"IMPURITY PROFILING OF DROTAVERINE API AND IT'S FORMULATION BY RP-HPLC"

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

BY

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May 2012

CERTIFICATE

This is to certify that the dissertation work entitled "Impurity profiling of Drotaverine API and it's formulations by RP-HPLC" submitted by Ms. SWETA CHECHANI with Regn. No. (10MPH309) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Analysis" is a bonafide research work carried out by the candidate at the Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Impurity profiling of Drotaverine API and it's formulations by RP-HPLC", is based on the original work carried out by me under the guidance of Dr. Priti J. Mehta, Professor & Head, Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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ACKNOWLEDGEMENT

"God is like a big circle, whose centre is everywhere but circumference is nowhere"

With these words I thank Almighty God, who began this work and carried it to completion. It is he who has bless me with the people whose names I feel privileged to mention here.

It gives me immense pleasure today when I take an opportunity to acknowledge all those personalities who contributed directly or indirectly to my project. This research would not have been possible without the whole hearted encouragement, guidance, support, and cooperation of my beloved family, teachers, friends, well-wishers and relatives. Probably I would have never achieved this without their support and blessings. With profound appreciation, I acknowledge to one and all.

I wish to express my sincere thanks, with a deep sense of gratitude, to my respected guide **Dr. Priti J. Mehta**, Professor & Head, Dept. of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University for initiating and suggesting the theme of work, for her valuable guidance, supervision, creative suggestions and meticulous attention, sustained interest, immense guidance, dedicated support. She has bestowed upon me for the timely completion of this work. I am extremely indebted to her for her motivational inspiration, kind expertise during the writing up of my thesis and the scientific attitude she has nurtured in me which will definitely stand in all my future endeavors.

I would like to thank especially to **Mr. Omkar sir** and to my respected co-guide **Mr. Nrupesh R. Patel** for their selfless support, co-operation and valuable suggestion.

I am extremely grateful to **Dr. Charmy Kothari, Deepak Khatri sir & Tejas sir,** Dept. of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University for their continuous encouragement and everlasting support throughout the course of this dissertation work.

I am grateful to **Dr. Manjunath Ghate**, Director, Institute of Pharmacy, Nirma University for providing all necessary help and facility for my work and also for his constant support and encouragement.

My friends are like ropes which have pulled me up from my lows and held me down firmly in my highs.

I acknowledge all of my batch mates **Kewal, Ajay, Margi, Kartik, Shikha, Jay, Ankit, Barkat, Pooja, Sonika, Urvashi, Rupak, Chirag, Pawan, Yesha, Krishna and Sunil** for their amicable support and help. A special word of gratitude to my friends **Sangeeta, Deepak, Shivangi, Priyanka, Bhavna and Neha** who were always there besides me with the hand of support and encouragement.

I am also thankful to my juniors and all of the others for helping me as and when required.

I also wish to acknowledge **Satejbhai, Shreyasbhai, Bipinbhai, Rohitbhai, Jigneshbhai** for providing me all the materials required in my work. I sincerely thanks to **Mrs. Lalita mam**, for library facilities and constant encouragement during my work.

Last but not least I am indebted infinitely to love, care and trust being showered on me by my grandfather **Mr. Jankilal Chechani**, my grandmother **Mrs. Sundardevi Chechani,** my father **Mr. Mahesh Chechani,** my mother **Mrs. Uma Chechani,** my Brother **Ravi Chechani** without their consistent prayers, affectionate blessings, selfless care and endless confidence in me, I would have never come to the stage of writing this acknowledge.

Date: Sweta Chechani

ABBREVIATIONS

CHEMICALS:

DRT	Drotaverine hydrochloride
ACN	Acetonitrile
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HBr	Hydrobromide
NaOH	Sodium hydroxide
MeOH	Methanol
H_2O	Water
KH_2PO_4	Potassium di hydrogen ortho phosphate
OPA	Ortho phosphoric acid

SYMBOL:

%	Percentage
R	Resolution
k’	Capacity Factor
α	Selectivity
N	Number of Theoretical Plates
As	Peak Asymmetric Factor
T	Tailing Factor
λmax	Wavelength of maximum absorption
<	Less than
>	Greater than
µg	Microgram (S)
µ L	Micro liter (S)
µm	Micro meter (S)
cm	Centimeter (S)
i.d.	Internal diameter
M	Molar

mg	Milligram (S)
Min	Minute (S)
ml	Milliliter (S)
mM	Milimolar
mm	Millimeter (S)
nm	Nanometer (S)
r^2	Correlation coefficient
Sec	Second (S)
Temp	Temperature
°C	Degree centigrade
No.	Number
Sr. No.	Serial number
N	Normal
v/v	Volume by volume
w/w	Weight by weight
Conc.	Concentration
ppm	Parts per million
h	Hour

OTHERS:

API	Active Pharmaceutical Ingredient
RSD	Relative Standard Deviation
SD	Standard Deviation
USP	United States Pharmacopoeia
ICH	International Conference on Harmonization
UV	Ultraviolet
IR	Infrared
TLC	Thin Layer Chromatography
HPTLC	High Performance Thin Layer Chromatography

HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
GC-MS	Gas Chromatography- Mass Spectroscopy
LC-MS	Liquid Chromatography -Mass Spectroscopy
CE	Capillary Electrophoresis
PDA	Photo Diode Array
PDE	Phosphodiesterase Enzymes
FR	Flow Rate
AOAC	American Association of Official Analytical Chemists
pKa	Dissociation Constant
Rt	Retention Time
Rf	Retention factor
MP	Mobile phase
Unk	Unknown
DP	Drug Product
DS	Drug Substances
LOD	Limit of detection
LOQ	Limit of Quantitation
ISE	Ion Selective Electrode
PVC	Poly Venyl Chloride

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A related impurity method was developed and validated for determination and quantification of impurities of drotaverine hydrochloride in bulk drug and pharmaceutical formulations. The separation was accomplished on a phenomenex Luna C-18 (150 mm × 4.6 mm; particle size 5 µm) column under isocratic mode. The mobile phase was 0.0125 M potassium dihydrogen orthophosphate (pH 4.0): acetonitrile (57:43v/v), flow rate was 1.0 ml min^{-1} and a PDA detector set at 240 nm was used for detection.. The proposed method was validated in terms of accuracy, precision, reproducibility according to ICH guidelines and successfully applied to the analysis of commercial formulations. Three major impurities were found among all formulations. Quantification of IMP C of drotaverine hydrochloride was performed using Std. IMP C impurity sample and other two impurities were reported in percentage with respect to drotaverine. Forced degradation of drotaverine hydrochloride was also carried out under thermal, photo, acidic, alkaline and peroxide conditions and it was found that impurities generated due to degradation of drotaverine. Thus, the developed method can be used for impurity profiling as well as quality control of drotaverine hydrochloride in bulk drug and pharmaceutical formulations.

