"SIMULTANEOUS ESTIMATION OF ONDANSETRON HCL AND PROMETHAZINE HCL BY UV-SPECTROSCOPY AND ONDANSETRON HCL AND PARACETAMOL BY HPTLC IN BULK AND PHARMACEUTICAL DOSAGE FORMS"

Dissertation work submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai

in partial fulfillment for the award of degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted by

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Under the Guidance of

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MAY 2012

DEPARTMENT OF PHARMACEUTICAL ANALYSIS

R.V.S. COLLEGE OF PHARMACEUTICAL SCIENCES

SULUR, COIMBATORE – 641402 (TN)

CERTIFICATE

This is to certify that the research project work entitled " **Simultaneous Estimation of Ondansetron Hcl and Promethazine Hcl by UV-Spectroscopy and Ondansetron Hcl and Paracetamol by HPTLC in Bulk and Pharmaceutical Dosage forms"** is a bonafide work of **Mrs.R.PARIMALESHWARI (Reg.No.26106426)** carried out in the **Department of Pharmaceutical Analysis, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore**, in Partial fulfillment of the requirements for the award of degree in **Master of Pharmacy in Pharmaceutical Analysis** of **The Tamilnadu Dr.M.G.R.Medical University, Chennai**,was guided & Supervised by Dr.W.D.Sam Solomon during the academic year 2011-2012.

Place : Coimbatore

Date:

Dr.R. VENKATANARAYANAN, M.Pharm.,Ph.D

Principal

R.V.S. College of Pharmaceutical Sciences

Sulur, Coimbatore.

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Place : Coimbatore

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Place: Coimbatore **R.PARIMALESHWARI**

Date: **(26106426)**

EVALUATION-CERTIFICATE

This is to certify that the dissertation work entitled " **SIMULTANEOUS ESTIMATION OF ONDANSETRON HCL AND PROMETHAZINE HCL BY UV-SPECTROSCOPY AND ONDANSETRON HCL AND PARACETAMOL BY HPTLC IN BULK AND PHARMACEUTICAL DOSAGE FORMS"** is a bonafide research work done by **Mrs.R.PARIMALESHWARI (Reg.No.26106426)** in partial fulfilment of the requirement for the award of **MASTER OF PHARMACY** in Pharmaceutical Analysis, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore **.**

INTERNAL EXAMINER EXTERNAL EXAMINER

Place : Coimbatore

Date:

INTRODUCTION

Analytical chemistry[1] is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Analytical chemistry is a sub discipline of chemistry that has the broad mission of understanding the chemical composition of all matter and developing the tools to elucidate such compositions.

Types

Traditionally, analytical chemistry has been split into two main types, qualitative and quantitative.

Qualitative

- Qualitative analysis seeks to establish the presence of a given element, given functional group or inorganic or organic compound in a sample.

Quantitative

- Quantitative analysis seeks to establish the amount of a given element or compound in a sample.

METHOD DEVELOPMENT

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals.

Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. This presentation will discuss the development and validation of analytical method (Spectrophotometric, High performance liquid chromatography (HPLC), & High performance thin layer chromatography for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

Introduction

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

Basic criteria for new method development of drug analysis

- ♣ The drug or drug combination may not be official in any pharmacopoeias.
- ♣ A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- ♣ Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- ♣ Analytical methods for the quantification of the drug in biological fluids may not be available,
- ♣ Analytical methods for a drug in combination with other drugs may not be available,
- ♣ The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Introduction to UV - Spectrophotometric Method of Analysis for Drugs

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods.

The spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances, which potentially interfere in the assay. If the formula of the samples is known, the identity and concentration of the interfering substance are known and the extent of interference in the assay may be determined.

The basis of all the spectrophotometric techniques for multicomponent samples is the property that at all wavelengths

- The absorbance of a solution is the sum of absorbance of the individual components or
- The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell.
- There are various spectrophotometric methods are available which can be used for the analysis of a combination samples. Following methods can be used Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry
- Solvent extraction method

Simultaneous Equation Method

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the lmax of the other (as shown in figure 1. λ_1 andλ$_2$), it may be possible to determine both drugs by the technique of simultaneous equation (Vierodt's method) provided that certain criteria apply.

The information's required are:

- The absorptivities of X at λ_1 and λ_2, a_{x1} and a_{x2} respectively.
- The absorptivities of Y at λ_1 andλ_2, a_{y1} and a_{y2} respectively.
- The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Let **Cx** and **Cy** be the concentration of **X** and Y respectively in the diluted samples.

Two equations are constructed based upon the fact that at λ_1 andλ_2, the absorbance of the mixture is the sum of the individual absorbance of X and Y.

$$C_x = (A_2\, a_{y1} - A_1\, a_{y2}) / (ax_2\, a_{y1} - a_{x1}\, a_{y2})$$

$$C_y = (A_1\, a_{x2} - A_2\, a_{x1}) / (ax_2\, a_{y1} - a_{x1}\, a_{y2})$$

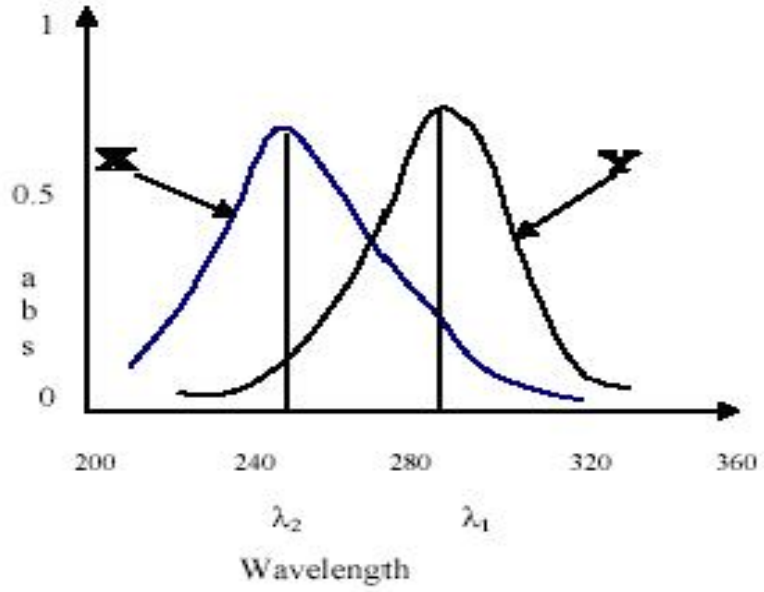


Fig.1 the overlain spectra of substance X and Y, showing the wavelength for the Q-Absorbance Method (Absorbance Ratio Method)

Q-Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbance's at any two wavelengths is a constant value independent of concentration or path length. For example, two different dilution of the same substance give the same absorbance ratio A_1/A_2. In the USP, this ratio is referred to as Q value.

In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbance's are measured at two wavelengths. One being the λmax of one of the component (λ_2) and the other being a wavelength of equal absorptivities of the two components (As shown in figure 2) i.e. an isoabsorptive point.

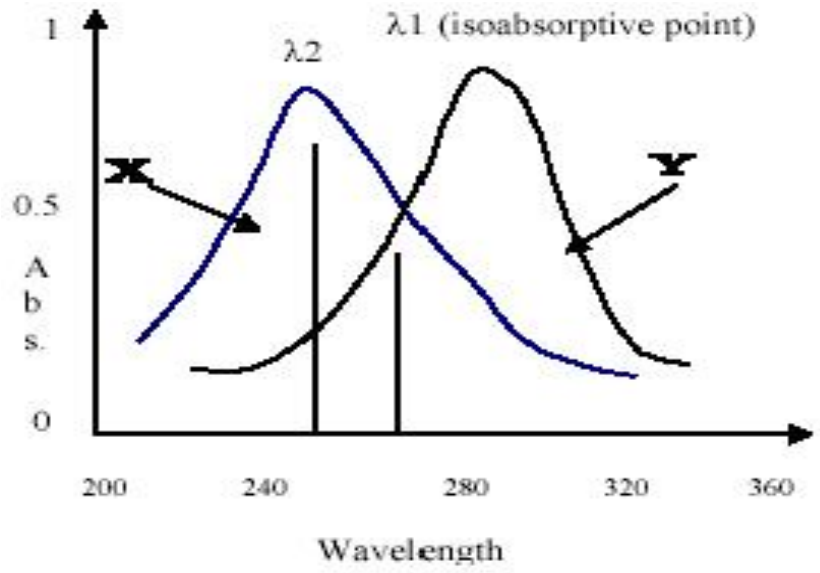


Fig.2 Wavelength for the assay of substances X and Y in admixture by the method of absorbance ratio method.

$C_x = (Q_M - Q_Y) A_1 / (Q_X - Q_Y) a_{x1}$

$Cy = (Q_M - Q_x) A_1 / (Q_X - Q_Y) a_{x1}$

Above equation gives the concentration of X and Y in terms of absorbance ratios, the absorbance of mixture and the absorptivities of the compounds at the isoabsorptive wavelength.

Derivative Spectroscopy

For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy[15].

Derivative spectrophotometry involves the conversions of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or D^0 spectrum.

The first derivative D^1 spectrum is a plot of the rate of change of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ vs. λ. The maximum positive and maximum negative slopes respectively in the D spectrum correspond with a maximum and a minimum respectively in the D^1 spectrum. The λmax in D spectrum is a wavelength of zero slope and gives $dA/d\lambda = 0$ in the D^1 spectrum.

The second derivative D^2 spectrum is a plot of the curvature of the D spectrum against wavelength or a plot of $d^2A/ d\lambda^2$ vs. λ. The maximum negative curvature in the D spectrum gives a minimum in the D^2 spectrum, and the maximum positive curvature in the D spectrum gives two small maxima called satellite bands in the D^2 spectrum. The wavelength of maximum slope and zero curvature in the D spectrum correspond with cross-over points in the D^2 spectrum.

These spectral transformations confer two principal advantages on derivative spectrophotometry. Firstly, an eve order spectrum is of narrower spectral bandwidth than its fundamental spectrum. A derivative spectrum therefore shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the λmax of the individual bands. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. All the amplitudes in the derivative spectrum are proportional to the concentration of the analyte, provided that Beer's law is obeyed by the fundamental spectrum.

The second technique to generate derivative spectra is electronic differentiation of the spectrophotometer analog signal. Resistance capacitance (RC) modules may be incorporated in series between the spectrophotometer and recorder to provide differentiation of the absorbance, not with respect to wavelength, but with respect to time, thereby producing the signal dA/dt. If the wavelength scan rate is constant (**$d\lambda/dt = C$**), the derivative with respect to wavelength is given by

$dA/ d\lambda = (dA/dt) / (d\lambda /dt) = (dA/dt)(1/C)$

Derivative spectra obtained by RC modules are highly dependent on instrumental parameters, in particular the scan speed and the time constant. It is essential, therefore, to

employ a standard solution of the analyte to calibrate the measured value the instrumental conditions selected.

The third technique is based upon microcomputer differentiation. Microcomputers incorporated into or interfaced with the spectrophotometer may be programmed to provide derivative spectra during or after the scan, to measure derivative amplitudes between specified wavelengths and to calculate concentrations and associated statistics from the measured amplitude.

Introduction to HPTLC Method of Analysis for Drugs [2]

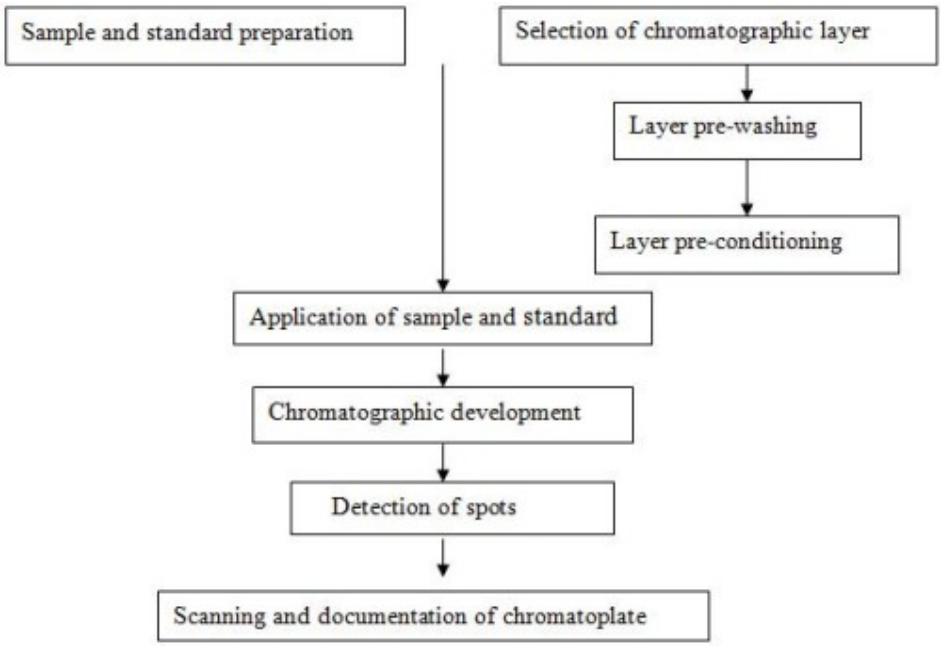
HPTLC (High Performance Thin Layer Chromatography) is a well known and versatile separation method which shows a lot of advantages in comparison to other separation techniques.

Layer of Sorbent	100µm
Efficiency	High due to smaller particle size generated
Separations	3 - 5 cm
Analysis Time	Shorter migration distance and the analysis time is greatly reduced
Solid support	Wide choice of stationary phases like silica gel for normal Phase and C8 , C18 for reversed phase modes
Development chamber	New type that require less amount of mobile phase
Sample spotting	Auto sampler
Scanning	Use of UV/ Visible/ Fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and the scanner is an advanced type of densitometer

Features of HPTLC

1. Simultaneous processing of sample and standard - better analytical precision and less need for Internal Standard
2. Several analysts work simultaneously
3. Lower analysis time and less cost per analysis
4. Low maintenance cost
5. Simple sample preparation - handle samples of divergent nature
6. No prior treatment for solvents like filtration and degassing
7. Low mobile phase consumption per sample
8. No interference from previous analysis - fresh stationary and mobile phases for each analysis - no contamination
9. Visual detection possible - open system
10. Non UV absorbing compounds detected by post-chromatographic derivatization

Steps involved in HPTLC



Sample and Standard Preparation

- To avoid interference from impurities and water vapours.
- Low signal to noise ratio
- Straight base line
- Improvement of LOD
- Solvents used are Methanol, Chloroform: Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90: 0:1), Methylene chloride: Methanol (1:1), 1%Ammonia or 1% Acetic acid
- Dry the plates and store in dust free atmosphere

Activation of pre-coated plates

- Freshly open box of plates do not require activation
- Plates exposed to high humidity or kept on hand for long time to be activated By placing in an oven at 110-120ºc for 30' prior to spotting,
- Aluminum sheets should be kept in between two glass plates and placing in oven at 110-120ºc for 15 minutes.

Application of sample and standard

- Usual concentration range is 0.1-1µg / µl
- Above this causes poor separation
- Linomat IV (automatic applicator) - nitrogen gas sprays sample and standard from syringe on TLC plates as bands
- Band wise application
- Better separation
- High response to densitometer

Selection of mobile phase

- Trial and error
- One's own experience and Literature

Normal phase

- Stationary phase is polar
- Mobile phase is non polar
- Non-polar compounds eluted first because of lower affinity with stationary phase
- Polar compounds retained because of higher affinity with the stationary phase

Reversed phase

- Stationary phase is non polar
- Mobile phase is polar
- Polar compounds eluted first because of lower affinity with stationary phase
- Non-Polar compounds retained because of higher affinity with the stationary phase
- 3-4 component mobile phase should be avoided
- Multi component mobile phase once used not recommended for further use and solvent composition is expressed by volumes (v/v) and sum of volumes is usually 100
- Twin trough chambers are used only 10 -15 ml of mobile phase is required
- Components of mobile phase should be mixed introduced into the twin trough chamber

Pre- conditioning (Chamber saturation)

- Un- saturated chamber causes high Rf values
- Saturated chamber by lining with filter paper for 30 minutes prior to development
- Uniform distribution of solvent vapours
- Less solvent for the sample to travel
- Lower Rf values.

Chromatographic development and drying

- After development, remove the plate and mobile phase is removed from the plate
- To avoid contamination of lab atmosphere
- Dry in vacuum desicator
- Avoid hair drier

- Essential oil components may evaporate

Detection and visualization

- Detection under UV light is first choice
- Non destructive Spots of fluorescent compounds can be seen at 254 nm (short wave length) or at 366 nm (long wave length)
- Spots of non fluorescent compounds can be seen
- Fluorescent stationary phase is used
- Silica gel GF
- Non UV absorbing compounds like ethambutol, dicylomine etc
- dipping the plates in 0.1% iodine solution
- When individual component does not respond to UV
- derivatisation required for detection

Quantification

- Sample and standard should be chromatographed on same plate
- After development chromatogram is scanned
- Camag TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode
- Scanning speed is selectable up to 100 mm/s
- Spectra recording are fast - 36 tracks with up to 100 peak windows can be evaluated
- Calibration of single and multiple levels with linear or non-linear regressions are possible when target values are to be verified such as stability testing and dissolution profile single level calibration is suitable
- Statistics such as RSD or CI report automatically
- Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors.

