DEVELOPMENT AND VALIDATION OF RP – HPLC METHOD, UV SPECTROPHOTOMETRIC SIMULTANEOUS EQUATION METHOD OF DROTAVERINE AND MEFENAMIC ACID IN COMBINED DOSAGE FORM

DEVELOPMENT AND VALIDATION OF HPLC METHOD, UV SPECTROPHOTOMETRIC SIMULTANEOUS EQUATION METHOD OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE IN COMBINED DOSAGE FORM

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Mr. M. Chandran, M.Pharm.,
The Professor and Head
Department of Pharmaceutical Chemistry
Madurai Medical College
Madurai – 625 020.

CERTIFICATE

This is to certify that dissertation entitled **"Development of Validation of RP – HPLC Method, UV – Spectrophotometric Simultaneous Equation Method of Drotaverine Hydrochloride and Mefenamic acid in Combined Tablet Dosage From". "Development and Validation of RP – HPLC method, UV – Spectrophotometric Simultaneous Equation Method of Olmesartan Medoxomil and Hydrochlorothiazide in Combined Tablet Dosage Form."** by **Mrs. J. Anudeepa** in the Department of Pharmaceutical Chemistry, Madurai Medical College, Madurai – 625 020 in partial fulfillment of the requirement for the award of degree of Master of Pharmacy in Pharmaceutical Chemistry during the year 2008-2009.

This dissertation is forward to the controller of Examination, The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

Station   : Madurai   **(M. CHANDRAN)**
Date   :

Mr. R. Tharabai, M.Pharm.,
Assistant Reader
Department of Pharmaceutical Chemistry
Madurai Medical College
Madurai – 625 020.

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Station   : Madurai   **(R.THARABAI)**
Date   :

INTRODUCTION

"Analytical Chemistry is what analytical chemist do". Analytical Chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both Qualitatively (what is present) and Quantitatively (how much is present).

Analytical techniques hold the key to the design development standardization and quality control of medical products. They are equally important in pharmacokinetics and in drug metabolism studies both of which are fundamental to the assessment of bioavailability and the duration of clinical response.

Analytical instrumentation plays an important role in the production and evaluation of new products and in the production of consumers and the environment.

Chemical analysis is generally applied in two areas.

- Quantitative analysis
- Qualitative analysis

Quantitative analysis:

The realm of quantitative analysis is to determine how much of each of component or of a specified component is present in the sample.

Qualitative analysis:

The realm of qualitative analysis is to determine the component present if the sample or perhaps confirming its presence (or) absence in the sample.

SPECTROSCOPY

It is the measurement and interpretation of electromagnetic radiations absorbed or emitted when the molecules or atoms or ions of the sample undergo transition from one energy state (ground state) to another (excited state).

UV- Visible spectroscopy

It involves the measurement of amount of UV radiation absorbed by a substance in the solution. The wavelength between 190-390 nm (practically 200-400 nm) is considered to be UV radiations / region.

Wavelength is defined as the distance between any two consecutive parts of the wave whose vibrations are in phase. For example from the crest of one wave to that of the next. Its symbol is λ. Coloured compounds absorb in visible range i.e. 400 – 800 nm. The wavelength at which maximum absorbance takes place is called as λmax.

The assay of the absorbing substance can be carried out by using:

- Standard absorbtivity value
- Use of calibration graph
- Single point standardization
- Standard absorbtivity value

This procedure is adopted by official compendia for the stable substance that have reasonably broad absorption bands and which are practically unaffected by variation of instrumental parameters. The use of standard A(1% 1cm) value avoids the need to prepare a standard solution of the reference substance in order to determine its absorpitivity.

Use of calibration graph:

In this procedure the absorbance of a number typically (4 – 6) of standard solution of the reference substance at concentrations encompassing sample concentration are measured and the calibration graph is constructed.

The concentration of the analide in the sample solution is read from the graph as a concentration corresponding to the absorbance of the solution.

Single point standardization

This procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The standard and sample solutions are prepared in the reference manner. Ideally of the concentration of the standard solution should be close to that sample solution. The concentration of the substance in the sample is calculated using

$$C_{test} = \frac{A_{text} \times C_{standard}}{A_{standard}}$$

Where C_{test} and $C_{standard}$ are the concentrations in the sample and standard solution A_{text} and $A_{standard}$ are the absorbance of the sample and standard solution respectively. The use of UV and visible spectroscopy for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength. The analysis of mixtures of two or more complements is facilitated by activity of absorbance. Other applications include measurement of absorption of complexes to establish their composition of all chromogenic compounds are not suitable for quantitative measurement i.e., the choice of the system and procedure depends largely on the chemistry of the species to be determined.

Assay of substances in multi component samples they are:

- Simultaneous Equation method
- Absorbance ratio method
- Derivative spectroscopy method
- Chemical derivatisation method
- Multi – component mode of analysis

Simultaneous Equation method:

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λmax of the other. It may be possible to determine the quantity of both drugs by the technique of simultaneous equation (or) Vierodt's method.   Criteria   for   obtaining

maximum precision based upon absorbance ratios have been suggested that place limits on the relative concentrations of the component of the mixture.

$$\frac{A2/A1}{ax_2/ax_1} \qquad \text{and} \qquad \frac{ay_1/ay_2}{A2/A1}$$

Where

ax_1, ax_2	= Absorptivities of X at λ_1 and λ_2
ay_1, ay_2	= Absorptivities of Y at λ_1 and λ_2
A_1, A_2	= Absorbances of the diluted Sample at λ_1 and λ_2

The ratio should lie outside the range f $0.1 - 2.0$ for the precise determination of (Y and X) two drugs respectively.

These criteria are satisfactory only when the λ_{max} of the two components is reasonable dissimilar. The additional criteria includes that the two additional criteria do not interact chemically thereby negating the initial assumption that he total absorbance is the sum of the individual absorbance's.

Derivative spectroscopic method:

This method involves the conversion of normal spectrum to its first, second or higher derivative spectrum the transformations that occur in the derivation spectra are understood by reference to an ideal absorption band. For the purpose of spectral analysis to relate chemical structure to electronic transition and for analysis situations in which mixtures contribute interfering absorption. A method of manipulating the spectral data called derivative spectroscopy was developed.

In this technique spectra are obtained by plotting the first or higher derivation of absorbance or transmittance with respect to wavelength versus wavelength. Often these plots reverse spectral details which is lost in an ordinary spectrum. In addition concentration measurement of an analyte in the presence of their interference can something be made easily or more accurately.

Chemical Derivatisation Method:

Indirect spectrophotometic assays are based on the conversion of the analyte by a

chemical reagent to a derivative that has different spectral properties.

This method is employed:

If the analyte absorbs weekly in the uv-region, a more sensitive method of assay is obtained by converting the substance to a derivative with a more intensely absorbing chromophore.

- The interference from irrelevant absorption may be avoided by converting the analyte to its derivative which absorbs in the visible region where irrelevant absorption is negligible.
- Indirect Spectrophotometric procedure is also used to improve the selectivity of the assay of an ultra violet absorbing substance in a sample that contains other UV-absorbing components.

CHROMATOGRAPHY

Modern pharmaceutical formulations are complex mixtures containing one or more therapeutically active ingredients., to a number of inert materials like diluents, disintegrants, colourants and flavours in order to ensure quality and stability of the final product, the pharmaceutical analyst must be able to separate the mixtures into individual components prior to quantitative analysis. Amongst the most powerful techniques available to the analyst for the separation of these mixtures, a group of highly efficient methods which are collectively called as chromatography. It's group of technique which works on the principle of separation of components of a mixture into individual components, depending on their affinities for the solutes between two immiscible phases. One of the phases is affixed bed of large surface area, while the other is a fluid, which moves through the surface of the fixed phase. The fixed phase is called stationary phase and the other is termed as the mobile phase. Depending on the type of chromatography employed the mobile phase may be a pure liquid or a mixture of solutions (eg.buffer) or it may be gas (pure or homogenous mixture).

Classification of chromatography:

It can be classified according to the nature of the stationary and mobile phases.

The different types of chromatography are

- Adsorption chromatography
- Partition chromatography
- Ion exchange chromatography
- Size exclusion or gel permeation chromatography

The modern instrumental techniques GLC, HPLC and HPTLC provide excellent separation and allow accurate assay of very low concentrations of wide variety of substance in complex mixtures.

High Performance liquid chromatography:

HPLC is one among most useful tools available for quantitative analysis. Reverse phase chromatography refers to the use of a polar mobile phase with a non polar stationary phase in contract to normal phase being employed with a non polar mobile phase.

Liquid chromatography is based upon the phenomenon that, under the same conditions, each component in a mixture interacts with its environment differently from other components. Since HPLC is basically a separating technique analysis. It is always used in conjunction with another analytical tool for quantitative and qualitative analysis. The mode of operation of this system is isocratic. i.e. one particular solvent or mixture is pumped throughout the analysis for some determinations the solvent composition may be altered gradually to give gradient elution.

The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography. Advances in column technology high pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation.

Column Detector Interface
Loop
Data station

Injection valve Pump
Mobile phase (eluent)
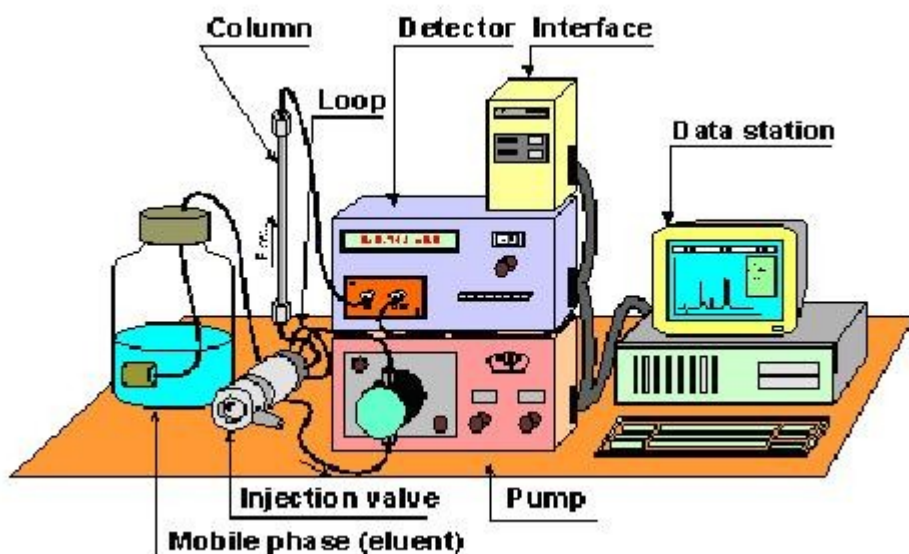
Modes of separation:

In normal phase mode the stationary phase (e.g. silica gel) is polar in nature and the mobile phase is non-polar. In this technique non-polar compounds travel faster and are eluted first. This is because less affinity between solute and stationary phase. Polar compounds are retained for longer time in the column because more affinity towards stationary phase and takes more time to be eluted form the column. This is not advantageous in pharmaceutical applications since most of the drug molecules are polar in nature and takes longer time to be eluted and detected. Hence this technique is not widely used in pharmacy.

Importance of polarity in HPLC:

Polarity is a term that is used in chromatography as an index of the ability of compounds to interact with one another. It is applied very freely to solutes. Stationary and mobile phase HPLC, the eluting power or solvent strength of the mobile phase is mainly determined by its polarity. If the polarities of stationary phase and the mobile phase are similar, it is likely that the interactions of solute with each phase may also be similar, resulting in poor separation. Retention of solutes is usually altered by changing the polarity of the mobile phase.

Packing materials and mobile phases:

Many of the packing materials used in HPLC are based on materials used in classical column chromatography. The commonly used packing materials and their associated solvent systems in each of the chromatographic modes are
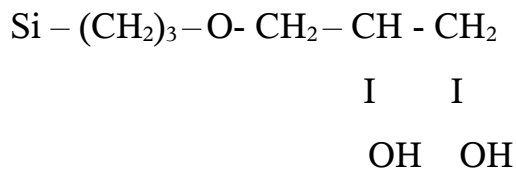
1) Partition HPLC
2) Adsorption HPLC

Adsorption HPLC:

Adsorbents used in classical column chromatography, unmodified silica has proved to be the most widely used in HPLC. The functional group responsible for adsorption is the silanol (Si-OH) group which interacts with the sample solutes by hydrogen bonding. There is therefore increasing retention of solutes with increasing solute polarity. Alumina is used as an adsorbent less frequently than silica, although for some separations in particular of aromatic substances and of structural isomers, greater selectivity is obtained with alumina.

Partition HPLC:

Packing materials based on silica are also used in partition chromatography. Early applications of partition HPLC involved coating the silica with a polar liquid stationary phase. Eg. Ethane – 1, 2-diol. Such packing materials have now been replaced by silica to which polar phases are chemically bonded. Example,
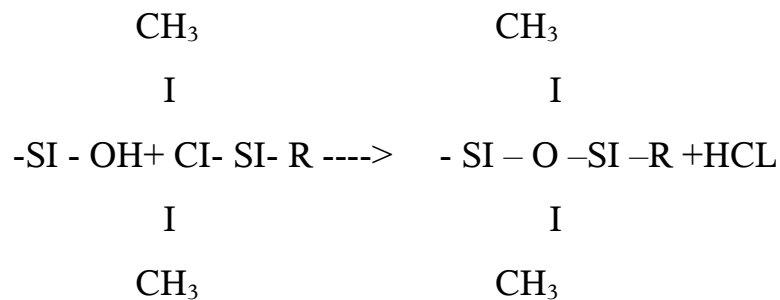
$$Si – (CH_2)_3 – O\text{-} CH_2 – CH \text{ - } CH_2$$
$$\qquad\qquad\qquad\qquad\quad | \qquad |$$
$$\qquad\qquad\qquad\qquad\quad OH \quad OH$$

Eg. LiChrosorb Diol

$$Si – (CH_2)_3 – CN \qquad\qquad e.g.\ Q\ Bond\ pak\ CN$$

Si – (CH$_2$)$_3$ – NH2   e.g. Polygosil NH$_2$

In reversed – phase partition HPLC the relative polarities of the stationary and mobile phase are the opposite to those in normal phase HPLC, i.e., the stationary phase is less polar than the mobile phase, and consequently the solutes are eluted in the order of their decreasing polarities. The stationary phase is silica, chemically bonded through a siloxame (si-o-si-o) linkage to a low polar functional group. These phases are prepared by treating the surface silanol groups of silica with an organochlorosilane reagent

$$\begin{array}{ccc}
CH_3 & & CH_3 \\
I & & I \\
-SI - OH + CI - SI - R ----> & & - SI - O - SI - R + HCL \\
I & & I \\
CH_3 & & CH_3
\end{array}$$

Where R   = C$_6$H$_{13}$ (Hexyl)

C$_8$H$_{17}$ (Octyl)

C$_{18}$H$_{37}$ (Octadecyl)

Untreated silanol groups may be 'capped' by treatment with trimethlchlorosilane to eliminate adsorption effects. The mobile phase in reversed- phase HPLC generally comprises water and a less polar organic solvent modifier. e.g. methonal or acetonitrile. Separations in these systems are considered to be due to different degrees of hydrophobicity of the solutes. The less polar solutes partitioning to a greater extent into the non-polar stationary phase and consequently being retained on the column longer than the more polar solutes. The rate of elution of the components is controlled by the polarity of the organic modifier and its proportion in the mobile phase. The rate of elution is increased by reducing the polarity, e.g. by increasing the

proportion of the organic solvent or by using acetonitrile instead of methanol. The simple alteration of the composition of the mobile phase or of flow rate allows the rate of elution of the solutes to be adjusted to an optimum value and permits the separation of a wide range of chemical types.