1.1 Impurity and impurity profiling

Impurity as something that is impure or makes something else impure. Impurities are any materials that affects the purity of the material of interest, viz., an active pharmaceutical ingredient (API) or drug substance. (Ahuja S., 2007)

To assure the quality of drugs, impurities must be monitored carefully. It is important to understand what constitutes an impurity and to identify potential sources of such impurities.

Impurity profiling, is the common name of analytical activities with the aim of detecting, identifying or elucidating the structure and quantitatively determining organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations. The development of chromatographic and spectroscopic methods and their combinations helps in identification and quantitative determination of individual impurities. This is why impurity profiling has become the most important activity in assuring the high quality of drugs. (Bari et al., 2007)

1.1.1 Classification of impurities: (ICH Q3B, 2006)

Impurities may be classified into the following categories:

• Organic impurities (process- and drug -related)

• Inorganic impurities

• Residual solvents

Organic impurities may arise during the manufacturing process and/or storage of the new drug substance. They may be identified or unidentified, volatile or non-volatile and it includes following:

• **Starting materials**: These are the materials that are used to begin the synthesis of an API.

• **By-products:** The unplanned compounds produced in the reaction are generally called by-products. It may or may not be possible to theorize all of them. Hence, they present a challenging problem.

• **Intermediates:** The compounds produced during synthesis of the desired material are called intermediates, especially when they have been isolated and characterized.

• **Degradations product:** These are the compounds produced because of decomposition of the material of interest or active ingredient. This term also include those products produced from degradation of other compounds that may be present as impurities in the drug substance.

Inorganic impurities: It includes following:

- Salts
- Catalysts
- Ligands
- Heavy metals or other residual metals.

Residual solvents:

Residual solvent come as an impurity in the form of organic and inorganic liquids used during production and/or crystallization.

1.1.2 Sources of impurities: (Ahuja S., 2007)

- **Synthesis-related impurities:** Impurities in a drug substance or a new chemical entity originate mainly during the synthetic process from raw materials, solvents, intermediates, and by-products. Similarly, solvents used in the synthesis are likely to contain a number of impurities that may range from trace levels to significant amounts that can react with various chemicals used in the synthesis to produce other impurities.
- **Formulation-related impurities:** A number of impurities in a drug product can arise out of interactions with excipients used to formulate a drug product. Furthermore, in the process of formulation, a drug substance is subjected to a variety of conditions that can lead to its degradation or other deleterious reactions. For example, if heat is used for drying or for other reasons, it can facilitate degradation of thermally labile drug substances.
- **Degradation-related impurities:** A number of impurities can be produced because of API degradation or other interactions on storage. Therefore, it is very important to conduct stability studies to predict, evaluate, and ensure drug product safety. The stability studies under various exaggerated conditions

of temperature, humidity, and light can help us determine what potential impurities can be produced by degradation reactions.

1.1.3 Analytical methods for impurity profiling: (Baertschi S. W., 2006)

The safety of a new pharmaceutical compound or drug requires that it meet the established purity standards as well as excellent stability throughout its shelf life. These requirements demand that the analytical methodology that is used be sensitive enough to measure low levels of impurities. This has led to analytical methods that are suitable for determination of trace/ultratrace levels, i.e., sub-microgram quantities of various chemical entities.

A variety of methods are available for monitoring impurities.

- Ultraviolet (UV)
- Infrared (IR)
- Nuclear magnetic resonance (NMR)
- High performance liquid chromatography (HPLC)
- Mass spectrometry (MS)

1.1.4 General scheme for drug impurity profiling:

The procedure of impurity profiling, begins with the detection of the impurities using the thin layer chromatography, high performance liquid chromatography or gas chromatography. Procurement of standard impurity samples from the synthetic organic chemists which include, last intermediate of the synthesis, products of predictable side reactions, degradation products if any etc.

The most reasonable way to determine the structure of impurity starts with the investigation of the UV spectra, easily obtainable with the aid of the diode array detector in the case of HPLC and the quantification with the help of densitometer. In exceptional cases, with the full knowledge of the synthesis of drug material, the structure of the impurity can be generated on the basis of NMR spectral data.

If the obtainable information from the UV spectrum is not sufficient, the next step in the procedure of impurity profiling is to take the mass spectrum of the impurity. The major disadvantage of this method is the volatility and thermal stability problems of the impurities. The use of derivatization reactions widely used in GC/MS analysis is

problematic because the side-products of the derivatization reaction can be confused with the impurities. The next step in the impurity profiling is the synthesis of the material (impurity standard) with the proposed structure.

The possibilities of spectroscopic techniques in drug impurity profiling without chromatographic separation are also worth mentioning. Spectra obtained by using high resolution, high sensitive NMR spectrometers with APCI/ESI facilities are suitable to provide a fingerprint picture regarding the purity of the sample. (Gorog S., 2006)

1.1.5 Regulatory requirements of impurity profiling:

The requirements specified by ICH for identification and qualification thresholds of impurities are given in following guidance documents:

- Q1A (R) stability testing of new drug substances and products
- Q3A (R) impurities in drug substances
- Q3B (R) impurities in drug products
- Q3C impurities: residual solvents
- Q6A specifications: test procedure and acceptance criteria for new drug substances and new drug products; chemical substances.

The identification and qualification thresholds of impurities are provided as per ICH guidelines in table 1.1.

Table 1.1: Thresholds for impurities in pharmaceuticals as per ICH recommendation. (ICH Q3A, 2006)

