International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 5(2): March-April 2015

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Review Article……!!!**

Received: 19-02-2015; Revised: 23-02-2015; Accepted: 01-03-2015

METHOD DEVELOPMENT & VALIDATION OF ESCITALOPRAM OXALATE & ETIZOLAM BY UV- SPECTROPHOTOMETRY

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Keywords:

UV spectrophotometry, simultaneous estimation, escitalopram oxalate & etizolam

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ABSTRACT

UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the UV visible region are called ultraviolet-visible spectrophotometers. Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods. The spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances, which potentially interfere in the assay. If the formula of sample is known, identity and concentration of the interfering substance is known then the extent of interference in the assay may also be determined. Further more validation work is to be carried out considering various parameters like ,specificity Accuracy ,Linearity, Precision, Ruggedness, Robustness, limit of detection(LOD), limit of quantitation(LOQ).

INTRODUCTION

1.1 Analytical Method Development

Development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method. Method development usually requires selecting the method requirements and deciding on what type of instrumentation is to be utilized.

There are several valid reasons for developing new methods of analysis:

- There may not be a suitable method for a particular analyte in the specific sample matrix.
- Existing methods may be too expensive, time consuming, or energy intensive, or they may not be easily automated.
- Newer instrumentation and techniques may have evolved that would provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision, or better return on investment.
- There may be a need for an alternative method to confirm, for legal or scientific reasons, analytical data originally obtained by existing methods.

To develop a method, it is necessary to consider the properties of the analyte(s) of interest that may be used to establish the optimal ranges of analyte parameter values.Once the instrumentation has been assembled and analyte parameters have been considered, standards should be used for the continuous development, optimization, and preliminary evaluation of the method.

It is important that method development should be performed using only analytical standards that have been well identified and characterized and whose purity is already known. Such precautions will prevent problems in the future and will remove variables when one is trying to optimize or improve the initial conditions during the method development [1].

Analytical methods for the drugs, as per instrumentation criteria include; [2]

a. Non-instrumental methods of analysis
   i. Gravimetery
   ii. Titrimetry
   iii. Voltametry
b. Instrumental methods of analysis
   i. Electrical method
   ii. Optical method

iii. Emission method

iv. Chromatography

1.2 Classification of analytical methods

Analytical methods can be broadly classified into following types:

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

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

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

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

Analytical methods as per USP are:

Category I: Analytical methods for quantitation of major components of bulk drug substances or active ingredients including preservatives in the finished pharmaceutical products.

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

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

Category IV: Identification tests.[3]

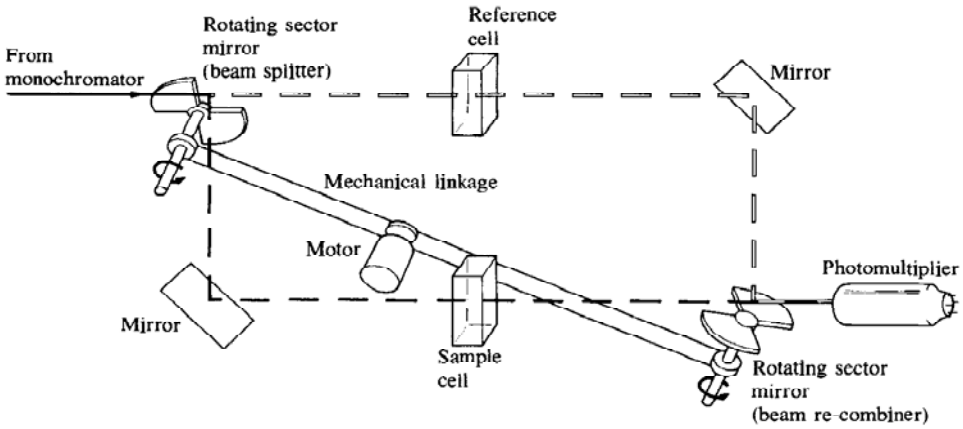
1.3 Analytical method development by UV-Visible spectrophotometry

1.3.1 Introduction to UV Visible Spectroscopy

1.3.1.1 Criteria for Method Development by UV Spectroscopy

a) Compound must fall in the absorbance range of 200-400 nm.
b) Compound should have a chromophore, or the chromophore should be reactive to some derivatizing agents.
c) Compound should be unsaturated.
d) Compound should follow Lambert-Beers law
e) To follow Lambert-Beers law, concentration of the compound should be very low.
f) All molecules have absorption bands; therefore solvent taken must be transparent within the wavelength range being examined.

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the UV visible region are called ultraviolet-visible spectrophotometers. Flow scheme of UV spectrophotometry is shown in **figure 1**.[4]



1.2 The Beer-Lambert's Law

Absorbance (A) = Constant x Concentration x Cell length Or,

Molar Absorptivity, $\varepsilon = A / c\, l$

Where, A= absorbance, c = sample concentration in moles/liter and l = length of light path through the sample in cm.