Detectors:

The detection of the separated components in the eluate from the column is based upon the bulk property or the solute property of the individual components. The most commonly used detectors in the HPLC are:

I. Photometric detectors:

These normally operate in the ultraviolet region of spectrum and are the most extensively used detectors in pharmaceutical analysis. They are of five principal types :

- Single wavelength detectors
- Multi-wavelength detectors
- Variable wavelength detectors
- Programmable detectors
- Diode array detectors

ii. **Fluorescence detectors:**

These are essentially filter fluorimeters or spectrofluorimeters equipped with grating monochromators and micro flow cells. Their sensitivity depends on the fluorescence properties of the components in the elute.

iii. **Refractive index detectors:**

These are differential refractometers which respond to the change in the bulk property of the refractive index of the solution of the component in the mobile solvent system. The sensitivity of the refractive index detector is much less than that of specific solute property detectors.

iv. Electrochemical detectors :

These are based on standard electrochemical principles involving amperometry voltammetry and polarography. These detectors are very sensitive for substances that are eletroactive.

Validation [8, 9,10]

Validation means assessment of validity or action of proving effectiveness. Method validation is the process of proving that in analytical method is acceptable for its intended purpose. For pharamaceutical methods guidelines from the united states pharmacopeia (USP), international conference on harmonization (ICH) and the food and drug administration (FDA) provide a frame work for performing such validations

Parameters used for assay validation

The validation of the assay procedure was carried out as per ICH guidelines using the following parameters.

Specificity:

Specificity is the ability to asses unequivocally the analyte in the presence of components which may be expected to be present, lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. For the chromatographic methods developing a separation involves demonstrating

specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components (placebo formulation, process impurities, etc.) is compared with the response of a solution containing only the analyte. Specificity criteria for an assay method is that the analyte peak will have baseline chromatographic resolution of at least 1.5 from all other sample components. If this cannot be achieved the unresolved components at there maximum expected level will not affect the final assay result by more than 0.5%

Linearity :

       Linearity of an analytical procedure is its ability (within a given range) to obtained test results, which are directly proportional to the concentration (amount) of analyte in the sample. For assay methods, this study is generally performed by preparing standard solutions at five concentration levels. Five levels are required to allow detection of curvature in the plotted data acceptability of linearity data is often judged by examining   the correlation and y – intercept of the linear regression line for the response versus concentration plot. A correlation coefficient of >0.999 is generally considered as evidence of acceptable fit of the data to the regression line. The y- intercept should be less than a few percent of the response obtained for the analyte at the target level.

Accuracy:

       The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of a method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined in one of four ways.

1. Accuracy can be assessed by analyzing a sample of known   concentration and comparing the measured value to the true value.

2. To compare test results from the new method with results from an existing alternate method that is known to be accurate.

3. To most widely used recovery study, is performed by spiking analyte in blank matrices. For assay methods, spiked samples are prepared in triplicate at three levels over a range of 50-150% of the target concentration.

4. The technique of standard additions, which can also be used to determine recovery of spiked analyte. This approach is used if it is not possible to prepare a blank sample matrix without the presence of the analyte. Accuracy criteria for an assay method is that the mean recovery will be 100+2% at each concentration over the range of 80-120% of the target concentration.

Range:

Range of analytical procedure is the interval between the upper and lower concentration amounts of analyte in the sample including these concentration for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range is determined using data from the linearity and accuracy studies. Range criteria for an assay method is that the acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained per previously discussed criteria and that yields a precision of 3% RSD.

Precision:

The precision of an analytical procedure express the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed the variance, standard deviation or coefficient of variance of a series of measurements. The first type of precision study is instrument precision or injection repeatability. A minimum of 10 injections of one sample solution is made to test the performance of the chromatographic instrument. The second type is repeatability or intra-assay precision. Intra- assay precision data are obtained on one day. Aliquots of a homogenous sample, each of which has been independently prepared according to the method procedure from these precision studies, the sample preparation procedure the number of replicate samples to be prepared, and the number of injections required for each sample in the final method is that the instrument precision (RSD) will be 1% and the intra assay precision will be 2%

Detection limit

The detection limit of an individual analyte procedure is the lowest amount of analyte in a sample which can be detected but not necessarily qualitated as an exact

value. Detection limit based on the standard derivation of the response and the slope.

Detection limit (or) limit of detection may be expressed as,

$$DL = \left[\frac{3.3 \times \sigma}{m} \right]$$

Where

σ = standard deviation of the response

m = slope of the calibration curve (of the analyte)

Quantitation limit:

The quantitation of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation limit based on the standard deviation of the response and the slope. It can be expressed as,

$$QL = \left[\frac{10 \times \sigma}{m} \right]$$

Where

σ = standard deviation of the response

m = slope of the calibration curve (of the analyte)

Ruggedness:

The ruggedness of an analytical method is the degree of reproducibility of test result obtained, the analysis of conditions such as different laboratories, different analysis using different instrument, on different days. Different source of reagent,

elapsed assay times, assay temperature conditions. Ruggedness is a measure of reproducibility of test result under the variation in conditions normally expected from analyst to analyst. The criteria of the ruggedness is the RSD should be not more than 2%.

Robustness:

The robustness of a method is its ability to remain unaffected by small changes in parameter such as percent organic content and p^H of the mobile phase, buffer concentration and temperature and injection volume. These method parameters may be evaluated one factor at a time or simultaneously as part of a factorial experiment. The criteria for robustness is the RSD should be not more than 2%.

System suitability testing:

System suitability testing is an integral part of many analytical procedures the tests are based on the concept that the equipment, electronics, analytical operation and samples to be analyzed constitute an integral system that can be evaluated as such. Typically the process involves making five injections of a standard solution and evaluating several chromatographic parameters such as resolution, area % reproducibility, number of theoretical plates and tailing factor.

Retention time: (R_t)

This is the time of emergence of the peak maximum of a compound after injection.

Retention volume: (RV)

The volume of mobile phase requires to elute one half of the compound from the column as indicated by the peak maximum and is given by $R_v = R_t$ x flow rate

Asymmetry factor (or) tailing factor (T)

$$T = \frac{Y_x}{2A}$$

where,

Y_x = the width of the peak at one twentieth of the peak height.

A = the distance between the perpendicular dropped from the peak

  Maximum and the leading edge of the peak at one twentieth of the

  peak height.

The value lies between 0.95 to 1.05.

Number of theoretical plates (N)

$$N = 5.54 \times \frac{(R_t)^2}{W_{h/2}}$$

The assessment of performance of efficiency of a column is in terms of the number of theoretical plates.

R_t = retention time

W = width of peak at half height

Resolution (R):

$$R = \frac{2 (R_{t2} - R_{t1})}{W_{t1} + W_{t2}}$$

This gives the resolution between the measured peaks on the chromatogram.

Where

Rt_1 and Rt_2 = retention time of two component.

Wt_1 and Wt_2 = the respective peak width. Resolution between measured peaks on the chromatogram must be > 1

STATISTICAL PARAMETERS

Statistics consists of a set of methods and rules for organizing and interpreting observations.

Lineanrity coefficient (γ)

$$Y = \frac{\sum (X-\bar{X})(\bar{Y}-Y)}{\sum (X-\bar{X})^2 (\bar{Y}-Y)^2}$$

Slope (m)

$$m = \frac{\sum (\bar{X}-X)(\bar{Y}-Y)}{\sum (X-\bar{X})^2}$$

Standard deviation (σ)

$$(\sigma) = \frac{\sum (X-\bar{X})^2}{n-1}$$

$$R.S.D\ (\%) = \frac{S.D}{X} \times 100$$

Where

$\sum$	=	Sum of observation
$\bar{X}$	=	Mean of arithmetic average ($\sum x/n$)
X	=	Individual observed value.
x-x	=	Deviation of a value from the mean
N	=	Number of observation
R.S.D	=	Relative standard deviation

Now in advanced type of analytical instrument the number of theoretical plate per column/ meter and tailing factor will be displayed automatically.

DEVELOPMENT OF REVERSE PHASE HPLC METHOD6

Information on Sample

```
┌─────────────────────────────────────┐
│        Define Separation Goals       │
└─────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────┐
│    Need for Special HPLC Procedure,  │
│      Sample Pretreatment, etc ?      │
└─────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────┐
│   Choose Detector and Detector Settings │
└─────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────┐
│   Choose HPLC Method, Preliminary Run, │
│    Estimate Best Separation Conditions │
└─────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────┐
│      Optimize Separation Conditions  │
└─────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────┐
│        Check for problems ( or)      │
│    Requirement for special procedure │
└─────────────────────────────────────┘
         │           │            │
         ▼           ▼            ▼
```