Maximum daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

[1] The amount of drug substance administered per day

[2] Higher reporting thresholds should be scientifically justified

[3] Lower thresholds can be appropriate if the impurity is unusually toxic

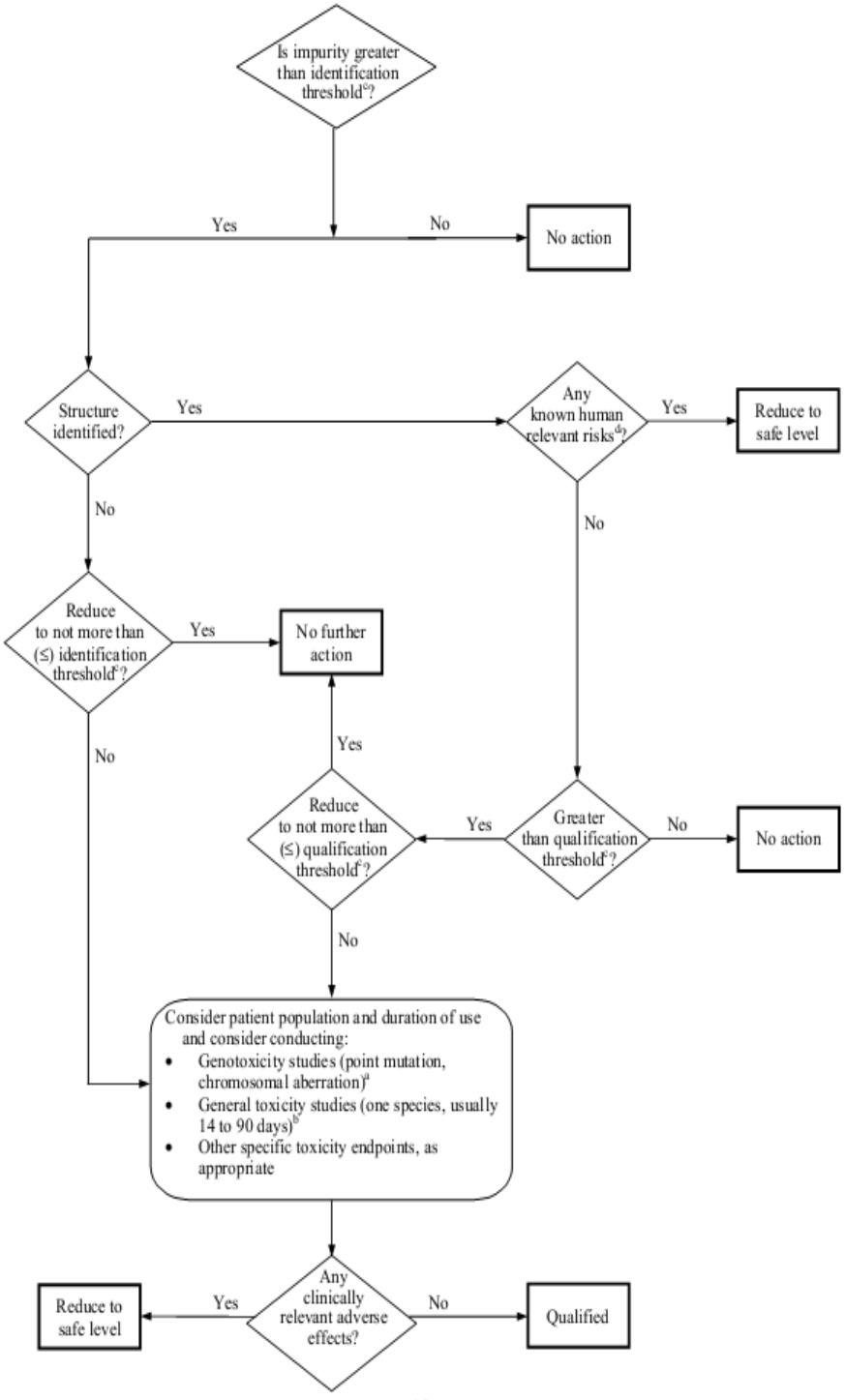


Figure 1.1: Decision tree for identification and qualification of impurities (ICH Q3B (R2), 2006)

1.2 Role of Phosphodiesterase Enzymes (PDEs) & their Inhibitors:

PDE enzymes break a phosphodiester bond. PDEs comprise of group of enzymes that degrade the phosphodiester bond in the 2nd messenger molecule like cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP). 14 different subtypes of PDEs have been identified. They regulate the localization, duration and amplitude of cyclic nucleotide signalling within sub cellular domain. PDEs are therefore important regulators of signal transduction mediated by these 2nd messengers. Inhibitors of PDEs can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE.

A phosphodiesterase inhibitor is a drug that blocks one or more subtypes of the enzyme phosphodiesterase (PDE), therefore preventing the inactivation of the intracellular second messengers cAMP and cGMP by the respective PDE subtype(s). (Rang H.P., 2006)

Classification of PDEs Inhibitors: (Rang H.P., 2006)

a) Non-selective phosphodiesterase inhibitors

- **Methylated xanthines and derivatives:**
  - Caffeine, a minor stimulant.
  - IBMX (3-isobutyl-1-methylxanthine), used as investigative tool in pharmacological research.
  - Paraxanthine.
  - Aminophylline.
  - Pentoxifylline, a drug that has the potential to enhance circulation and may have applicability in treatment of diabetes, fibrotic disorders, peripheral nerve damage, and microvascular injuries.
  - Theobromine.
  - Theophylline, a bronchodilator.

- **Methylated xanthines act as both:**

- Competitive nonselective phosphodiesterase inhibitors which raise intracellular cAMP, and leukotriene synthesis, and reduce inflammation and innate immunity.
- Nonselective adenosine receptor antagonists.

b) Selective phosphodiesterase inhibitors

- **PDE I selective inhibitors**
  - Vinpocetine.
- **PDE II selective inhibitors**
  - EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine).
  - Anagrelide.
- **PDE III selective inhibitors**
  - Enoximone and milrinone, used clinically for short-term treatment of cardiac failure. These drugs mimic sympathetic stimulation and increase cardiac output.

PDE III is sometimes referred to as cGMP-inhibited phosphodiesterase.

- **PDE IV selective inhibitors**
  - Drotaverine.
  - Mesembrine, an alkaloid from the herb *Sceletium tortuosum.*
  - Rolipram, used as investigative tool in pharmacological research.
  - Ibudilast, a neuroprotective and bronchodilator drug used mainly in the treatment of asthma and stroke. It inhibits PDE IV to the greatest extent, but also shows significant inhibition of other PDE subtypes, and so acts as a selective PDE IV inhibitor or a non-selective phosphodiesterase inhibitor, depending on the dose.
  - Piclamilast, a more potent inhibitor than rolipram.
  - Luteolin, supplement extracted from peanuts that also possesses IGF-1 properties.

PDE IV is the major cAMP-metabolizing enzyme found in inflammatory and immune cells. PDE IV inhibitors have proven potential as anti-inflammatory drugs, especially in inflammatory pulmonary diseases such as asthma, COPD, and rhinitis. They suppress the release of cytokines and other inflammatory signals,

and inhibit the production of reactive oxygen species. PDE IV inhibitors may have antidepressive effects and have also recently been proposed for use as antipsychotics.

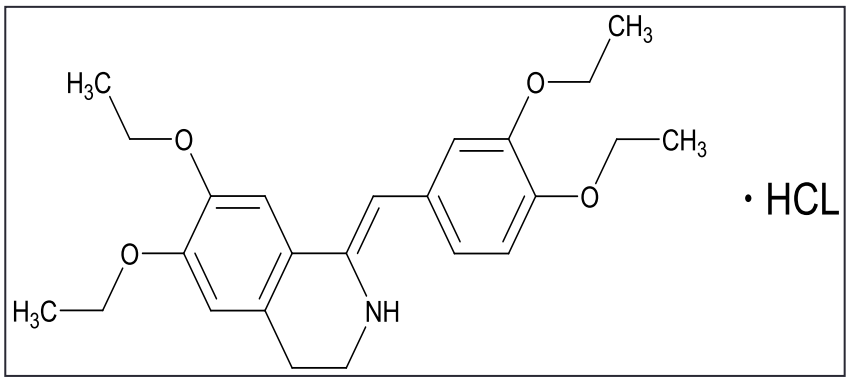
- **PDE V selective inhibitors**
  - Sildenafil, tadalafil, vardenafil, and the newer udenafil and avanafil selectively inhibit PDE V, which is cGMP-specific and responsible for the degradation of cGMP in the corpus cavernosum. These phosphodiesterase inhibitors are used primarily as remedies for erectile dysfunction, as well as having some other medical applications such as treatment of pulmonary hypertension.
  - Dipyridamole also inhibits PDE V. This results in added benefit when given together with nitric oxide or statins.