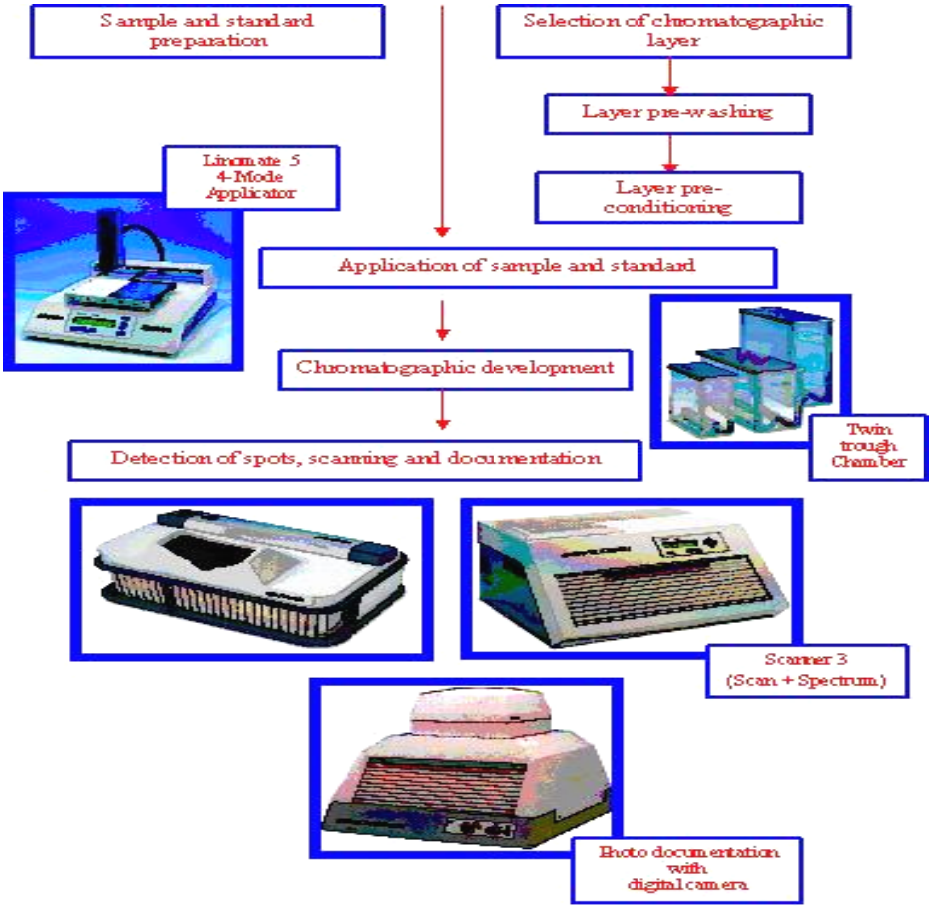


Fig.9 Schematic procedure for HPTLC.

HPTLC Method design and development

Set the analytical objective first that may be quantification or qualitative identification or separation of two components/multicomponent mixtures or optimization of analysis time before starting HPTLC. Method for analyzing drugs in multicomponent dosage forms by HPTLC demands primary knowledge about the nature of the sample, namely, structure, polarity, volatility, stability and the solubility parameter. An exact recipe for HPTLC, however, also same like HPLC cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, with what kind of mobile phase.

1. Retention factor (R_f)

Retention factor (R_f) is defined as the amount of separation due to the solvent migration through the sorbent layer as shown in the formula. It depends on time of development and velocity coefficient or solvent front velocity.

$$R_f = \frac{\text{Migration distance of substance}}{\text{Migration distance of solvent front from origin}}$$

2. Peak purity

The null hypothesis "these spectra are identical" can in this case (purity) with two sided significance. During the purity test the spectrum taken at the first peak slope is correlated with the spectrum of peak maximum [r(s,m)] and the correlation of the spectra taken at the peak maximum with the one from the down slope or peak end [r(m,e)] which is used as a reference spectra for statistical calculation. An error probability of 1% only is rejected if the test value is greater than or equal to 2.576.

VALIDATION OF ANALYTICAL METHOD

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc.

When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.

The various validation parameters are

1. Accuracy,
2. Precision(Repeatability and Reproducibility),
3. Linearity and range,
4. Limit of detection (LOD)/ limit of quantization (LOQ),
5. Selectivity/ specificity,
6. Robustness/ ruggedness and
7. Stability and system suitability studies.

Advantages of Analytical method Validation

The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user.

Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end.

Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

ANALYTICAL METHOD VALIDATION

Key Parameters of the Analytical Method Validation

It is important for one to understand the parameters or characteristics involved in the validation process. The various Performance parameters, which are addressed in a validation exercise, are grouped as follows.

(1) Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the

analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.

Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value.

(2) Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at

- Different Occasions,
- Different Laboratories,
- Different Batch of Reagent,
- Different Analysts,
- Different Equipments.

Determination of Repeatability

Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time.

It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. (RSD below 1% for built drugs, RSD below 2% for assays in finished product).

Determination of reproducibility

Reproducibility means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, putatively identical samples taken from the same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments, or by carrying out the analysis at different times can also provide valuable information.

(3) Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

The linear range of detectability that obeys Beer's law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range. The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression. Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation y= ax + b together with the correlation coefficient of determination r. For the method to be linear the r value should be close to1.

The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

(4) Limit of Detection and limit of Quantification:-

Limit of detection**:-**

The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analyte concentration is above or below a certain level.

The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio.

For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (S_a) which may be related to LOD and the slope of the calibration curve, b, by

$LOD = 3 S_a / b$

Limit of quantification

Limit of quantification is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantification is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are

attainable where the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantification. In many cases, the limit of quantification is approximately twice the limit of detection.

(5) Selectivity and Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific.

Hence one basic difference in the selectivity and specificity is that, while the former is restricted to qualitative detection of the components of a sample, the latter means quantitative measurement of one or more analyte.

Selectivity may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared the results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared to the results obtained on the same analyte without added substances. When the other components are all known and available, selectivity may be determined by comparing the test results obtained on the analyte with and without the addition of the potentially interfering materials. When such components are either unidentified or unavailable, a measure of selectivity can often be obtained by determining the recovery of a standard addition of pure analyte to a material containing a constant level of the other components.

(6) Robustness and Ruggedness

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.

For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

(7) Stability and System suitability tests

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis.

System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. The nature of the test and the acceptance criteria will be based upon data generated during method development optimization and validation experiments.

Data Elements Required For Assay Validation

There are various analytical methods used for the examination of pharmaceutical materials. Not all the characteristics referred above will need to be considered in all cases. Analytical methods may be broadly classified as Per WHO as follows:

Class A Tests designed to establish identity, whether of bulk drug substances or of a particular ingredient in a finished dosage form.

Class B Methods designed to detect and quantitative impurities in a bulk drug substance or finished dosage form.

Class C Methods used to determine quantitatively the concentration of a bulk drug substance or of a major ingredient in a finished dosage form.

Class D Methods used to assess the characteristic of finished dosage forms, such as dissolution profiles and content uniformity.

Table: Characteristic that should be considered for different types of analytical procedure:

	Class A	Class B		**Class C**	**Class D**
		Quantitative tests	**Limit tests**		
Accuracy		X		X	X
Precision		X		X	X
Robustness	X	X	X	X	X
Linearity and range		X		X	X
Selectivity	X	X	X	X	X
Limit of Detection	X		X		
Limit of Quantification		X			

Where, X indicates the tests to be performed.

As per USP

Category I

Analytical methods for quantification of major components of bulk drug substances or active ingredients including preservatives in finished pharmaceutical products.

Category II

Analytical methods for determination of impurities in bulk drugs or for determination of degradation compounds in finished pharmaceutical products.

Category III

Analytical methods for determination of performance characteristics (e.g. dissolution, drug release).

Category IV

Identification tests.

Table: Data Elements Required for Assay Validation

Analytical Performance Characteristics	**Assay Category I**	Assay Category II		**S**	**Assay Category IV**
		Quantitative tests	**Limit tests**		
Accuracy	X	X	May be	May be	
Precision	X	X		X	
Specificity	X	X	X	May be	X
Limit of Detection			X	May be	
Limit of Quantification		X		May be	
Linearity	X	X		May be	
Range	X	X	May be	May be	

Where, X indicates the tests to be performed.

Conclusion

The efficient development and validation of analytical methods are critical elements in the development of pharmaceuticals. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance. Experience is one of these factors both the experience level of the individual scientists and the collective experience level of the development and validation department.

REVIEW OF LITERATURES

1. LIST OF ANALYTICAL METHOD AVAILABLE FOR ONDANSETRON HYDROCHLORIDE

- **Mehta kalpesh K**[7], et al., reported Spectrophotometric methods for formulation and process optimization of Gastro Retentive floating tablets of ondansetron HCL.Absorbance of each solution was measured at 310 nm using Shimadzu UV-VIS double beam spectrophotometer.

- **S Pillai**[23]**,**et al., reported Spectrophotometric simultaneous estimation of ranitidine hydrochloride and ondansetron hydrochloride from tablet formulation. First developed method involves formation and solving of simultaneous equations at 267.2 nm and 314.4 nm. Second method was developed making use of first order derivative spectroscopy using 340.8 nm and 276.0 nm as zero crossing points for estimation of ranitidine hydrochloride and ondansetron hydrochloride respectively. Third method is based on two wavelength calculation, wavelengths selected for estimation of ranitidine hydrochloride were 266.1 nm and 301.8 nm and for ondansetron hydrochloride 305.7 nm and 319.2 nm.

- **Sradhanjali Patra**[13],et al., reported Spectrophotometric method for ondansetron hydrochloride. It shows maximum absorbance at 310 nm with water.

- **Steffen Bauer**[24],et al., reported Simultaneous determination of ondansetron and tropisetron in human plasma using HPLC with UV detection. The lower limits of quantification (LOQ) were 0.62 ng/mL for ondansetron and 1.25 ng/mL or tropisetron. Intra- and inter-assay coefficients of variation ranged from 1.5 to 7.5% and 5.3 to 13.7%, respectively.

- **Jawed Akhtar**[25], et al., reported Spectrophotometric Methods for Simultaneous Estimation of Rabeprazole Sodium and Ondansetron. For simultaneous equation method RAB shows maximum absorbance at 284.20 nm and OND shows at 246.0 nm.

- **P Yeole**[8], .et al., reported A Validated HPTLC Method for Determination of Ondansetron in Combination with Omeprazole or Rabeprazole in Solid Dosage Form. The method involved separation of components by TLC on a precoated silica gel 60 F 254 using a mixture of dichloromethane:methanol (9:1) as a mobile phase. Detection of spots was carried out at 309 nm and 294 nm .

- **Raval P B**[26]., et al., reported A validated HPTLC method for determination of ondansetron in combination with omeprazole or rabeprazole in solid dosage form. The method involved separation of components by TLC on a precoated silica gel 60 F(254) using a mixture of dichloromethane:methanol (9:1) as a mobile phase. Detection of spots was carried out at 309 nm and 294 nm.

- **P. Ravi Kumar**[9]., et al., reported Derivative Spectrophotometric Estimation of Ondansetron and Paracetamol. Ondansetron and paracetamol have λmax at 302 nm and 246 nm respectively in methanol.

- **Lobhe GA**[27]**.**, et al., reported Simultaneous Spectrophotometric methods for estimation of Ondansetron Hydrochloride and Omeprazole in tablets. In ethanol, ondansetron hydrochloride and Omeprazole showed λmax at 246.2 nm and 301 nm,respectively.

- **Ali Mujtaba**[12] et al., reported Development of HPTLC method for the estimation of ondansetron hydrochloride in bulk drug and sublingual tablets. The mobile phase composition was chloroform: ethyl acetate: methanol: ammonia (9:5:4:0.1 *v*/*v*). Spectrodensitometric analysis of ondansetron was carried out at 254 nm.

- **Patra**[13]**,** et al., reported Spectrophotometric method for ondansetron hydrochloride. It shows maximum absorbance at 310 nm with water. Beer's law obeys in the concentration range of 5-15 [micro]g/ml

- **Bauer S**[14]., et al., reported Simultaneous determination of ondansetron and tropisetron in human plasma using HPLC with UV detection. The lower limits of quantification (LOQ) were 0.62 ng/mL for ondansetron and 1.25 ng/mL or tropisetron. Intra- and inter-assay coefficients of variation ranged from 1.5 to 7.5% and 5.3 to 13.7%, respectively.

- **Srikant Nayak**[27].,et al., reported UV-Spectrophotometric method for simultaneous estimation of Paracetamol and Ondansetron in Bulk and their formulation. 267 nm for Ondansetron and 248 nm for Paracetamol.solution –Methanol: double distilled water (9:1). Concentration range 2-20µg/ml for Paracetamol and 0.1-1µg/ml for Ondansetron.Recovery: 99.36% for Paracetamol and 99.99% for Ondansetron .

2. LIST OF ANALYTICAL METHOD AVAILABLE FOR PROMETHAZINE HYDROCHLORIDE

- **Muhammad Jawwad Saif**[11] .,et al., reported A new spectrophotometric method for the determination of promethazine–HCl from pure and pharmaceutical preparation.absorbance at 515 nm.

3. LIST OF ANALYTICAL METHOD AVAILABLE FOR PARACETAMOL

- **M Gandhimathi**[28]. Et al., reported High performance thin layer chromatographic method for Simultaneous estimation of Paracetamol and Valdecoxib in tablet dosage from india. Chloroform:Isopropyl alcohol:Glacial acetic acid (9.5:1:0.2 v/v/v) as mobile phase.

- **Caitlin Sullivan and Joseph Sherma**[29]., reported Development and Validation of an HPTLC-Densitometry method for Assay of Caffeine and Acetaminophen in Multi component Extra Strength Analgesic Tablets.

- **Thenmozhi**[15]**.**, et al., reported HPTLC Method For Simultaneous Estimation Of Tramadol Hydrochloride And Paracetamol In Combined Tablet Dosage. In this method pre coated silica gel 60 GF 254 TLC plate was used as stationary phase and the chromatogram was developed using Toluene: Ethylacetae: Methanol: Glacial acetic acid (40:30:28:2 % v/v/v/v) as mobile phase. The plate was scanned and quantified at 276 nm using Camag TLC Scanner.

- **Khatal LD**., et al., reported Validated HPTLC method for simultaneous quantitation of paracetamol, diclofenac potassium, and famotidine in tablet formulation. The method used HPTLC aluminum plates precoated with silica gel 60F254 as the stationary phase, and the mobile phase consisted of toluene-acetone-methanol-formic acid (5 + 2 + 2 + 0.01, v/v/v/v). Densitometric evaluation of the separated zones was performed at 274 nm.

- **C. Roosewelt**[17] .,et., reported Simultaneous Estimation and Validation of Tramadol and Paracetamol by HPTLC in Pure and Pharmaceutical Dosage Form. It was performed on TLC plate pre-coated with silica get 60F254 as a stationary phase using mobile phase comprising of chloroform:methanol:glacial acetic acid (90:20:1) and the detection was carried out of 270 nm.

- **Santosh V**[18]**.**, et al., reported Simultaneous HPTLC determination of nabumetone and paracetamol in combined tablet dosage form. The separations were carried out on Merck aluminum plates precoated with silica gel 60 F_{254}, using toluene:2-propanol:acetic acid (8:2:0.1, v/v/v) as mobile phase. Quantitative determination of bands was done by densitometric scanning at 236 nm.

- **Kanchan Raut**[19]., et al ., reported Validated HPTLC Method for Simultaneous Estimation of Paracetamol and Etoricoxib in Bulk Drug and Formulation. Chromatographic separation of the drugs were performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of toluene: ethyl acetate: methanol in the ratio of 6: 4: 1 (v/v/v).Densitometric evaluation of the separated zones was performed at 263 nm.

- **K. G. Baheti**[20] ., et al., reported Validated Simultaneous Estimation of Paracetamol and Etoricoxib in Bulk and Tablet by HPTLC Method. A good linear relationship with regression coefficient 0.998 and 0.995 in the concentration range of 100-600 ng/spot and 200-1200 ng/spot for paracetamol and etoricoxib respectively was obtained. The recovery study was carried out by standard method and results were in the range of 98 to 102 %.

- **Janhavi R**[21] ., et al., reported Simultaneous HPTLC Determination of Paracetamol and Dexketoprofen trometamol in pharmaceutical dosage form. Silica gel G 60F254 plates were used as stationary phase and toluene: ethyl acetate: acetic acid (6: 4: 0.2 v/v/v) as mobile phase. Wavelength selected for analysis was 256 nm.

DRUG PROFILE[3-4]

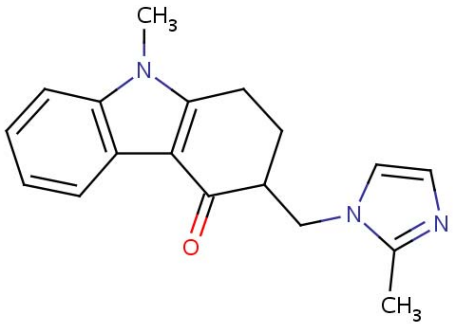
ONDANSETRON HYDROCHLORIDE

Official in Indian Pharmacopeia

Chemical Name

Chemically acebrophylline is 1, 2, 3, 6-tetrahydro-1, 3-dimethyl-2, 6-dioxo-7H purine-7-acetic acid with trans- 4-[(2-amino-3, 5-dibromophenyl) methyl] amino] cyclohexanol.

Structure



Molecular formula

$C_{18}H_{19}N_3O.HCl.2H_2O$

Molecular weight

365.86

Description

White to off white colour

Solubility

Very soluble in acid soloution.Sparingly soluble in water

Mode of Action

Ondansetron is a potent, highly selective 5 HT3 receptor –antagonist. Its precise mode of action in the control of nausea and vomiting is not known. Chemotherapeutic agents and radiotherapy may cause release of 5HT in the small intestine initiating a vomiting reflux by

activating vagal afferents via 5 HT3 receptors. Ondansetron blocks the initiation of this reflex. Activation of vagal afferents may also cause a release of 5 HT in the area postrema located on the floor of the fourth ventricle ,and this may also promote emesis through a central mechanism. Thus , the effect of ondansetron in the management of the nausea and vomiting induced by cytotoxic chemotherapy and radiotheraphy is due to antagonism of 5HT3 receptors on neurones located both in the peripheral and central nervous system. The mechanisms of action in post-operative nausea and vomiting are not known but there may be common pathways with cytotoxic induced nausea and vomiting .In psychomotor testing ondansetron does not impair performance nor cause sedation. Ondansetron does not alter plasma prolactin concentrations.

A study in cloned human cardiac ion channels has shown ondansetron has the potential to affect cardiac repolarisation via blockade of HERG potassium channels. The clinical relevance of this finding is uncertain.

Therapeutic Category

Prevention and treatment of nausea and vomiting

Pharmacological Action

Ondansetron is a selective 5-HT3 receptor –antagonist.Chemotherapeutic agents and radiotherapy may cause release of 5-HT in the small intestine initiating a vomiting reflex by activating vagal afferents via 5-HT3 receptors. The initiation of this reflex is blocked by ondansetron. Activation of vagal afferents may also cause a release of 5HT in the area postrema,located on the floor of the fourth ventricle,and this may also promote emesis through a central mechanism. Thus the effect of ondansetron in the management of the nausea and vomiting induced by chemotheraphy and radiotheraphy may be due to the antagonism of 5-HT3 receptors on neurons located both in the peripheral and central nervous system.

In psychomotor testing,ondansetron does not cause sedation nor impair performance.