The law is only true for monochromatic light, that is, light of a single wavelength or narrow band of wavelengths, provided that the physical or chemical state of the substance does not change with concentration. When monochromatic radiation passes through a homogeneous solution in a cell, the intensity of the emitted radiation depends upon the thickness (l) and the concentration (c) of the solution. Transmission of monochromatic radiation through sample is shown in **figure 2**.[5]

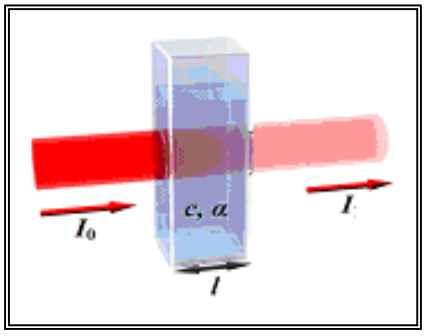


Figure 2: Transmission of monochromatic radiation through sample

Io is the intensity of the incident radiation and I is the intensity of the transmitted radiation. The ratio I/Io is called as transmittance of radiant energy. This is sometimes expressed as a percentage and is referred as % transmittance.

Mathematically, absorbance is related to percentage transmittance T by the expression:

$$A = \log10 (Io/I) = \log10 (100/T) = kcl$$

Where, l = length of the radiation path through the sample,

c = concentration of absorbing molecules in that path, and

k = extinction coefficient - a constant which depend only on the nature of the molecule and the wavelength of radiation.

Absorbance in older literature is sometimes referred to as 'extinction' or 'optical density'. If, in the expression A = kcl, c is expressed in molar^{-1}and l in m, then k is replaced by the symbol T and is called the molar absorption coefficient.

The units of T are mol^{-1}m^2. T was formerly called the molar extinction coefficient and concentrations were often expressed as mol.l^{-1}, mol dm^{-3} or M and the cell length in cm to give units mol^{-1}cm^{-1}, m^{-1}dm^{3}cm^{-1} and M^{-1} cm^{-1} respectively. [5, 6]

Chromatography

Chromatography, the process by which the components of a mixture can be separated, has become one of the primary analytical methods for the identification and quantification of compounds in the gaseous or liquid state.

The basic principle is based on the concentration equilibrium of the components of interest, between two immiscible phases. One is called the stationary phase, because it is immobilized within a column or fixed upon a support, while the second, called the mobile phase, is forced through the first.

The phases are chosen such that components of the sample have differing solubility's in each phase. The differential migration of compounds leads to their separation.

"Methods used primarily for the separation of the components of a sample, in which the components are distributed between two phases, one of which is stationary while other moves". The word chromatography is derived from Greek word '**chroma**' meaning color and '**graphein**' meaning to write. Chromatography is group of methods for separating molecular mixtures. Solutes are separated by a differential migration process in a system consisting of two or more phases. One of which moves continuously in the given direction called as mobile phase and the individual components exhibits different mobilities by reason of different adsorption, partition, solubility, vapour pressure, molecular size or ionic charge density. The

other is stationary phase may be a porous or finely divided solid or liquid that has been coated in a thin layer on an inert supporting material [3].

Partition Chromatography

It involves predominantly a simple partitioning between the immiscible phases, one stationary and the other mobile.

Adsorption Chromatography

It is the technique, where physical surfaces are mainly involved in the retentive ability of the stationary phase.

Ion exchange Chromatography

It involves separation of ionic components of the sample, by selective exchange with counter ions of the stationary phase.

1.4. Quantitative Analysis by UV-visible Spectrophotometry

1.4.1 Methods of estimation of single component formulations

Quantitative spectrophotometric assay of medicinal substance as a single entity can be carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength, most preferably the wavelength maxima (λ_{max}). The concentration of the absorbing substance can be calculated from the measured absorbance using one of the four principle procedures. [7]

- Use of standard absorptivity value
- Use of calibration graph
- Single or double point standardization
- Chemical derivatization Method

1.5 Methods of estimation of multi-component formulations

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods. [8] The spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances

is required in samples known to contain other absorbing substances, which potentially interfere in the assay. If the formula of sample is known, identity and concentration of the interfering substance is known then the extent of interference in the assay may also be determined. [9]

A number of modifications to the simple spectrophotometric procedure are available to the analyst, which may eliminate certain sources of interference and permit the accurate determination of all the absorbing components. Each modification of the basic procedure may be applied if certain criteria are satisfied.

The basis of all the spectrophotometric techniques for multi component samples is the property that at all wavelengths:

- the absorbance of a solution is the sum of absorbance of the individual components or
- the measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell. [10]

There are various spectrophotometric methods available which can be used for the analysis of a combination samples. Following methods can be used

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry
- Solvent extraction method
- Two wavelength method
- Geometric correction method
- Orthogonal polynomial method
- Difference spectrophotometry
- Area under curve method

(A) Simultaneous Equation Method

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ max of the other (as shown in figure 3. λ_1 and λ_2), it may be possible to determine both drugs by the technique of simultaneous equation (Vierodt's method) provided that certain criteria apply.

The informations required are:

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

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

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

At λ_1 $A_1 = a_{x1}bC_x + a_{y1}bC_y$ ..... (1)

At λ_2 $A_2 = a_{x2}bC_x + a_{y2}bC_y$ ……. (2)

For measurements in 1 cm cells, b =1.

Rearrange equation (2)

$C_y = (A_2 - a_{x2} C_x) / ay2$

Substituting for C_y in eq. (1) and rearranging gives

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

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

The overlain spectra of substance X and Y in admixture by the simultaneous equation method is shown in **figure 3**.