1.3 INTRODUCTION TO DRUG PROFILE: DROTAVERINE HYDROCHLORIDE (DRT): (Martindale, 2009 and Merck Index, 2006)

Molecular Formula: $C_{24}H_{31}NO_4.HCl$.

Molecular Weight: 433.97

Structural Formula:



IUPAC Name: **1.**6,7,3,4'-Tetraethoxy-1-benzal-1,2,3,4-tetrahydroisoquinoline hydrochloride.

2.(Z)-1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4 tetrahydroisoquinoline.

3.1-(3,4-Diethoxybenzylidene)-6,7-diethoxy-1,2,3,4 tetrahydroisoquinoline hydrochloride.

CAS No.: 985-12-6

Category: Antispasmodic drug

Official Status: Not official in IP, BP, USP. But official in:

1. Martindale-the complete drug reference; Pharmaceutical Press USA, 2009, 36th edition, 2298.

2. The Merck Index, An encyclopedia of chemicals, drugs and biological, Merck research laboratories, USA, 2006, 14th edition, 3455.

Physicochemical Properties:

- **Appearance:** Yellowish and greenish shade crystalline powder.
- **Solubility:** Moderately soluble in water, soluble in 96% ethanol, easily soluble in chloroform.
- **Melting Point:** 208-212 ºC
- **Storage:** In an airtight container, protected from light.

Pharmacological Actions:

Mechanism of Action: DRT is an analogue of papaverine with the smooth muscle relaxant properties. It is non-anticholinergic & antispasmodic, which selectively inhibits phosphodiesterase IV and is accompanied by a mild calcium channel-blocking effect.

Pharmacokinetics: (Bolaji et al., 1996)

- **Peak concentrations**: 1 to 3 hours after oral dose.
- **Oral bioavailability**: 25 to 91% and its metabolites are 80% to 95% protein bound volume of distribution 193 to 195 litres.
- **Metabolism**: Hepatic.
- **Half life**: 7 to 12 hours.
- **Excretion**: Fecal and renal.
- **Routes of administration**: Oral, intravenous

Drug Interactions: May attenuate the action of levodopa. Additive beneficial effect with concurrent use of analgesics, antimuscarinics or benzodiazepines.

Indications:

- DRT can be used in **acute colicky pain** caused by renal and ureteric stones.
- DRT can be used for **augmentation of labour**.
- DRT is used for acceleration of labour and relief of labour pains. There are no adverse fetal effects, but atonic postpartum haemorrhage is more common.
- Efficacy of DRT is seen in **irritable bowel syndrome**.
- **Relaxant effect** of DRT is seen in **human isolated ureteral rings.**

Contraindications:

- In glaucoma.
- Drotaverine interacts with the L-type Ca^{2+} channel in pregnant rat uterine membranes.
- Severe renal/hepatic/cardiac dysfunction, porphyria.
- May attenuate the action of levodopa.

Adeverse Effects: Diarrhoea, nausea, dyspepsia, abdominal pain, dizziness, rashes. **Potentially Fatal:** Severe GI bleeding; nephrotoxicity; blood dyscrasias.

Dosage:

- Adults: 40-80 mg thrice 3 daily.
- Children**:** 1-5 years 20mg, 3-4 times daily. 6-12 year : 40mg, thrice daily.

Overdosing:

- AV blockade, cardiac arrest, respiratory center paralysis.

Marketed preparation :

Table 1.2: Marketed preparation of DRT

Brand Name	Dosage form	Strength	Manufacturer
Drotin	Injection	20mg/ml	Martin and Harris Pharmaceutical Pvt. Ltd.
Drotikind	Injection	20mg/ml	Lifestar Pharmaceutical Pvt. Ltd.
Verine	injection	20mg/ml	Corona remedies Pvt. Ltd.
Drotin	Tablet	80mg	Martin and Harris Pharmaceutical Pvt. Ltd.
Drotikind	Tablet	80mg	Lifestar Pharmaceutical Pvt. Ltd.
Doverine	Tablet	80mg	Intas Pharmaceutical Pvt. Ltd.
Drovera	Tablet	80mg	Maneesh pharmaceuticals

1.4 INTRODUCTION TO HPLC

Liquid Chromatography is an analytical technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase, the different solute will interact with the other phase to differing degree due to difference in adsorption, ion exchange, partitioning or size. These differences allow the mixed component to be separated from each other by using these differences to determine the transit time of solutes through column. (Sethi P. D., 2001)

Methods in chromatography

1) **Adsorption chromatography**

2) **Partition chromatography**

3) **Size exclusion chromatography**

Mechanism of reverse phase chromatography:

In reverse phase mode retention of molecules achieved by interaction of stationary phases non polar hydrocarbon chain with non polar parts of the sample molecules.

HPLC instrument consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column.

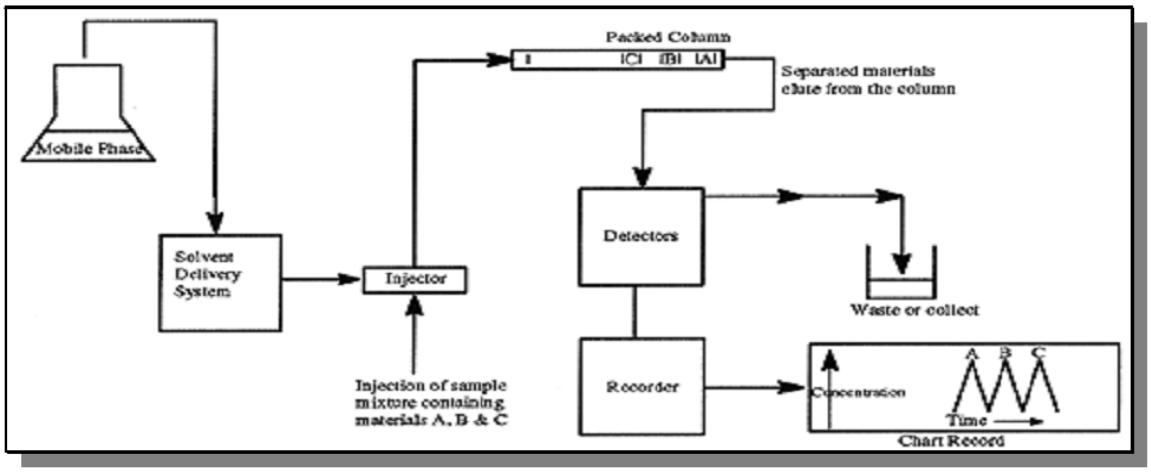


Figure 1.2: Schematic diagram of HPLC (Sethi P. D., 2001)

System suitability parameters:

- Resolution (Rs),
- Capacity factor (k'),
- Selectivity (α),
- Column efficiency (N) and
- Peak asymmetry factor (As).

