible, sensitive, specific and robust. The developed method was free from interferences due to other active ingredients and excipients present in the formulation and thus can be conveniently applied for the routine analysis of the selected drug in its pharmaceutical dosage form. The results of market sample analysis suggests the applicability, reproducibility and utility of the method for the estimation of the drugs in quality control laboratories.

6.3 TOXICITY PREDICTION

The chemical structure was drawn in OSIRIS property explorer to show the biological properties of the compound. Properties like high risks of undesired effects like mutagenicity, tumorigenicity and reproductive effect are shown in red. Green colour indicates the drug conform behaviour. It was found that the degraded product is non-mutagenic, non-tumorigenic and has no irritability and reproductive effects as shown in **Figure 18.**

with UV light for 5hrs. Drug was found to be stable when subjected to thermal degradation at 105ºC for 5hrs.

TNT was found to be stable in acid, alkali, thermal and photolytic conditions. A degradation product was formed in oxidative stress conditions at the R_t of 28.355mins. The chromatogram of TNT under various stress conditions are shown in **Figures 6 to 10**.

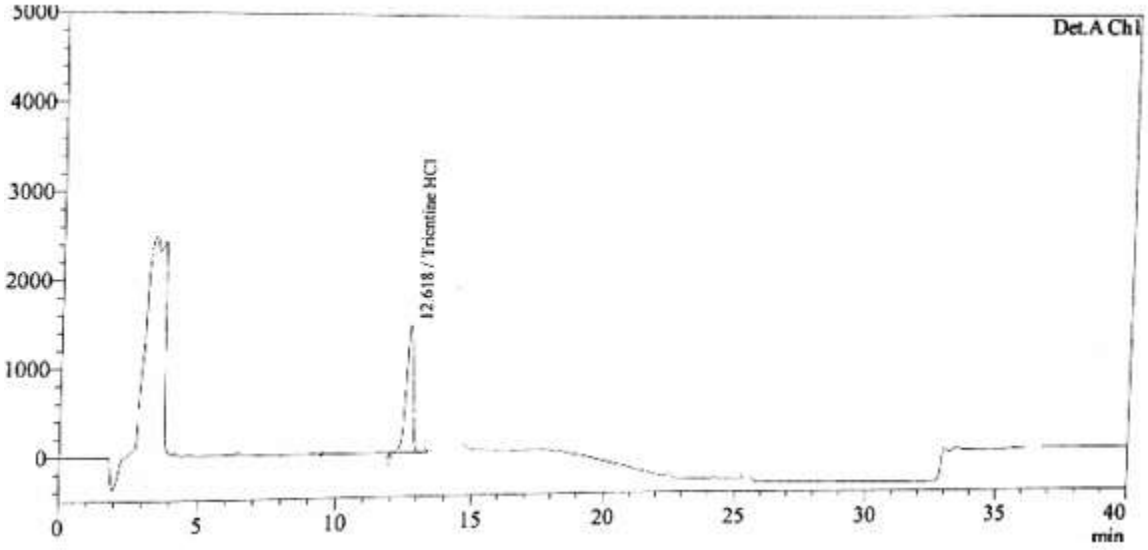


Figure 6. Acid degradation chromatogram of TNT

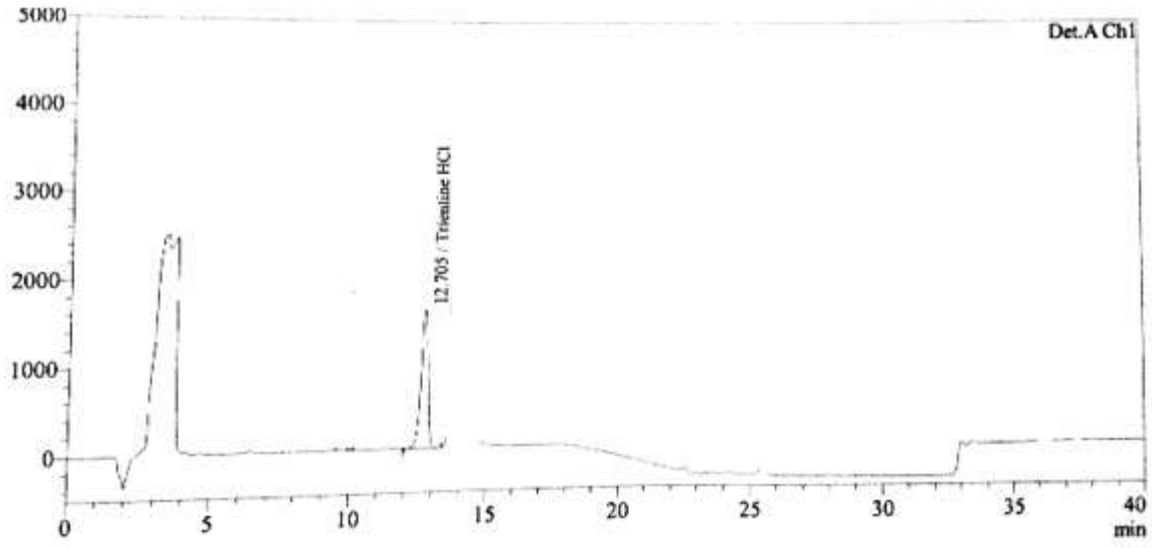


Figure 7. Base degradation chromatogram of TNT

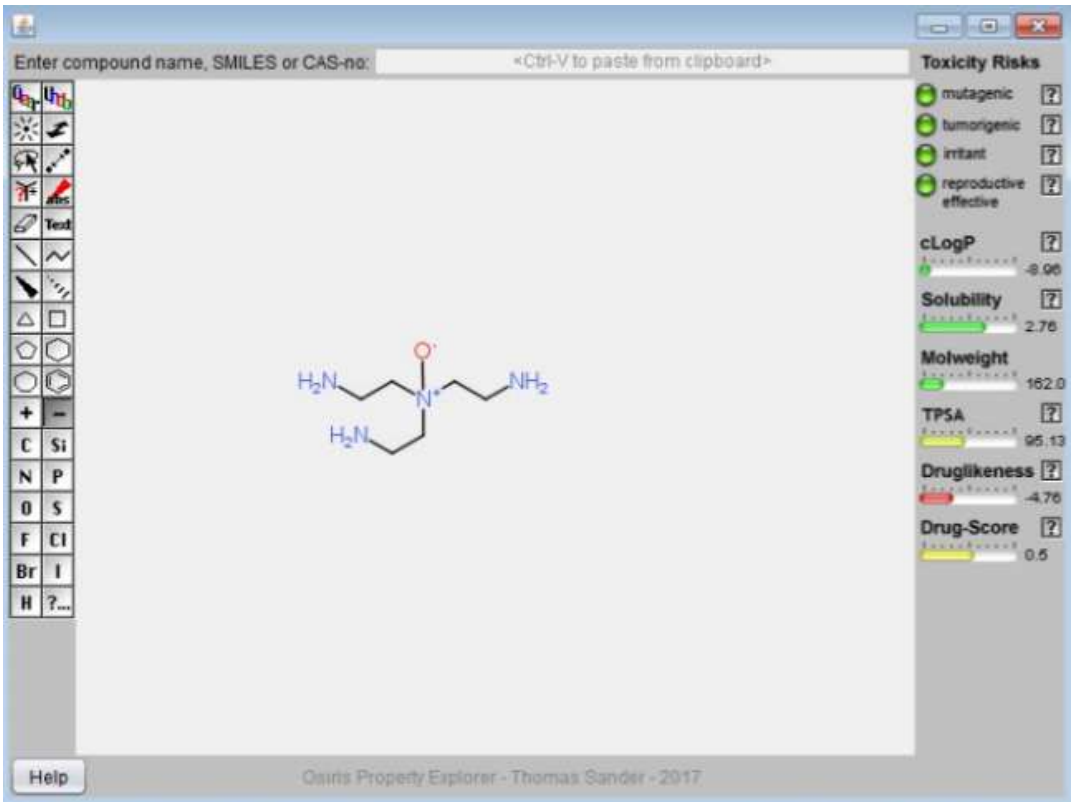


Figure 18. Toxicity prediction of degraded product of TNT


Conclusion

CHAPTER 7

SUMMARY AND CONCLUSION

In the present study a new stability indicating RP-HPLC method has been developed and validated for the quantification of Trientine hydrochloride. The developed method was validated as per ICH guidelines. The parameters which are validated are linearity, accuracy, precision, robustness and system suitability parameters.