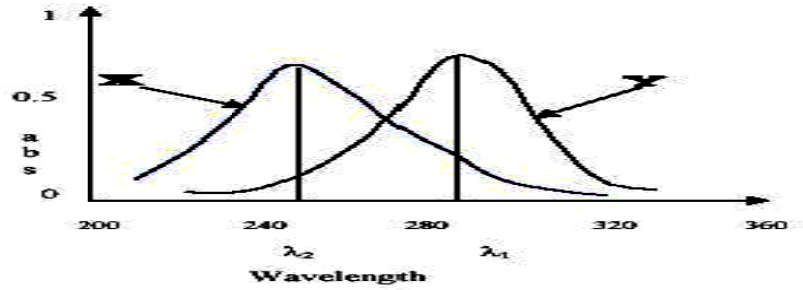


Figure 3: The overlain spectra of substance X and Y showing their wavelength in admixture by the method of simultaneous equation.

Criteria for obtaining maximum precision have been suggested by Glenn. According to him absorbance ratio place limits on the relative concentrations of the components of the mixture.

$(A_2/A_1) / (a_{x2}/a_{x1})$ and $(a_{y2}/ay_1)/ (A2/A_1)$

The criteria are that the ratios should lie outside the range of 0.1- 2.0 for the precise determination of X and Y respectively. These criteria are satisfied only when the λmax of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically, thereby the initial assumption is that the total absorbance is the sum of the individual absorbance. The additive of the absorbance should always be confirmed in the development of a new application of this technique. [11]

(B) Q-Absorbance Method (Absorbance Ratio Method)

Q-Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength. The ratio of absorbances at any two wavelengths is constant value

independent of concentration or path length. For example, two different dilution of the same substance give the same absorbance ratio A_1/A_2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbances are measured at two wavelengths. One being the λmax of one of the component and the other being a wavelength of equal absorptivities of the two components (as shown in **figure 4**) i.e. an isoabsorptive point. [11]

Two equations are constructed as described for simultaneous equation method.

$A_1 = a_{x1}C_x + a_{x1}C_y$ ..... (1)

$A_2 / A_1 = (a_{x2}C_x + a_{y2}C_y) / (ax_1C_x + a_{x1}C_y)$

Divide each term by $C_x + C_y$ and let $Fx = C_x / (C_x + C_y)$ and $Fy = C_y / (C_x + C_y)$ i.e. Fx and Fy are the fraction of X and Y respectively in the mixture:

$A_2 / A_1 = (a_{x2} F_x + a_{y2}F_y) / (ax_1F_x + a_{x1}F_y)$

But $Fy = 1 - Fx$

$A_2 / A_1 = (ax2\, F_x - F_x\, a_{y2} + a_{y2)} / a_{x1}$

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

Wavelength for the assay of substances X and Y in admixture by the absorbance ratio method is shown in **figure 4**.

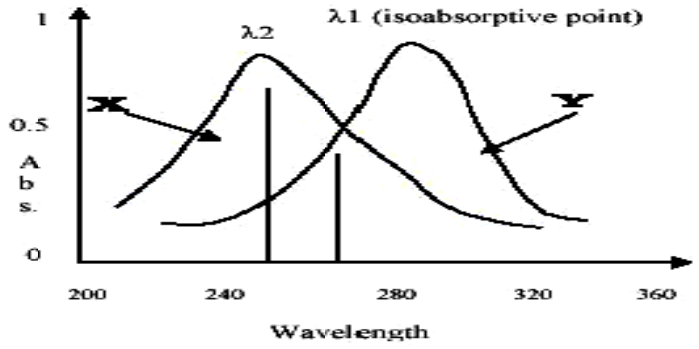


Figure 4: Wavelength for the assay of substances X and Y in admixture by the absorbance ratio method.

Let $Q_X = a_{x2} / a_{x1,}$ $Q_Y = a_{y2}/ a_{y1,}$ and $Q_M = A_2 / A_1$

$Q_M = F_x (Q_X - Q_Y) + Q_Y$

$F_x = (Q_M - Q_Y) / (Q_X - Q_Y)$ ..... (2)

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Divide each term by $C_x + C_y$ and let $Fx = C_x / (C_x + C_y)$ and $Fy = C_y / (C_x + C_y)$ i.e. Fx and Fy are the fraction of X and Y respectively in the mixture:

$A_2 / A_1 = (a_{x2} F_x + a_{y2}F_y) / (ax_1F_x + a_{x1}F_y)$

But $Fy = 1 - Fx$

$A_2 / A_1 = (ax2\, F_x - F_x\, a_{y2} + a_{y2}) / a_{x1}$

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

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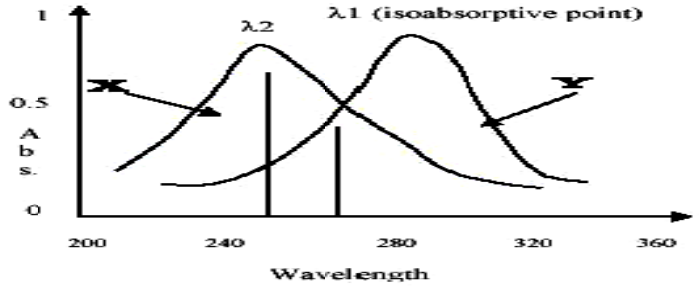

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$Q_M = F_x (Q_X - Q_Y) + Q_Y$

$F_x = (Q_M - Q_Y) / (Q_X - Q_Y)$ ..... (2)

spectrum correspond with cross-over points in the D^2 spectrum.