Recover Purified Material   Quantitative Calibration   Qualitativemethod

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         │           │            │
         ▼           ▼            ▼
┌─────────────────────────────────────┐
│    Validation for Release to laboratory │
└─────────────────────────────────────┘
```

"Validation does not make a method good or efficient: it merely demonstrates that an analytical method performs in accordance with the claims made for it".

REVIEW OF LITERATURE

1. **EI – saharty-ys et al.,** carried out the study describing the application of new membrane selective electrodes for the determination of drotaverine hydrochloride in tablets and plasma is presented, including method validation [17].

2. **Girgis – EH et al.,** carried out the ion – pair reversed phase liquid chromatographic identification and quantitation of papaverine congeners[18].

3. **Regdon – G et al.,** formulated and prepared drotaverine hydrochloride (No – spa) suppository with excellent drug release and storage characteristics. Determination of release of active Principle by membrane diffusion method[19].

4. **Rutz – Coudray – MH et al;** developed the compartmental analysis for the development of long acting dosage forms experience with drotaverine hydrochloride in humans[20].

5. **Ushbaev – KU et al;** developed a spectrophotometric method for the determination of drotaverine hydrochloride (No spa) in tablets [21].

6. **Bindiya Gupta et al;** Studied the augmentation of labour by Drotaverine hydrochloride versus hyoscine – N – bytyl bromide[22].

7. **Metwally FH et al;** Carried out the simultaneous determination of nifuroxazide and drotaverine hydrochloride in pharmaceutical Preparations by bivariate and multivariate spectral analysis[23].

8. **EI – sheikh R, etcl;** carried out the spectrophotometric determination of some anti-tussive and anti-spasmodic drugs through ion-pair complex formation with thiocyanate and cobalt (II) or Molybdenum(V)[24].

9. **AminAset al;** carried ot the spectro photometric determination of pipazethate Hcl, dextromethorphan HBr and drotaverine Hcl in their Pharmaceutical preparations. Using chromotrope 2B (C2B) and chromotrope 2R (C2R). The 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 drotaverine HCI. The ion-associates exhibit absorption maxima at 528, 540 and 532 nm with C2B and at 526, 517 and 522 nm with C2R for pipazethate HCI, dextromethorphan HBr and drotaverine HCI, respectively. The calibration curves resulting from the measurements of absorbance-concentration relations (at the optimum reaction conditions) of the extracted ion-pairs are linear over the concentration range 4.36-52.32 microg mL (-1) for pipazethate, 3.7-48.15 , microg mL(-1) for dextromethorphan and 4.34-60.76 microg mL(-1) for drotaverine, respectively[25].

10. **Metwally FH, et al;** Determination of nifuroxazide and drotaverine hydrochloride in pharmaceutical preparations by three in dependent analytical method. The first method was spectrophotometric, which allowed determination of 1 in the presence of II using a zero – order spectrum with an analytically useful maximum at 364.5 nm that obeyed Beer's law over a concentration range of 2-10 microg/mL with mean percentage recovery of 100.08+/- 0.61. Determination of II in presence of I was obtained by second derivative spectrophotometry at 243.6 which obeyed Beer's law over a concentration range of 2-10 microg/mL with mean recovery of 99.82+/- 1.46%. The second method was spectrodensitometry, with which both drugs were separated on a silica gel plate using chloroform-acetone-methanol-glacial acetic acid (6+3+0.9+0.1) as the mobile phase and untraviolet (UV) detection at 365 nm over a concentration range of 0.2-1 microg/band for both drugs, with mean recoveries of 99.99+/- 0.15 and 100.00 +/- 0.34% for I and II, respectively. The third method was reversed phase liquid chromatography using acetonitrile-water (40+60, v/v; adjusted to pH 2.55 with orthophosphoric acid) as the mobile phase and pentoxifyline as the internal standard at a flow rate of 1 mU/min UV detection at 285 nm at ambient temperature over a concentration range of 2-10 microg/mL for both drugs, with mean recoveries of 100.24+/- 1.51 and 100.08 +/- 0.78% for I and II, respectively

11. **EI – saharty ys et al;** studied the application of new membrane selective electrodes for the determination of drotaverine hydrochloride in tablets and plasma[27].

12. **Daabees, H.G. et al;** carried out the selective Differential spectrophotometric Methods for Determination of Niclosamide and Drotaverine hydrochloride. Differential (DEL TAA) second derivative (DELTAD2) AND third derivative (DELTAD3) differential untraviolet spectrophotometric methods have been presented for the quantitation of niclosamide and drotaverine hydrochloride in pure forms and in their pharmaceutical formulations. For niclosamide, the method is based on measuring DELTADELTA A, DELTA DEL TAD2, DELTA DELTAD3 of niclosamide in alkaline solutions against heir neutral ethanolic solutions as blanks. For drotaverine hydrochloride, the acidic solutions of the drug are measured against their alkaline solutions as blanks[28].

13. **Fadia H Metwally et al;** studied the application of derivative, derivative ratio and multivariate spectral analysis and thin layer chromatography – densitometry for determination of a ternary mixture containing drotaverine hydrochloride, caffeine and Paracetamol[29].

14. **Ruiz TP et al;** carried out the analysis of binary mixtures of flufenamic, meclofenamic and mefenamic acids by derivative synchronous fluorescence spectrometry. The method is based on the intrirsic fluorescence of these compounds in chloroform, Serum samples are treated with trichloroacetic acid to remove the proteins, an the analytes are extracted in chloroform Prior to determination[30].

15. **Nebot C et al;** Studied the Quantification of human pharmaceuticals in water samples of high performance liquid chromatography – tandem mass spectrometry [31].

16. **Hung cy et al;** carried out the Analysis of Ketoprofen and mefenamie acid by high – performance liquid chromatography with molecularly imprinted polymer as the stationary phase, A simple sensitive HPLC method for simultaneous determination of the above said, HPLC with UV detection (220nm) was performed on an analytical column packed with molecularly imprinted polymer (MIP) as the stationary phase[32].

17. **Yogini jaiswal et al;** Studied the application o HPLC for the simultaneous determination of Ethamsylate and mefenamic acids in Bulk Drugs and Tablets used with PDA detection. Ibuprofen was used as the internal standard, which made the analyses more accurate[33].

18. **P.P.Dahivelkar   et   al;** carried   out   the   simultaneous   derivative   and

multicomponent spectrophotometric determination of drotaverine hydrochloride and mefenamic acid in tablets. Both the drugs obey the Beer's law in the Concentration range employed for these methods[34].

19. **Bier- MC et al;** carried out the determination of olmesartan medoxomil in tablets by UV – Vis spectrophotometry. The Solutions of standard, tablets and synthetic tablets were prepared in acetonitrile an NaoH – water, at 258 nm and 250 nm respectively. The method was validated. The linearity range of method was 1.0 – 70.0 mg tml -1 and 1.0 – 75.0 mg tmL-1 for acetonitrile and NaoH respectively[35].

20. **Jiang – JJ et al;** evaluated the relative bioavilability of olmesartan medoxomil tablet (test) versus capsule (reference). 20 healthy male volunteers received a single crossover dose of test and reference olmesartan medoxomil 20mg. The plasma concentrations of olmesartan were determined by HPLC[36].

21. **CHEN – m et al;** carried out the High performance liquid chromatography determination of the olmesartan medoxomil and hydrochlorothiazide tablets, with Potassium hydrogen phosphate (PH 3.6 adjusted with phosphoric acid) acetonitrile (50:50) with flow rate of lml [37].

22. **Murakami T et al;** studied the identification of degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC Hyphenated techniques [38].

23. **Liv D et al;** Carried out the Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry (HPLC – MS / MS) Solid phase extraction (SPE) was used to isolate the compounds from biological matrix followed by injection of the extracts onto a C(18) column with isocratic elution [39].

24. **Celebier et al;** Carried out the determination of olmesartan medoxomil in tablets by UV – Visible spectrophotometry [40].

25. **Najma sultana et al;** carried out the simultaneous determination of olmesartan medoxomil and irbesartan and hydrochlorothiazide in pharmaceutical formulations and human serum using high performance liquid chromatography. Good chromatographic separation was achieved using a μ-BondPak, C18 column The ultraviolet detector was set at a wave – length of 260 nm [41].

26. **Valizedh H et al;** developed a simple and rapid high performance liquid chromatography method with UV detection at 280 nm for simultaneous quantitation of furosemide and hydrochlorothiazide along with phenol red as a non absorbable marker for in situ permeability studies in anathetized rats[42].

27. **Ramakrishna NV et al;** developed a sensitive liquid chromatography tandem mass spectrometery method for quantification of hydrochlorothiazide in human plasma, tamsulosin was internal standard extracted by liquid – liquid extraction with diethyl ether – dichloromethane (70:30, v/v) using a glas – col malti pulse Vortexer [43].

28. **Henion JD et al;** carried out the qualitative and quantitative analysis of hydrochlorothiazide in equine plasma and urine by high performance liquid chromatography. Thin layer chromatography is used to screen for the presence of the drug in unknown samples. An internal standard, trichloromethiazide is used to derive quantitative data at concentrations as low as 25ng / ml for plasma disappearance curves and urinary excretion rates [44].

29. **Zendelovska D et al;** Developed a solid phase extraction method and its application for determination of hydrochlorothiazide in human plasma using HPLC [45].

30. **Abdel Razak O et al;** developed a electrochemical study of hydrochlorothiazide

and its determination in urine and tablets. The voltametric study of (HCTZ) at glassy carbon electrode was carried out, the drug in Britton – Robinson buffer (PH 3.3) is oxidized at +1040mv giving rise to a well defined peak. Cyclic Voltametric study indicates that the oxidation process is irreversible and diffusion controlled [46].

31. **Garg G et al;** developed a sepectrophotometric and column high Performance liquid chromatographic methods for simultaneous estimation of metoprolol for simultaneous estimation of metoprolol tartrate and hydrochlorothiazide in tablets Paracetamol was used as internal standard [47].

32. **Lusis ML et al;** carried out the Simultaneous spectrophotometric determination of diuretics by using multivariate calibration methods, under the optimum conditions thus established, synthetic mixtures of the analytes can be resolved with errors and RSD less than 4.5 and 1.0% respectively [48].

33. **Maggio RM et al;** developed a multivariate approach for the simultaneous determination of losartan Potassium and hydrochlorothiazide in a combined pharmaceutical tablet formulation[49].

34. **Obando MA et al;** carried out the simultaneous determination of hydrochlorothiazide and lorsartan Potassium in tablets by high Performance low Pressure chromatography using a multi syringe burette coupled to a monolithic column. The system comprised a multi syringe module, three low Pressure solenoid Valves, a monolithic c(18) column and a diode array detector [50].

35. **Menon GN et al;** carried out the simultaneous determination of hydrochlorothiazide and triamterene in capsule formulations by high performance liquid chromatography. Recoveries of the two drugs added to placebos ranged from 98.4 to 101.7 1% [51].

36. **Vrani CE et al;** studied the influence of tablet splitting on content uniformity of lisinopril / hydrochlorthiazide tablets. The aim of this study was to tablets. The aim of this study was to establish possible influence of tablet splitting on content uniformity of lisinopril and hydrochlorthiazide was carried out by HPLC Method. The results of content uniformity studies for halves of tablets containing combination of lisinopril – hydrochlorthiazide (supposed to contain 50% of stated 20 / 12.5 mg in the whole tablet) were 49.60 + /- 3.29% and 49.29 +/- 0.60% *lisiropril); 50.33 +/- 3.50% and 50.69 +/- 1.95% (hydrochlorthiazide) [52].

37. **Uchida Y et al;** carried out the multi channel liquid chromatography tandem mass spectrometry cocktail method for comprehensive substrate characterization of multidrug resistance – associated Protein 4 transporter [53].

38. **Song M et al;** carried out the simultaneous determination of amiloride and hydrochlorothiazide in human plasma by liquid chromatography / tandem mass spectrometry with Positive / negative ion switching electrospray ionization [54].

39. **Gao J et al;** developed a liquid chromatography / negative ion electrospray tandem mass spectrometry method for the quantification of rosuvastatin in human plasma, application to a pharmacokinetic study, using hydrochlorothiazide as internal standard[55].

40. **L I H et al;** developed liquid chromatography / tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorthiazide in human plasma [56].

41. **Nirogi RV et al;** developed a simple and rapid HPLC / UV method for the

simultaneous quantification of theophylline and etofylline in human plasma, the analytes and internal standard hydrochlorothiazide were separated using an isocratic mobile phase on a reverse phase C18 column [57].

42. **Vonapartic A et al;** studied the development and validation of a liquid chromatographic / electrospray ionization mass spectrometric method for the determination of benazepril, benazeprilat and hydrochlorothiazide in human plasma[58].

43. **Quaglia MG et al;** carried out the determination of losartan and hydrochlorthiazide in tablets by CE and CEC (capillary electrophoresus) and capillary Electrochromatography respectively. The mobile phase was a mixture of 50 mm ammonium acetate pH 7, water acetonitrile (1/1.5 / 7.5) [59].

44. **Jonczyk A et al;** studied the determination of hydrochlorothiazide, triamterene and propranolol hydrochloride by the spectrophotometric method and high performance liquid chromatography [60].

45. **Shah SA et al;** studied the simultaneous determination of losartan and hydrochlorothiazide in combined dosage forms by first derivative spectroscopy and high performance thin layer chromatography The mobile phase of chloroform – methanol – acetone – formic acid was used in HPTLC, 2 well separated and sharp peaks for LST AND HCTZ were obtained at Rf values of 0.61 +/- 0.02 and 0.41 +/- 0.02 respectively [61].

46. **Franolic JD et al;** Determined the isolation of a 2:1 hydrochlorothiazide formaldehyde adduct impurity in hydrochlorothiazide drug substance by preparative chromatography and characterization by electrospray ionization LC – MS [62].

47. **Satana E et al;** carried out the simultaneous determination of valsaratan and