Dose

4 mg 3 times a day

DRUG PROFILE[4-5]

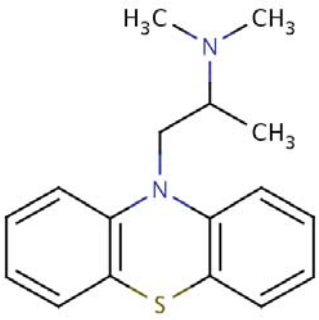
PROMETHAZINE HYDROCHLORIDE

Official in Indian Pharmacopeia.

Chemical Name

Dimethyl [1-(10H-phenothiazin-10-yl)propan-2-yl]amine

Structure



Molecular Formula

$C_{17}H_{20}N_2S$

Molecular Weight

419.45 g / mol

Description

White to faint yellow crystalline powder that is practically odorless.

Solubility

It is very soluble in water, freely soluble in alcohol and in chloroform and practically insoluble in ether.

Mode of Action

Competitively blockts H1 receptors. Possesses sedative ,antemetic and anticholinergic properties. The relief of motion sickness and nausea/vomiting appear to be related to central anticholinergic actions and may implicate activity on the medularry chemoreceptor trigger zone.

Therapeutic Category

Antiemetic and anticholinergic

Pharmacological Action

Promethazine is a phenothazine derivative which differs structurally from the antipsychotic phenothazines by the presence of a branched side chain and no ring substitution. It is thought that this configuration is responsible for its relative lack (1/10 that of chlorpromazine) of dopamine antagonist properties.

Promethazine is an H1 receptor blocking agent. In addition to its antihistamininc action,it provides clinically useful sedative and antiemetic effects.

Promethazine is well absorbed from the gastrointestinal tract. Clinical effects are apparent within 20 minutes after oral administration and generally last four to six hours,although they may persist as long as 12 hours. Promethazine is metabolized the liver to a variety of compounds; the sulfaoxides of promethazine and N-desmethyl promethazine are the predominant metabolites appearing in the urine.

Dose

The recommended dose of promethazine for treating allergies in most people is 25 mg before bedtime. For the treatment of nausea and vomiting, guidelines call for 12.5 to 25 mg, taken every four to six hours as needed. The standard recommended amount for treating morning sickness is 25 mg twice daily. Dosing for children is determined by the child's weight.

DRUG PROFILE[6]

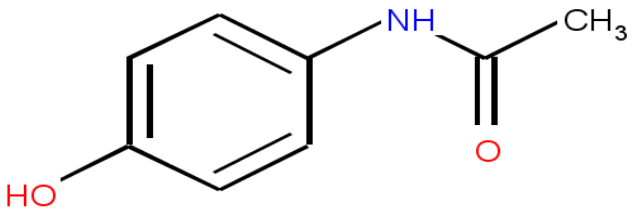
PARACETAMOL

Official in Indian Pharmacopeia.

Chemical Name

Chemically it is N-(4-hydroxyphenyl)acetanilide.

Structure



Molecular Formula

$C_8H_9NO_2$

Molecular Weight

151.16 g / mol

Description

A White solid crystalline powder.

Solubility

Slightly Soluble in water, soluble in organic solvents such as methanol,ethanol,insoluble in petroleum ether,benzene.

Mode of Action

The mechanism by which Paracetamol reduces fever and pain is still a source of

debate. The reason for this confusion has largely been due to the fact that paracetamol reduces the production of prostaglandins.

Therapeutic Category

Paracetamol is non steroid anti inflammatory drug

Pharmacological Action

Acetaminophen is thought to act primarily in the CNS increasing the pain threshold by inhibiting both is forms of cyclooxygenase; COX-1, COX-2 and COX-3 enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDs, acetaminophen does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory affects. While aspirin acts as an irreversible inhibitor of COX and directly blocks the enzyme's active site,studies have found that acetaminophen indirectly blocks COX, and that this blockade is in effective in the presence of peroxides. This might explain why acetaminophen is effective in the central nervous system and in endothelial cells but not in platelets and immune cells which have high levels of peroxides. Studies also report data suggesting that acetaminophen selectively blocks a poorly understood, but future research may provide further insight into how it works. The antipyretic properties of acetaminophen are likely due to direct effects on the heat-regulating centres of the hypothalamus resulting in peripheral vasodilation,sweating and hence heat dissipation.

Dose

0.5 to 1 gm 4 to 6 hours as necessary

AIM AND OBJECTIVE

Literature survey revealed that various analytical methods like UV-Spectrophotometry,HPLC and HPTLC methods, have been reported for the estimation of Ondansetron Hcl, Promethazine Hcl and Paracetamol, individually and combination with some other drugs. No methods for estimation of Ondansetron Hcl with Promethazine by using UV-Spectroscopy and Ondansetron Hcl with Paracetamol by using HPTLC.

The review of literature prompted us to develop a new validated UV Spectroscopic method for the estimation of Ondansetron Hcl with Promethazine Hcl and a HPTLC method for Ondansetron with Paracetamol in pharmaceutical dosage forms.

Aim:

1. To develop a UV-Spectroscopic method for the Simultaneous estimation of Ondansetron Hcl with Promethazine Hcl in formulation.
2. To develop and validate a HPTLC method for Simultaneous estimation of Ondansetron Hcl and Paracetamol in formulation.

Objective:

1. To procure pure samples from Bulk drug industry
2. To find the linearity range of the drugs to be estimated with suitable solvents & mobile phase using UV and HPTLC.
3. To prepare the formulation dilution inside the linearity range to evaluate the drug content.
4. To validate the developed method according to FDA, USP & ICH guidelines.

LIST OF INSTRUMENTS USED

S. No.	Instruments
1.	Elico SL-164 UV-VIS Spectrophotometer
2.	Camag HPTLC Instrument
3.	Camag TLC Scanner 3
4.	Camag Linnomate V Automatic Sample Applicator
5.	Twin-trough chamber (10×10 cm)
6.	Hamilton syringe (20 µl)
7.	Electronic Balance
8.	Ultra sonicator
9.	pH Analyzer
10.	Camag–Reprostar –3 instrument

LIST OF CHEMICALS AND SOLVENTS

S. No	Chemicals and Solvents
1.	Ondansetron Hydrochloride working standard
2.	Promethazine Hydrochloride working standard
3.	Paracetamol working standard
4.	Chloroform (AR Grade)
5.	Distilled water (AR Grade)
6.	Dichloromethane (AR Grade)
7.	Methanol (AR Grade)

SIMULTANEOUS ESTIMATION OF ONDANSETRON HYDROCHLORIDE AND PROMETHAZINE HYDROCHLORIDE BY UV – SPECTROSCOPY IN BULK AND PHARMACETICAL DOSAGE FORM

Study of spectra and selection of wave length

The aliquot portion of standard stock solution of Ondansetron Hcl and Promethazine Hcl were diluted appropriately with Chloroform to obtain 10 µg/ml each respectively and the solutions were scanned in the range of 200-400 nm in 1.0 cm cell against chloroform as blank and the λ max for Ondansetron Hcl and Promethazine Hcl was found to be 310 nm and 250 nm respectively.

Standard Solution

a) Ondansetron Hydrochloride standard stock solution

An accurately weighed quantity of Ondansetron Hcl equivalent to 100mg was dissolved in chloroform and volume was made up to 100ml with same solvent to produce 1000µg/ml.

b) Promethazine Hydrochloride standard stock solution

An accurately weighed quantity of Promethazine Hcl equivalent to 100mg was dissolved in Chloroform and volume was made up to 100ml with same solvent to produce 1000µg/ml.

Study of Beer Lambert's law

The aliquots portion of standard stock solutions of Ondansetron Hcl and Promethazine Hcl were diluted to 5-25 µg/ml and 10-50 µg/ml respectively and absorbance was measured at 310 nm and 250 nm. (Fig.1-10)

Determination of Absorptive value and Concentration in Pure Drug

By Simultaneous Equation Method (or) Vierodt's Method

The absorbance at the 310 nm and 250 nm for Ondansetron Hcl and Promethazine Hcl respectively were measured and calibration curves were plotted. The absorptive values for the drugs are then calculated by ax_1, ax_2, ay_1 and ay_2. The absorbance of the sample mixture was measured at λ_1 and λ_2 and recorded as A_1 and A_2 by following equation. The absorbance graphs of pure drug samples of Ondansetron Hcl and Promethazine Hcl were given. (Fig.1-10)

$$\mathbf{Cx = A_1ax_2\text{-}A_2ax_1/ax_2ay_1\text{-}ax_1ay_2} \quad \text{---------- 1}$$

$$\mathbf{Cy = A_2ax_1\text{-}A_1ax_2/ax_2ay_1\text{-}ax_1ay_2} \quad \text{---------- 2}$$

Where,

ax_1 = the absorptivity valve of Ondansetron at 310.0 nm.

ax_2 = the absorptivity valve of Ondansetron at 250.0 nm.

ay_1 = the absorptivity valve of Promethazine at 310.0 nm.

ay_2 = the absorptivity valve of Promethazine at 250.0 nm.