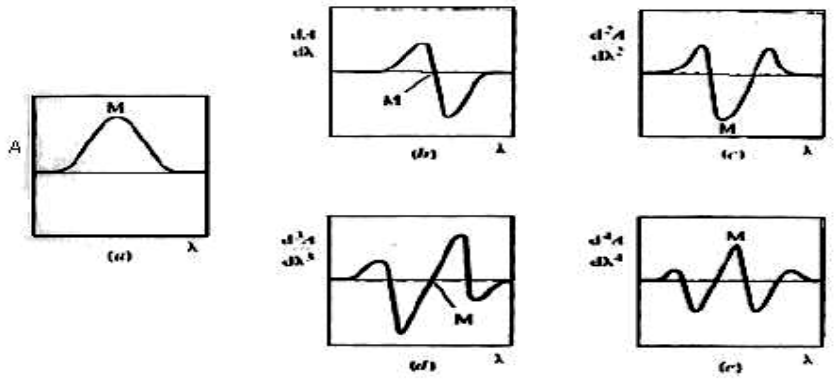


Figure 5: Spectrum of (a) Gaussian peak, (b) First, (c) Second, (d) Third and (e) Fourth derivative Spectrum.

(D) Solvent Extraction Method

In solvent extraction method, quantitation of individual drugs in combinations is performed by separating of individual drugs on basis of their selective solubility followed by spectrophotometric measurement. If the interference from the other absorbing substances is large, it may be possible to separate the absorbing interferent from the analyte by solvent extraction procedure. These are particularly appropriate for acidic or basic drugs whose state of ionisation determines their solvent partitioning behavior. The judicious choice of pH of the aqueous medium may affect the complete separation of the interferents from the analyte, the concentration of which may be obtained by a simple measurement of absorbance of the extract containing the analyte. [11]

(E) Two wavelength method

The method is used to calculate the concentration of component of interest along with some unwanted interfering components in a mixture. The absorption difference between two points on the mixture spectra is directly proportional to the concentration of the component to be determined irrespective of the interfering component. [11]

(F) Geometric correction method

A number of mathematical correction procedures have been developed which reduces or eliminates the background irrelevant of absorption that may be present in the samples of biological origin. The simplest of this procedure is the three-point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected. This procedure is simply algebraic calculations of what the baseline technique in infrared spectrophotometry does graphically. [11]

(G) Orthogonal polynomial method

The technique of orthogonal polynomials is another mathematical correction procedure, which involves complex calculation than the three-point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions. [11]

(H) Difference spectrophotometry

Difference spectrophotometry provides a sensitive method for detecting small changes in the environment of a chromophore or it can be used to demonstrate ionization of a chromophore leading to identification and quantitation of various components in a mixture. The essential feature of difference spectrophotometric assay is that the measured value is the difference in the absorbance (ΔA) between two equimolar solutions of the analyte in different chemical forms, which exhibits different spectral characteristics. [11]

(I) Area under curve method

In this method, the absorptivity values (ε_1 and ε_2) of each of the two drugs were determined at the selected wavelength range. Total area under curve of a mixture at wavelength range is equal to the sum of area under the individual component at that wavelength range. This method is applicable when the λ_{max} of the two components are reasonably dissimilar, both two components do not interact chemically and must be soluble in same solvent. [12]

1.6 Determination of optical parameters [13]

The molecular absorptivity and Sandell's sensitivity are calculated as,

Molecular Absorptivity (Є) = AM / Ct

Where A = Absorbance, M = Molecular weight, C = Concentration of Sample, t = Path length

Sandell's Sensitivity = M / Є

Where M = Molecular weight, Є = Molecular Absorptivity

Other optical parameters i.e. Beer's limit, Slope, Intercept and Correlation Co-efficient are calculated from calibration curve.

1.7 Validation of analytical method

Validation is an act of proving that when any procedure, process, equipment, material, activity or system is performed as expected under given set of conditions then it should give the required accuracy, precision, sensitivity, ruggedness to the method /system etc.

When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc. [14, 15]

The various validation parameters used as per ICH guideline are:

- accuracy,
- precision(intraday and interday precision, repeatability and reproducibility),
- linearity,
- range,
- limit of detection(LOD) and limit of quantitation(LOQ),
- selectivity/ specificity,
- robustness/ ruggedness

The various validation parameters used as per ICH guideline are shown in table 1.

Advantages of Analytical method Validation

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

Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end. Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

Key parameters of the analytical method validation: The various performance parameters, which are addressed in a validation exercise, are grouped as follows.

1.7.1 Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often expressed as percent recovery by the assay of a known amount of analyte added.

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

The ICH document recommends that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicates of each concentration).

1.7.2 Precision

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

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, different batch of reagent, different analysts, and different equipments.

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

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

The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e. three concentrations and three replicates of each concentration or using a minimum of six determinations at 100% of the test concentration).

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

1.7.5 Linearity and Range

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

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

1.7.6 Limit of Detection and Limit of Quantitation

Limit of detection: It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing the test results from the samples having known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. Limit of quantitation*:* Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in the finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when required procedure is applied in a solution of acetyl salicylic acid (ASA), then injected into HPLC system.