hydrochlorothiazide in tablets by first derivative ultraviolet spectrophotometry and LC. The derivative procedure was based on the linear relationship between the drug concentration and the first derivative amplitudes at 270.6 and 335 nm for valsartan and hydrochlorothiazide respectively [63].

48. **EI Gindy et al;** studied the application of LC and HPTLC densitometry for the simultaneous determination of benapril hydrochloride and hydrochlorothiazide [64].

49. **Er.K N et al;** developed the Analysis of binary mixtures of losartan Potassium and hydrochlorothiazide by using high performance liquid chromatography, ratio derivative spectrophotometric and compensation technique. The first method based on HPLC on a RP column using mobile phase 0.01 N sodium di hydrogen phosphate : methanol : acetonitrile (8:2:1 v/v/v) PH 5.5 with detection at 265.0 nm. The second method involves the ratio spectra at 238.360 nm and at 230, 425nm were selected to determine losartan Potassium and HCTZ. The third method based on compensation technique is presented for the derivative technique is presented for the derivative spectrophotometric determination of binary mixtures with overlapping spectra [65].

50. **Zecevic M et al;** studied the use of a response surface methodology on HPLC analysis of methyldopa amiloride and hydrochlorothiazide in tablet dosage form [66].

51. **Banaglu E et al;** Studied the Dissolution tests of benazepril – Hcl and hydrochlorothiazide in Commercial tablets: Comparison of spectroscopic and high performance liquid chromatography methods[67].

52. **Belal F et al;** developd A stability indicating LC Method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms. Detection was affected spectrophotometrically at 210nm. Clobazam was used as an internal standard[68].

53. **Meduedovici A et al;** developed a liquid extraction and HPLC– DAD assay of hydrochlorothiazide from plasma for a bioequivalence study at the lowest therapeutic dose. The main parameters considered in optimizing the liquid extraction and quantitative assay were the yield, precision limit of quantification, time required for extraction and oncentration and quanity of solvent. The influence on these parameters of the following factors was examined; nature of the extracting solvent, quantity of sovent, co-extraction solvent, and duration of stirring. Instead of equilibrium parameters of the involved thermodynamic system, a kinetic approach was preferred in terms of the effective partition 'constant' which is not really constant but a function of time and extraction condition [69].

54. **Zecevic M et al;** development the statistical optimization of a reversed phase liquid chromatographic method for the analysis of amiloride and hydrochlorothiazide in tablets [70].

55. **Kartal M et al;** carried out the simultaneous determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative spectrophotometry and high performance liquid chromatography [71].

56. **Panderi IE et al;** carried out the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide in tablets by second – order derivative spectrophotometry[72].

57. **Panderi IE et al;** carried out the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide by micro–bore liquid chromatography [73].

58. **Forthing D et al;** developed a simple method for determination of hydrochlorothiazide in human urine by high performance liquid chromatography utilizing narrowbore chromatography [74].

59. **Revelle LK et al;** studied the indentification of chlorothiazide and

hydrochlorothiazide UV – A photolytic decomposition products [75]

60. **Richter K et al;** developed a new sensitive method for the determination of hydrochlorothiazide in human serum by high performance liquid chromatography with electrochemical detection[76]

61. **el walily et al;** carried out the simultaneous determination of enalapril maleate and hydrochlorothiazide by first derivative ultraviolet spectrophotometry and high performance liquid chromatography with mobile phase acetonitrile – water (20:80, v/v) (PH 3.8) with Programmable detection at 215 and 275 nm [77]

62. **Thomas BR et al;** developed and validated micellar electrokinetic capillary chromatography method for quality control of the drug substance hydrochlorothiazide and chlorothiazide[78]

63. **de Vries et al;** carried out the simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography[79].

64. **Kuo BS et al;** developed column switching high performance liquid chromatographic (HPLC) determination of hydrochlorothiazide in rat dog and human plasma. The method involves direct injection of plasma to the extraction column for sample clean up followed by switching onto the analytical column. Good Precision accuracy and linearity were obtained over a range of 25 to 2000ng/ml in rat, dog and human plasma[80].

65. **Ba chman wj et al;** carried out the HPLC photolysis electrochemical detection in pharmaceutical analysis, application to the determination of spironolactone and hydrochlorothiazide in tablets[81].

66. **Stewart JT et al;** developed the liquid chromatographic determination of quanethidine salts and hydrochlorothiazide using electrochemical detection and ion pair techniques[82].

67. **Barbhaiya RH et al;** carried out the High Pressure liquid chromatographic

determination of chlorothiazide and hydrochlorothiazide in plasma and urine: preliminary results of clinical studies[83].

68. **Ouyang J et al;** carried out the flow injection of hydrochlorothiazide applying sensitized chemileminescence detection, optimisation in view of narrow bore – HPLC [84].

69. **Khedr A et al;** studied 3 – bromo methyl – prophenazone as a new derivatization reagent for high performance liquid chromatography of captopril and hydrochlorothiazide with UV detection [85].

OBJECTIVE AND PLAN OF WORK

❖ Drotaverine Hydrochloride and Mefenamic acid an Antispasmodic and Analgesic drug combination in combined dosage form, is being considered for analysis by UV spectrophotomeric simultaneous equation method as λ max of drug is dissimilar and their absorbance ratio lies outside the range 0.1 to 2. The next aim is to develop and validate a reverse phase high performance liquid chromatography in accordance with USP and ICH guidelines for the assay of the active ingredients which would be simple, rapid, efficient and reliable for the analysis of both the drugs in combined dosage form.

❖ Olmesartan Medoxomil and Hydrochlorothiazide an Antihypertensive and Diuretic drug combination in combined dosage form, is being considered for analysis by UV spectrophotomeric simultaneous equation method as λ max of drug is dissimilar and their absorbance ratio lies outside the range 0.1 to 2. The next aim is to develop and validate a reverse phase high performance liquid chromatography in accordance with USP and ICH guidelines for the assay of the active ingredients which would be simple, rapid, efficient and reliable for the analysis of both the drugs in combined dosage form.

SCHEME OF WORK

a)

Drotaverine Hydrochloride and Mefenamic acid in Combined dosage form

UV Spectrophotometric Simultaneous Equation Method

RP HPLC Method

Recovery Studies

Validation

b).

OLMESARTAN Medoxomil And Hydrochlorothiazide in Combined dosage form

UV Spectrophotometric Simultaneous Equation Method

RP HPLC Method

Recovery Studies

Validation
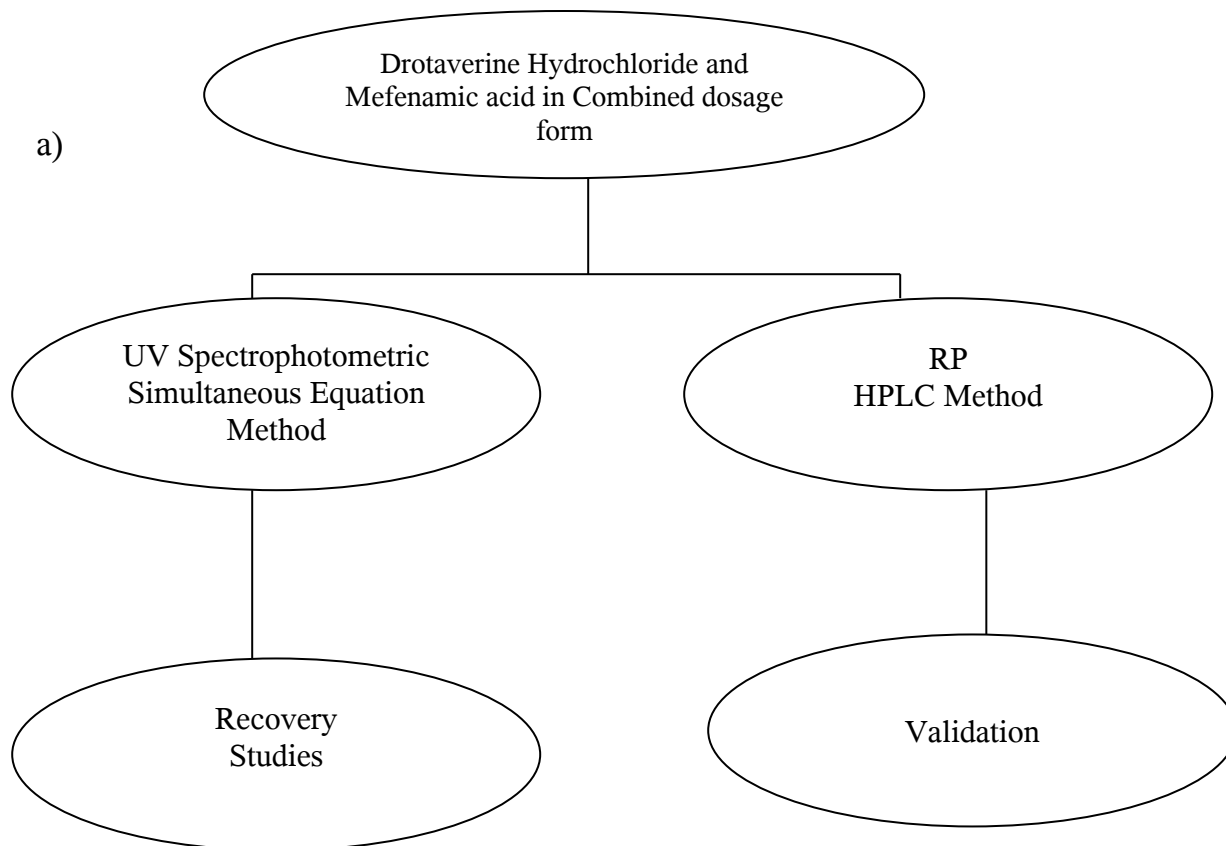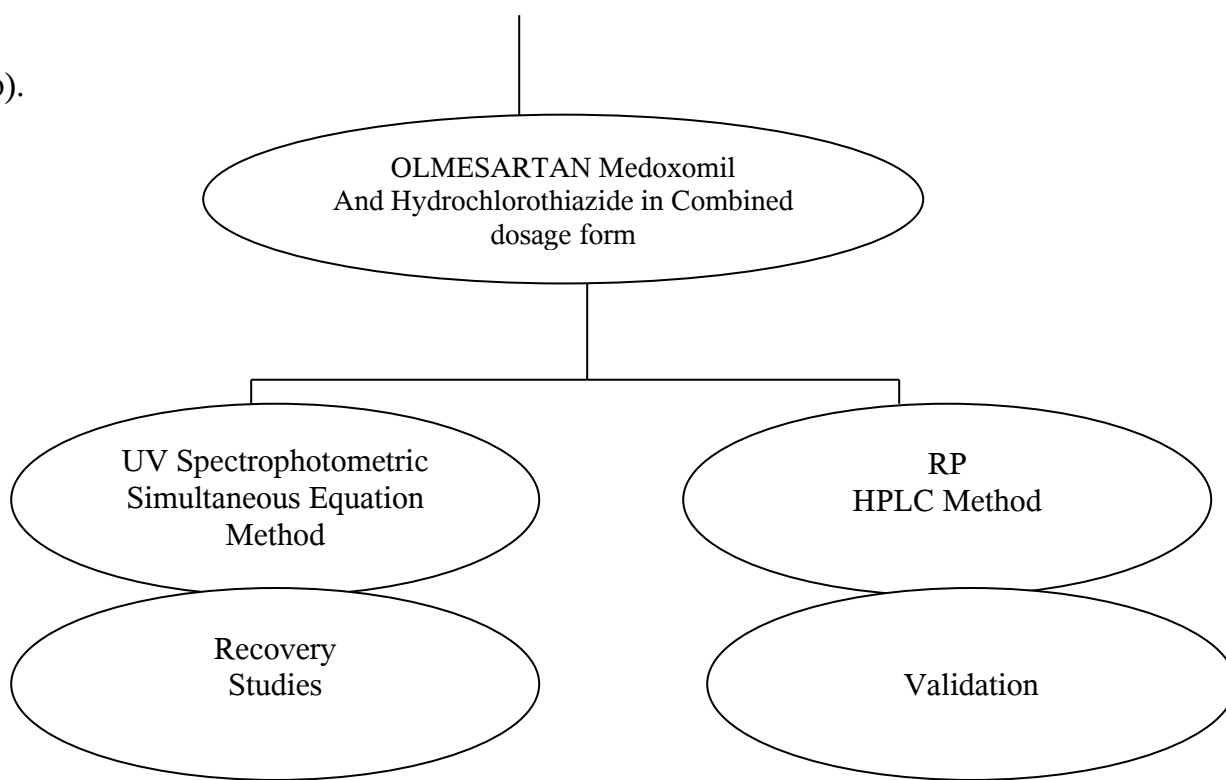
DRUG PROFILE
DROTAVERINE HYDROCHLORIDE

Molecular Structure
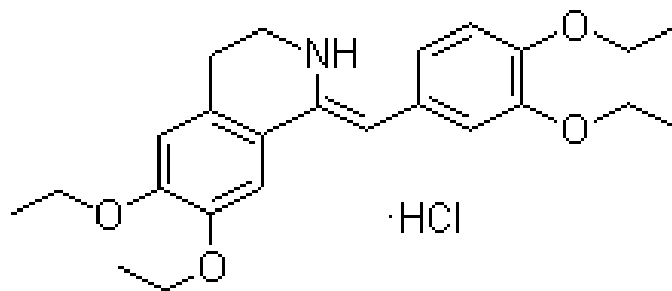
- ❖ **Chemical Name**

    1,2,3,4-Tetrahydro-6,7-diethoxy-1-((3,4-diethoxyphenyl) methylene)-
    isoquinoline hydrochloride

- ❖ **Molecular Formula**

    $C_{24}H_{31}NO_4.HCl;C_{24}H_{32}ClNO_4$

- ❖ **Molecular Weight**

    433.97

- ❖ **Physical State**

    Yellow crystalline Powder

- ❖ **Solubility**

    Insoluble in water and soluble in 0.1N NaoH, Methonal

- ❖ **Therapeutic Category**

    Isoquinolone, Antispasmodic agents

- ❖ **Mechanism of Action**

    Drotaverine has antispasmodic affect medicated via inhibition of phosphodiesterase–IV specific for smooth muscle. It has a rapid and direct action on the smooth muscle. It acts to correct cyclic AMP and Ca imbalance at the spastic site thereby relieving smooth muscle spasam and pain.

MEFENAMIC ACID

Molecular Structure
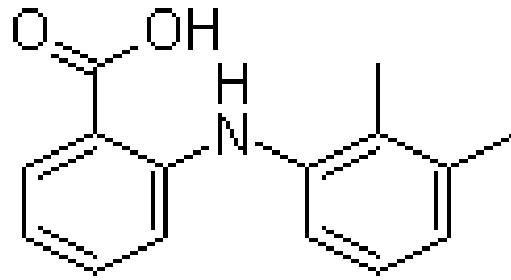
❖ **Chemical Name**

      Dimethylphenyl)amino]benzoic acid;

      N-(2,3-Xylyl)anthranilic acid

❖ **Molecular Formula**

      $C_{15}H_{15}NO_2$

❖ **Molecular Weight**

      241.29

❖ **Physical State**

      White Powder

❖ **Solubility**

      Insoluble in water and soluble in 0.1N NaoH, 0.1N HCL

❖ **Therapeutic Category**

      Analgesic & NSAID

❖ **Mechanism of actions**

      Mefenamic acid inhibits the enzymes cycloxygenase Cox-1 and Cox-2 and reduce the formation of prostaglandin and leukotrienes. It also act as an antagonist at prostaglandin receptor sites. It has Analgesic and Antipyretic properties with minor anti inflammatory activity.

UV SPECTROPHOTOMETRIC SIMULTANEOUS EQUATION METHOD OF MEFENAMIC ACID AND DROTAVERINE HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM

PRINCIPLE:

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other. It may be possible to determine the quantity of both drugs by the technique of simultaneous equation (or) Vierodt's method.

Criteria for obtaining maximum precision, based upon absorbance ratios have been suggested that place limits on the relative concentrations of the component of the mixture.

$$\frac{A_2 / A_1}{ax_2 / ax_1} \qquad \text{and} \qquad \frac{ay_2 / ay_1}{A_2 / A_1}$$

Where ax_1, ax_2 = Absorptivities of X at λ_1 and λ_2

ay_1, ay_2 = Absorptivities of X at λ_1 and λ_2

A_1, A_2 = Absorbances of the diluted sample at λ_1 and λ_2.

The ratio should lie outside the range of $0.1 - 2.0$ for the precise determination of (Y and X) two drugs respectively.

These criteria are satisfactory only when the λ_{max} of the two components is reasonably dissimilar. The additional criteria includes that two components do not interact chemically, there by negating the initial assumption that the total absorbance is the sum of the individual absorbance's.

MATERIALS

Market Sample: DROTIN -M

WALTER BUSHNELL

Label claim:

Mefenamic acid 250mg

Drotaverine hydrochloride 80mg

Equipments Used:

➢ ATCO Balance

➢ SHIMADZU UV - & spectrophotometer double beam digital UV-1700

Solvent Used:

➢ Methanol AR

λ_{max}

The wavelength at which maximum absorption takes is place called λ_{max}

Determination of Absorption Maximum(λ_{max}) for   Mefenamic acid

Procedure:

30mg of authentic   Mefenamic acid sample was accurately weighed and transferred to 50ml volumetric flask and   methanol was added, dissolved and the volume was made upto 50ml with methanol.

5ml of this stock solution was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

The absorbance of solution was measured against solvent blank in UV-region of 200-400nm. The λ_{max} was found to be 236nm. This spectrum of maximum absorbance is shown in graph.

BEER'S LAW PLOT FOR MEFENAMIC ACID

PROCEDURE

100mg of authentic Mefenamic acid sample was accurately weighed and transferred to 100ml volumetric flask and methanol was added, dissolved and the volume was made up to 100ml with methanol.

From this aliquots of 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml, 4ml, 4.5ml, 5 ml was pipetted out in to separate 100ml volumetric flask. Then the volume was made upto 100ml with methanol. The absorbance of each solution was found out at 236nm against a reagent blank. The readings are presented in Table-1 and graph.

Table – 1

DATA FOR BEER'S LAW PLOT FOR MEFENAMIC ACID (Linearity)

S.No.	Concentration µg/ml	Absorption
1	5	0.1192
2	10	0.2382
3	15	0.3573
4	20	0.4766
5	25	0.5956
6	30	0.7147
7	35	0.8338
8	40	0.9529
9	45	1.0720
10	50	1.1911

Linearity Co-efficient (γ) = 0.999

SLope (m)   = 0.023820

 Intercept(c)   = - 0.000056

DEVIATIONS FROM BEER'S LAW

For the drug Mefenamic acid maximum deviation was found in the Concentration range above 50μg/ml. The readings are presented in Table -2 and graph.

Table -2

DATA FOR DEVIATIONS FORM BEER'S LAW PLOT FOR

MEFENAMIC ACID

S.No.	Concentration μg/ml	Absorbance
1.	5	0.1192
2.	10	0.2382
3.	15	0.3573
4.	20	0.4766
5.	25	0.5956
6.	30	0.7147
7.	35	0.8338
8.	40	0.9529
9.	45	1.0720
10.	50	1.1911
11	55	1.3104
12	60	1.2603
13	65	1.2601
14	70	1.2599

Determination of Absorption Maximum (λ_{max}) Drotaverine hydrochloride

Procedure:

100mg of authentic **D**rotaverimne hydrochloride sample was accurately weighed and transferred to 50ml volumetric flask and methanol was added, dissolved and the volume was made upto 50ml with methanol

5ml of this stock was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol

The absorbance of solution was measured against solvent bank in UV-region of 200-400 nm. The λ_{max} was found to be 245nm. This spectrum of maximum absorbance is shown in graph.

BEER'S LAW PLOT FOR DROTAVERINE HYDROCHLORIDE

PROCEDURE

100mg of authentic Drotaverine Hydrochloride sample was accurately weighed and transferred to 100ml volumetric flask and methanol was added, dissolved and the volume was made up to 100ml with methanol.

From this aliquots of 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml, 3.ml, 3.5ml, 4ml, 4.5ml,5ml, was pipetted out in to separate 100ml volumetric flask. Then the volume was made upto 100ml with methanol. The absorbance of each solution was found out at 245nm against a reagent blank. The readings are presented in Table-3 and graph

TABLE-3

DATA FOR BEER'S LAW PLOT FOR DROTAVERINE

HYDROCHLORIDE

S.No.	Concentration µg/ml	Absorbance
1.	5	0.07645
2.	10	0.1529
3.	15	0.2293
4.	20	0.3058
5.	25	0.3822
6.	30	0.4587
7.	35	0.5351
8	40	0.6116
9	45	0.6880
10	50	0.7645

Linearity coefficient γ = 0.9999

Slope(m) = 0.01527

Intercept(c) = -0.00053

DEVIATIONS FROM BEER'S LAW

For the drug, Drotaverine Hydrochloride maximum deviation was found in the concentration range above 55µg/ml .The readings are presented in Table –4 and graph.

Table – 4

Data for Deviations from Beer's law plot for Drotaverine

Hydrochloride

S.No.	Concentration µg/ml	Absorption
1	5	0.07645
2	10	0.1529
3	15	0.2293
4	20	0.3058
5	25	0.3822
6	30	0.4587
7	35	0.5351
8	40	0.6116
9	45	0.6880
10	50	0.7645
11	55	0.6920
12	60	0.6915
13	65	0.6910
14	70	0.6909

Preparation of Mefenamic acid standard solution

100mg of authentic Mefenamic acid sample is accurately weighed and transferred to 100ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made upto 100ml with methanol.

From this 1ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

Preparation of Drotaverine hydrochloride standard solution

100mg of authentic Drotaverine hydrochloride sample is accurately weighed and transferred to 100ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made upto 100ml with methanol.

From this 1ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

The absorbance and absorptivity values are shown un Table-5a & 5b

Preparation of sample solution

Twenty tablets are weighed and average weight was calculated. The tablets are ground to a fine powder. A powder equivalent to 250mg of Mefenamic acid and 80mg of Drotaverine hydrochloride was accurately weighed and transferred to 50ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made upto 50ml with methanol. This solution was filtered through whatmann filter paper.