Fig.1. Ondansetron Hcl 5 µg/ml at 310 nm

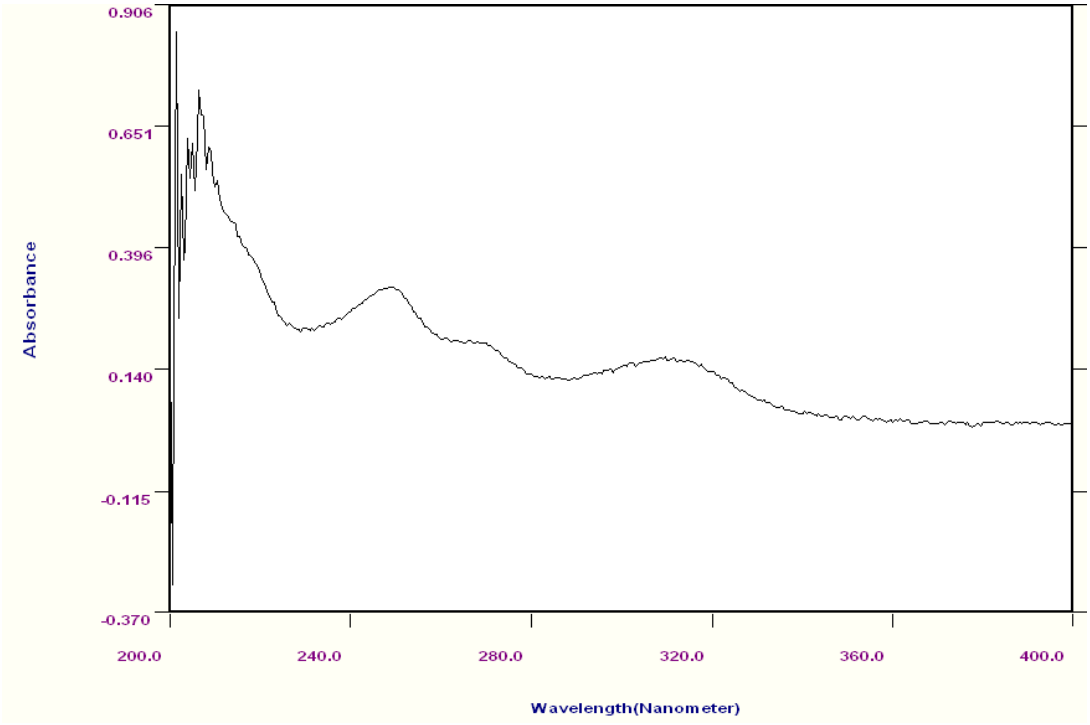


Fig. 2 Ondansetron Hcl 10 µg/ml at 310 nm

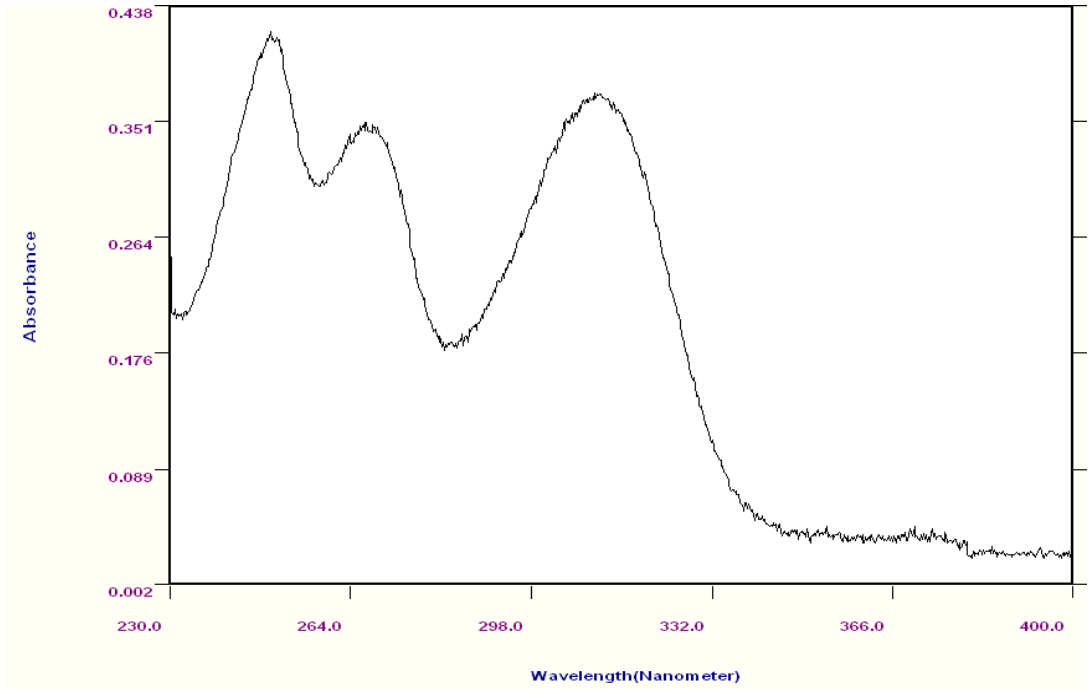


Fig.3 Ondansetron Hcl 15 µg/ml at 310 nm

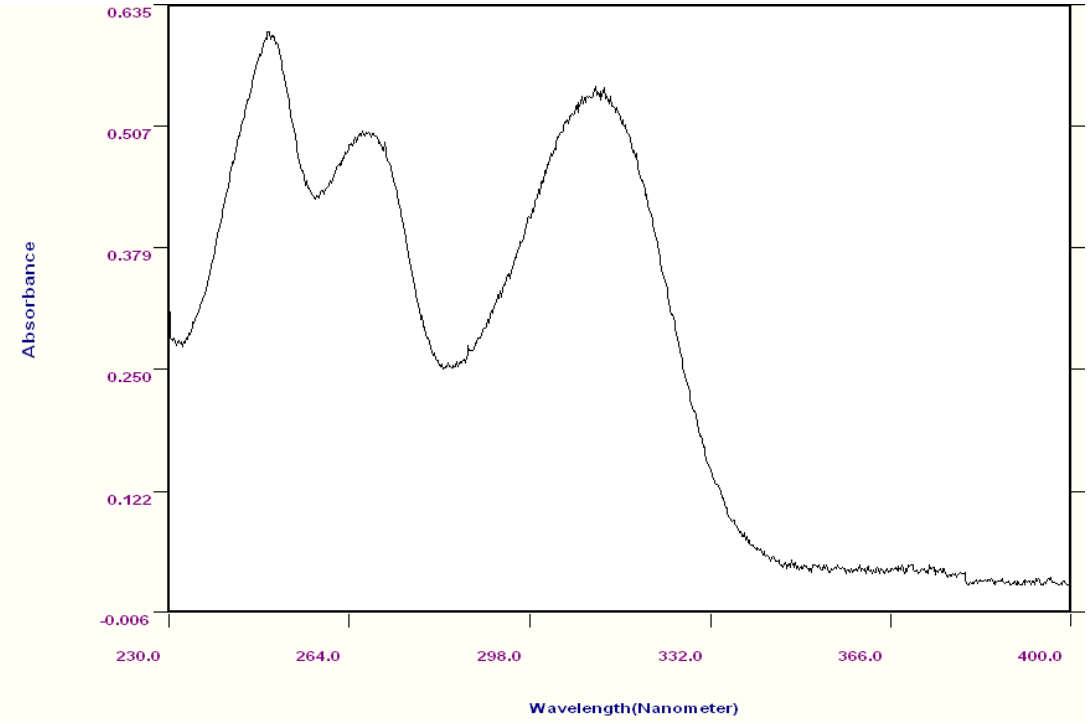


Fig.4 Ondansetron Hcl 20 µg/ml at 310 nm

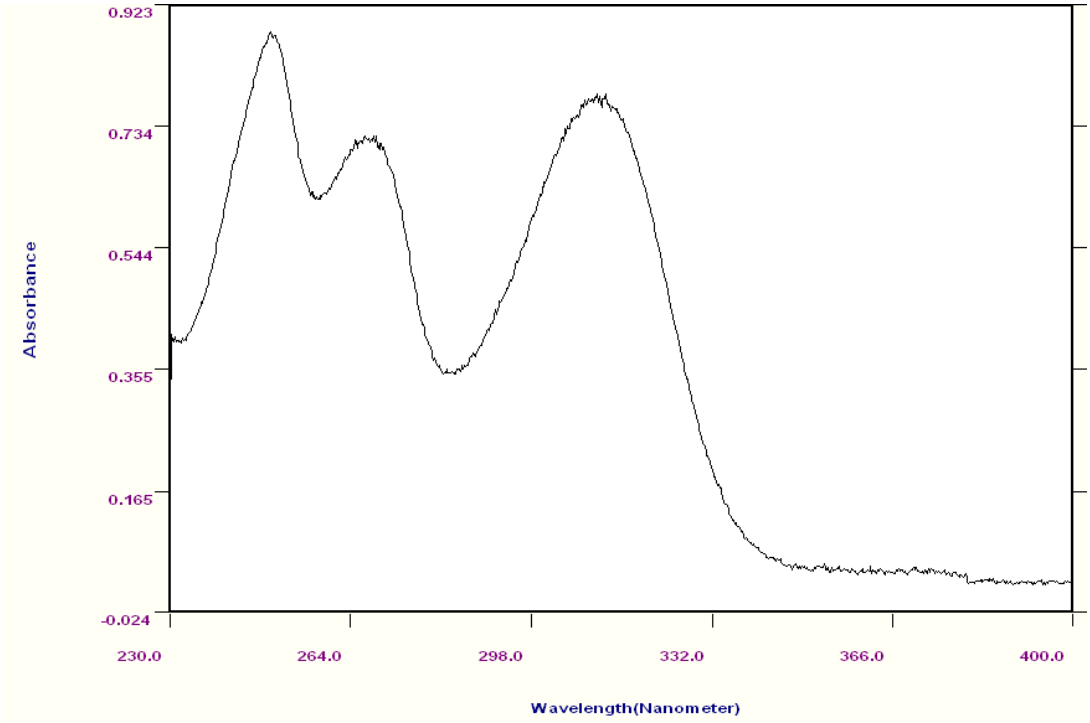


Fig.5 Ondansetron Hcl 25 µg/ml at 310 nm

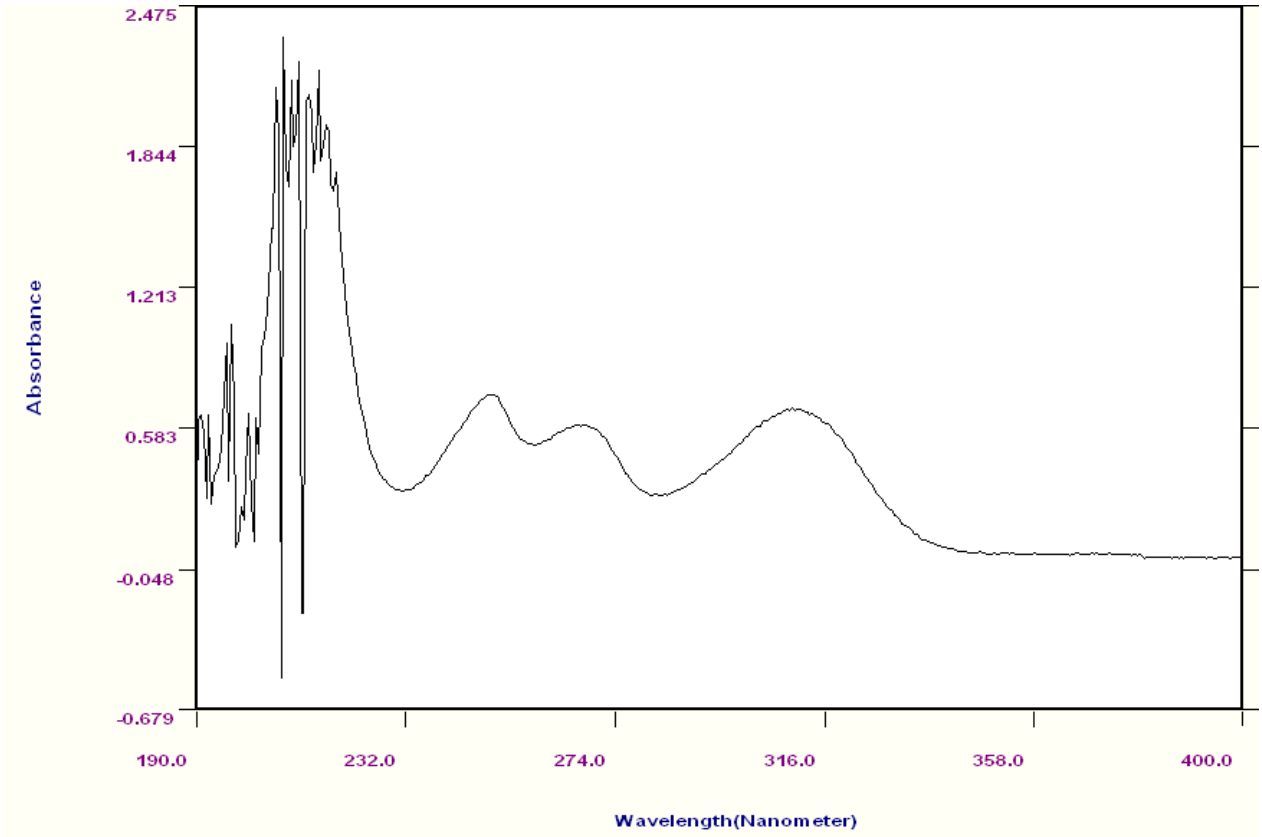


Fig.6 Promethazine Hcl 10 µg/ml at 250 nm

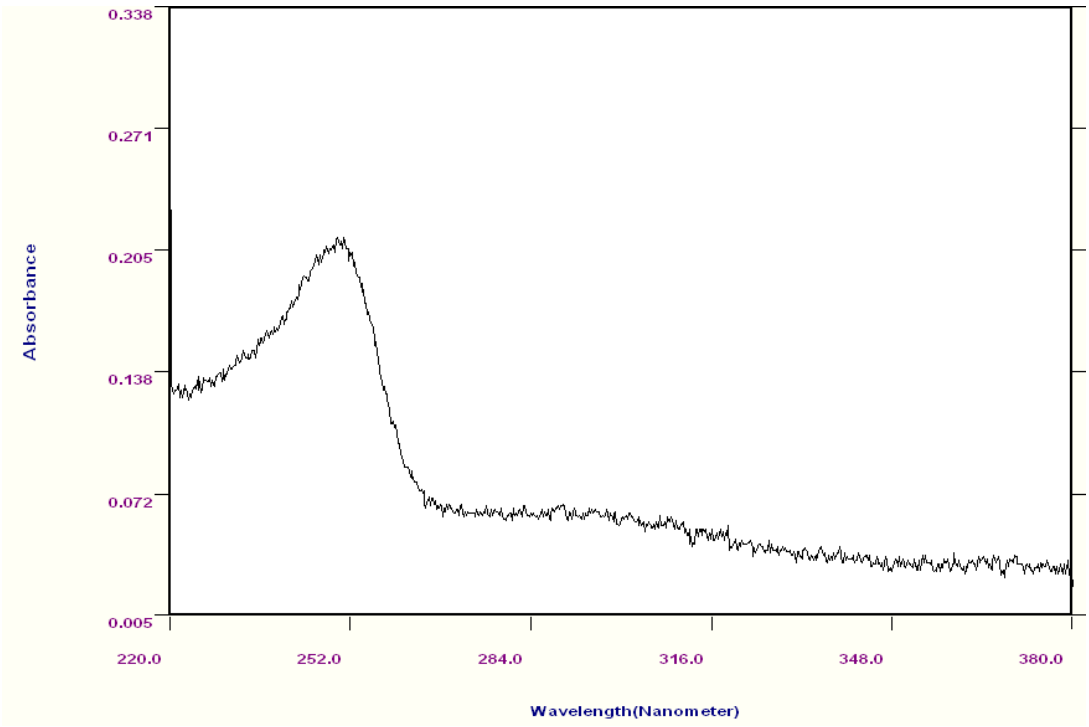


Fig.7 Promethazine Hcl 20µg/ml at 250 nm

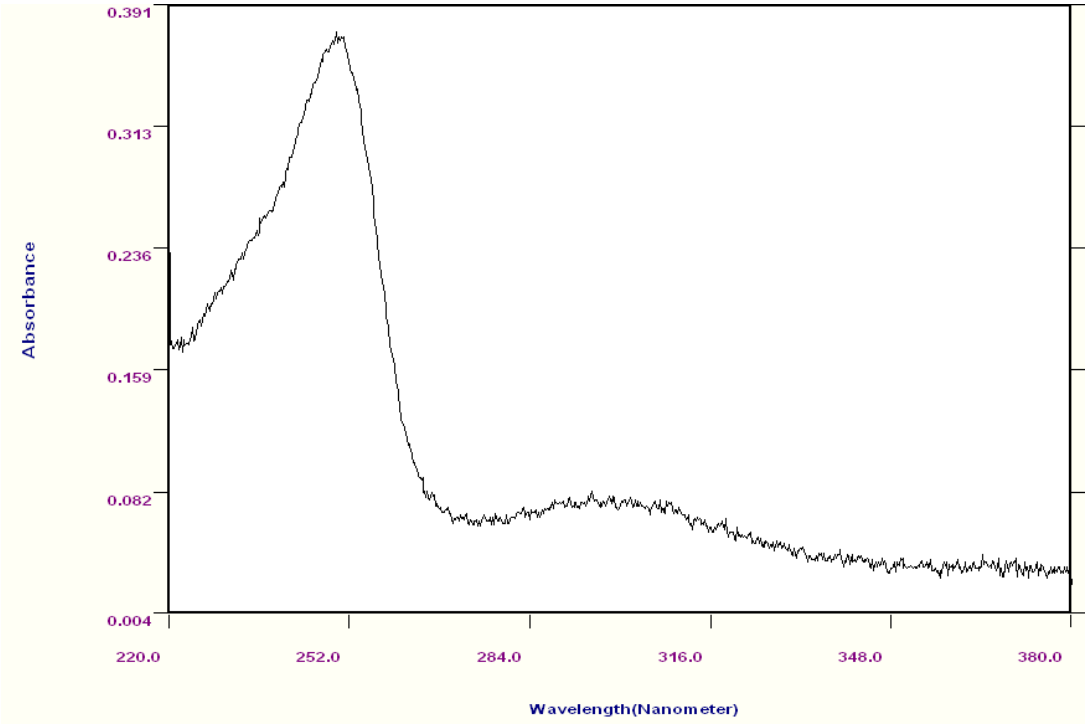


Fig.8 Promethazine Hcl 30 µg/ml at 250 nm

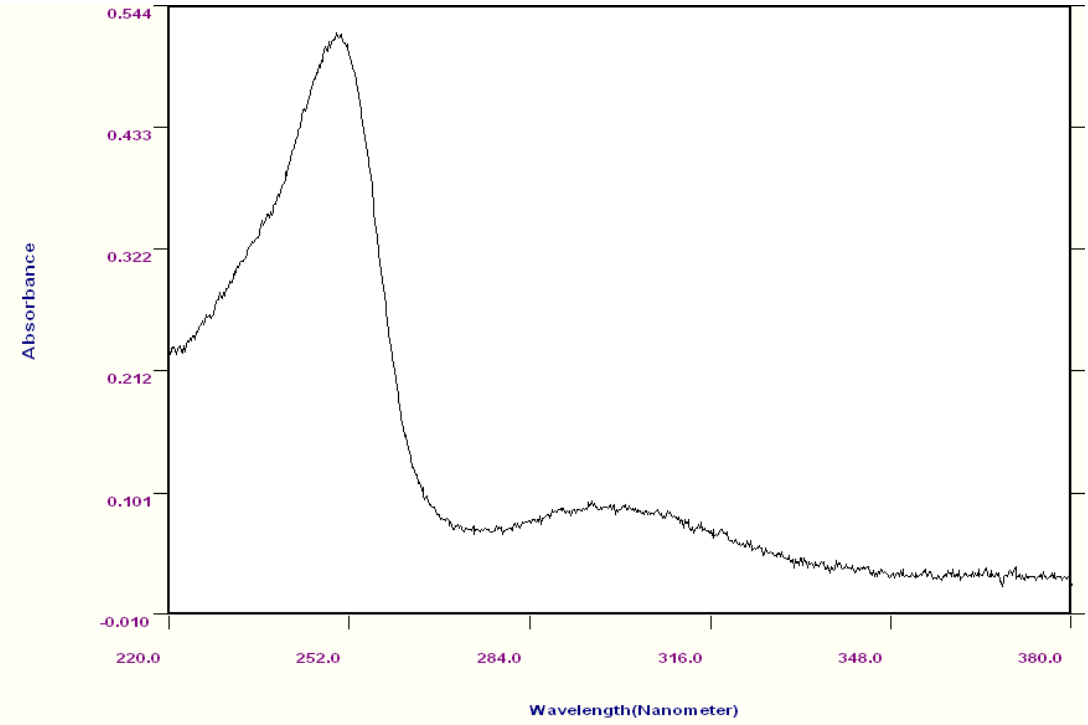


Fig.9 Promethazine Hcl 40 µg/ml at 250 nm

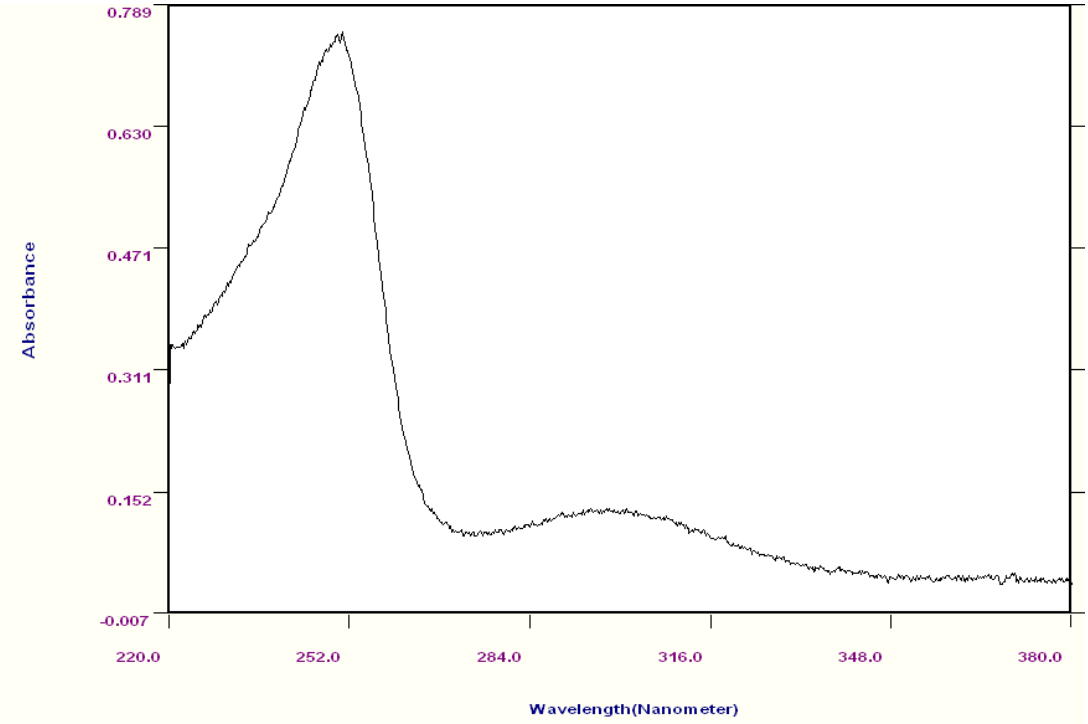
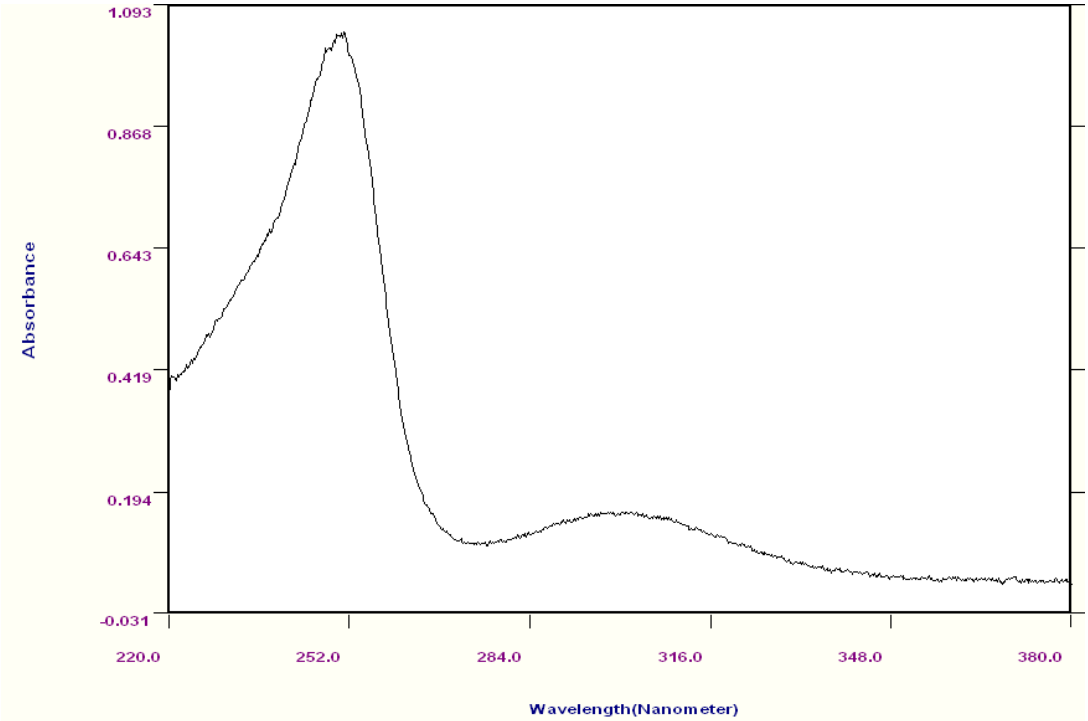


Fig.10 Promethazine Hcl 50 µg/ml at 250 nm



Analysis of Formulation

Twenty tablets are weighed and powdered. A quantity equivalent to 4 mg of Ondansetron and 25 mg of Promethazine was taken in 100 ml volumetric flask, dissolved in Chloroform and sonicated for 10 min. The volume was then made up to the mark using same solvent. The resulting solution was filtered through Whatmann filter paper grade I and from filtrate 1ml was taken and the volume was made up to 100 ml to get the approximate concentration of 20 µg/ml of Ondansetron . Absorbances of 10 µg/ml sample solution were recorded at 310.0 nm and 250.0 nm and the concentration of two drugs in the sample were determined by using simultaneous equation. The results are shown in Fig.11 and table.