The chromatographic conditions were optimized before the development of the chromatogram. The mobile phase consisted of a mixture of pH 5.3 ammonium formate buffer and acetonitrile (90:10v/v) under gradient mode of elution. The system suitability parameters such as theoretical plates, tailing factor and peak symmetry were determined to check the validity of the developed RP-HPLC method. The developed chromatographic method proved to be superior to most of the reported methods in terms of accuracy, precision and sensitivity. The data obtained was subjected to statistical analysis. The proposed method was successfully applied to the determination of the selected drug in its pharmaceutical dosage form.

The stability indicating RP-HPLC method is useful to understand the degradation behaviour of Trientine hydrochloride. Trientine hydrochloride was subjected to forced degradation study. TNT was found to degrade in oxidative stress conditions and the degradation product was characterised by IR, Mass and NMR spectroscopic techniques.

Toxicity of the degradation product was checked by using Osiris software. It was found that the degradation product is non-toxic. Prediction results are colour coded in which the red colour shows high risks with undesired effects like mutagenicity or poor intestinal absorption and green colour indicates drug-conform behaviour.

The proposed method is accurate, selective, sensitive and reproducible.The method is relatively free from any interference produced from common tablet excipients. Hence, the recommended procedure is well suited for the assay and evaluation of TNT in pharmaceutical quality control. The present work can be

extended for the quantification of the selected drug in bioavailability, bioequivalence, pharmacokinetics, *in-vitro* and *in-vivo* correlation studies.