From this 1ml was pipetted out in to separate 100ml volumetric

flask and the volume was made up to 100ml with methanol.

The absorbance of each solution was found out at 236nm (λ_{max} of Mefenamic acid) and 245nm (λ_{max} of Drotaverine hydrochlororide) against a reagent blank.

The analysis values are given in Table-6.

Calculation:

λ_1 = 236nm (λ_{max} of Mefenamic acid)

λ_2 = 245nm (λ_{max} of Drotaverine hydrochloride)

X – Mefenamic acid

Y – Drotaverine hydrocholoride

ax_1 and ax_2 – Absorptivities of Mefenamic acid at λ_1 and λ_2

ay_1 and ay_2 – Absorptivities of Drotaverine hydrocholoride at λ_1 and λ_2

Cx and Cy – Concentration of Mefenamic acid and Drotaverine hydrochloride (Sample) in grams per 100ml

A1 and A2 – Absorbance of sample at λ_1 and λ_2

$$\text{Absorptivity (a)} = A/bc = \frac{\text{Absorbance}}{b \times \text{concentration of substance}}$$

Determination of Cx and Cy

$$Cx = \frac{A_2 ay_1 - A_1 ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$Cy = \frac{A_1 ax_2 - A_2 ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Table-5a

Absorbance values for standard and sample

Wavelength	Olmesartan medoxomil	Hydrochlorothiazide	Sample
236 λ_1 Mefenamic acid	0.2383 (X_1)	0.1021 (Y_1)	0.8452 (A_1)
245 λ_2 Drotaverine hydrochloride	0.1447(X_2)	0.1529(Y_2)	0.6024 (A_2)

Table-5b

Absorptivity values for Mefenamic acid and Drotaverine

Hydrochloride

Parameter	Absorptivity at 236nm		Absorptivity at 245nm	
	Mefenamic acid	Drotaverine Hydrochloride	Mefenamic acid	Drotaverine Hydrochloride
*Mean	ax1=238.2	ay1=102.1	ax2= 144.1	ay2=152.9
SD	0.3791	0.2455	0.4207	0.1645

* Absorptivity values are the mean of six determinations. S.D. is standard deviation. ax1 and ax2 are absorptivities of Mefenamic acid at 236nm and 245nm respectively; ay1 and ay2 are absorptivities of Drotaverine hydrochloride at 236nm and 245nm respectively.

CRITERIA FOR OBTAINING MAXIMUM PRECISION

$$(A_2 / A_1)/ (ax_2 / ax_1) \text{ and } (ay_2 / ay_1)/ (A_2 / A_1)$$

was found to be 0.043098 & 2.06735 respectively. This ratio should lie outside the range 0.1-2.0 for the precise determination.

Table-48
Analysis data of tablet formulations

Parameters	UV-spectrophotometry	
	Mefenamic acid	Drotaverine Hydrochloride
Label Claim	250mg	80mg
Amount found	2.49mg	79.96mg
*% Drug content	99.8	99.95
S.D.	0.2739	1.1516
% R.S.D	0.2724	1.1698

RECOVERY STUDIES

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 80, 100 and 120% level. From the total amount of drug found the percentage recovery was calculated. The results are reported in Table-7

Table 7

Recovery studies

Mefenamic acid

Range	Amount found	*Recovery	% R.S.D
80%	249.55mg	99.80%	0.0995
100%	249.58mg	99.80%	0.1892
120%	249.59mg	99.83%	0.09

Drotaverine Hydrochloride

Range	Amount found	*Recovery	% R.S.D
80%	79.98mg	99.90%	0.3213
100%	79.96mg	99.95%	0.1219
120%	79.92mg	99.90%	0.1928

*Recovery is the mean of three estimations

DEVELOPMENT AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF DROTAVERINE HYDROCHLORIDE AD MEFENAMIC ACID IN COMBINED TABLET DOSAGE FORM

INSTRUMENTS

- Shimadzu
- UV Visible SPD – 20A
- LC 20AT Isocratic
- Rheodyne
- C18.

Regents and Chemicals

- Acetonitrile
- HPLC grade water
- Buffer
- Methanol

REFERENCE STANDARDS :

Drotaverine hydrochloride and mefenamic acid

These two reference standards were obtained as gift samples from Dr. Ceeal Lab, Chennai. The authenticity and purity of the sample was certified by the same.

Sample Tablet brand used : **DROTIN – M**

        Dr. Ceeal Lab, Chennai.

Label claim : Drotaverine hydrochloride 80 mg

        Mefenamic acid 250 mg

METHOD DEVELOPMENT AND OPTIMIZATION :

SELECTION OF WAVELENGTH :

The known concentration of Drotaverine hydrochloride and Mefenamic acid were taken and dissolved in methanol. The wavelength were tried at 250nm, 260nm, 270nm, and the Peaks of the drugs were showing fronting and tailing. 240nm were selected for the analysis.

OPTIMIZATION OF CHROMATOGRAPHIC PARAMETERS

(a) SELECTION OF MODE OF OPERATION:

As both the drugs were are polar in nature, a RP-HPLC method was proposed.

(b)SELECTION AND STANDARDISATION OF MOBILE PHASE :

DROTIN –M is combination of Drotaverine hydrochloride 80 mg and mefenamic acid 250 mg. The method development of Drotaverine hydrochloride and mefenamic acid required adequate resolution of two drug peaks in the chromatogram.

DIFFERENT COMBINATIONS OF BUFFER AND SOLVENTS:

Buffer Potassium di hydrogen phosphate and acetonitrile (70:30)

Buffer Potassium di hydrogen phosphate and acetonitrile (50:50)

Buffer Potassium di hydrogen phosphate acetonitrile (50:50)

Buffer potassium di hydrogen : Methanol:acetonitrile (50:15:35)

Peaks of Drotaverine Hydrochloride and mefenamic acid were well resolved with solvent system. Buffer Potassium di hydrogen phosphate : methanol: acetonitrile (50:15:35) and it is shown in figure-1.

SELECTION OF FLOW RATE :

The Flow rate for Drotaverine HCL and Mefenamic acid were tried with 0.4ml, 0.5ml, and 0.7ml and the peaks of the drugs were showing fronting and tailing with 0.4ml and 0.7ml respectively and finally 1.5ml per minute was selected for the analysis.

PREPARATION OF BUFFER SOLUTION :

Buffer solution was prepared by using 1.3609gm of Potassium di hydrogen of HPLC grade water, filtered through 0.45u nylon membrane and degassed.

PREPARATION OF MOBILE PHASE :

Mix the Buffer, methanol: and acetonitrile in the ratio of 50:15:35 and degass it. Filtered through 0.45u membrane.

DILUENT

Mobile phase is used as diluent.

DETERMINATION OF RETENTION TIME :

(A) PREPARATION OF STANDARD SOLUTION OF DROTAVERINE HYDROCHLORIDE

Accurately 0.08g Drotaverine hydrochloride was taken in a 100ml volumetric flask and the volume was adjusted to 100ml with mobile phase. 5ml was taken in a separate 50ml volumetric flask and the volume was adjusted to 50 ml with mobile phase to get concentration of 80 µgl/ml Drotaverine hydrochloride of 20µl of this solution was injected and chromatogram was obtained and it is in shown Figure-3.

(B) PREPARATION STANDARD SOLUTION OF MEFENAMIC

ACID

Accurately 0.250g was taken in a 100ml volumetric flask and the volume was adjusted to 100ml with mobile phase. 5 ml was taken in a separate volumetric flask of 50ml and the volume was adjusted to 50ml with mobile phase to get a concentration of 250 µg/ml. 20µl of this solution was injected and the chromatogram was recorded and shown in Figure-2.

(C) PREPARATION OF MIXED STANDARD SOLUTION :

0.08g of Drotaverine hydrochloride and 0.250g of Mefenamic acid was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 20ml of mobile phase and it was sonicated. Then the volume was adjusted to 100ml with mobile phase. From the stock solution 5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50ml with mobile phase to get a concentration of 80µg/ml of Drotaverine hydrochloride and 250µg/ml of Mefenamic 20µl if the resulting solution was injected and chromatogram was recorded shown in Figure-4.

FIXED CHROMATOGRAPHIC CONDITIONS :

INSTRUMENT   :   Shimadzu Prominence model

                                    LC 20 AT Isocratic

COLUMN   :   C18

WAVELENGTH   :   240nm

TEMPERATURE   :   Ambient temperature

FLOW RATE   :   1.5ml / mit

INJECTION VOLUME :   20μl

MOBILE PHASE   :   Buffer Potassium di hydrogen phosphate
Methanol and acetonitrile 50:15:35

RETENTION TIME   :   3.0   for Mefenamic Acid   6.4 for drotaverine

QUANTITATIVE DETERMINATION OF THE DRUGS OF THE DRUGS BY USING THE DEVELOPED METHOD

Sample   :   Drotaverine Hydrochloride and
Mefenamic acid

Label Claim   :   Drotaverine hydrochloride 80mg

Mefanamic acid 250mg

METHOD :

Twenty tablets were weighed and powdered. Average weight 593.2g of sample tablet DROTIN – M (equivalent to 80mg of Drotaverine Hydrochloride and 250mg Mefenamic acid was taken into 100ml dried volumetric flask. The powder was first dissolved in 20ml mobile phase and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 80μg/ml of Drotaverine Hydrochloride and 250 μg /ml of Mefenamic acid. 20μl of the solution was injected and the chromatogram obtained is shown in Figure 5.

The amount of Drotaverine Hydrochloride and Mefenamic acid present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Table-8.

Amount of drug present in the tablet:

$$\frac{\text{Sample area}}{\text{Standard Area}} \; x \; \frac{\text{Standard dilution}}{\text{Sample dilution}} \; x \; \frac{\text{Potency}}{100} \; x \; \text{Average weight}$$

$$\text{Percentage purity} = \frac{\text{Amount present}}{\text{Label claim}} \; x \; 100$$

Table – 8
Quantitative Estimation

S.No.	Brand Name	Content	Label Claim (mg)	Peak area	Amount present (mg)	Percent Purity % w/v
1.	Drotin-M	Drotaverine Hydro chloride	80 mg	868.77	79.56	99.45%
		Mefenamic Acid	250 mg	2615.75	250.12	100.05%

Acceptance criteria : 98-102% w/v

Assay for Drotaverine HCL

Amount Present =

$$\frac{868.77}{869.25} \; x \; \frac{0.08}{0.5946} \; x \; \frac{99.83}{100} \; x \; 593.2 \; = 79.56 \text{ mg}$$

Percentage Purity =   79.56 / 80 x 100 = 100.20 %

Assay for mefenamic acid

Amount Present =

$$\frac{0.250}{0.5946} \; X \; \frac{99.68}{100} \; X \; \frac{593.2}{79.56 \text{mg}} = 250.12 \text{mg}$$

Percentage Purity
        250.12 / 250 x 100 = 100.05 %

VALIDATION

Validation of an analytical method is a process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Design of experiment:

- Specificity
- Linearity and range
- Limit of quantification
- Limit of detection
- Accuracy
- Precision
    - System precision
    - Method precision
- Robustness
- Ruggedness
- System suitability studies
    - Resolution
    - Number of theoretical plates
    - The tailing factor.

SPECIFICITY

The specificity of an analytical methods is ability to measure accurately and specifically the analytes in the presence of compounds that may be expected to be present in the sample matrix.

Determination

The specificity of the analytical methods was determined by injecting the placebo solution under the same experimental conditions as the assay.

Preparation of placebo

Placebo is prepared by mixing all the expcipients without active ingredients

Procedure

➢ 225 mg placebo was accurately weighted and transferred into 25 ml volumetric flask and the volume as made to 25 ml with the mobile phase. The solution was filtered through Millipore filter paper and degassed. 20 µl of this solution was injected and chromatogram was recorded and shown in figure- 6.

➢ 80 mg drotaverine, hydrochloride and 250 mg mefenamic acid were weighed and transferred into a 100 ml volumetric flask and volume was adjusted to 100 ml with mobile phase. Further 5 ml of the solution was taken and the volume was made upto 50 ml to get a concentration of 80 µg/ml Drotaverine hydrochloride & 250 µg/ml Mefenamic acid to this solution 90 mg of placebo was added and it was sonicated, filtered through a Millipore filter paper. 20 µl of this solution was injected, chromatogram was recorded and shown in Figure-7 and the report are shown in Table 9 & 10.

TABLE – 9

SPECIFICITY FOR DROTAVERINE HYDROCHLORIDE

S.No	Sample	Area Obtained
1	Standard	869.25
2	Standard + Placebo	877.44
3	Placebo	0

TABLE – 10

SPECIFICITY FOR MEFENAMIC ACID

S.No	Sample	Area Obtained
1	Standard	2599.03
2	Standard + Placebo	2596.22
3	Placebo	0

LINEARITY AND RANGE

Linearity of an analytical method is its ability to elicit test result that are directly proportional to the concentration of analyte in samples within a given range.

Determination

The linearity of the analytical method was determined by mathematical treatment of test result obtained by analysis of sample with analyte concentration across the claimed range. Area was plotted graphically as a function of analyte concentration. Percentage curve fitting was calculated.

Method

Preparation of mixed standard stock solution

Accurately weighted 80 mg drotaverine hydrochloride 250 mg mefenamic acid were transferred in to the 100 ml of standard flask and it was dissolved with mobile phase and the volume was made upto 100ml with mobile phase. From the resulting solution 4,4.5, 5, 5.5 6 ml were transferred into 5 different 50 ml volumetric flask. The volume was made with mobile phase to get a final concentration of 64, 72, 80, 88, 96 µg/ml drotaverine hydrochloride 200, 225 250, 275 300 µg/ml of Mefenamic acid. 20µl up of the resulting solution was injected and chromatogram was recorded.

The chromatograms of Drotaoverine hydrochloride and Mefenamic acid are shown in figure 8-12.

Acceptance Criteria

Correlation coefficient should not be less than 0.99

The linearity datas and analytical performance parameters of Drotaoverine hydrochloride and Mefenamic acid are shown in Table 11-13 and calibration curve are shown in graph.

TABLE – 11

LINEARITY DATA

DROTAVERINE HYDROCHLORIDE

S.NO	CONCENTRATION (μg/ml)	PEAK AREA
1	64	698.37
2	72	789.02
3	80	872.05
4	88	969.24
5	96	1058.00

Table – 12

MEFENAMIC ACID

S.NO	CONCENTRATION (μg/ml)	PEAK AREA
1	200	2039.59
2	225	2272.73
3	250	2513.72
4	275	2767.59
5	300	3016.84

TABLE – 13
ANALYTICAL PERFORMANCE PARAMETERS

S.NO	DRUG NAME	LINEAR DYNAMIC RANGE (U/ML)	CORRELATION COEFFICIENT	SLOPE	INTERCEPT
1	Drotaverine Hydrochloride	64-96	0.9997	11243.5	-22.144
2	Mefenamic Acid	200-300	0.9998	9797.44	72.734

ACCURACY

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value. Accuracy may often be

expressed as percent recovery by the assay of known added amount of analyte.

Determination

The accuracy of the analytical method was determined by applying the method to the analysed samples to which known amounts of analyte had been added. The accuracy was calculated from the test results as the percentage of analyste recovered by the assay.

Acceptance criteria :

Percentage recovery should be within 98-102 %

PROCEDURE

Mixed standard stock solution 2 ml and sample stock solution 2 ml were mixed together in 50 ml volumetric flask and the volume was made upto 50 ml with mobile phase to get 80 % range. Similarly 100 % and 120 % range

was prepared 20 μl of this solution was injected three times and chromatograms were shown in Fig. 13 to 21 and values in table 14 and 15.

TABLE -14

RECOVERY STUDY OF DROTAVERINE HYDROCHLORIDE

S.No	Range	Area Obtained	Amount Recovered (mg)	% Recovery
1		699.74	80.17	100.21
		700.13	80.21	100.27
	80 %	699.98	80.20	100.25
2		877.44	80.42	100.53
		878.20	80.50	100.62
	100 %	878.09	80.48	100.60
3		1057.09	80.74	100.90
		1057.12	80.75	100.90
	120 %	1056.21	80.67	100.80

TABLE – 15

RECOVERY STUDY MEFENAMIC ACID

S.No	Range	Area Obtained	Amount Recovered (mg)	% Recovery
1		2095.22	250.45	100.18
		2087.95	249.58	99.83
	80 %	2093.45	250.24	100.09
2		2596.22	248.26	99.30
		2606.04	249.20	99.68
	100 %	2597.13	248.35	99.30
3		3129.21	249.36	99.74
		3120.22	248.64	99.45
	120 %	3117.10	248.40	99.36

PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analystical method is usually expressed as the standard deviation and relative standard deviation.

Determination :

The precision of the analytical method was determined by assaying sufficient number of sample and relative standard deviation was calculated.

The precision of the instrument was determined by assaying the samples consecutively, number of time and relative standard deviation was calculated.