1.5 ANALYTICAL METHOD VALIDATION: (ICH Q2(R1), 2005)

The main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. By performing thorough analytical method validation; overall knowledge of the capabilities of the analytical procedure can be evaluated.

1.5.1 Validation parameters:

1. **Specificity**

Specificity is ability of an analytical method to measure the analyte free from interference due to other components.

Acceptance criteria.

- Peak Purity of main peak (check by PDA or LC-MS) – 0.999

2. Linearity

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

Table 1.3: Linearity acceptance Criteria

Sr. No.	Test name	r	y-intercept	SD
1	Related Substances Level < 0.2%	0.990 DP 0.998 DS	≤ 25%	≤ 20%
	Level< 0.2-< 0.5%	0.990 DP 0.998 DS	≤ 15%	≤ 10%
	Level< 0.5-< 5.0%	0.990 DP 0.998 DS	≤ 10%	≤ 5%
	Level≥5%	0.990 DP 0.998 DS	≤ 5%	≤ 2.5%

3. Range

The interval between the upper and lower concentrations of analytes in the sample that have been demonstrate to have a suitable level of precision, accuracy, and linearity.

Table 1.4: Range Acceptance Criteria

Sr. No.	Test name	Minimum specified range
1	Related substances	LOQ to 120 % of the limit specified

4. Accuracy

Closeness of the test results obtained by the method to the true value. It should be established across specified range of analytical procedure.

Table 1.5: Accuracy acceptance criteria

Sr.No.	Test name	% Recovery	S_{rel} n = 9 at atleast three conc.
1	Related Substances Level < 0.2%	70-130	≤ 15%
	Level< 0.2-< 0.5%	80-120	≤ 10%
	Level< 0.5-< 5.0%:	90-110	≤ 5%
	Level≥ 5%	95-105	≤ 2.5%

5. Precision

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample. It should be investigated using homogeneous, authentic samples.

Table 1.6: Precision acceptance criteria

Sr.No	Test name	Repeatability S_{rel} (n≥6)	Intermediate Precision S_{rel} (n≥4)
1	Related Substances Level < 0.1%	≤ 30%	≤ 40%
	Level< 0.1-< 0.2%	≤ 20%	≤ 30%
	Level< 0.2-< 0.5%	≤ 10%	≤ 15%
	Level 0.5< 5%	≤ 5%	≤ 7.5%
	Level≥5%	≤ 2.5%	≤ 4%

6. Sensitivity

Limit of Detection (LOD) Lowest amount of analyte in a sample that can be detected but not necessarily quantitated.

Limit of Quantitation (LOQ) Lowest amount of analyte in a sample that can be quantified with suitable accuracy and precision.

LOD=3.3s\S

LOQ=10s\S

S = slope of calibration curve

s = standard deviation of intercept

7. Robustness

Capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Acceptance Criteria:

The results should be less than 2.0% for intentionally altered sensitive parameters from the normal unchanged parameters.

8. System Suitability

System suitability testing is an integral part of many analytical procedures**.** The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

- System suitability requirements

Parameters	**Recommendations**
R	R > 2, between the peak of interest and the closest potential interfere (degradant, internal standard, impurity, excipient, etc.)
T	T ≤ 2
N	In general N > 2000
Repeatability RSD	≤ 2.0% (n ≥ 5)

Table 2.1: Reported analytical method for estimation of drotaverine (DRT):

Sr. No.	Title of article	Description	Reference
1	Application of UV spectrophotometric method for estimation of Drotaverine hydrochloride in bulk & tablets.	Detection : 354 nm Linearity range : 4 - 24 µg/ml	Mahajan et al., 2006
2	Spectrofluorometric determination of Drotaverine hydrochloride in pharmaceutical preparations	Fluorescence of the DRT measured in 0.1M H_2SO_4 Emission: 465 nm Excitation: 295 nm	Wasseef et al., 2008
3	A new method for high performance liquid Chromatographic determination of Drotaverine in plasma	Plasma samples : pH 1.5 Extraction with chloroform Stationary Phase : SiO_2 Mobile Phase : n-heptane-dichloromethane-diethylamine (50:25:2) Detection with variable-wavelength UV detector set at 302 nm	Mezei et al., 1984
4	Modified high- performance liquid chromatographic method for analysis of Drotaverine in human plasma.	Internal standard: papaverine LOD: 50 ng ml^{-1}	Lalla et al., 1993
5	High-performance liquid chromatographic method for the determination of Drotaverine in human plasma and urine	Stationary Phase : C18-column Mobile Phase : 0.02 M sodium dihydrogen phosphate—methanol (30:70, v/v) containing perchlorate ion at pH 3.2 Detection : 254 nm LOD : 6 ng/ml	Bolaji et al., 1993

6	An ion-selective electrode for quantitative analysis of therapeutic formulations of Drotaverine hydrochloride	By using Ion Selective Electrode (ISE). By direct potentiometry and potentiometric depositional titration. ISE reacted reversibly to changes in the potential determining ion concentration over the range 5×10^{-2} to 7.9×10^{-6} M, the detection limit was $(4.3 \pm 0.2) \times 10^{-6}$ M electrode function slope was 58 ± 2 mV/Pc	EI-Saharty et al., 2008
7	Application of new membrane selective electrodes for the determination of Drotaverine hydrochloride in tablets and plasma	Poly Vinyl Chloride (PVC) membrane sensors are used. PVC based on the use of the ion association complexes of drotaverine cation with sodium phosphotungestate (Dro-PTA) or ammonium reineckate (Dro-R) counter anions as ion exchange sites in the PVC matrix. Linearity Range : 10^{-5}-10^{-2} M	El-Saharty et al., 2006
8	Cathodic adsorptive stripping voltammetry of Drotaverine hydrochloride and its determination in tablets and human urine by differential pulse voltammetry	By using Hanging mercury drop electrode. Linearity Range : 21.70–257.34 ng/ml LOD : 3.15 ng/ml LOQ : 10.50 ng/ml	Zayed S. I. M. and Issa Y. M., 2009
9.	Potentiometric flow injection analysis of Drotaverine hydrochloride in pharmaceutical preparations	By using five poly vinyl chloride (PVC) membrane electrodes. The membranes of these electrodes consist of drotaverinium-silicotungstate (Dv-ST),	Issa Y.M. et al., 2005

		silicomolybdate (Dv-SM), phosphotungstate (Dv-PT), phosphomolybdate (Dv-PM), or tetraphenylborate (Dv-TPB) ion associations dispersed in PVC matrix with dibutyl phthalate plasticizer. Electrodes showed near-Nernstia response in range of 2.0×10^{-6} to 1.0×10^{-2} M DRT LOD- 0.87 µg/ml	

Table 2.2: Reported analytical method for estimation of drotaverine in their combined dosage form:

1.	Spectrophotometric methods for simultaneous estimation of Nimesulide and Drotaverine.	**1 method**: Q-absorbance equation- 349 nm (isoabsorptive point) and 298.5 nm (λmax of Nimesulide) **2 method**: Simultaneous equation method- two wavelengths 298.5 nm (λmax of Nimesulide) and 245 nm (λmax of DRT) in ethanol **3 method**: Multicomponent mode absorbance. two wavelengths 298.5 nm (λmax of Nimesulide) and 362.5 nm (λmax of DRT) Linearity : 5 -30 µg/ml for both drugs	Chitlange S. et al., 2009
2.	Simultaneous determination of Drotaverine hydrochloride and Aceclofenac in tablet dosage form by spectrophotometry	**1 method** is Absorbance Ratio Method. 230 nm as λ_1 (Isobestic point) and 242 nm as λ_2 (λ max of DRT) **2nd** is Simultaneous equation method. λ max for DRT and	Vivek S. et al., 2009

		Aceclofenac is 242 nm and 273 nm respectively. **3rd method** is based on first order derivative spectroscopy. 250 nm and 226 nm were selected for the estimation of the DRT and Aceclofenac respectively. Linearity : 10-50µg/ml for both drugs	
3.	Simultaneous determination of Nifuroxazide and Drotaverine hydrochloride in pharmaceutical preparations by bivariate and multivariate spectral analysis.	**Method A**: Bivariate spectrophotometric analysis. (The absorption spectra measured at 229 and 287.5 nm) **Method B**: Multivariate spectral analysis. (absorbencies of mixtures were measured between 200 and 400 nm) Linearity : 2-10µg/ml	Metwally F., 2008
4.	Spectrophotometric determination of Pipazethate HCl, Dextromethorphan HBr and Drotaverine HCl in their pharmaceutical preparations.	Involves use of chromotrope 2B & chromotrope 2R. Method consists of extracting the formed ion-associates into chloroform in the case of Pipazethate HCl and Dextromethorphan HBr or into methylene chloride in the case of DRT Ion-associates exhibit absorption maxima at 528, 540 and 532 nm with chromotrope 2B and at 526, 517 and 522 nm with chromotrope 2R for pipazethate HCl, dextromethorphan HBr and drotaverine HCl, respectively.	El-Sheikh A. et al., 2007

		Linearity : For Pipazethate 4.36–52.32 µg/ml, 3.7–48.15 µg/ml for Dextromethorphan and 4.34–60.76 µg/ml for DRT	
5.	Selective differential spectrophotometric methods for determination of Niclosamide and Drotaverine hydrochloride.	**Method A**: Differential spectroscopy. **Method B**: second derivative spectroscopy. **Method C:** Third derivative spectroscopy	Daabees H. G.
6.	High performance liquid Chromatographic estimation of Drotaverine hydrochloride and Mefenamic acid in human plasma.	Extraction: Dichloromethane and isopropyl alcohol in the ratio 80:20 (v/v). Stationary Phase: Thermo BDS Hypersil C_8 (25.0 cm×4.6 mm, 5 µm particle size). Mobile Phase: Acetonitrile and Ammonium acetate buffer (20 mM, pH 3.5 ± 0.05 adjusted with 85% phosphoric acid) in a ratio of 55: 45 (v/v). Flow rate : 1 ml min^{-1} UV detection at 230 nm. Diclofenac sodium (internal standard) 32-960 ng/ml LOD & LOQ 32 ng/ml for DRT and 100-3000 ng/ml LOD & LOQ 100 ng/ml for Mefenamic acid	Dahivelkar P. et al., 2009
7.	Development and validation of an RP- HPLC method for simultaneous analysis of Drotaverine and Omeprazole in a tablet dosage form.	Stationary Phase: C_{18} column Mobile Phase : 60:40 (v/v) Methanol & Ammonium acetate (0.1 M , pH 5, adjusted with OPA)	Topagi K. et al., 2010

		Flow rate: 1.5 ml min^{-1} UV detection: 319 nm Linearity: 5-40 ng/ml for DRT & 5-50 ng/ml for Omeprazole LOD:16.2 and 4.8 ng/ml & LOQ 49.0 ng/ml and 14.5 ng/ml for DRT and Omeprazole respectively.	
8.	Determination of Nifuroxazide and Drotaverine hydrochloride in pharmaceutical preparations by three independent analytical methods.	**Method A:** Spectrophotometry **Method B:** Spectrodensitometry method Stationary phase: silicagel Mobile phase: chloroform : acetone : methanol: glacial acetic acid (6:3:0.9:0.1) **Detection** : 365 nm **Method C**: RP HPLC Mobile phase: ACN : Water (40:60) pH- 2.55 with OPA Flow rate:1ml/min Detection: 285 nm	Fadia H., 2006
9.	Application of derivative, derivative ratio, and multivariate spectral analysis and thin- layer chomatography-densitometry for determination of a ternary mixture containing Drotaverine hydrochloride (I) , Caffeine (II) and Paracetamol (III)	**Method A**:First (D1) and third (D3) derivative spectrophotometry at 331 and 315 nm for the determination of (I) and (III) **Method B** : simultaneous use of the first derivative of the ratio spectra (DD_1) with measurement at 312.4 nm for determination of (I) **Method C** :Thin-layer chromatography Stationary Phase: silica gel Mobile phase: Ethyl acetate-Chloroform-	Metwally et al., 2007

		Methanol (16 + 3 + 1, v/v/v); The spots were scanned at 281, 272, and 248 nm for the determination of (I), (II), and (III), respectively.	
10.	Spectrophotometric and spectrodensitometric determination of Paracetamol and Drotaverine HCl in combination.	**Method A**: TLC densitometric method Mobile Phase : Ethyl acetate: methanol: NH_3 (100:1:5 v/v/v); spots of the two drugs were scanned at 249 and 308 nm respectively. **Method B**: First derivative spectroscopic, measurements at zero-crossing points 259 and 325 nm **Method C**: ratio spectra derivative spectrophotometry, measurements at 246 and 305 nm **Method D**: Vierordt's method	Hisham E., 2007
11.	A comparative study on various spectrometries with thin layer chromatography for simultaneous analysis of Drotaverine and Nifuroxazide in capsules	Three spectrophotometric methods **Method A**: Vierordt's method **Method B**: Ratio spectra derivative **Method C**: Derivative spectroscopy **Method D** :Thin layer chromatography (TLC)-UV densitometric method Mobile phase: Ethyl acetate:methanol: ammonia 33% (10 : 1 : 0.1 v/v/v) Detection: Densitometrical area were measured at 308 and 287 nm.	Ayad M. M. et al., 2006

Table 2.3: Reported Stability indicating method for DRT combinations:

1.	Stability indicating HPLC method for simultaneous determination of Drotaverine and Aceclofenac	RP- C_{18} column mobile phase- 0.1% trifluoro acetic acid: acetonitrile (45: 55 %v/v), pH 3.4 adjusted with 1% triethyl amine. Retention time of drotaverine and ace clof- enac 5.8 and 10.5 min, respectively. Both the drugs were subjected to acid ,alkali and neutral hydrolysis, oxidation,dry heat, and photolytic degradation.	Azhlwar S. and Kochupappy T., 2011
2.	Stability-indicating HPTLC method for simultaneous estimation of Drotaverine and Nimesulide in pharmaceutical dosage form	Stationary phase- Precoated silica gel 60 F_{254} Plate. Mobile phase- Cyclohexane:Methanol: ethyl acetate (6:2:2 v/v/v) Densitometric scanning at 295 nm Rf values 0.15 for DRT and 0.53 for NIM. Forced degradation by acid, alkali, oxidation and dry heat.	Chitlange S. et al., 2009

Drotaverine hydrochloride is an antispasmodic drug. It is selective PDE IV inhibitor. Drotaverine is used in the management of biliary-tract, urinary-tract, and gastrointestinal spasm. It has also been used to accelerate labour. (Singh K. C. et al.,2004; Romics I. et al., 2003)

Based on the literature review, it was found that a number of analytical methods involving measurement of DRT concentration by HPLC with UV detector, thin layer chromatography, spectrophotometry, spectrofluorimetry, coulometry titration, potentiometry, voltammetry for individual/simultaneous estimation and/or separation in formulations/biological specimen have been developed. Stability indicating HPTLC and HPLC method also has been reported for the determination of the DRT in the presence of nimesulide and aceclofenac respectively from its combined dosage preparations.

Literature survey revealed that not a single reported method is available for impurity profiling of DRT. DRT is available in tablet as well as in injectable forms so it was thought to develop and validate related impurity method for quantification of impurity from Drotaverine API and its formulations using available standard impurity sample.

Hence the major objectives of the present work include:

- ✓ To develop and validate related impurity method for DRT according to ICH guidelines.
- ✓ To quantify impurities from DRT API and its formulations.
- ✓ To determine potential source of impurities in DRT API and its formulations.

4.1 Instrumentation

4.1.1 High performance liquid chromatography, with model no. JASCO 200 series, manufactured by Jasco Inc., JAPAN with Pump (Jasco PU 2080 plus); Mixer (Jasco MX 2080-31); Injector (Rheodyne valve with 20 µ L fixed loop); Detector (Jasco MD-2015 plus); Software (Borwin Jasco) and Column (Phenomenex Luna C_{18} (150 mm × 4.6 mm; 5µm particle size)).

4.1.2 UV-visible spectrophotometer, with model no. UV-2450; Double beam, manufactured by Shimadzu, Japan.

4.1.3 Fourier Transform Infrared spectrometer (FT-IR), with model no. JASCO FT/IR-6100 series, manufactured by Jasco Inc., JAPAN.

4.1.4 Analytical balance, with model CITIZEN Scale CX-220, manufactured by CITIZEN Private Ltd.; India having weighing capacity of 10mg to 220mg.

4.1.5 Sonicator, with model Trans-o-sonic, D compact 936 having capacity of 2 litres.

4.1.6 pH meter, manufactured by Lutron, Taiwan; model pH206 with pH-mV-temperature measurement probe.

4.1.7 Melting point apparatus, T0603160; manufactured by EIE Instruments Pvt. Ltd., Ahmedabad India.

4.1.8 Hot air oven, EIE 108, manufactured by EIE Instruments Pvt. Ltd., Ahmedabad India.

4.1.9 Water bath, manufactured by EIE Instruments Pvt. Ltd., Ahmedabad India.

4.1.10 Vaccum pump, manufactured by Rockers/ Shah brothers.

4.2 Reagents and Materials

4.2.1 The pure sample of the drug Drotaverine hydrochloride (DRT) was obtained as a gift sample from Troikaa Pharmaceutical Pvt Ltd.

4.2.2 Standard impurity sample (Std. IMP C) was available at institute of Pharmacy, Nirma University.

4.2.3 Acetonitrile, methanol, Potassium dihydrogen ortho-phosphate, ortho-phosphoric acid and ammonia used for mobile phase preparation were of HPLC grade, Merck Specialties Pvt. Ltd, Worli, Mumbai.

4.2.4 Hydrochloric acid, hydrogen peroxide (30%,w/v), sodium hydroxide pellets used for stress degradation studies are of analytical reagent grade, CDH Chemicals, Delhi, India.

4.2.4 De-ionized water prepared using Milli-Q plus purification system (Bradford, USA) was used throughout study.

4.2.5 The membrane filter paper 0.22 µm used for mobile phase filteration were supplied by Millipore Ltd. Banglore.

4.2.6 All the glasswares volumetric flask, beaker, measuring cylinder, pipette were of Class A borosilicate glass.

4.2.7 Marketed formulations used were procured from local market.

- Drotin injection containing 20 mg/ml of Drotaverine HCl of Martin and Harris Pharmaceutical Pvt. Ltd. (Formulation A)
- Drotikind injection containing 20 mg/ml of Drotaverine HCl of Lifestar Pharmaceutical Pvt Ltd.(Formulation B)
- Verine injection containing 20 mg/ml of Drotaverine HCl of Corona Remedies Pvt Ltd. (Formulation C)
- Drotin Tablet containing 80 mg of Drotaverine HCl of Martin and Harris Pharmaceutical Pvt. Ltd. (Formulation D)
- Drotikind Tablet containing 80 mg of Drotaverine HCl of Lifestar Pharmaceutical Pvt Ltd. (Formulation E)
- Doverin Tablet containing 80 mg of Drotaverine HCl of Intas Pharmaceutical Pvt Ltd. (Formulation F)
- Drovera Tablet containing 80 mg of Drotaverine HCl of Maneesh Pharmaceuticals (Formulation G)

Identification of drug Drotaverine hydrochloride (DRT) was carried out by Melting point, UV-Visible spectroscopy and FT-IR spectroscopy methods.