Fig.11 Ondansetron Hcl 10 µg/ml in Formulation (Vomicare P)

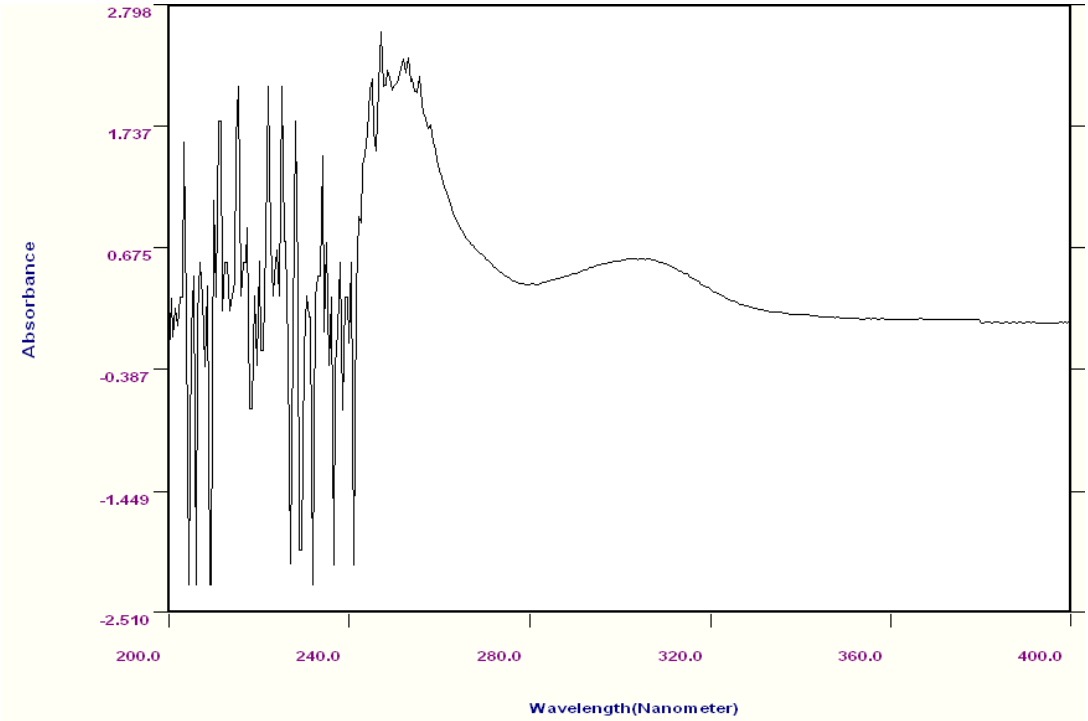


Table.1 Analysis of Formulation

Formulation	Label claim	Amount found % RSD*	% Assay RSD*
Vomicare-P tablets	Ondansetron Hcl 4 mg	3.95	98.75
	Promethazine Hcl 25 mg	24.92	99.68

* RSD for Three Determinations

Method Validation

The developed method was validated in terms of accuracy, precision and stability. The validation parameters are shown in table.2

Table.2 Validation Parameters

Parameters	Ondansetron Hcl 310 nm	Promethazine Hcl 250nm
Linearity Range(µg/ml)	5-25	10-50
Coefficient of Correlation	0.998	0.999
Slope	0.038	0.018
Intercept	0.011	0.009
Repeatability (% RSD) Intraday precision Interday precision	0.39-1.47 0.13-1.38 0.22-0.96	0.23-77 0.29-1.08 0.25-1.38

Linearity and Range

The aliquot portion of stock standard solutions of Ondansetron Hcl and Promethazin Hcl were diluted with Chloroform to get the series of concentration 5-25µg/ml and 10-50 µg/ml respectively. To construct Beer's law plot for Ondansetron Hcl and Promethazine Hcl, a standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Fig.12 and 13. The drug has obeyed Beer's law. The regression coefficient for Ondansetron Hcl and Promethazine Hcl was found to be 0.998 at 310 nm and 0.999 at 250 nm. The results were shown in table.3 and Fig 12 and 13.

Table.3 Linearity and Range

SL. No	Concentration µg/ml		Absorbance at	
	Ondansetron Hcl	Promethazine Hcl	Ondansetron Hcl 310 nm	Promethazine Hcl 250 nm
1	5	10	0.210	0.192
2	10	20	0.411	0.380
3	15	30	0.567	0.568
4	20	40	0.790	0.730
5	25	50	0.960	0.900

Fig.12 Calibration curve of Ondansetron Hcl

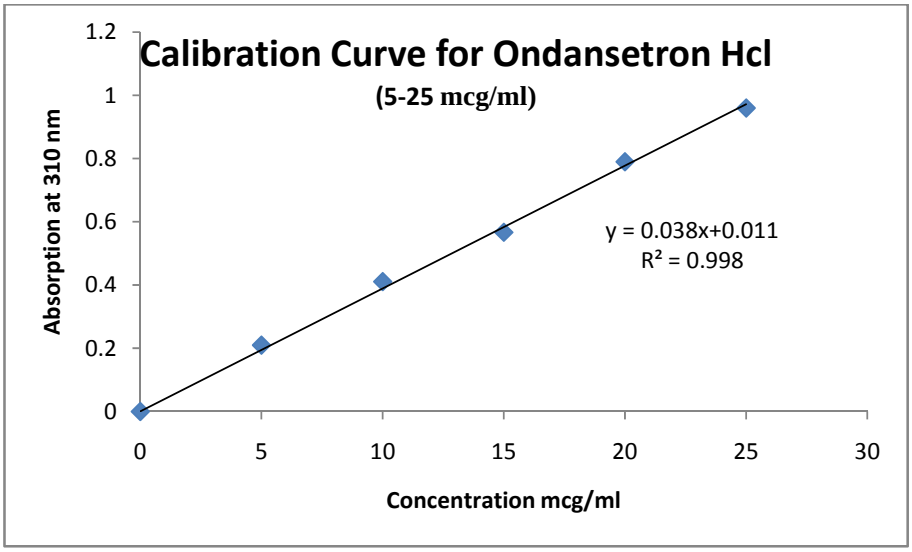
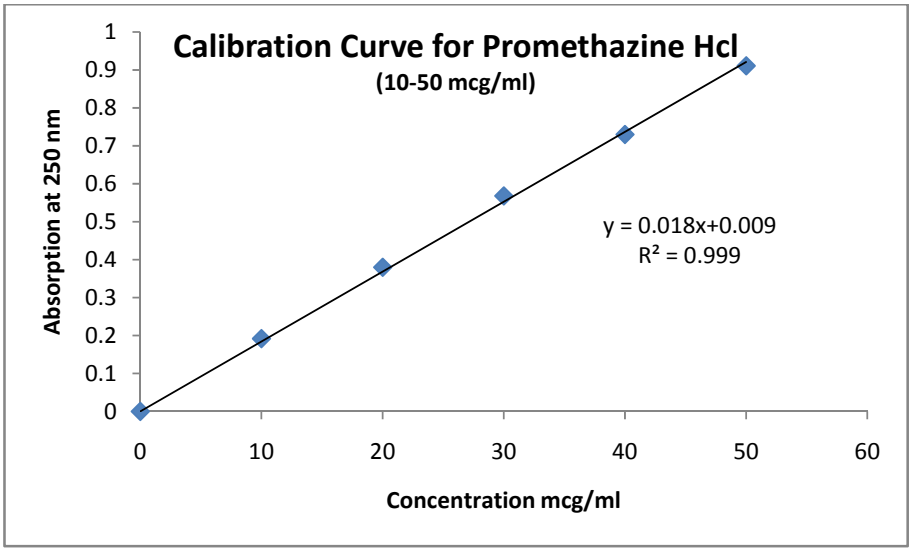


Fig.13 Calibration curve of Promethazine Hcl



Accuracy

To study the accuracy and reproducibility of the proposed methods, recovery studies were carried out by adding known amount of drugs to pre-analyzed sample at three levels and the percentage recovery was calculated. The results are summarized in table.4

The % recovery was calculated by using the following formula

$$\%\text{Recovery} = \frac{\text{Amount of drug found after addition of standard drug} - \text{Amount of drug found in sample before addition of standard drug}}{\text{Amount of standard drug added}} \times 100$$

Table.4 Recovery Studies

Recovery Level		Amt. of drug added (mg)		Amt. of drug found (mg)		Mean		% Recovery * +%RSD	
		Ond Hcl	Pro Hcl	Ond Hcl	Pro Hcl	Ond Hcl	Pro Hcl	Ond Hcl	Pro Hcl
Level 1	80%	3.2	20	3.18	20.21	3.2	20.08	100.0%±0.05	100.40%±0.11
				3.19	20.07				
				3.23	19.98				
Level 2	100%	4	25	4.04	24.90	4.01	25.03	100.25%±0.03	100.12%±0.17
				4.01	25.23				
				3.98	24.97				
Level 3	120%	4.8	30	4.81	29.97	4.80	30.03	100.1%±0.005	99.91%±0.07
				4.80	30.02				
				4.81	30.11				

* RSD for three Determinations

Ond = Ondansetron, Pro = Promethazine

Precision

Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging analytical procedures by their results.

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The term 'set' is defined as referring to a number (n) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of squares of deviations of individual results for the mean, divided by one less than the number of results in the set. The standard deviation S, is given by

$$S = \sqrt{\frac{1}{n-1}\sum_{i-1}^{n}(x_i - \bar{x})^2}$$

Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (S^2). Relative standard deviation is the standard deviation expressed as a fraction of the mean, i.e., S/x. It is some times multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

% Relative standard deviation = S x 100 / x

Table 5. Repeatability studies for Ondansetron Hcl

Concentration in µg/ml	Absorbance	%RSD
1	0.210	1.47
	0.218	
	0.219	
	0.217	
	0.217	
	0.216	
5	0.960	0.39
	0.953	
	0.951	
	0.950	
	0.955	
	0.951	

Table 6. Repeatability studies for Promethazine Hcl

Concentration in µg/ml	Absorbance	%RSD
1	0.192	0.77
	0.190	
	0.191	
	0.193	
	0.189	
	0.190	
5	0.900	0.23
	0.902	
	0.899	
	0.905	
	0.902	
	0.901	

Table 7: Inter-day precision for Ondansetron Hcl

Concentration µg/ml)	Day	Absorbance	%RSD
1	1	0.213	0.96
	2	0.215	
	3	0.212	
	4	0.209	
	5	0.211	
	6	0.213	
5	1	0.960	0.22
	2	0.963	
	3	0.958	
	4	0.957	
	5	0.961	
	6	0.960	

Table 8: Inter-day precision for Promethazine Hcl

Concentration µg/ml)	Day	Absorbance	%RSD
1	1	0.193	1.38
	2	0.188	
	3	0.195	
	4	0.194	
	5	0.190	
	6	0.191	
5	1	0.902	0.25
	2	0.903	
	3	0.899	
	4	0.904	
	5	0.900	
	6	0.905	

Table 9. Intra-day precision for Ondansetron Hcl

Concentration µg/ml	6 times in a day	Absorbance	%RSD
1	1	0.201	0.70
	2	0.200	
	3	0.201	
	4	0.199	
	5	0.203	
	6	0.202	
5	1	0.912	0.13
	2	0.913	
	3	0.910	
	4	0.911	
	5	0.913	
	6	0.913	

Table. 10 Intra-days precision for Promethazine Hcl

Concentration µg/ml	6 times in a day	Absorbance	%RSD
1	1	0.185	1.14
	2	0.182	
	3	0.181	
	4	0.179	
	5	0.180	
	6	0.182	
5	1	0.900	0.90
	2	0.901	
	3	0.901	
	4	0.902	
	5	0.905	
	6	0.907	

Stability

The stability studies of the drugs solution were carried out. The drugs solution was found to be stable at room temperature and results were shown in table.11

Table.11 Stability Study

Drug Concentration (µg/ml)		Time(min.)	Absorbance's	
OND Hcl	**PRO Hcl**		**OND Hcl**	**PRO Hcl**
10µg/ml	10µg/ml	30	0.411	0.192
		60	0.416	0.198
		90	0.418	0.193
		120	0.411	0.190
		150	0.414	0.191

SIMULTANEOUS ESTIMATION OF ONDANSETRON HCL AND PARACETAMOL BY HPTLC IN BULK AND PHARMACETICAL DOSAGE FORM

METHOD DEVELOPMENT:

1. Selection of Mobile Phase:

The selection of mobile phase was done by trial and error method in which several mobile phases were tried. The best mobile phase was selected based on the R_f value of the drugs. The mobile phase tried were listed below,

1. Chloroform :Isopropyl alcohol : Glacial acetic acid (8:1:1)
2. Chloroform: Methanol:Glacial acetic acid (8:1:1)
3. Dichloromethane: Methanol: Glacial acetic acid (8:1:1:)
4. Dichloromethane:Methanol (9:1)

2. Fixed Mobile Phase:

For Ondansetron and Paracetamol Dichloromethane:Methanol (9:1% v/v/) was selected as mobile Phase.

3. Activation of Pre-Coated Plates:

Activated by placing in oven at110-120ºc for 30 minutes after sample spotting

4. Sample Preparation:

PROCEDURE

The given Formulation Mixture (Ondansetron Hcl-Paracetamol 1 : 125 Ratio) was weighed in an Electronic balance (Afcoset) and dissolved in 90 : 10 Methanol-water centrifuged and the supernatant liquid was taken for the HPTLC studies. This solution contains 4µg of Ondansetron and 500µg of Paracetamol drug sample in 20µl Methanol-water 90 : 10 used as test solution for quantitative analysis of Ondansetron Hcl and Paracetamol from Formulation Mixture.

Standard ONDANSETRON preparation

The given standard Ondansetron 100µg was dissolved in 1000µl with Methanol-water 90 : 10, this solution used as working standard solution (1µg/10µ or 100ng/µl) for the analysis.

Standard PARACETAMOL preparation

The given standard Paracetamol 1mg was dissolved in 100µl with Methanol-water 90 : 10, this solution used as working standard solution (10µg/1µl) for the analysis.

5.Sample & Standard loading

2µl of Sample solution (Formulation Mixture 800) and a series of 1µl, 2µl, 3µl, 4µl & 5µl Standard of Ondansetron Hcl solution and 1µl, 2µl, 3µl, 4µl & 5µl Standard of Paracetamol solution were loaded as 5mm band length in the 10 x 10 Silica gel $60F_{254}$ TLC plate using 100µl Hamilton syringe and CAMAG-LINOMAT-5 instrument.

6.Spot development

The Sample and standards loaded plate was kept in Twin trough chamber 10 x 10cm with respective mobile phase up to 15min for Chamber saturation. After completion of chamber saturation, the plate was kept in mobile phase for development up to 90mm.

7.Photo-documentation

The developed plate was dried by hot-air to evaporate solvents from the plate and the plate was kept in Photo-documentation chamber. The images of developed plate were captured at white light, UV 254nm and UV 366nm using CAMAG-REPROSTAR-3 instrument.

8.Scanning

The developed plate was scanned before derivatization in UV 254nm wavelength for Ondansetron Hcl compound and Paracetamol compound using CAMAG-TLC SCANNER-3 instrument. The Baseline display, Peak densitogram & Peak table of each track were obtained. The Ondansetron Hcl and Paracetamol content present in the loaded sample was evaluated by Peak assignment with 5 level Ondansetron and Paracetamol standards.

9.Spectrum scanning

The assigned peaks of standards and sample were scanned in spectrum of UV region (200nm-400nm) and found the λmax value of Ondansetron Hcl and Paracetamol.

Mobile phase

Dichloromethane-Methanol (9 : 1)

Detection

Blackish brown coloured quenching zone at UV 254nm mode (Rf-0.35) before derivatization appeared in the given standard and sample track indicates the presence of Ondansetron Hcl and Dark black coloured quenching zone at UV 254nm mode (Rf 0.90) before derivatization showed in the given standard and sample track indicates the presence of Paracetamol.

METHOD VALIDATION:

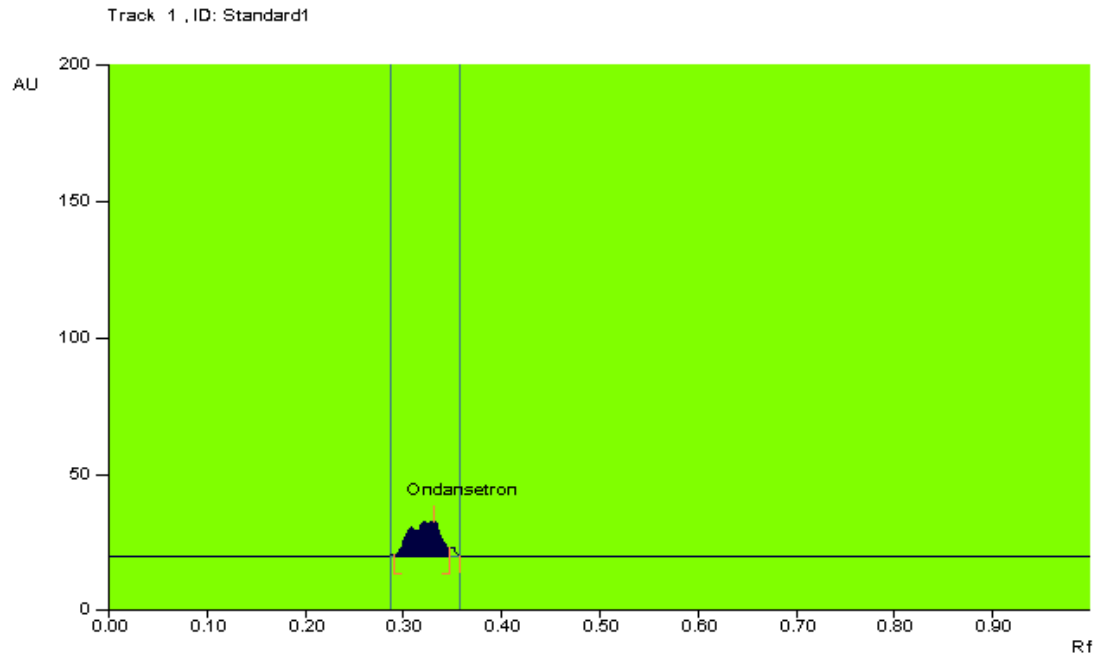
1. Linearity:

The linearity of both the drugs was determined by calibration curves and the linearity based on the area observed in the range of 10-50 µg/ml for Paracetamol and 100 –500ng/ml for Ondansetron Hcl. The regression co-efficient value for Ondansetron Hcl and Paracetamol is . The result shown in table 12

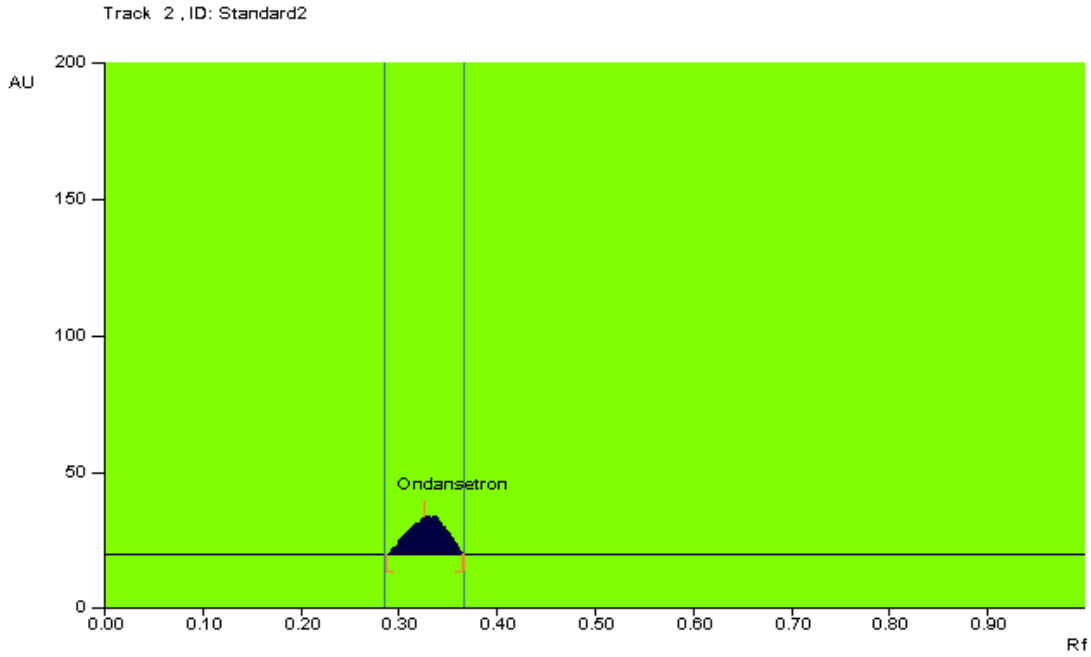
Table. 12

Parameters	**Ondansetron Hcl**		**Paracetamol**	
Linearity Range (µg/ml)	100-500 ng/ml		10-50 µg/ml	
	Concentration	Peak area	Concentration	Peak area
	100	910.2	10	8900.2
	200	2008.7	20	17100.6
	300	3000.0	30	27283.4
	400	3844.3	40	36809.8
	500	4899.09	50	45210.9
Coefficient Correlation	0.999		0.999	
Slope	9.781		905.9	
Y-intercept	0.80		0.35	