Acceptance Criteria

The relative standard deviation should be with in 2 %

SYSTEM PRECISION

Procedure

Accurately weighed 80 mg drotaverine hydrochloride and 250 mg mefenamic acid were transferred into 100 ml standard flask and it was dissolved with mobile phase. From the resulting solution 50 ml was transferred into 50 ml volumetric flask. The volume was made up with mobile phase to 50 ml.

The system precision was evaluated by measuring 6 successive injection of 20μl of standard solution. The peak response were measured from the chromatogram shown in   figure 22-27 and system precision data area shown in table 16 & 17.

Method Precision

Procedure

Twenty tables were weighted and powdered. Average weight 593.2 of sample tablet DROTIN-M (equivalent to 80 mg Drotaverine hydrochloride and 250 mg of mefenamic acid) was taken into 100 ml dried volumetric flask. The power was first dissolved in 20ml of mobile phase and sonicated and finally the volume was adjusted to 100 ml with mobile phase. From this solution 5ml transferred to 50 ml volumetric flask and volume was adjusted to 50 ml with mobile phase to get a concentration of 80µg/ml of drotaverine hydrochloride and 250 µg/ml of mefenamic acid 0.20µl of the solution was injected and the chromatogram obtained is shown in Figure 28-33 and method precision data are shown in table 18-20.

TABLE – 16
SYSTEM PRECISION DATA

S.No	Area Drotaverine Hydrochloride	Area of Mefenamic acid
1	877.44	2623.8
2	877.03	2615.65
3	883.41	2599.03
4	876.06	2598.06
5	877.59	2598.10
6	878.29	2614.96
Mean	878.30	2608.27
S.D.	2.6071	11.2722
% RSD	0.2968	0.4321

TABLE -17

Relative standard deviation	Drotaverine Hydrochloride	Mefenamic acid	Acceptance Criteria
	0.2968	0.4321	2 %

TABLE – 18
METHOD PRECISION OF DROTAVERINE HYDROCHLORIDE

S.No	Area Obtained	Amount Recovered	Purity % w/v
1	877.00	78.63	99.54
2	876.98	79.61	99.59

3	879.99	79.36	99.20
4	877.11	79.77	99.71
5	877.53	79.67	99.58
	878.24	79.67	99.58
	MEAN		99.53
	STANDARD DEVIATION		1.1705
	RELATIVE STAND DEVIATION		0.133

Table – 19 Method Precision of Mefenamic acid

S.No	Area Obtained	Amount Recovered	Purity % w/v
1	2619.07	248.08	99.20
2	2618.65	248.82	99.53
3	2615.77	250.13	100.05
4	2619.53	250.59	100.23
5	2617.84	250.41	100.16
6	2618.06	248.80	99.52
	MEAN		99.78
	STANDARD DEVIATION		1.3243
	RELATIVE STAND DEVIATION		0.05

Table – 20 Method Precision report for Drotaverine Hydrochloride and Mefenamic Acid

Relative standard deviation	Drotaverine Hydrochloride	Mefenamic acid	Acceptance Criteria
	0.133	0.05	2 %

LIMIT OF DETECTION (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessary quantities as an exact value under the stated, experimental conditions. The detection limit is usually expressed as the concentration of analyte.

It is given by

LOD = 3.3 xσ

----

m

σ = standard deviation of the response

m = slope of the calibration curve

TABLE – 21

LIMIT OF DETECTION

DRUG	STANDARD DEVIATION	SLOPE	L.O.D μg/ ml
Drotaverine Hydrochloride	2.6071	11243.5	0.00076519
Mefenamic acid	11.2722	9797.44	0.003796

LIMIT OF QUANTITATION

The Quantitation limit of an analytical procedure is the lowest amount of analytic which can be quantitatively determined with suitable precision and Accuracy.

It is given by

$$L.O.Q = \frac{10 \times \sigma}{m}$$

σ= Standard deviation of the response

m=slope of the calibration curve

TABLE – 22

LIMIT OF QUANTITATION

DRUG	STANDARD DEVIATION	SLOPE	L.O.Q µg/ ml
Drotaverine Hydrochloride	2.6071	11243.5	0.002318
Mefenamic acid	11.2722	9797.44	0.011505

RUGGEDNESS

The Ruggedness of an analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under a variety of normal test condition, such as different laboratories, different analyst different assay temperature, different days etc.

Ruggedness is normally expressed as the lack of influence on test result of operational and environmental variables of the analytical method.

Determination :

The ruggedness of an analytical method was determined by analysis of aliquots from homogenous lots by different analysis using operations and environmental conditions that may different but were still with in the specified parameters of the assay. The degree of reproducibility of test result was then determined as a function of the assay variables. This reproducibility was then determined as a function of the assay variables. This reproducibility was assayed under normal conditions to obtain a measure of the ruggedness of analytical method.

The assay Drotaverine hydrochloride and mefenamic acid were performed in different conditions like different analyst on different days.

METHOD

The standard and sample solutions were prepared by different analysts on different days and the resulting solution were injected and chromatograms are recorded and shown in Figure 34-37 and ruggedness of the method and report of drotaverine hydrochloride and mefenamic acid as shown in Table 23.

Table -23
RUGGEDNESS

Analyst	Date	Amount Found		% purity	
		Drotaverine hydrochloride	Mefenamic acid	Drotaverine hydrochloride	Mefenamic acid
I	4Nov 2008	79.58 mg	248.53 mg	99.48	99.41
II	5Nov 2008	79.66 mg	250.14mg	99.58	100.05

ROBUSTNESS

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indications of its reliability during normal usage.

Determination

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by different physical parameters that may differ but were still within the specified parameter of the assay for example change in physical parameters like flow rate and lambda max.

Method

Standard solution preparation

80 mg Drotaverine hydrochloride and 250 mg mefenamic acid were transferred into a 100 ml volumetric flask, volume was adjusted to 100 ml with mobile phase. Further 5 ml of the solution was taken and the volume was made upto 50 ml to get a concentration of 80 µg/ml of drotaverine hydrochloride and 250 µg/ml of mefenamic acid.

Sample preparation

Twenty tablets were weighted and powered. Average weight 593.2g of sample tablet DROTIN – M (equivalent to 80 mg Drotaverine and 250 mg mefenamic acid was taken into 100 ml dried volumetric flask. The powder was first dissolved in 20 ml of mobile phase. From this solution 5 ml was transferred in to 50 ml volumetric flask and volume was adjusted to 100 ml with mobile phase. 20µl of this solution was injected and individual chromatograms were recorded and they are in figure 38-45 and datas are shown in Table 24-31.

TABLE 24

Chromatographic conditions : - change in flow rate 1.3 ml/ min)

Change in flow rate	1.3 ml / min
Column	CN
Wave length	240 nm
Temperature	Ambient 25^{0}c
Injection volume	20 µl

TABLE 25

Change in flow rate (1.3 ml/ min

S.No	Drug	standard area	sample area	% Purity w/v
1	Drotaverine hydrochloride acid	1347.69	1345.39	99.41
2	Mefenamic acid	3896.62	3920.51	100.02

TABLE -26

Chromatographic condition :- change in flow rate (1.7 ml/ min)

Change in flow rate	1.7 ml / min

Column	CN
Wave length	240 nm
Temperature	ambient 25^{0}c
Injection volume	20 μl

TABLE 27

Change in flow rate (1.7 ml/ min)

S.No	Drug	standard area	sample area	% Purity w/v
1	Drotaveraine hydrochloride acid	809.02	806.51	99.28
2	Mefenamic acid	2305.51	2328.59	100.41

TABLE -28

Chromatographic condition :- change in Lambda Max 242 nm

Column	CN
Wave length	240nm
Temperature	Ambient 25^0.c
Injection Volume	20 μl
Flow rate	1 ml/ min

TABLE 29

Change in Lambda max 242 nm

S.No	Drug	standard Area	sample area	% Purity w/v
1	Drotaverine hydrochloride	712.532	711.27	99.41
2	Mefenamic acid	2074.91	2097.42	100.44

TABLE -30

CHROMATOGRAPHIC CONDITIONS : - CHANGE IN LAMBDA MAX 238 NM

Column	CN
Wave length	240 nm
Temperature	Ambient 25^0.c
Injection Volume	20 μl
Flow rate	1 ml/ min

TABLE 31

Change in Lambda Max   238 nm

S.No	Drug	Standard area	sample area	% Purity w/v
1	Drotoverine hydrochloride	867.93	866.32	99.3
2	Mefenamic acid	2584.84	2560.68	98.48

SYSTEM SUITABILITY PARAMETERS

System suitability testing is an integral part of many analytical procedures. The test based on the concept that the equipment, electronics, analytical operation and sample to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particulars procedure depend on the type of procedure being validated.

Method

A solution of 80µg/ml of Drotaverine Hydrochloride and 250µg/ml Mefenamic acid were prepared by diluting with mobile phase and same was injected and a chromatogram was recorded and they are shown in figure 46 and system suitability report are shown in the following.
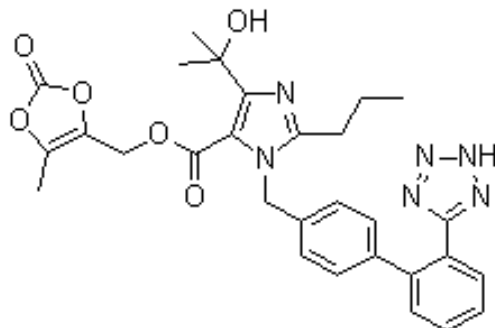
TABLE – 32
SYSTEM SUITABILITY PARAMETERS

S.No	Parameters	Drotaverine Hydrochloride	Mefenamic acid
1	Theoretical plates	12,082,1306	8,361.6466
2	Tailing Factor	1.00	1.00
3	Resolution	6.2	

DRUG PROFILE
OLMESARTAN MEDOXOMIL

❖ **Molecular Structure**

❖ **Chemical Name**

4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-carboxylic acid (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl ester

❖ **Molecular Formula**

$C_{29}H_{30}N_6O_6$

❖ **Molecular Weight**

558.59

❖ **Physical State**

White to off white powder

❖ **Solubility**

Soluble in 0.1N HCL, 0.1N NaoH, Methanol, Insoluble water

❖ **Therapeutic Category**

Antihypertensive Agent

❖ **Mechanism Of Action**

Olmesartan is an nonpeptic AT_1 angiotension II receptor antagonist exerts antihypertensive activity by preventing angiotension II to from binding to AT_1 receptors thus inhibiting the vasoconstriction and aldosterone – secreting effect of angiotension II.

HYDROCHLOROTHIAZIDE

❖ **Molecular Structure**
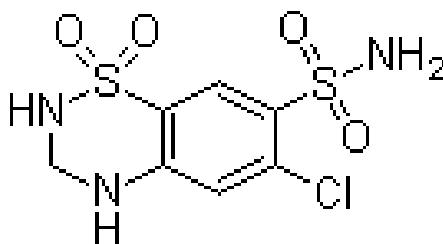
❖ **Chemical Name**

3,4-Dihydro-6-chloro-7-sulfamoyl-1,2,4-

benzothiadiazine-1,1-dioxide; 6-Chloro-3,4-dihydro-7-

sulfamoyl-2H-1,2,4-benzothiadiazine-1,1-dioxide; 3,4-

Dihydrochlorothiazide

❖ **Molecular Formula**
$C_7H_8ClN_3O_4S_2$

❖ **Molecular Weight**

297.73

❖ **Physical State**

White Powder

❖ **Solublity**
Soluble in 0.1N HCL, 0.1N NaoH, Methanol, Insoluble
distilled H_2O

❖ **Therapeutic Category**

Diuretic,Antihypertensive Agent

❖ **Mechanism Of Action**
Hydrochlorothiazide inbbitis the reabsorbtion of Na and chloride
at the beginning of the distal convoluted tubule. It causes
natriuretic effect mainly by decreasing Na and chloride
reabsorbtion in the cortical segment of the ascending limb of the
loop of Henle by inhibition of a specific $Na^+ Cl^-$ Co- transporter.

UV SPECTROPHOTOMETRIC SIMULTANEOUS EQUATION METHOD OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET DOSAGE FORM

PRINCIPLE:

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other. It may be possible to determine the quantity of both drugs by the technique of simultaneous equation (or) Vierodt's method.

Criteria for obtaining maximum precision, based upon absorbance ratios have been suggested that place limits on the relative concentrations of the component of the mixture.

$$\frac{A_2 / A_1}{ax_2 / ax_1} \qquad \text{and} \qquad \frac{ay_2 / ay_1}{A_2 / A_1}$$

Where $ax_1, ax_2 =$ Absorptivities of X at λ_1 and λ_2

$ay_1, ay_2 =$ Absorptivities of X at λ_1 and λ_2

$A_1, A_2 =$ Absorbances of the diluted sample at λ_1 and λ_2.

The ratio should lie outside the range of 0.1 – 2.0 for the precise determination of (Y and X) two drugs respectively.

These criteria are satisfactory only when the λ_{max} of the two components is reasonably dissimilar. The additional criteria includes that two components do not interact chemically, there by negating the initial assumption that the total absorbance is the sum of the individual absorbance's.

MATERIALS

Market Sample: OLMEZEST-H

SUN PHARMA

Label claim:

Olmesartan Medoxomil 20mg

Hydrochlorothiazide 12.5mg

Equipments Used:

➢ ATCO Balance

➢ SHIMADZU UV - & spectrophotometer double beam digital
   UV-1700

Solvent Used:

➢ Methanol AR

FIXATION OF VARIOUS PARAMETERS

(Olmesartan Medoxomil) λmax

The wavelength at which maximum absorption takes is place called λmax

Determination of Absorption Maximum (λ_{max}) for

Olmesartan Medoxomil

Procedure:

30 mg of authentic Olmesartan Medoxomil sample was accurately weighed and transferred to 50ml volumetric flask and methanol was added, dissolved and the volume was made upto 50ml with methanol.

5ml of this stock solution was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

The absorbance of solution was measured against solvent blank in UV-region of 200-400nm. The λ_{max} was found to be 215nm. This spectrum of maximum absorbance is shown in graph.

BEER'S LAW PLOT FOR OLMESARTAN MEDOXOMIL

The intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. More simply it is stated that the absorbance is proportional to the concentration

$$Log \ 10/IT = KC$$

Beer's law plot was constructed by measuring the absorbance of various concentration of drug against solvent blank.

PROCEDURE

100mg of authentic Olmesartan Medoxomil acid sample was accurately weighed and transferred to 100ml volumetric flask and methanol was added, dissolved and the volume was made up to 100ml with methanol.

From these aliquots of 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml, 4ml, 4.5ml, 5 ml was pipetted out in to separate 100ml volumetric flask. Then the volume was made upto 100ml with methanol. The absorbance of each solution was found out at 215nm against a reagent blank. The readings are presented in Table-1 and graph.

Table – 1

DATA FOR BEER'S LAW PLOT FOR Olmesartan Medoxomil

(Linearity)

S.No.	Concentration µg/ml	Absorption
1	5	0.1336
2	10	0.2672
3	15	0.4008
4	20	0.5344
5	25	0.668
6	30	8014
7	35	0.9352
8	40	1.0688
9	45	1.2004
10	50	1.336

Linearity Co -efficient (γ) = 0.999

Slope (m)   = 0.0267

Intercept(c)   = -0.00075

DEVIATIONS FROM BEER'S LAW

A System is said to be obey Beer's law where, a plot of concentration vs. absorbance gives a straight line by using a line of best fit, when a straight line is not obtained; the system is said to be deviated from Beer's Law.

For the drug Olmesartan Medoxomil maximum deviation was found in the Concentration range above 50µg/ml. The readings are presented in Table -2 and graph.

Table -2

DATA FOR DEVIATIONS FORM BEER'S LAW PLOT FOR

OLMESARTAN MEDOXOMIL

S.No.	Concentration µg/ml	Absorbance
1.	5	0.1336
2.	10	0.2672
3.	15	0.4008
4.	20	0.5344
5.	25	0.668
6.	30	8014
7.	35	0.9352
8.	40	1.0688
9.	45	1.2004
10.	50	1.336
11	55	0.9357
12	60	0.9341
13	65	0.9293
14.	70	0.9210

Determination of Absorption Maximum (λ_{max}) for Hydrochlorothiazide

Procedure:

100mg of authentic Hydrochlorothiazide sample was accurately weighed and transferred to 50ml volumetric flask and methanol was added, dissolved and the volume was made upto 50ml with methanol

5ml of this stock was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol

The absorbance of solution was measured against solvent bank in UV-region of 200-400 nm. The λ_{max} was found to be 226nm. This spectrum of maximum absorbance is shown in graph.

BEER'S LAW PLOT FOR HYDROCHLOROTHIAZIDE

PROCEDURE

100mg of authentic Hydrochlorothiazide sample was accurately weighed and transferred to 100ml volumetric flask and methanol was added, dissolved and the volume was made up to 100ml with methanol.

From these aliquots of 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml, 4ml, 4.5ml, 5ml was pipetted out in to separate 100ml volumetric flask. Then the volume was made upto 100ml with methanol. The absorbance of each solution was found out at 226 nm against a reagent blank. The readings are presented in Table-3 and graph