1.7.7 Selectivity and Specificity

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

1.7.8 Robustness

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

Table 1: Validation parameters as per ICH guidelines Q2 (R1)[18-21]:

Parameters		Acceptance criteria
Specificity		Interference ≤ 0.5%
Linearity		Correlation Coefficient (r^2) ≥ 0.999
Range		80 – 120 % of target concentration which can be detected accurately (100±2%) & precisely (RSD ≤2%)
Precision	**Repeatability**	RSD ≤ 2%
	Intermediate Precision	RSD ≤ 2%
Accuracy		Recovery (98-102%). Spike with 80%, 100% and 120%
Detection Limit		S/N > 2 or 3
Quantitation Limit		S/N >10, RSD ≤ 20%
Robustness		Various conditions for U.V. as follows: Change in λ_{max}, change in scanning speed, change in room temperature

2. Review of literature:

2.1 Official Methods for Determination of Drug:

(A) Escitalopram oxalate: It is official in Indian Pharmacopoeia 2010. There is official method for determination by HPLC.

(B) Etizolam: It is official in British Pharmacopoeia 2012, United states Pharmacopoeia 2011. There is official method for determination by HPLC[15-18].

2.2 Other Reported Methods:

Dighe V. V.et al (2012)[19] developed and validated, simple, sensitive, precise and NP-HPLC method for simultaneous estimation of Escitalopram oxalate and R-enantiomer of Escitalopram oxalate as API and tablet dosage form using uv detector at 240 nm. The developed method was able to separate R-enantiomer of Escitalopram oxalate from its bulk drug within 25 min. The chromatographic separation was carried out using column of cellulose based chiral stationary phase (Chiralcel-OD 250mm x 4.6mm x 10µm) with a mobile phase comprising of n-heptane, isoropanol, diethylamine (94.5:5:0.5, v/v/v) at a flow rate of 1.0 ml/min at 25°C temperature. The limit of detection (LOD) and limit of quantitation (LOQ) of R-enantiomer of escitalopram oxalate were found to be 0.16 µg/ml and 0.50 µg/ml respectively. The linearity of response of R-citalopram oxalate was in the range of 0.05 µg/ml to 7.5 µg/ml with $r^2 \leq 0.9999$. The percentage recovery of the escitalopram oxalate for bulk drug sample ranged between 98.20 to 98.97 and for tablet ranged between 97.07 to 99.61. The percentage recovery of the R-citalopram oxalate from escitalopram oxalate bulk drug sample ranged between 97.41 to 100.79 and for escitalopram oxalate tablet ranged between 98.82 to 99.61. The method was validated and found to be suitable for estimation of escitalopram and determination of R-citalopram from bulk drug and tablet dosage form.

Chakole R.D. et al (2012)[20] developEd and validated RP-HPLC method for simultaneous estimation of Escitalopram and clonazepam in tablet dosage form. The process was carried out on on a 250 A— 4.6 mm, 5I¼, C18 column. The flow rate was 1ml/min and eluent was monitored by absorbance at 248 nm using a mixture of methanol and Buffer (pH 4.0) in the ratio of 90:10 (v/v). The retention time of Escitalopram and Clonazepam was found to be 3.22 and 4.29 min respectively. The total run time was 10 min. The proposed method was validated by testing its linearity, recovery, specificity, system suitability, precision (inter day and intraday), robustness and LOD/LOQ values and it was successfully employed for the simultaneous estimation of Escitalopram and clonazepam in pharmaceutical tablet formulations.

Bhimanadhuni C.N. et al (2012)[21] developed and validated a simple, efficient and reproducible RP-HPLC method was for the simultaneous determination of Escitalopram oxalate and Clonazepam in combined tablet dosage form. The separation was effected on a Hypersil ODS C18 column (250 mm X 4.6mm; 5 µ) using a mobile phase mixture of buffer and acetonitrile in a ratio of 50:50 v/v at a flow rate of 1.0 ml/min. The detection was made at 240 nm. The retention time of Escitalopram oxalate and Clonazepam was found to be 2.840± 0.007 min and 4.007±0.006 min. Calibration curve was linear over the concentration range of 20-120 µg/ml and 1-6µg/ml for Escitalopram oxalate and Clonazepam. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method was also found to be precise, accurate, specific, robust and rapid for the simultaneous determination of Escitalopram oxalate and Clonazepam in tablet dosage forms.

Kakde Rajendra B. et al (2012)[22] developed and validated, specific stability-indicating reversed-phase liquid chromatographic (LC) method for the quantitative determination of Escitalopram oxalate and Clonazepam and their related substances in bulk drugs and tablet dosage forms in the presence of degradation products. Forced degradation studies were performed on the pure drugs of Escitalopram oxalate and Clonazepam, as per ICH guideline using acid, base, oxidation, thermal stress and photolytic degradation to show the stability-indicating power of the method. The detection was conducted at 268 nm. The limit of detection and the limit of quantitation for Escitalopram oxalate and Clonazepam were established.

AIM & OBJECTIVE

The review of literature revealed that Escitalopram oxalate is official in Indian Pharmacopeia and HPLC method is reported for its estimation Etizolam is official in the British Pharmacopoeia and U.S. Pharmacopeia. IP, BP and USP describe HPLC method for its estimation. Many methods have been reported for estimation of Escitalopram oxalate and Etizolam individually and in combination with other drugs.

However, no method has been reported in any literature so far for simultaneous estimation of Escitalopram oxalate and Etizolam in combination by UV-spectrophotometry.