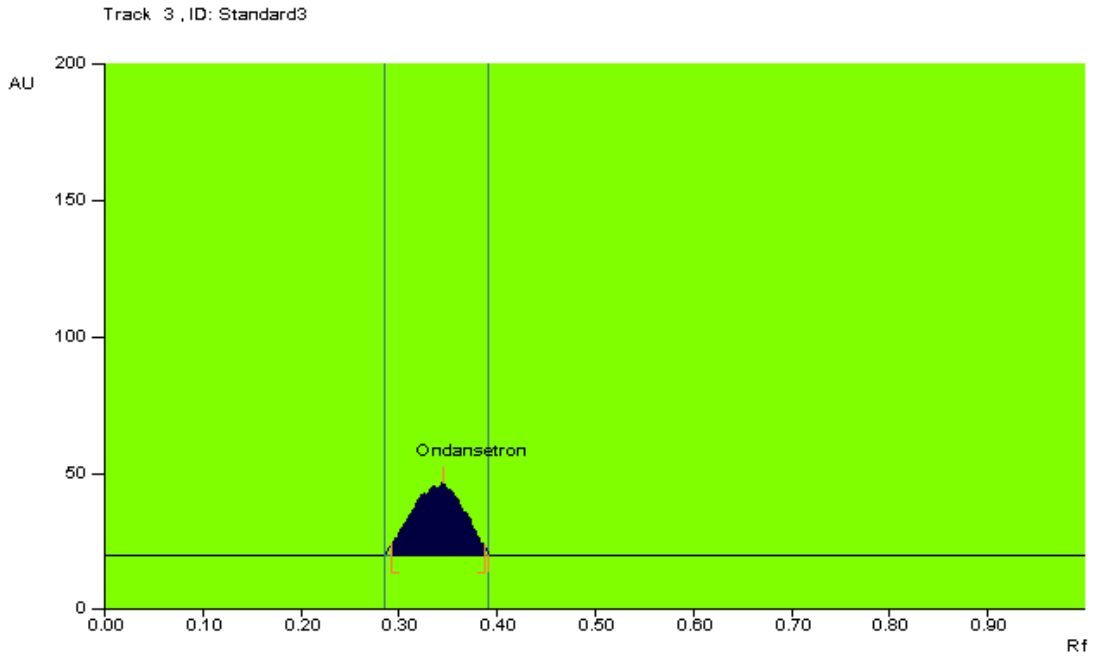
Track O1- Ondansetron Hcl standard Scanning Peak densitogram display @ 254nm



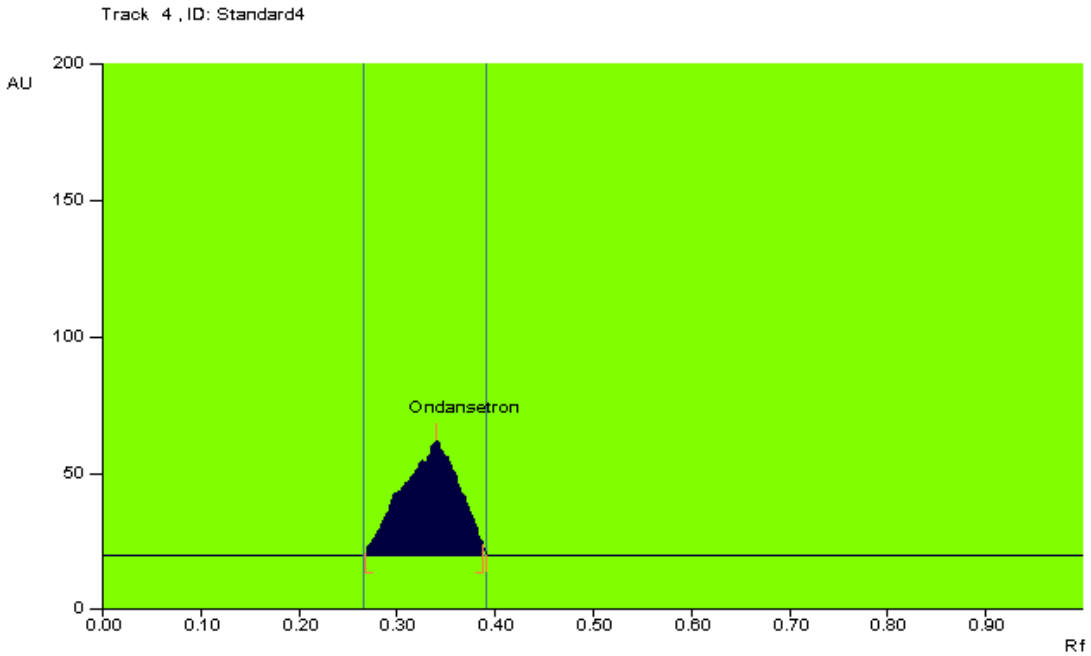
Track O2- Ondansetron Hcl standard Scanning Peak densitogram display @ 254nm



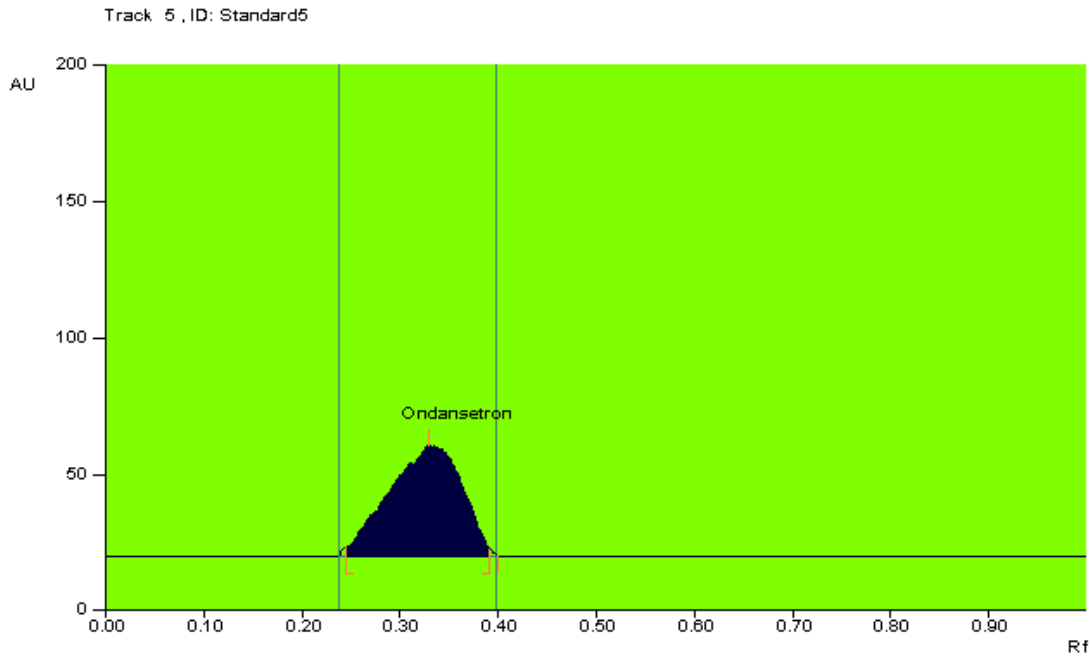
Track O3 – Ondansetron Hcl standard Scanning Peak densitogram display @ 254nm



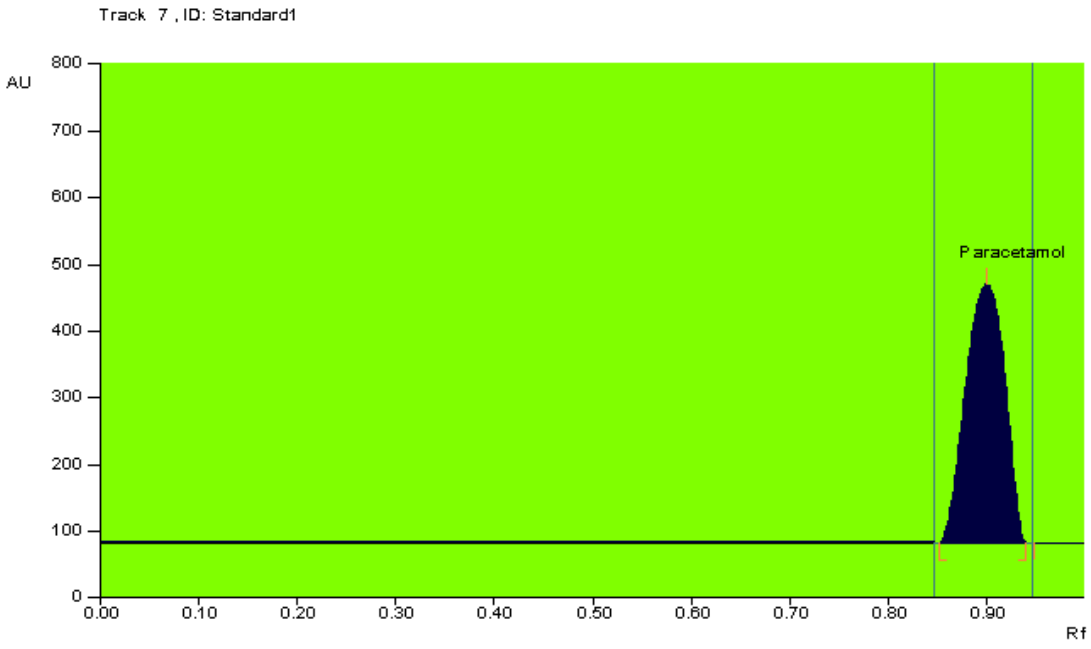
Track O4 – Ondansetron Hcl standard Scanning Peak densitogram display @ 254nm



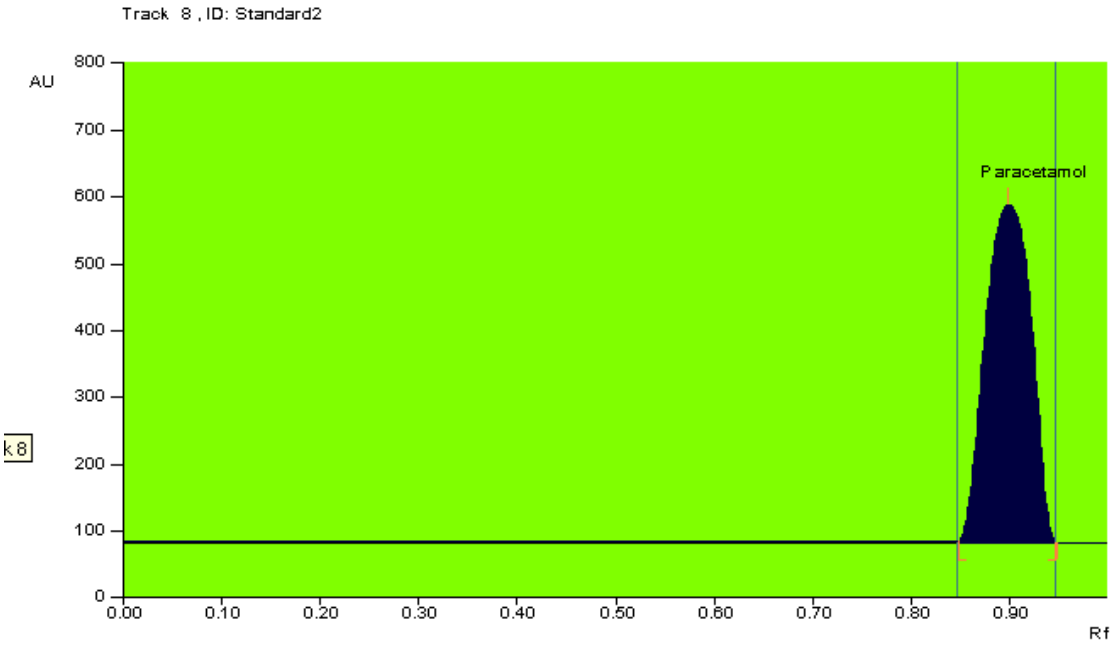
Track O5 – Ondansetron Hcl standard Scanning Peak densitogram display @ 254nm

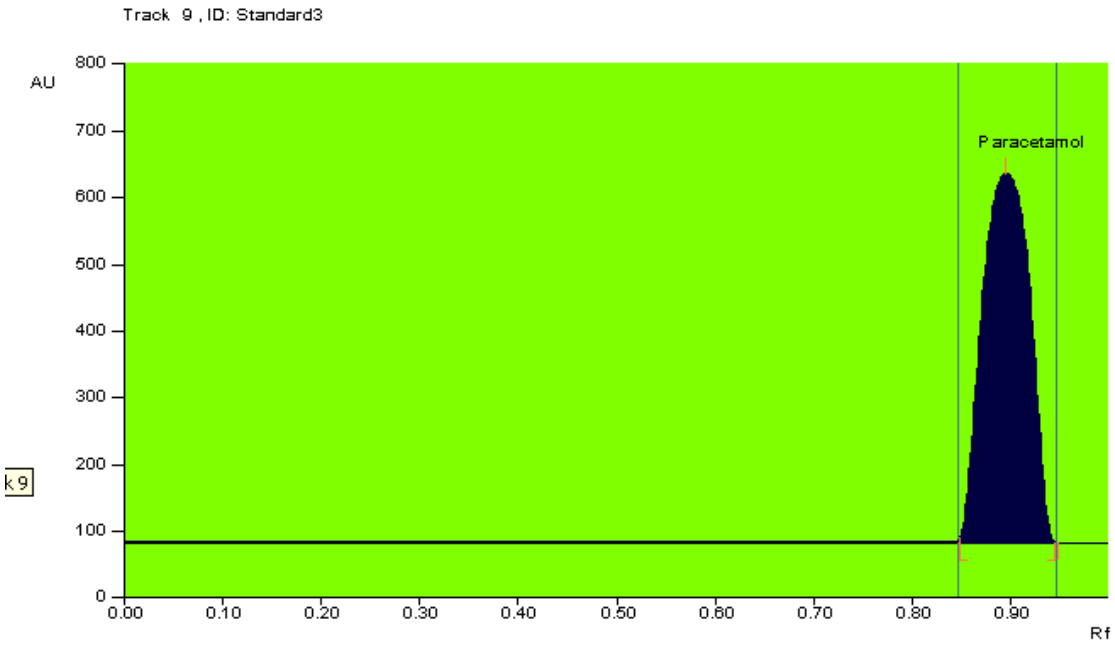


Track P1- Paracetamol standard Scanning Peak densitogram display @ 254nm



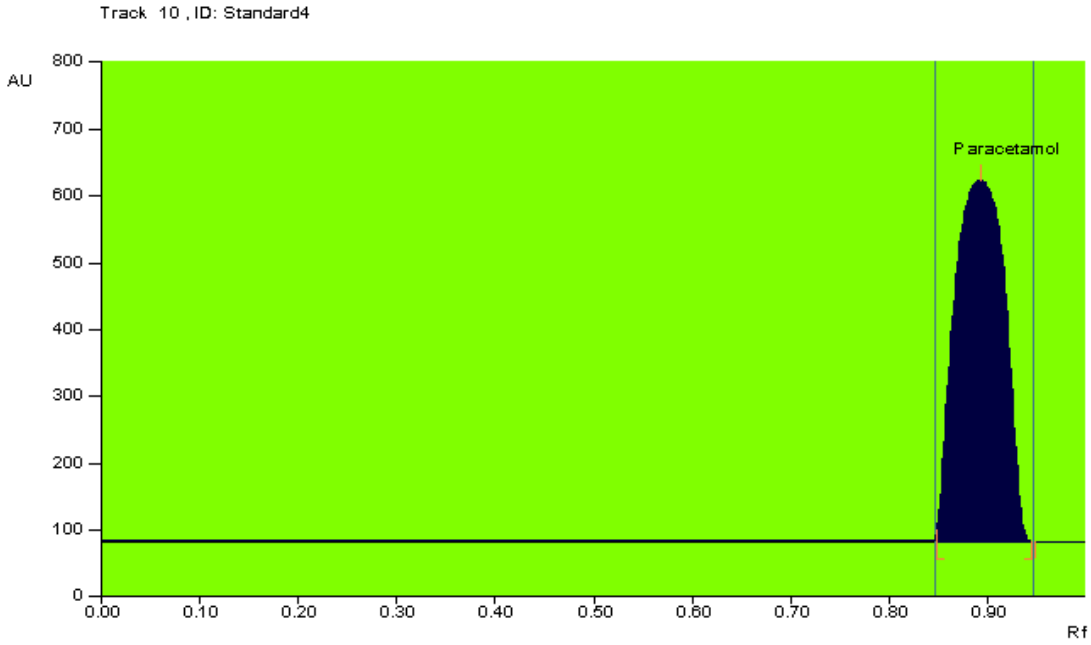
Track P2 – Paracetamol standard Scanning Peak densitogram display @ 254nm