TABLE-3

DATA FOR BEER'S LAW PLOT FOR HYDROCHLOROTHIAZIDE

S.No.	Concentration µg/ml	Absorbance
1.	5	0.1809
2.	10	0.3618
3.	15	0.5427
4.	20	0.7236
5.	25	0.9045
6.	30	1.0854
7.	35	1.2663
8	40	1.4472
9	45	1.6281
10	50	1.809

Linearity coefficient γ = 0.9998

Slope(m)　　　　　= 0.03617

Intercept(c)　　　　=-0.00005

DEVIATIONS FROM BEER'S LAW

For the drug, Hydrochlorothiazide maximum deviation was found in the concentration range above 55µg/ml .The readings are presented

in Table –4 and graph.

Table – 4

Data for Deviations from Beer's law plot for Hydrochlorothiazide

hydrochloride

S.No.	Concentration µg/ml	Absorption
1	5	0.1809
2	10	0.3618
3	15	0.5427
4	20	0.7236
5	25	0.9045
6	30	1.0854
7	35	1.2663
8	40	1.4472
9	45	1.6281
10	50	1.809
11	55	1.253
12	60	1.251
13	65	1.250
14	70	1.248

Preparation of olmesartan medoxomil standard solution

100mg of authentic olmesartan medoxomil sample is accurately weighed and transferred to 100ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made upto 100ml with methanol.

From this 1ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

Preparation of Hydrochlorothiazide standard solution

100mg of authentic Hydrochlorothiazide sample is accurately weighed and transferred to 100ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made upto 100ml with methanol.

From this 1ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

The absorbance and absorptivity values are shown un Table-5a & 5b

Preparation of sample solution

Twenty tablets are weighed and average weight was calculated. The tablets are ground to a fine powder. A powder equivalent to 20mg of olmesartan medoxomil and 12.5mg of hydrochlorothiazide was accurately weighed and transferred to 50ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made upto 50ml with

methanol. This solution was filtered through whatmann filter paper.

From this 1ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

The absorbance of each solution was found out at 215nm (λ_{max} of olmesartan medoxomil) and 226nm (λ_{max} of hydrochlorothiazide) against a reagent blank.

The analysis values are given in Table-6.

Calculation:

λ_1 = 215nm (λ_{max} of olmesartan medoxomil) λ_2 = 226nm (λ_{max} of hydrochlorothiazide)

X – olmesartan medoxomil

Y – hydrocholorothiazide

ax_1 and ax_2 – Absorptivities of olmesartan medoxomil at λ_1 and λ_2

ay_1 and ay_2 – Absorptivities of hydrocholorothiazide at λ_1 and λ_2

Cx and Cy – Concentration of olmesartan medoxomil and hydrochlorothzide
(Sample) in grams per 100ml

A1 and A2 – Absorbance of sample at λ_1 and λ_2

$$\text{Absorptivity (a)} = A/bc = \frac{\text{Absorbance}}{b \text{ x concentration of substance}}$$

Determination of Cx and Cy

$$Cx = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 \ ay_1 - ax_1 \ ay_2}$$

$$Cy = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 \ ay_1 - ax_1 \ ay_2}$$

Table-5a

Absorbance values for standard and sample

Wavelength	Olmesartan medoxomil	Hydrochlorothiazide	Sample
215 λ_1 olmesartan medoxomil	0.1336 (X_1)	0.1403 (Y_1)	0.3535 (A_1)
226 λ_2 hydrochlorothiazide	0.1102($X2$)	0.1809(Y_2)	0.3560 (A_2)

Table-5b
Absorptivity values for Olmesartan Medoxomil and
Hydrochlorothiazide

Parameter	Absorptivity at 215nm		Absorptivity at 226nm	
	Olmesartan Medoxomil	Hydro Chlorothiazide	Olmesartan Medoxomil	Hydro chlorothizide
*Mean	ax1=267.2	ay1=280.6	ax2= 220.4	ay2=361.8
SD	0.3719	0.2455	0.3152	0.1220

* Absorptivity values are the mean of six determinations. S.D. is standard deviation. ax1 and ax2 are absorptivities of Olmesartan Medoxomil at 215nm and 226nm respectively; ay1 and ay2 are absorptivities of hydrochlorothiazide at 215nm and 226nm respectively.

CRITERIA FOR OBTAINING MAXIMUM PRECISION

$$(A_2 / A_1)/ (ax_2 / ax_1) \text{ and } (ay_2 / ay_1)/ (A_2 / A_1)$$

was found to be 0.035 & 3.12 respectively. This ratio should lie outside the

range 0.1-2.0 for the precise determination.

Table-6
Analysis data of tablet formulations

Parameters	UV-spectrophotometry	
	Olmesartan Medoxomil	Hydro chlorothiazide
Label Claim	20mg	12.5mg
Amount found	19.9mg	12.35mg
*% Drug content	99.9	98.87
S.D.	0.1806	0.2097
% R.S.D	0.1790	0.2128

*value for % Drug content are mean of five estimations

RECOVERY STUDIES

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 80, 100 and 120% level. From the

total amount of drug found the percentage recovery was calculated. The

results are reported in Table-7

Table 7
Recovery studies

Olmesartan Medoxomil

Range	Amount found	*Recovery	% R.S.D
80%	19.8mg	99.8%	0.1104
100%	19.9mg	99.9%	0.1192
120%	19.85mg	99.9%	0.0995

Hydrochlorothiazide

Range	Amount found	*Recovery	% R.S.D
80%	12.4mg	98.9%	0.1011
100%	12.35mg	98.82%	0.1015
120%	12.35mg	99.0%	0.1929

*Recovery is the mean of three estimations

DEVELOPMENT AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE

INSTRUMENTS

- ➤ Shimadzu
- ➤ UV Visible SPD – 20A
- ➤ LC 20AT Isocratic
- ➤ Rheodyne
- ➤ C18.

Regents and Chemicals

- ➤ Acetonitrile
- ➤ HPLC grade water
- ➤ Buffer
- ➤ Methanol

REFERENCE STANDARDS :

Olmesartan Medoxomil and hydrochlorothiazide

These two reference standards were obtained as gift samples from Dr.Ceeal Lab, Chennai   The authenticity and purity of the sample was certified by the same.

Sample Tablet brand used   : **OLMEZEST – H**

        Dr. Ceeal Lab, Chennai

Label claim   : Olmesartan Medoxomil 20 mg

        Hydrochlorothiazide 12.5 mg

METHOD DEVELOPMENT AND OPTIMIZATION :

SELECTION OF WAVELENGTH :

The known concentration of Olmesartan Medoxomil and Hydrochlorothiazide acid were taken and dissolved in methanol. The wavelength were tried at 250nm, 260nm, 270nm, and the Peaks of the drugs were showing fronting and tailing. 230 nm were selected for the analysis.

OPTIMIZATION OF CHROMATOGRAPHIC PARAMETERS

(a) SELECTION OF MODE OF OPERATION:

As both the drugs were are polar in nature, a RP-HPLC method was proposed.

(b) SELECTION AND STANDARDISATION OF MOBILE PHASE :

OLMEZEST - H is combination of Olmesartan Medoxomil 20 mg and hydrochlorothiazide 12.5 mg. The method development of Olmesartan Medoxomil and hydrochlorothiazide required adequate resolution of two drug peaks in the chromatogram.

DIFFERENT COMBINATIONS OF BUFFER AND SOLVENTS:

Buffer Sodium di hydrogen phosphate and acetonitrile (70:30)

Buffer Sodium dihydrogen phosphate and acetonitrile (50:50)

Buffer (Sodium dihydrogen phosphate) and acetonitrile (50:50) methanol:acetonitrile (50:30:20)

Peaks of Olmesartan Medoxomil and Hydrochlorothiazide were well resolved with solvent system. Buffer Sodium di hydrogen phosphate : methanol: acetonitrile (60:20:20) and it is shown in figure-1.

SELECTION OF FLOW RATE :

The Flow rate for Olmesartan Medoxomil and Hydrochlorothiazide were tried with 0.4ml, 0.5ml, and 0.7ml and the peaks of the drugs were showing fronting and tailing with 0.4ml and 0.7ml respectively and finally 1 ml per minute was selected for the analysis.

PREPARATION OF BUFFER SOLUTION :

Buffer solution was prepared by using 1.3609gm of sodium di hydrogen of HPLC grade water, filtered through 0.45μ nylon membrane and degassed.

PREPARATION OF MOBILE PHASE :

Mix the Buffer, methanol: and acetonitrile in the ratio of 60:20:20 and degass it. Filtered through 0.45μ membrane.

DILUENTS

Mobile phase is used as diluents.

DETERMINATION OF RETENTION TIME :

(A) **PREPARATION OF STANDARD SOLUTION OF OLMESARTAN MEDOXOMIL**

Accurately 0.200g Olmesartan Medoxomil was taken in a 100ml volumetric flask and the volume was adjusted to 100ml with mobile phase. 5ml was taken in a separate 50ml volumetric flask and the volume was adjusted to 50 ml with mobile phase to get concentration of 200 µg/ml of Olmesartan Medoxomil. 20µl of this solution was injected and chromatogram was obtained and it is in shown Figure-2.

(B) PREPARATION OF STANDARD SOLUTION OF HYDROCHLOROTHIAZIDE.

Accurately 0.125g of hydrochlorothiazide was taken in a 100ml volumetric flask and the volume was adjusted to 100ml with mobile phase. 5 ml was taken in a separate volumetric flask of 50ml and the volume was adjusted to 50ml with mobile phase to get a concentration of 125 µg/ml. 20 µl of this solution was injected and the chromatogram was recorded and shown in Figure-3.

(C) PREPARATION OF MIXED STANDARD SOLUTION :

0. 200g of Olmesartan Medoxomil and 0.125g of hydrochlorothiazide was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 20ml of mobile phase and it was sonicated. Then the volume was adjusted to 100ml with mobile phase. From the stock solution

5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50ml with mobile phase to get a concentration of 200µg/ml of Olmesartan Medoxomil and 125 µg/ml of hydrochlorothiazide. 20µ if the resulting solution was injected and chromatogram was recorded shown in Figure-4.

FIXED CHROMATOGRAPHIC CONDITIONS :

INSTRUMENT : Shimadzu Prominence model

LC 20 AT Isocratic

COLUMN : CN18

WAVELENGTH : 230nm

TEMPERATURE : Ambient temperature

FLOW RATE : 1 ml / mit

INJECTION VOLUME : 20µl

MOBILE PHASE : Buffer (Sodium dihydrogen phosphate Methanol and acetonitrile 60.20:20

RETENTION TIME : 4.383 for Olmesartan Medoxomil for 3.150 for hydrochlorothiazide

QUANTITATIVE DETERMINATION OF THE DRUGS OF THE DRUGS BY USING THE DEVELOPED METHOD

Sample   : Olmesartan Medoxomil
              Hydrochlorothiazide

Label Claim   : Olmesartan Medoxomil 20mg

              Hydrochlorothiazide 12.5 mg

METHOD :

Twenty tablets were weighed and powdered. Average weight 593.2g of sample tablet OLMEZEST - H (equivalent 20 mg of Olmesartan Medoxomil and 12.5 mg of hydrochlorothiazide was taken into 100ml dried volumetric flask. The powder was first dissolved in 20ml mobile phase and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 200µg/ml of Olmesartan Medoxomil and 125 µ/ml of hydrochlorothiazide.   20µl of the solution was injected and the chromatogram obtained is shown in Figure 5.

The amount of Olmesartan Medoxomil and hydrochlorothiazide present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Table-8.

Amount of drug present in the tablet:

$$\frac{\text{Sample area}}{\text{Standard Area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{Average weight}$$

$$\text{Percentage purity} = \frac{\text{Amount present}}{\text{Label claim}} \times 100$$

Table – 8
Quantitative Estimation

S.No.	Brand Name	Content	Label Claim (mg)	Peak area	Amount present (mg)	Percent Purity % w/v
1.	Olmezest -H	Olmesartan Medoxomil	20 mg	2346.125	20.08 mg	100.40 %
		Hydrochloro Thiazide	12.5 mg	4563.539	12.49 mg	99.9 %

Acceptance critearia : 98-102% w/v

Assay for Olmesartan Medoxomil

Amount Present =

$$\frac{2346.125}{2333.090} \times \frac{200}{1449} \times \frac{99.85}{100} \times 144.9 = 20.08 \text{ mg}$$

Percentage Purity = 20.08/20 x 100 = 100.40

Assay for Hydrochlorothiazide

Amount Present = $\frac{4563.539}{4560.786} \times \frac{125}{1449} \times \frac{99.93}{100} \times 144.9 = 12.49 \text{ mg}$

Percentage Purity = 12.49 / 12.5 x 100 = 99.9 %

VALIDATION

Validation of an analytical method is a process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Design of experiment :

- Specificity

- Linearity and range

- Limit of quantification

- Limit of detection

- Accuracy

- Precision

    - System precision

    - Method precision

- Robustness

- Ruggedness

- System suitability studies

    - Resolution

    - Number of theoretical plates

    - The tailing factor.

SPECIFICITY

The specificity of an analytical methods is ability to measure accurately and specifically the analytes in the presence of compounds that may e expected to be present in the sample matrix.

Determination

The specificity of the analytical methods was determined by injecting the placebo solution under the same experimental conditions as the assay.

Preparation of placebo

Placebo is prepared by mixing all the excipients without active ingredients

Procedure

➢ 225 mg placebo was accurately weighted and transferred into 25 ml volumetric flask and the volume as made to 25 ml with the mobile phase. The solution was filtered through Millipore filter paper and degassed. 20 µl of this solution was injected and chromatogram was recorded and shown in figure-6.

➢ 200 mg olmesartan medoxomil and 125 mg hydrochlorothiazide were weighed and transferred into a 100 ml volumetric flask and volume was adjusted to 100 ml with mobile phase. Further 5 ml of the solution was taken and the volume was made upto 50 ml to get a concentration of 200/µg/ml olmesartan medoxomil & 125µg/ml hydrochlorothiazide to this solution 90 mg of placebo was added and it was sonicated, filtered through a Millipore filter paper 20 up of this solution was injected, chromatogram was recorded and shown in Figure-7 the report are shown in Table 9 & 10.

TABLE – 9

SPECIFICITY FOR OLMESARTAN MEDOXOMIL

S.No	Sample	Area Obtained
1	Standard	2332.090
2	Standard + Placebo	2330.642
3	Placebo	0

TABLE – 10

SPECIFICITY FOR HYDROCHLOROTHIAZIDE

S.No	Sample	Area Obtained
1	Standard	4560.786
2	Standard + Placebo	4561.253
3	Placebo	0

LINEARITY AND RANGE

Linearity of an analytical method is its ability to elicit test result that are directly promotional to the concentration of analyte in samples within a given range.

Determination

The linearity of the analytical method was determined by mathematical treatment of test result obtained by analysis of sample with analyst concentration across the claimed range. Area was plotted graphically as a function of analyst concentration. Percentage curve fitting was calculated.

METHOD
Preparation of mixed standard stock solution

Accurately weighted 200   mg olmesartan medoxomil   and 125   mg hydrochlorothiazide were transferred in to the 100 ml of standard flask and it was dissolved with mobile phase and the volume was made upto 100ml with mobile phase. From the resulting solution 4,4.5, 5, 5.5 6 ml were transferred into 5 different 50 ml volumetric flask. The volume was made with mobile phase to get a final concentration of   160, 180, 200,220, 240μg/ml of olmesartan   medoxomil   100,   112.5,   125,   137.5,   150μg/ml   of hydrochlorothiazide. 20 μl   of the resulting solution was injected and chromatogram was recorded.

      The   chromatograms   of   Olmesartan   Medoxomil   and hydrochlorothiazide are shown in figure 8-12.

 Acceptance Criteria

Correlation coefficient should not be less than 0.99

The linearity datas and analytical performance parameters of olmesartan medoxomil and hydrochlorothiazide are shown in Table 11-13 and calibration curve are shown in graph.

TABLE – 11

LINEARITY DATA

OLMESARTAN MEDOXOMIL

S.No	Concentration µg/ml	Peak Area
1	160	1840.700
2	180	2109.02
3	200	2328.729
4	220	2556.345
5	240	2812.535

Table – 12

HYDROCHLOROTHIAZIDE

S.No	Concentration µg/ml	Peak Area
1	100.0	3654.119
2	112.5	4104.276
3	125.0	4555.297
4	137.5	5015.889
5	150.0	5472.902

TABLE – 13

ANALYTICAL PERFORMANCE PARAMETERS

S.No	Drug name	Linear dynamic range (µg/ml)	Correlation coefficient	Slope	Intercept
1	Olmesartan Medoxomil	160-240	0.9994	11.95143	-60.6784
2	Hydrochlorothiazide	100-150	0.9999	36.3934	11.3176

ACCURACY

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amount of analyte.

Determination