DRUG PROFILE

Drug profile

4.1 Drug Profile of Escitalopram oxalate

Table-2:- Physiochemical Properties of Escitalopram oxalate: [16 – 18]

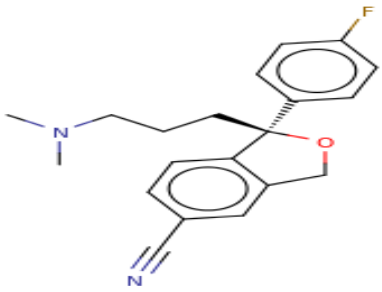
S. No.	Properties	Specifications
1.	Chemical Structure	 $(COOH)_2$
2.	IUPAC Name	(1S)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile
3.	Molecular formula	$C_{20}H_{21}FN_2O \cdot C_2H_2O_4$
4.	Mol. Wt.	414.40
5.	CAS No.	128196-01-0
6.	Solubility	Escitalopram is freely soluble in methanol and dimethylsulfoxide (DMSO), sparingly soluble in water, ethanol, slightly soluble in ethyl acetate and insoluble in n- heptanes
7.	Appearance	White to slightly-yellow powder
8.	Melting point	146-149 °C
9.	Storage condition	Store in a cool and dry place at a temperature of 20-25°C.

Table 3 - Pharmacological Properties of Escitalopram Oxalate:

S. No.	Properties	Description
1.	Categories	*Serotonin uptake inhibitors antidepressive agents,*
2.	Pharmacokinetic	The absolute bioavailability of Escitalopram is about 80% relative to an intravenous dose. Volume of distribution is 12l/kg,protein binding of 56%. Metabolite.
3.	Mechanism of action	The antidepressant actions of Escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. Escitalopram blocks the reuptake of serotonin at the serotonin reuptake pump of the neuronal membrane & enhancing the actions of serotonin on 5-HT1A auto receptors.
4.	Clinical uses	Used in treatment of depression and induces sleep .
5.	Adverse effects	Hyponatremia, constipation, diarrhoea, dizziness, dry mouth, fatigue**,** decrease libido, increased sweating, insomnia nausea, abdominal pain and loss of appetite.

4.2 Drug Profile of Etizolam:[13-15]

Table-4:- Physiochemical Properties of Etizolam :

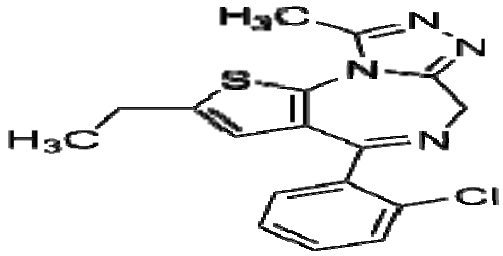
S. No.	Properties	Specifications
1.	Chemical Structure	
2.	IUPAC Name	7-(2-Chlorophenyl)-4-ethyl-13-methyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.02,6] trideca-2(6),4,7,10,12-pentaene
3.	Molecular formula	$C_{17}H_{15}ClN_4S$
4.	Mol. Wt.	342.07
5.	CAS No.	40054-69-1
6.	Solubility	Soluble in methanol, ethanol and chloroform, Practically insoluble in water.
7.	Appearance	Crystalline white powder
8.	Melting point	147-148°C
9.	Storage condition	Store at a room temperature of 15-30°C.

Table 5 - Pharmacological Properties of Etizolam:

S.No.	Properties	Description
1.	Categories	Anxiolytic, anticonvulsant, hypnotic, sedative & skeletal muscle relaxant
2.	Pharmacokinetic	Rapidly absorbed from GI tract. Etizolam is absorbed fairly rapidly, with peak plasma levels achieved between 30 min and 2 hrs, and its pharmacologically active metabolite alpha-hydroxyetizolam which has the same potency as of Etizolam, is eliminated more slowly, with a mean half life of just over 8 hours.
3.	Mechanism of action	Etizolam is a derivative of benzodiazepine. It is used for the short-term treatment of insomnia and anxiety disorder.
4.	Clinical uses	Used for the treatment of depression & used as a sedative hypnotics and also used in the management of anxiety, agitation or tension.
5.	Adverse effects	Drowsiness, sedation, muscle weakness and ataxia, vertigo, headache, confusion, depression, slurred speech, changes in libido, visual disturbances, urinary retention, GI disturbances, changes in salivation and amnesia.

INSTRUMENT AND DRUG CHARACTERIZATION

5.1.INSTRUMENTS INFORMATION- OVERVIEW

Table 6

S. No.	Name of instrument	Instrument model	Name of manufacturer
1.	UV-Visible double beam spectrophotometer	UV 1800	Shimadzu. Corp. Japan.
2.	Digital weighing balance	GR 200	A & D Company Ltd
3.	Attenuated total reflectance (ATR) spectrophotometer	ATR-Alpha	Alpha Bruker Ltd
4.	Whatmann filter paper-42	_____	Whatmann Int. Ltd.
5.	Ultra sonicator	--------	Entrech Electronics Ltd
6.	Melting point apparatus	--------	Surana Scientific, Jodhpur
7.	pH meter	Pen pH	Hanna Instruments

5.2 Chemicals / Reagents Used Table 7

S. No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Distilled water	----	----	In house laboratory.
2.	Methanol	---	A.R.	RENKEM (RFCL Ltd)

DRUG CHARACTERIZATION

5.3 Physiochemical characteristics

Description of Escitalopram oxalate: A fine, white to slightly-yellow powder.

Description of Etizolam: White crystalline powder.