Track P3 – Paracetamol standard Scanning Peak densitogram display @ 254nm

Track P4 – Paracetamol standard Scanning Peak densitogram display @ 254nm



Track P5 - Paracetamol standard Scanning Peak densitogram display @ 254nm

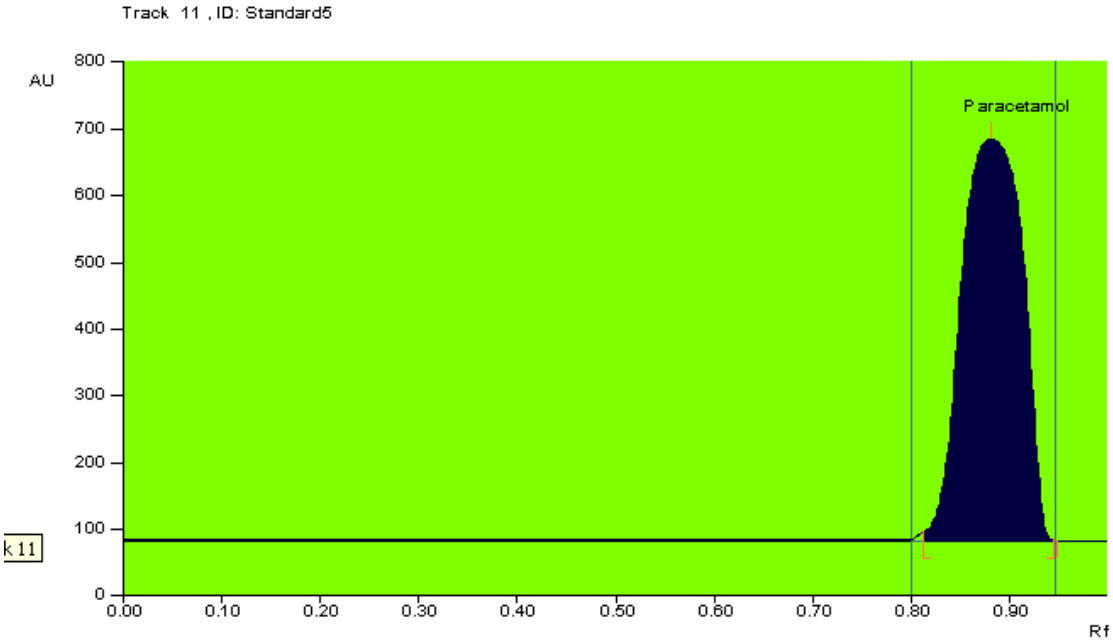


Fig 14 Calibration Curve of Ondansetron Hcl

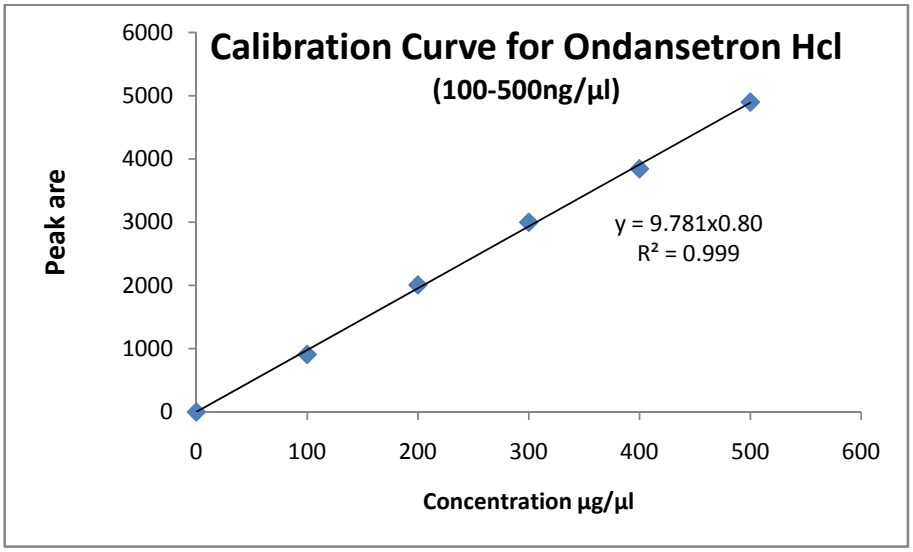
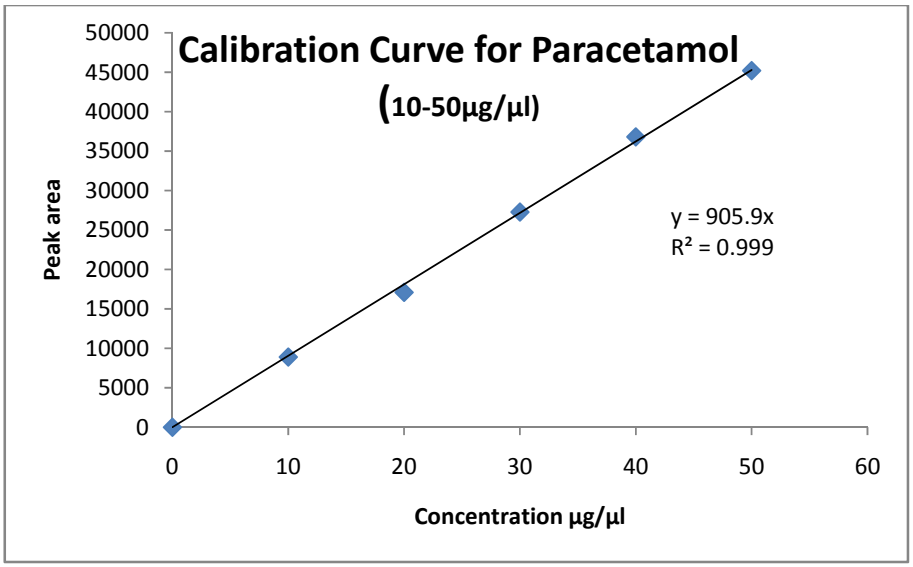


Fig.15 Calibration Curve of Paracetamol



2. Accuracy:

Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The results of the accuracy study are reported in Table 16. From the recovery study, it was clear that the method is very accurate for quantitative estimation of Ondansetron Hcl and Paracetamol in tablet dosage form because all the statistical results were within the acceptance range (i.e., % RSD <2.0).

Table. 13

Recovery Level		Amt. of drug added (mg)		Amt. of drug found (mg)		Mean		% Recovery * +%RSD	
		Ond Hcl	Para	Ond Hcl	Para	Ond Hcl	Para	Ond Hcl	Para
Level 1	80%	3.2	400	3.19	400.90	3.17	400.63	99.06%±0.05	100.15%±0.56
				3.11	399.98				
				3.22	401.01				
Level 2	100%	4	500	3.95	498.89	4.00	498.65	100.0%±0.07	99.72%±0.59
				3.98	499.09				
				4.09	497.98				
Level 3	120%	4.8	600	4.77	598.67	4.79	599.48	99.79%±0.02	99.91%±0.71
				4.81	600.011				
				4.80	599.76				

* RSD for three Determinations

3. Precision:

The precision of the method (system reproducibility) was assessed by spotting 3µl of drug solution six times on a TLC plate, followed by development of plate and recording the peak area for 6 spots. The % RSD for peak area values of Ondansetron Hcl and Paracetamol found to be 0.27% & 0.11%. The results were shown in Table-17.

The method reproducibility (intra-day precision) was determined by analyzing standard solution in the concentration range of 1 µg/spot to 5 µg/spot of drug for 3 times on

the same day and inter-day precision was determined by analysing corresponding standards daily for 3 day over a period of one week.

The intra-day and inter-day coefficients of variation (%RSD) are in range of 0.09 to 0.18, 0.006to 0.20 and 0.03 to 0.03, 0.003 to 0.02, respectively. The results were shown in Table-14, 15, 16,17,18,19.

Table – 14. Repeatability studies for Ondansetron Hcl

Sl.No	Concentration (ng/ spot)	Peak Area
1.	300	3000.0
2.	300	3000.03
3.	300	2997.98
4.	300	2978.90
5.	300	2996.34
6.	300	3000.56
Mean	-	2995.63
Percentage Relative Standard Deviation	**-**	0.27

Table – 15: Repeatability studies for Paracetamol

S.No	Concentration (µg/ spot)	Peak Area
1.	30	27283.4
2.	30	27224.6
3.	30	27312.0
4.	30	27313.7
5.	30	27285.1
6.	30	27275.4
Mean	-	27282.36
Percentage Relative Standard Deviation	**-**	0.11

Table – 16: Intra-day Precision of Ondansetron Hcl

S. No	Concentration (ng / spot)	Area	Mean	Standard Deviation	% RSD
1.	100	910.2	911.59	1.67	0.18
2.	100	911.12			
3.	100	913.45			
1.	300	3000.0	3002.74	4.66	0.15
2.	300	3000.09			
3.	300	3008.13			
1.	500	4899.09	4896.39	4.69	0.09
2.	500	4899.12			
3.	500	4890.98			

Table – 17: Intra-day Precision of Paracetamol

S. No	Concentration (µg / spot)	Area	Mean	Standard Deviation	% RSD
1.	10	8900.2	8910.99	18.26	0.20
2.	10	8900.7			
3.	10	8932.09			
1.	30	27283.4	27284.03	1.64	0.006
2.	30	27282.8			
3.	30	27285.9			
1.	50	45210.9	45207.65	6.58	0.01
2.	50	45200.08			
3.	50	45211.98			

Table – 18: Inter-day Precision of Ondansetron Hcl

S. No	Concentration (µng / spot)	Area	Mean	Standard Deviation	% RSD
1.	100	899.5	899.16	0.31	0.03
2.	100	899.09			
3.	100	898.89			
1.	300	2989.09	2988.51	0.74	0.02
2.	300	2988.77			
3.	300	2987.67			
1.	500	4800.78	4799.74	1.79	0.03
2.	500	4800.78			
3.	500	4797.67			

Table – 19: Inter-day Precision of Paracetamol

S. No	Concentration (µg / spot)	Area	Mean	Standard Deviation	% RSD
1.	10	8845.06	8842.79	2.51	0.02
2.	10	8840.08			
3.	10	8843.24			
1.	30	27200.67	27205.45	5.71	0.02
2.	30	27203.90			
3.	30	27211.78			
1.	50	45123.34	45122.6	1.47	0.003
2.	50	45120.90			
3.	50	45123.56			

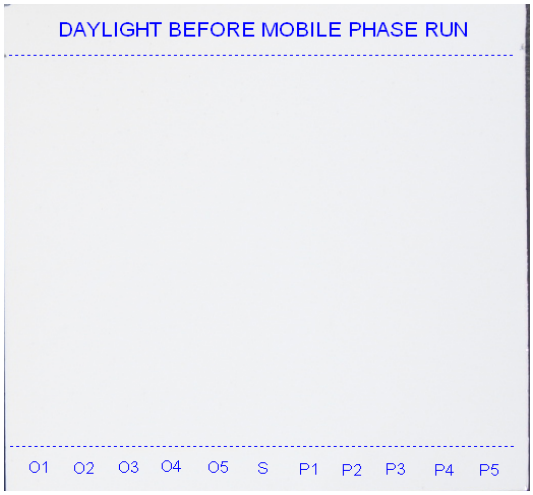
Table – 20 VALIDATION PARAMETERS

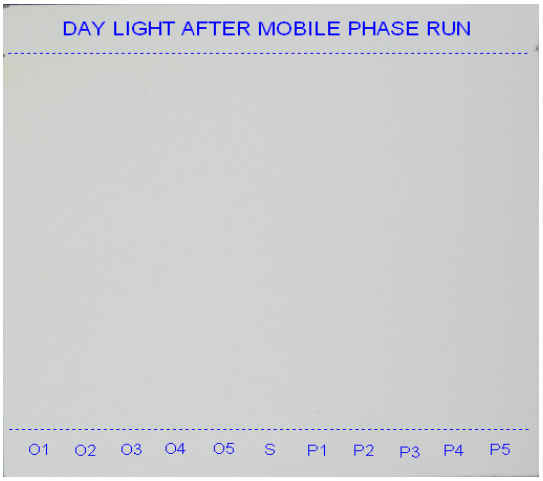
Parameters	**Ondansetron Hcl**		**Paracetamol**	
Linearity Range (µg/ml)	100-500 ng/ml		10-50 µg/ml	
	Concentration	Peak area	Concentration	Peak area
	100	910.2	10	8900.2
	200	2008.7	20	17100.6
	300	3000.0	30	27283.4
	400	3844.3	40	36809.8
	500	4899.09	50	45210.9
Coefficient Correlation	0.999		0.999	
Slope	9.781		905.9	
Y-intercept	0.80		0.35	
Rf	0.34		0.90	
Accuracy (% Recovery) (n = 3) %RSD 1. 80 % 2. 100% 3. 120%	99.79%±0.02 100.0%+0.07 99.79%+0.02		99.91%±0.71 99.72%+0.59 99.91%+0.71	
Precision (%RSD, n = 6)	0.27		0.11	
Intra-day(n = 3) (%RSD)	0.09-0.18		0.006-0.20	
Inter-day (n = 3) (%RSD)	0.02-0.03		0.003-0.02	

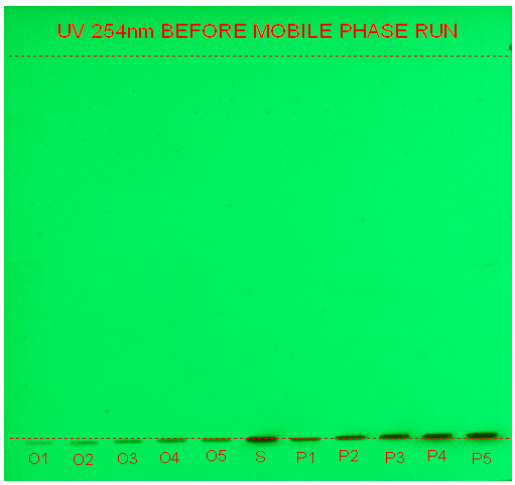
HPTLC CHROMATOGRAM OF SAMPLE OF ONDANSETRON HYDROCHLORIDE AND PARACETAMOL at UV 254 nm

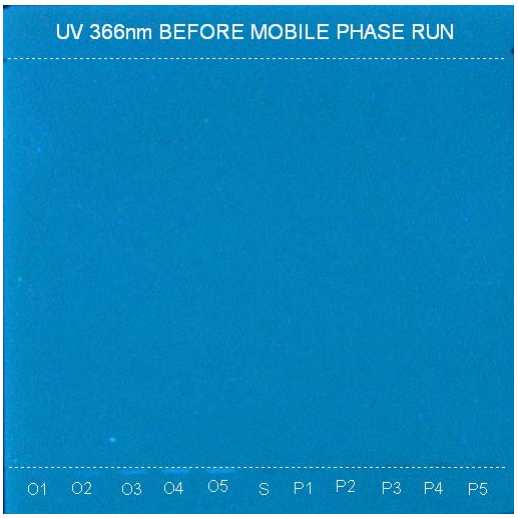
Chromatograms

Before derivatization

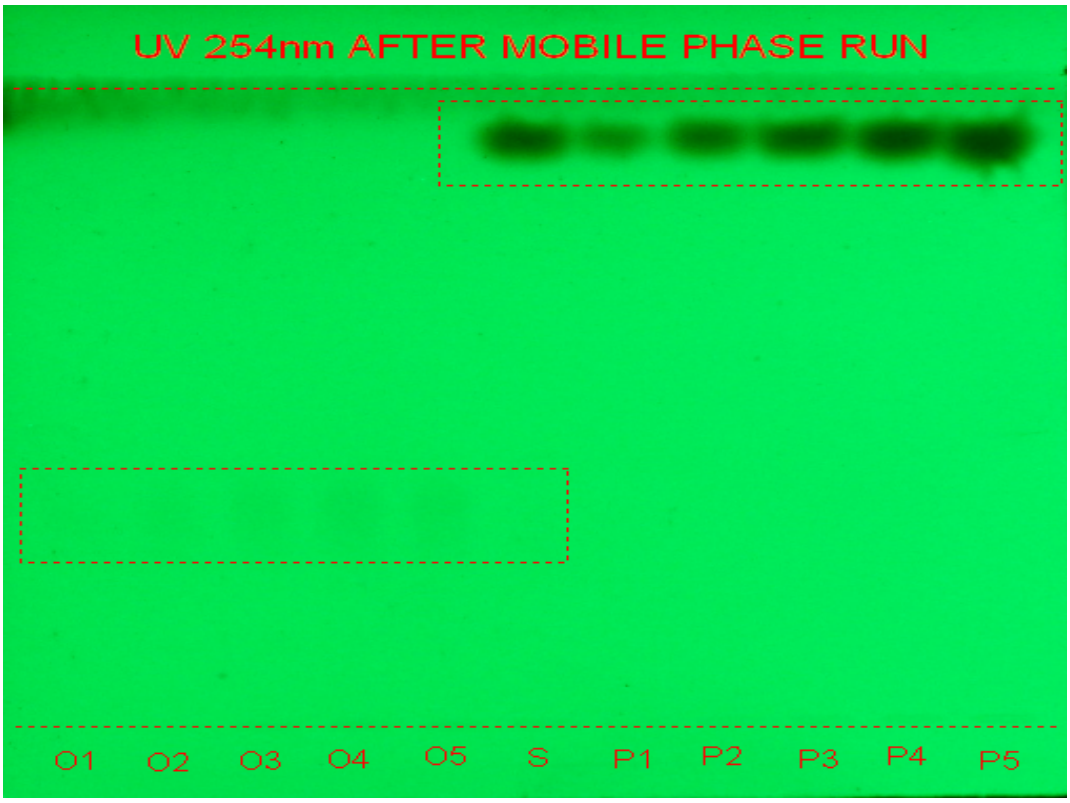









UV 366nm AFTER MOBILE PHASE RUN
O1
O2
O3
O4
O5
S
P1
P2
P3
P4
P5


UV 254nm AFTER MOBILE PHASE RUN
O1
O2
O3
O4
O5
S
P1
P2
P3
P4
P5

ANALYSIS OF FORMULATION

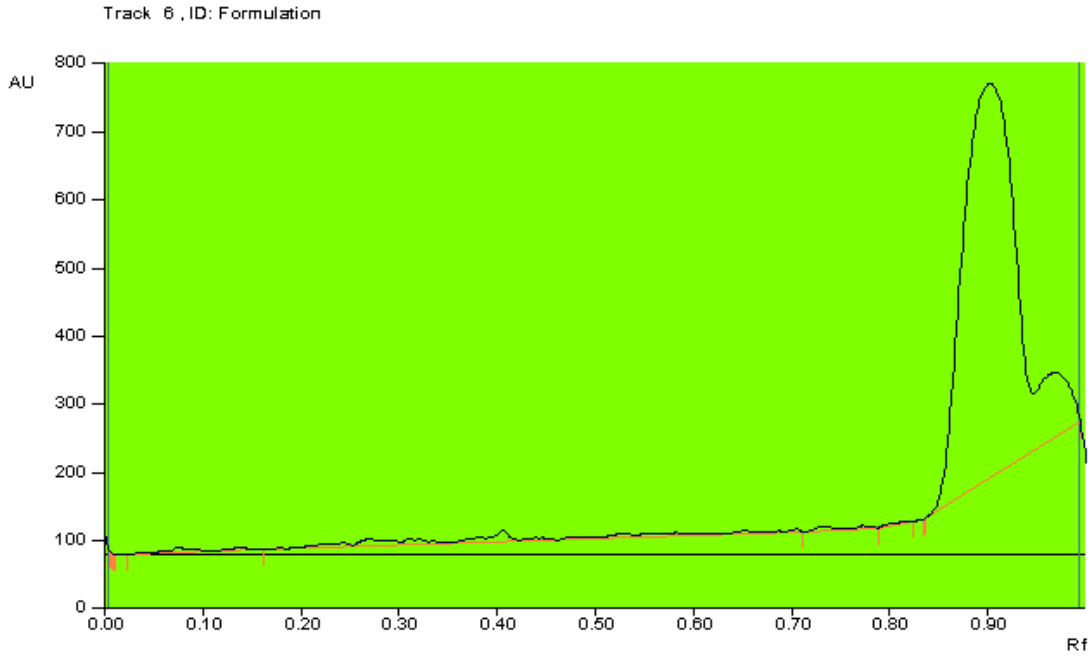
Twenty tablets (Brand name-Vomikind plus,manufactured by Mankind Pharma) were taken and their average weight was determined. The Vomikind plus tablets were powdered into fine powder using Pestle and Mortar. 100 mg of drug samples was dissolved in methanol:water (90:10) ,centrifuged and the supernatant liquid was made-up to 10 ml in a volumetric flask with Methanol:water. This solution contains 10 µg tablet in µl Methanol:water used as test solution for quantitative analysis of Ondansetron Hcl and Paracetamol from Vomikind Plus tablet.