The accuracy of the analytical method was determined by applying the method to the analysed samples to which known amounts of analyte had been added. The accuracy was calculated from the test results as the percentage of analyte recovered by the assay.

Acceptance criteria :

Percentage recovery should be within 98-102 %

PROCEDURE

Mixed standard stock solution 2 ml and sample stock solution 2 ml were mixed together in 50 ml volumetric flask and the volume was made upto 50ml with mobile phase to get 80 % range. Similarly 100 % and 120 % range was prepared 20μl of this solution was injected three times and chromatograms were shown in Fig. 13 to 21 and values in table 14 and 15.

TABLE -14

RECOVERY STUDY OF OLMESARTAN MEDOXOMIL

S.No	Range	Area Obtained	Amount Recovered (mg)	% Recovery
1		1851.636	19.81	99.05
		1851.698	19.81	99.05
	80 %	1850.730	19.80	99.00
2		2317.664	19.83	99.18
		2318.012	19.84	99.20
	100 %	2317.687	19.83	99.19
3		2807.483	20.02	100.12
		2806.632	20.01	100.09
	120 %	2807.455	20.02	100.12

TABLE – 15

RECOVERY STUDY HYDROCHLOROTHIAZIDE

S.No	Range	Area Obtained	Amount Recovered (mg)	% Recovery
1		3671.848	12.55	100.46
		3671.863	12.56	100.46
	80 %	3670.995	12.56	100.44
2		4549.086	12.45	99.60
		4549.099	12.45	99.70
	100 %	4549.102	12.45	99.60
3	120 %	5438.741	12.41	99.30
		5439.231	12.41	99.31
		5438.929	12.41	99.30

PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation.

Determination :

The precision of the analytical method was determined by assaying sufficient number of sample and relative standard deviation was calculated.

The precision of the instrument was determined by assaying the samples consecutively, number of time and relative standard deviation was calculated.

Acceptance Criteria

The relative standard deviation should be with in 2 %

SYSTEM PRECISION

Procedure

Accurately weighed 200 mg olmesartan medoxomil and 125 mg of hydrochlorothiazide acid were transferred into 100 ml standard flask and it was dissolved with mobile phase. From the resulting solution 50 ml was transferred into 50 ml volumetric flask. The volume was made up with mobile phase to 50 ml.

The system precision was evaluated by measuring 6 successive injection of 20μl of standard solution. The peak response were measured from the chromatogram shown in figure 22-27 and system precision data area shown in table 16 & 17..

Method Precision

Procedure

Twenty tables were weighted and powdered. Average weight 144.9 of sample tablet OLMEZST-H (equivalent to 200mg olmesartan medoxomil and 1250 mg of hydrochlorothiazide was taken into 100 ml dried volumetric flask. The power was first dissolved in 20ml of mobile phase and sonicated and finally the volume was adjusted to 100 ml with mobile phase. From this solution 5ml transferred to 50 ml volumetric flask and volume was adjusted to 50 ml with mobile phase to get a concentration of 200 μg/ml of olmesartan medoxomil and 125 μg/ ml of hydrochlorothiazide. 20μl of the solution was injected and the chromatogram obtained is shown in Figure 28-33 and method precision data are shown in table 18-20.

TABLE – 16

SYSTEM PRECISION DATA

S.No	Area of Olemesartan medoxomil	Area of Hydrochlorothiazide
1	2330.464	4560.789
2	2350.522	4576.226
3	2324.136	4572.686
4	2316.563	4581.903
5	2324.821	4571.016
6	2326.064	4553.828
Mean	2328.761	4569.408
S.D.	11.5708	10.3234
% RSD	0.4968	0.2259

Table -17

Relative standard deviation	Olmesartan Medoxomil	Hydrochlorothiazide	Acceptance Criteria
	0.496	0.2259	2 %

Table – 18 Method Precision of Olemesartan Medoxomile

S.No	Area Obtained	Amount Recovered in (mg)	Purity % w/v
1	2338.738	20.04	100.20
2	2347.046	19.94	99.70
3	2356.955	20.25	101.20
4	2349.124	20.25	101.25
5	2357.832	20.26	101.26
6	2358.983	20.25	101.26
	MEAN		100.82
	STANDARD DEVIATION		7.9269
	RELATIVE STAND DEVIATION		0.3371

Table – 19 Method Precision of hydrochlorothiazide

S.No	Area Obtained	Amount Recovered In (mg)	Purity % w/v
1	4563.542	12.49	99.99
2	4584.705	12.51	100.11
3	4547.645	12.42	99.38
4	4584.653	12.49	99.98
5	4573.801	12.49	99.99
6	4556.599	12.49	99.99
	MEAN		99.90
	STANDARD DEVIATION		15.1839
	RELATIVE STAND DEVIATION		0.3323

Table – 20 Method Precision report for Olmesartan Mexoxomil and Hydrochlorothiazide

Relative standard deviation	Olmesartan Medoxomil	Hydrochlorothiazide	Acceptance Criteria
	0.3371	0.3223	2 %

LIMIT OF DETECTION (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessary quantities as an exact value under the stated, experimental conditions. The detection limit is usually expressed as the concentration of analyte.

It is given by

$$LOD = \dfrac{3.3 \times \sigma}{m}$$

σ = standard deviation of the response

m = slope of the calibration curve

TABLE – 21

LIMIT OF DETECTION

DRUG	STANDARD DEVIATION	SLOPE	L.O.D µg/ ml
Olmesartan Medoxomil	11.5708	11.95143	3.194
Hydrochlor thiazide	10.3234	36.393432	0.93608

LIMIT OF QUANTITATION

The Quantitation limit of an analytical procedure is the lowest amount of analytic which can be quantitatively determined with suitable precision and Accuracy.

It is given by

$$L.O.Q = \frac{10x \, \sigma}{m}$$

σ= Standard deviation of the response

m= slope of the calibration curve

TABLE – 22

LIMIT OF QUANTITATION

DRUG	STANDARD DEVIATION	SLOPE	L.O.Q µg / ml
Olmesartan Medoxomil	11.5708	11.95143	9.6815
Hydrochlorothiazide	10.3234	36.3934	2.8366

RUGGEDNESS

The Ruggedness of an analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under a variety of normal test condition, such as different laboratories, different analyst, different assay temperature, different days etc.

Ruggedness is normally expressed as the lack of influence of test result of operations and environmental variables of the analytical method.

Determination :

The ruggedness of an analytical method was determined by analysis of aliquots from homogenous lots by different analysis using operations and environmental conditions that may different but were still with in the specified parameters of the assay. The degree of reproducibility of test result was then determined as a function of the assay variables. This reproducibility was then determined as a function of the assay variables. This reproducibility was assayed under normal conditions to obtain a measure of the ruggedness of analytical method.

The assay olmesartan medoxomil and hydrochlorothiazide were performed in different conditions like different analyst on different days.

METHOD

The standard and sample solutions were prepared by different analysts on different days and the resulting solution were injected and chromatograms are recorded and shown in Figure 35-42 and ruggedness of the method and report of olmesartan medoxomil and hydrochlorothiazide acid or shown in Table 26.

Table -23

RUGGEDNESS

Analyst	Date	Amount Found		% purity	
		Olmesartan Medoxomil	Hydrochlorothiazide	Olmesartan Medoxomil	Hydrochlorothiazide
I	19 Nov 2008	20.251 mg	12.498 mg	101.27	99.98
II	20 Nov 2008	20.254 mg	12.494 mg	101.27	99.95

ROBUSTNESS

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by different physical parameters that may differ but were within the specified parameter of the assay for example change in physical parameters like flow rate and lambda max.

Method

Standard solution preparation

200 mg olmesartan medoxomil and 125 mg hydrochlorothiazide were transferred into a 100 ml volumetric flask, volume was adjusted to 100 ml with mobile phase. Further 5 ml of the solution was taken and the volume was made upto 50 ml to get a concentration of 200 µg/ml of olmesartan medoxomil and 250 µg/ml of hydrochlorothiazide.

Sample preparation

Twenty tablets were weighted and powered. Average weight 144.9 g of sample tablet OLMEZEST-H (equivalent to 200 mg olmesartan medoxomil 125 mg hydrochlorothiazide) was taken into 100 ml dried volumetric flask. The powder was first dissolved in 20 ml of mobile phase. From this solution 5 ml was transferred in to 50 ml volumetric flask and volume was adjusted to 100 ml with mobile phase. 20µl of this solution was injected and individual chromatograms were recorded and they are in figure 38-45 and datas are shown in Table 24-31.

TABLE 24

Chromatographic conditions : - change in flow rate 0.8 ml/ min

Change in flow rate	0.8 ml / min
Column	CN
Wave length	230 nm
Temperature	Ambient 25^0.c
Injection volume	20 µl

TABLE 25

Change in flow rate 0.8 ml/ min

S.No	Drug	standard area	sample area	% Purity w/v
1	Olmesartan Medoxomil	2162.835	2195.655	101.36
2	Hydrochlorothiazide	4144.16	4146.914	99.99

TABLE -26

Chromatographic condition :- change in flow rate (1.2 ml/ min)

Change in flow rate	1.2 ml / min
Column	CN
Wave length	230 nm
Temperature	Ambient 25ºc
Injection volume	20 µl

TABLE 27

Change in flow rate 1.2ml/ min

S.No	Drug	Standard sample	sample area	% Purity w/v
1	Olmesartan Medoxomil	1615.951	1648.766	101.87
2	Hydrochlorothiazide	2516.911	2919.671	100.02

TABLE -28

Chromatographic condition :- change in Lambda Max 232 nm

Column	CN
Wave length	230 nm
Temperature	Ambient 25ºc
Injection Volume	20 μl
Flow rate	1 ml/min

TABLE 29

Change in flow rate 232 nm

S.No	Drug	standard area	sample area	% Purity w/v
1	Olmesartan Medoxomil	2220.564	2253.385	101.36
2	Hydrochlorothiazide	4422.845	4425.600	99.99

TABLE -30

Chromatographic conditions : - Change in Lambda Max 228 nm

Column	CN
Wave length	230 nm
Temperature	Ambient 25^0c
Injection Volume	20 μl
Flow rate	1 ml/ min

TABLE 31

Change in flow rate 228 nm

S.No	Drug	standard area	sample area	% Purity w/v
1	Olmesartan Medoxomil	2414.815	2447.130	101.21
2	Hydrochlorothiazide	4648.815	4649.571	99.94

SYSTEM SUITABILITY PARAMETERS

System suitability testing is an integral part of many analytical procedures. The test based on the concept that the equipment, electronics, analytical operation and sample to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particulars procedure depend on the type of procedure being validated.

Method

A solution of 200µg/ml of Olmesartan medoxomil and 125µg/ml hydrochlorothiazide were prepared by diluting with mobile phase and same was injected and a chromatogram was recorded and they are shown in figure 59 and system suitability report are shown in the following.

TABLE – 32
SYSTEM SUITABILITY PARAMETERS

S.No	Parameters	Olmesartan Medoxomil	Hydrochlorothiazide
1	Theoretical plates	8797	4280
2	Tailing Factor	1.444	1.783
3	Resolution	6.499	

RESULTS AND DISCUSSION

Drotaverine hydrochloride and mefenamic acid

UV Spectrophotometery and reverse phase high performance liquid chromatography were developed for analyzing Drotaverine hydrochloride and mefenamic acid in combined tablet dosage form.

For UV spectrophotometry linearity was obtained in the concentration range of 5 to 50 µg/ml for both drugs. In simultaneous equation method the % drug content was found to be 99.95 % 98.80 % for Drotaverine hydrochloride and mefenamic acid. Recovery experiments were performed and it was within 98-102 % and showed < 2 % RSD which is highly precises and accurate for the proposed methods.

In HPLC method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially various mobile phase were tried, to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, etc). The system with buffer (potassium di hydrogen phosphate) : methanol : Acetonitrile (50:15:35 v/v) with 1.5 ml/ / min flow rate is quite robust. The optimum wavelength for detection was 240 nm at which better detector response for drugs was obtained. The average retention times Drotaverine hydrochloride and mefenamic acid is 6.4 min and 3.0 min respectively.

According to USP system suitability test are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solution.

The calibration curve was found to be linear for both Drotaverine hydrochloride and mefenamic acid . The percentage purity was 100.20 % and 100.05 % for Drotaverine hydrochloride and mefenamic acid respectively.

The mean recoveries were found to be in the range of 98 % to 102 %.

Limit of detection for Drotaverine hydrochloride and mefenamic acid was found to be 0.00076519µg/ml and 0.003796µg/ml respectively.

Limit of quantitation for Drotaverine hydrochloride and mefenamic acid was found to be 0.002318µg/ml and 0.011505µg/ml respectively.

Robustness of the proposed method was determined by changing the wavelength and flow rate.

Ruggedness of proposed method was determined by analysis of aliquots from homogenous slot by different analyst in different days using similar operation environmental conditions. The results were within 98-102 %.

Olmesartan Medoxomil and hydrochlorothiazide

UV Spectrophotometery and reverse phase high performance liquid chromatography were developed for analyzing Olmesartan Medoxomil and hydrochlorothiazide in combined tablet dosage form.

For UV spectrophotometry linearity was obtained in the concentration range of 5 to 50 µg/ml for both drugs. In simultaneous equation method the % drug content was found to be 99.99 % and 98.82 % for Olmesartan Medoxomil and hydrochlorothiazide. Recovery experiments were performed and it was within 98-102 % and showed < 2 % RSD which is highly precises and accurate for the proposed methods.

In HPLC method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially various mobile phase were tried, to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, etc). The system with buffer (sodium di hydrogen phosphate) : methanol : Acetonitrile (60:20:20 v/v) with 1 ml/min flow rate is quite robust. The optimum wavelength for detection was 230 nm at which better detector response for drugs was obtained. The average retention times Olmesartan Medoxomil and hydrochlorothiazide is 4.383 min and 3.150 min respectively.

According to USP system suitability test are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solution.

The calibration curve was found to be linear for both Olmesartan Medoxomil and hydrochlorothiazide. The percentage purity was 100.40 % and 99.9 % for Olmesartan Medoxomil and hydrochlorothiazide respectively.

The mean recoveries were found to be in the range of 98 % to 102 % .

Limit of detection for Olmesartan Medoxomil and hydrochlorothiazide was found to be 3.194µg/ml and 0.93608µg/ml respectively.

Limit of quantitation for Olmesartan Medoxomil and hydrochlorothiazide was found to be 9.6815µg/ml and 2.8360µg/ml respectively .

Robustness of the proposed method was determined by changing the wavelength and flow rate.

Ruggedness of proposed method was determined by analysis of aliquots from homogenous slot by different analyst in different days using similar operation environmental conditions. The results were within 98-102 %.

SUMMARY AND CONCLUSION

Drotaverine hydrochloride and Mefenamic aid in combined tablet dosage form analyzed by UV – spectrophotometric simultaneous equation and Reverse phase high performance liquid chromatography.

On comparing these methods Reverse phase high performance liquid chromatography was found to be more precise, accurate, rugged, robust,simple, and rapid than UV spectrophotometric method.

Olmesartan medoxomil and hydrochlorothiazide in combined tablet dosage from was analyzed by UV – spectrophotometric simultaneous equation method and Reverse phase high performance liquid chromatography.

On comparing these methods Reverse phase high performance chromatography was found to be more precise, accurate, rugged robust, simple and rapid than spectrometric method.

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