A successful analyst must know what reactions are taking place during analysis and be able to understand and apply the theory upon which the method is dependent. The analyst must acquire skills of technique, patience, neatness and accuracy. Absolute integrity is demanded of every quantitative analyst. To become a sucessful analyst, one must realize that, analytical chemistry is not a simple routine procedure. Manipulative skill acquired by experience with the ability to follow directions under the supervision of a skilled analyst may enable one to carry out successfully certain analytical procedures.

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CHAPTER 8

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PAPER PUBLICATION

CHAPTER 9

PAPER PUBLICATIONS

Paper published

Title	**:**	Validated RP-HPLC (PDA) method for estimation of Trientine hydrochloride in Pharmaceutical dosage form
Journal	**:**	International Journal of Chemtech Research. 2018;12(1):87-92. (SCOPUS indexed)

Paper accepted for Publication

Title	**:**	Development of Novel Stability indicating RP-HPLC method for quantification of Trientine hydrochloride and characterization of its degradation product by spectroscopic techniques.
Journal	**:**	Research journal of Pharmaceutical, Biological and Chemical Sciences (SCOPUS indexed)
Date of acceptance	**:**	12.3.2019 (To be published in Vol 10, Issue No.3)

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ANNEXURE

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE, CHENNAI - 3.

WORKSHOP ON DRUG DESIGN

Certificate of Appreciation

This is to certify that ~~Mr.~~ / Ms / ~~Mrs.~~ A. VARALAKSHMI of COLLEGE OF PHARMACY, MMC has attended the WORKSHOP ON DRUG DESIGN AND HANDS ON TRAINING on 2nd March and 3rd March 2018 at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03.

Dr. N. Jayshree
Organizing Secretary,
College of Pharmacy, MMC

Dr. A. Jerad Suresh
Principal,
College of Pharmacy, MMC

DEPARTMENT OF PHARMACOGNOSY
SRI RAMACHANDRA COLLEGE OF PHARMACY
SRI RAMACHANDRA UNIVERSITY
(Declared under section 3 of the UGC Act, 1956)
Accredited by NAAC with 'A' Grade
Porur, Chennai - 600 116.

in association with
Society for Ethnopharmacology, Chennai Chapter

CERTIFICATE

This is to certify that Dr./Mr./Ms. A. Varalakshmi .....

has participated as a Resource person / Delegate in the Pre conference workshop on ***"Recent Advances in Analytical Techniques - Drugs & Pharmaceuticals"*** *held on 27th June 2017, organized by Sri Ramachandra University, Porur, Chennai.*

This carries 2 Credits.

D. Chamundeeswari
Dr. D. CHAMUNDEESWARI
Principal &
Co-ordinator, SFE - Chennai Chapter

K.V. Somasundaram
Dr. K.V. SOMASUNDARAM
Dean of Faculties

INDIAN PHARMACEUTICAL ASSOCIATION

NATIONAL CONVENTION 2017 - 18

Theme: Pharma Vision 2030: Planning the Future

Certificate of Participation

This is to certify that

Dr./Prof./Mr./Ms. A. VARALAKSHMI.

participated in the National Convention 2017 - 18 held at B. S. Abdur Rahman Crescent Institute of Science & Technology, Chennai, 10th - 11th February 2018

Dr. S. Manivannan	J. Jayaseelan	T. Sathish
Chairman - LOC	IPA Convention Co-ordinator - LOC	Hon. Secretary - LOC

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY

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Certificate

This is to certify that ~~Mr./Ms./Prof./Dr.~~ A. VARALAKSHMI *of* _____ *has actively participated as a Delegate in the National Seminar on* **"Impact of Intellectual Property Rights in Formulation and Development"** *organized by* ***Department of Pharmaceutics*** *on 23.02.2019 at* ***Arulmigu Kalasalingam College of Pharmacy,*** *Krishnankoil, Tamil Nadu.*

5 credit points awarded by The Tamil Nadu Dr.M.G.R. Medical University, Chennai.

Dr.R.Ramprasad	Dr.S.R.Senthilkumar	Dr.N.Venkateshan	Dr.S.Shasi Anand	Dr.K.Sridharan
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Certificate

This is to certify that Mr./Ms./Prof./Dr. A. Vanlaveboni *of* _____ *has actively participated in the International Seminar on* **"3D - Approaches in Combinatorial Chemistry : Challenges and Perspectives"** *organized by* ***Department of Pharmaceutical Chemistry*** *on 28.02.2018 at Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamil Nadu.*

5 credit points awarded by The Tamil Nadu Dr.M.G.R. Medical University, Chennai.

Dr.R.Rajapandi	Dr.J.Amutha Iswarya Devi	Dr.N.Venkateshan	Dr.S.Shasi Anand	Dr.K.Sridharan
Scientific Advisor	Organizing Secretary	Convenor	Secretary	Chairman