Table 8: Observed and reported melting point of Escitalopram oxalate and Etizolam

Drug Name	Reported melting point (ºC)	Observed melting point (ºC)
Escitalopram oxalate	146-149° C	148°C
Etizolam	147-148°C	147°C

ANALYTICAL METHOD DEVELOPMENT

6.1 Characterization and identification of Escitalopram oxalate and Etizolam

ATR spectrum analysis: The IR absorption spectrum of Escitalopram oxalate and Etizolam was obtained. These drugs was than scanned from 400-200 nm and the spectrum was recorded. IR spectrum of Escitalopram oxalate and Etizolam are shown in figure 6-9 and comparisons of recorded IR spectrum showing type of vibration and wave number are shown in table 11 and 12 .

6.1 Identification Escitalopram oxalate by ATR Spectra

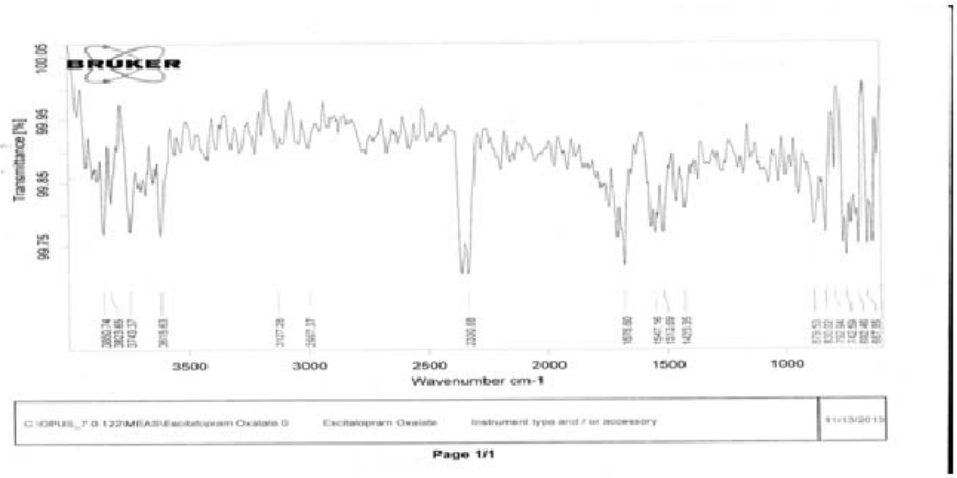


Figure 6: ATR Spectra of Escitalopram oxalate

Official Spectra of Escitalopram oxalate as per I.P.

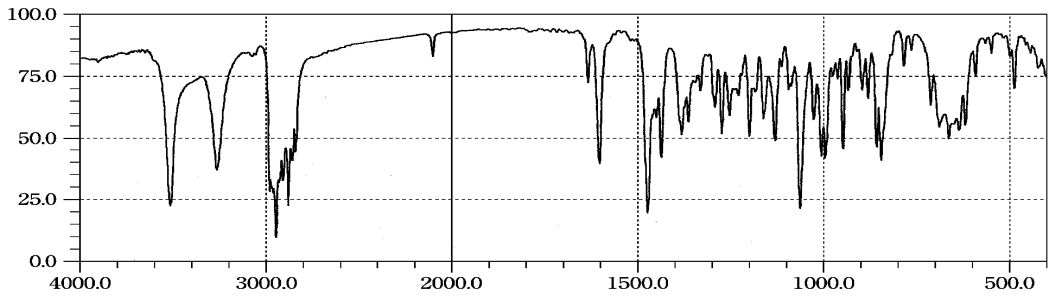


Figure 7: Official Spectra of Escitalopram oxalate

Table 9: Identification of peaks for Escitalopram oxalate

S.No.	ATR Functional group	Band	Theoretical Range	Observed Value
1.	Aromatic Ring	N-H	3000-3900	3897.56
2.	Alcohols & phenols	O-H	3000-3750	3747.77
3.	Alyne	C≡C-H $_{Str}$	3300-3100	3127.28
4.	Cycloalkenes	C=C-H $_{str}$	3100-3000	2997.37