From the peak area recorded the amount of the drug in the formulation was determined and reported in table no; 16

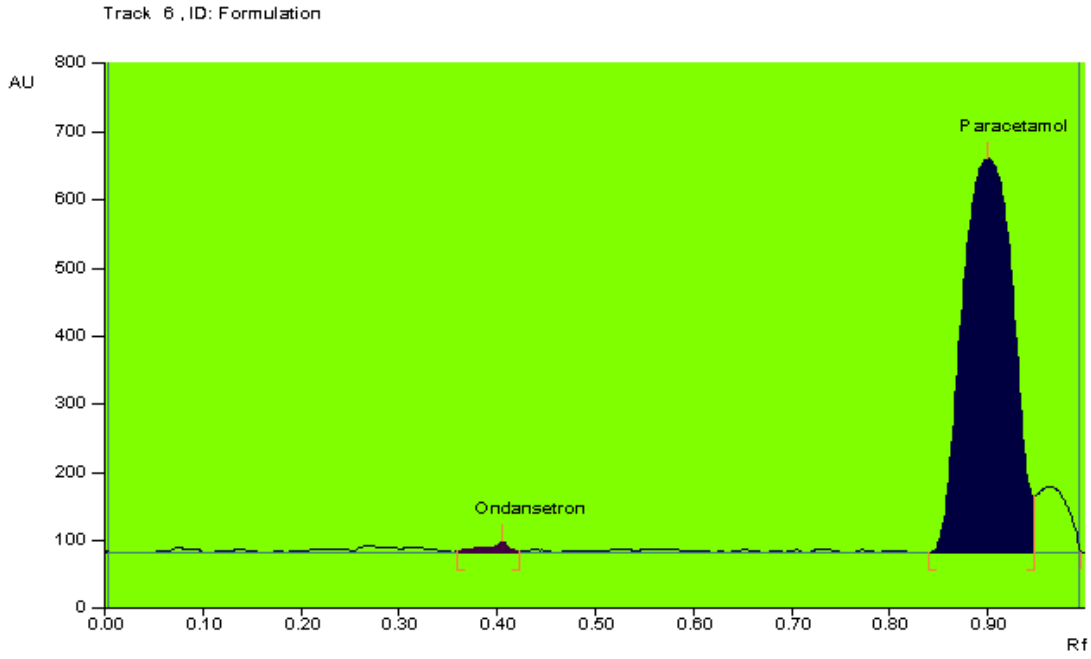
Formulation	Label Claim gm/tab	Amount found gm/tab ± RSD	% Assay ± RSD
Ondansetron Hcl	0.0.040	0.399±0.0001	99.75±0.0001
Paracetamol	0.500	501±0.200	100.20 ±0.200

RSD for three Determinations

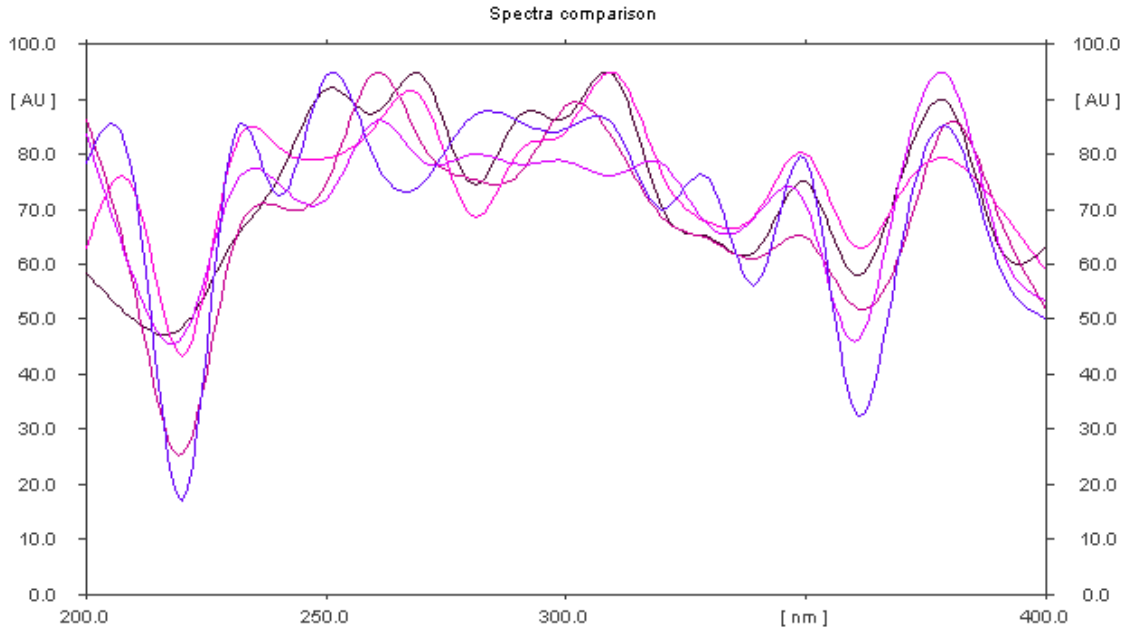
Track S – Formulation Mixture Scanning baseline display @ 254nm



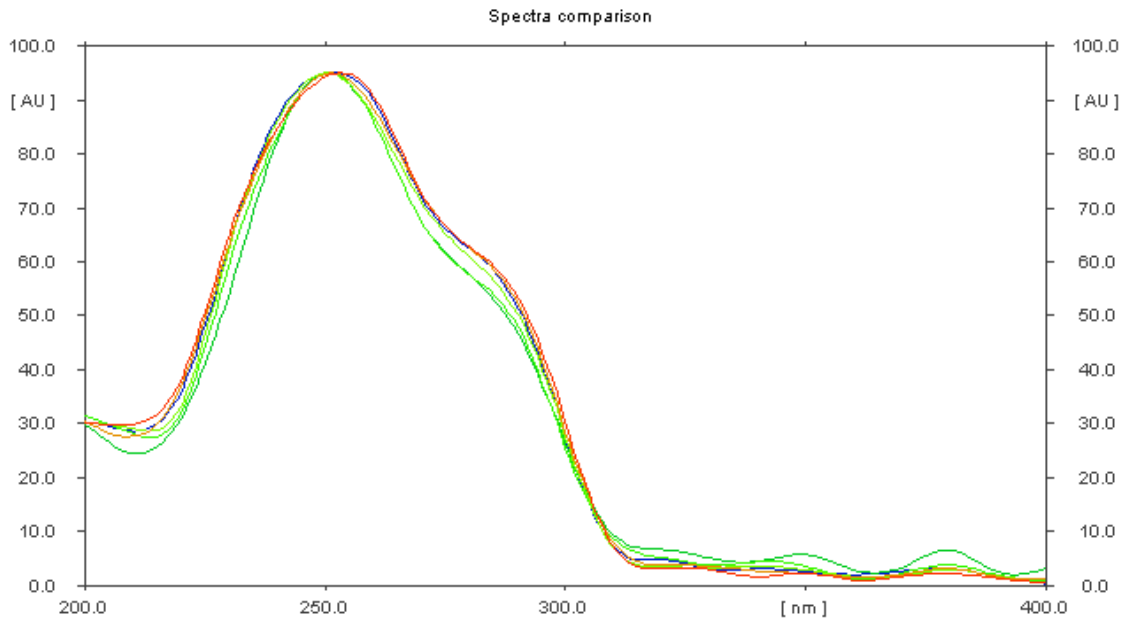
Track S – Formulation Mixture Scanning Peak densitogram display @ 254nm



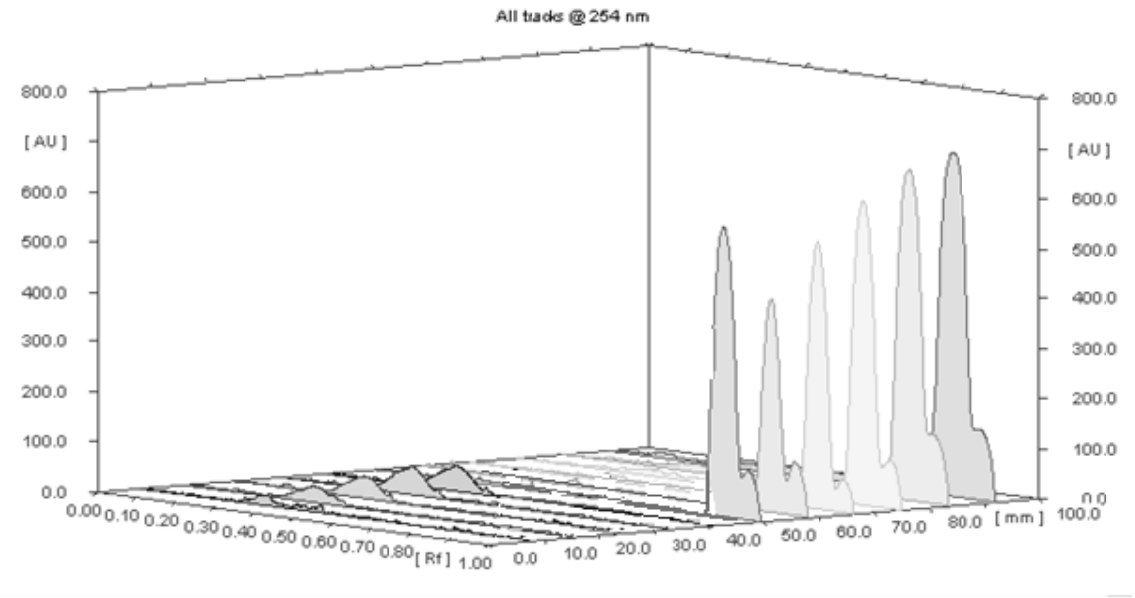
UV Spectrum of Ondansetron Hcl in Standards and Sample (λ max – 309nm)



UV Spectrum Of Paracetamol Hcl in Standards and Sample (λmax 252 nm)



3D display of Peak densitogram – All tracks (Ondansetron Hcl standards & Paracetamol standards and sample) @ 500nm



RESULT & DISCUSSION

UV SPECTROPHOTOMETRY

From the optical characteristics of the proposed methods, it was found that the λmax of Ondansetron Hcl and Promethazine Hcl was found to 310 nm and 250 nm. Ondansetron Hcl and Promethazine Hcl obey linearity within the concentration range of 5-25 µg/ml and 10-50 µg/ml respectively from the result shown in table 3. The linearity was shown by calibration curve and the correlation coefficient (r2) values are 0.998 & 0.999 for Ondansetron Hcl and Promethazine Hcl respectively. Fig .12 & 13. From the table.1 .it was found that the %RSD is less than 2 % which indicates that the method has good reprocibility. The % recovery values of pure drug from the preanalyzed formulations were in between 99-103 % indicates that the proposed method is accurate and reveals that the commonly used excipients and additives in formulations were not interpreting in proposed method. The results of validation parameters for the developed methods were reported in table.2. The analysis of formulation showed good result in concentration in range 98-101%.Table 4.

Table 17

Results on Application of UV-Spectroscopy

Recovery Level		**Amt. of drug added (mg)**		**Amt. of drug found (mg)**		**Mean**		**% Recovery *** +%RSD	
		Ond Hcl	**Pro Hcl**	**Ond Hcl**	**Pro Hcl**	**Ond Hcl**	**Pro Hcl**	**Ond Hcl**	**Pro Hcl**
Level 1	80%	3.2	20	3.18	20.21	3.2	20.08	100.0%±0.05	100.40%±0.11
				3.19	20.07				
				3.23	19.98				
Level 2	100%	4	25	4.04	24.90	4.01	25.03	100.25%±0.03	100.12%±0.17
				4.01	25.23				
				3.98	24.97				
Level 3	120%	4.8	30	4.81	29.97	4.80	30.03	100.1%±0.005	99.91%±0.07
				4.80	30.02				
				4.81	30.11				

RSD of three determinations

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

During the stage of method development different mobile phase were tried and the mobile phase comprising of Dichlormethane : Methanol in the proportion of 9:1 v/v for Ondansetron Hcl and Paracetamol respectively were found to be better and produced the Rf value of 0.34 & 0.90 for Ondansetron Hcl and Paracetamol respectively.

The linearity of both the drugs were determined by calibration curves and the linearity based on the area observed in the range of 100-500ng/µl & 10-50µg/µl for Ondansetron Hcl and Paracetamol respectively. Table 6.Fig 14 & 15.

Recovery studies were carried out for the accuracy parameter and were reported in table 13.

The validation parameters like Calibration Range 100-500ng/µl & 10-50µg/µl for Ondansetron Hcl and Paracetamol. Simultaneously, Correlation co efficient (r2), Repeatability, are tabulated in table 12.

The validate method was applied for the analysis of the tablets containing 4 mg and 500 mg mg of Ondansetron Hcl and Paracetamol respectively as label claim. An average quantity of Ondansetron Hcl and Paracetamol were 498.65±0.59 and 4.0±0.09 respectively.

The results are tabulated in table 13.

The methods developed were simple. It has showed a good peak and good Rf values. The Proposed methods are specific,precise,accurate,and reproducible. Hence the proposed method can be used for the routine assay Ondansetron Hcl and Paracetamol.

RESULTS

APPLICATION OF UV-SPECTROPHOTOMETRY FOR SIMULTANEOUS ESTIMATION

- **Instrument used-** Elico SL 164 UV-VIS Spectrophotometer
- **Solvent selected-**Chloroform
- **Wavelength used-**310 nm for Ondansetron Hcl & 250 nm for Promethazine Hcl

VALIDATION PARAMETERS

Parameters	Ondansetron Hcl 310 nm	Promethazine Hcl 250nm	Limit	Pass/Fail
Linearity Range(µg/ml)	5-25 µg/ml	10-50 µg/ml	No limit	Pass
Coefficient of Correlation	0.998	0.999	NLT 0.999	Pass
Precision (%RSD, n = 6) Intra-day(n = 3) (%RSD) Inter-day (n = 3) (%RSD)	0.39-1.47 0.13-1.38 0.22-0.96	0.23-77 0.29-1.08 0.25-1.38	 RSD NMT 2%	 Pass
Accuracy (% Recovery) 80% 100% 120%	 100.0%±0.05 100.25%±0.03 100.1%±0.005	 100.40%±0.11 100.12%±0.17 99.91%±0.07	 97-103 %	 Pass

APPLICATION OF HPTLC IN ONDANSETRON HCL AND PARACETAMOL

- **Instrument Used-**Camag
- **Mobile Phase-** Dichloromethane :Methanol (9:1)
- **Run time-**20 minutes
- **Wavelength used-**254 nm

VALIDATION PARAMETERS

Parameters	Ondansetron Hcl	Paracetamol	Limits	Pass/Fail
Linearity Range(µg/ml)	100-500 ng/µl	10-50 µg/µl	No limit	Pass
Coefficient of Correlation	0.999	0.999	NLT 0.999	Pass
Precision (%RSD, n = 6) Intra-day(n = 3) (%RSD) Inter-day (n = 3) (%RSD)	0.27 0.09 – 0.18 0.02 – 0.03	0.11 0.006-0.20 0.003-0.02	RSD NMT 2%	Pass
Accuracy (% Recovery) (n = 3) %RSD 80 % 100% 120%	 99.79%±0.02 100.0%+0.07 99.79%+0.02	 99.91%±0.71 99.72%+0.59 99.91%+0.71	97-103 %	Pass
Rf Value	0.34	0.90	Less than 1	Pass

SUMMARY AND CONCLUSION

In order to develop a UV and HPTLC effective most of the effort should be spent in method development and optimization as this will improve the final method performance. A well developed should be easy to validate. A method should be developed with the goal to rapidly preclinical sample, formulation prototype and commercial samples.

Review of the literature on Ondansetron Hcl and Promethazine Hcl & Ondansetron and Paracetamol strongly indicates that there is no method available for UV and HPTLC method for Simultaneous determination and validation of Ondansetron Hcl and Promethazine Hcl & Ondansetron Hcl and Paracetaml in bulk and pharmaceutical dosage forms,but individual and combination with other drugs methods are available.

- The analytical procedure described for assay represents a specific,linear,precise,accurate and system suitable for Simultaneous determination of Ondansetron Hcl and Promethazine Hcl by UV-Spectroscopy and Ondansetron Hcl and Paracetamol by HPTLC in bulk and pharmaceutical dosage form.
- By observing the validation parameters,accuracy,precision,expressed as % RSD,specificity,linearity (correlation –coefficient). The validation methods can be employed for routine analysis of bulk and formulation for assay.
- The formulations were successfully analyzed using the developed methods.
- The results obtained from the validation parameters met the ICH,FDA and USP requirements as well as obeys Beer's law.

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