5.1 UV spectra of drug:

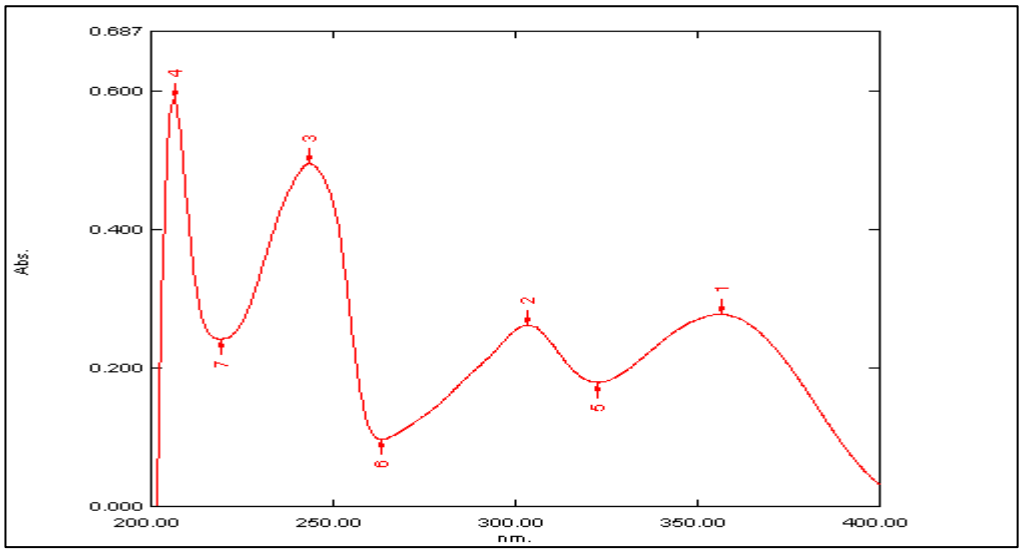


Figure 5.1 : UV spectrum of 10 ppm DRT in methanol

Table 5.1: comparison of reported λmax with obtained λmax of DRT

Drug	**Reported λmax** (Merck index, 2006)	**Obtained λmax**
DRT	241 nm	240 nm

5.2 FT-IR spectra of drug:

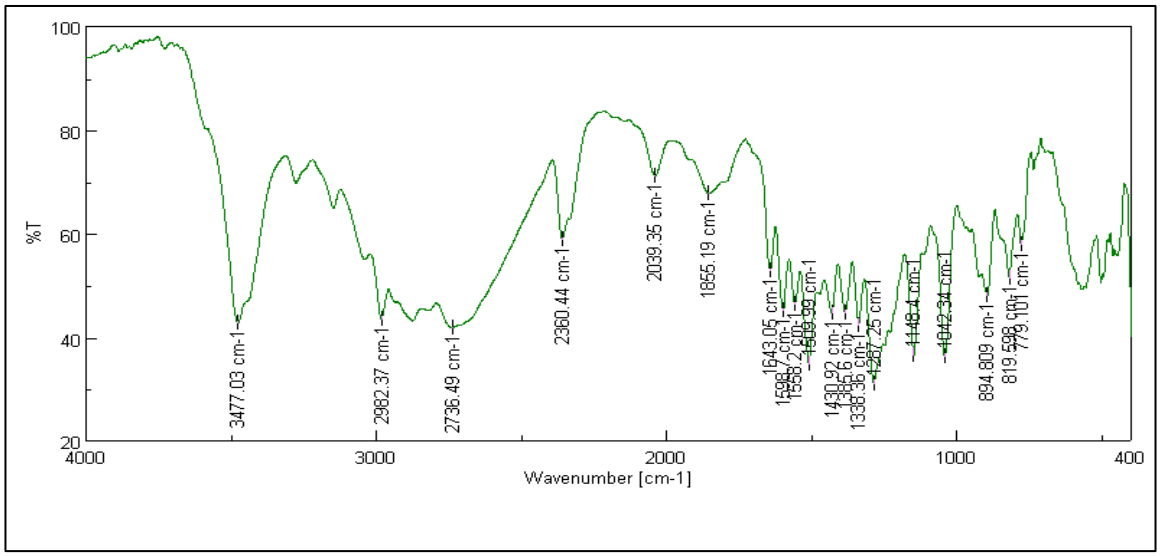


Figure 5.2: FT-IR spectra of DRT

Table 5.2: Important frequencies of DRT obtained in FT-IR spectra

Sr. No.	Functional group	Theoretical frequency (cm^{-1})	Observed frequency (cm^{-1})
1	Secondary Amines (-NH)	3500-3100	3477.03
3	Methyl (-CH_3)	3000-2850	2982.37
4	Aromaticity(benzene overtones)	1550-1650	1598.70
5	Amine (C-N)	1350-1000	1148.40
6	C-O ethoxy	1000-1100	1042.34

5.3 Determination of Melting point:

Table 5.3: Melting point of DRT

Reported melting Point (Martindale, 2009)	**Observed melting Point**
208-210^{o}C	206-208 ^{o}C

The related impurity method was developed and validated for quantification of impurity from drotaverine API and it's formulations. The developed related impurity method for DRT was simple, selective, sensitive and economical.

Drotaverine is available in market in two type of dosage form i.e. tablets and injections. First of all estimation of DRT from it's formulation was carried out using reported RP-HPLC method. During estimation, it was found that assay of formulation C having concentration of 10 µg/ml of DRT was 105.53%, show additional peak along with peak of DRT having area of 9.85% which cannot neglected. So a related impurity method was developed and validated for DRT using available impurity standard (Std. IMP C)

For related impurity method, reverse phase C_{18} column (Phenomenex Luna C-18, 150 × 4.5mm i.d., with 5µm particle size) equilibrated with mobile phase acetonitrile: 0.0125 M potassium dihydrogen orthophosphate (43:57, v/v; pH 4.0) was used on isocratic mode. Mobile phase flow rate was maintained at 1.0 ml/min and response was detected at 240 nm. The sample was injected using a 20 µ L fixed loop.

The related impurity method was validated according to ICH guidelines. The linearity of Std. IMP C was performed and it was in the range of 1-50 µg/ml respectively and the value of correlation coefficient (r^2) was found to be 0.999. LOD and LOQ values for IMP C were found to be 0.08 µg/ml and 0.24 µg/ml, respectively. The accuracy (%recovery) for Std. IMP C was found to be 94.89- 98.19%. During precision, robustness and solution stability study % RSD was found within 2%.

Three major impurities were found in formulations. Quantification of IMP C from formulations was performed using the impurity standard available and IMP A and IMP B were reported in percentage with respect to DRT.

The degradation study was also performed to find out potential source of impurities in marketed formulation of DRT. It was observed that DRT was susceptible to alkaline, oxidative, neutral and photolytic degradation but it was not susceptible to degradation under acidic and thermal conditions. Two degradation products DP-I and DP-IV were obtained at same Rt as of IMP A and IMP C and UV spectra of these degradation products were compared with UV spectra of IMP A and IMP C.

The developed related impurity method for DRT is simple, selective, sensitive and economical. The method could detect, separate and quantify all the found impurities in DRT formulations with sufficient resolution. Major three impurities were found among all formulations (IMP A, IMP B and IMP C) but no impurity was found in API. Quantification of IMP C from formulation was performed using the Std. IMP C and other two impurities were reported as percentage with respect to DRT. The reliability of the method was proved from acceptable results of all the validation parameters.

From quantification study it was concluded that

- Formulation C (Verine Injection) has high percentage of IMP A (1.93%).
- Formulation A (Drotin Injection) has high percentage of IMP B (0.24%).
- Formulation D (Drotin Tablet) has high percentage of IMP C (4.08%).
- Formulation D (Drotin Tablet) has high percentage of total impurities (4.29%).

From the degradation studies, it was observed that DP-I and DP-IV were obtained at same Rt as of IMP A and IMP C respectively. After Comparing the UV spectra of impurities and degradation products, it was concluded that impurities in formulation were formed may be due to degradation of DRT.

Overall, the developed method provides high throughput solution for the quantification of impurities from DRT API and its formulations and has application in the laboratory for routine quality check.

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