. **Identification of Etizolam by ATR Spectra**

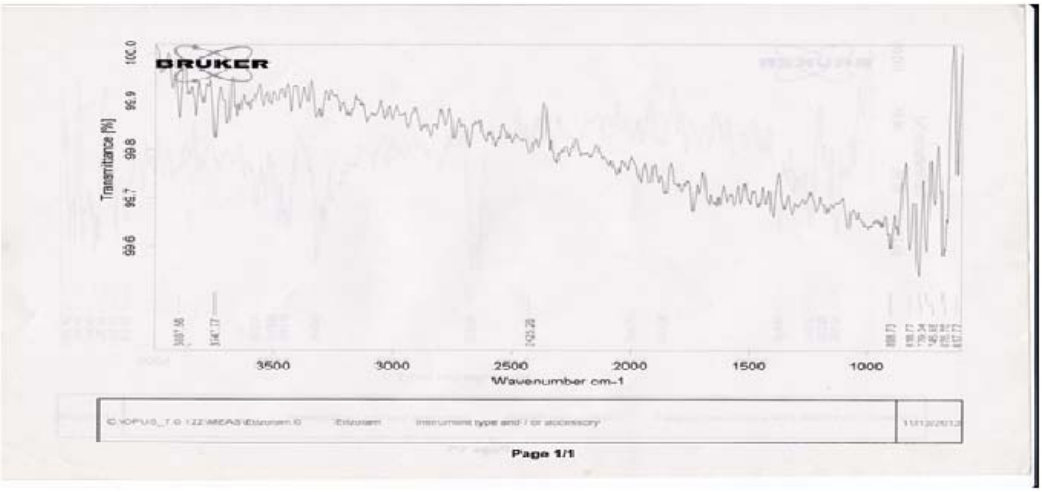


Table 8: Identification of peaks for Etizolam

S.No.	ATR Functional group	Band	Theoretical Range	Observed Value
1.	Aromatic Ring	N-H	3000-3900	3897.56
2.	Alcohols & phenols	O-H	3000-3750	3747.77
3.	Alkenes	C=C	2100-2660	2425.26
4.	Monosubstituted	C-H_{def}	900-700	898.73
5.	p- disubstituted	C-H_{def}	840-800	818.77
6.	m- disubstituted	C-H	800-750	745.45
7.	Chlorosubstituted	C-Cl	600-800	675.70

RESULT AND DISCUSSION

The IR spectrum of API showed the transmittance peaks. The spectrum showed similar peaks as reported in their respective functional groups. Comparisons of the obtained IR spectra with the reported IR spectra were displayed in table 11 and 12. Thus, both Escitalopram oxalate and Etizolam were found to be of acceptable quality.

6.2 METHOD DEVELOPMENT

6.1 Specificity

Method: The synthetic mixture of Escitalopram oxalate and Etizolam was prepared in ratio of 10:1. Accurately weighed 10 mg of Escitalopram oxalate and 1 mg of Etizolam were transferred to 100 ml volumetric flask and 70 ml of methanol was added to it. Common excipients used in the tablet formulation such as 8% starch, 7% magnesium stearate, 84% lactose, 1% talc (for 1000 µg/ml) were added into this mixture and the solution was sonicated for 20 minutes. Then solution was filtered through the Whatmann filter paper 42 and the residues were washed with methanol. The filtrate and washings were combined and volume

was made-up to 100 ml with the methanol. Then 10 ml of the solution was transferred to 100 ml volumetric flask and volume was made upto 100 ml with the methanol. Further 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml of resulted solution was transferred to a series of six volumetric flasks of 10 ml separately and volume was made up to the mark with methanol. These dilutions were calculated on the basis of Escitalopram oxalate (1000 µg/ml) and dilutions of Etizolam were obtained automatically in respective ratio. The absorbance of these solutions was measured at 238.0 nm and 248.6. nm. The decision of this ratio of drugs in the synthetic mixture was based upon the dosage strength of combination, which is available in the market. The specificity study results are shown in **table 11**.

Table 11: Specificity study for the synthetic mixture of Escitalopram oxalate and Etizolam in the ratio of 10 :1

Mix	Conc. (µg/ml)	λ_{max} (nm)	Before addition of excipients		After addition of excipients		% Interference
			Abs.	Conc. (µg/ml)	Abs.	Conc. (µg/ml)	
1	10	238.0	0.147	10.23	0.148	10.25	0.195
	1	248.6	0.104	1.03	0.102	1.04	0.970
2	20	238.0	0.292	20.47	0.293	20.49	0.097
	2	248.6	0.255	2.06	0.253	2.07	0.485
3	30	238.0	0.476	30.02	0.473	30.07	0.166
	3	248.6	0.301	3.23	0.303	3.25	0.619
4	40	238.0	0.663	40.16	0.664	40.17	0.024
	4	248.6	0.423	4.27	0.426	4.24	0.236
5	50	238.0	0.818	50.27	0.823	50.28	0.019
	5	248.6	0.523	5.46	0.525	5.45	0.382
6	60	238.0	0.971	60.33	0.973	60.37	0.082
	6	248.6	0.613	6.53	0.614	6.56	0.163
Mean	**ESC**						**0.461**
	ETI						**0.369**

RESULT AND DISCUSSION

The developed method was found to be specific as percent interference obtained was 0.461 and 0.369 for Escitalopram oxalate and Etizolam respectively, which is less than the prescribed limit (0.5%) as per ICH guidelines. Thus, it was also concluded that the addition of excipients had a very negligible change in the concentration.

6.2 Linearity and Range

Method: As assessed under previous section.

Linearity range was found to be 10.0 - 60.0 µg/ml for Eescitalopram oxalate at 238.0 nm and 248.6 nm. The correlation coefficient was found to be 0.9984 & 0.9992 which showed good linearity between the ranges. The slope was found to be 0.0169 & 0.0039 and intercept was found to be -0.0275 and -0.007.

For Etizolam at 238.0 nm and 248.6 nm, linearity range was found to be 1.0 – 6.0 µg/ml. The correlation coefficient was found to be 0.9999 & 0.9982 which showed good linearity between ranges. The slope was found to be 0.1009 and 0.1021 and intercept was found to be -0.0489 and 0.0061.

6.3 Range

Range of an analytical method is defined as the interval between upper and lower levels (including these levels). It includes working range, linearity range, target range and 100% concentration or the test concentration.

1. Working range: It begins from the limit of quantitation to the maximum concentration used for the development of the analytical method. For the developed method, the working range was found to be 08 to 60.0 µg/ml & 0.8 to 6.0 µg/ml for Escitalopram oxalate and Etizolam respectively.

2. Linearity range: It is the interval in which the response is directly proportional to the concentration between the upper and lower levels. It was found to be equal to 10.0 – 60.0 µg/ml & 1.0 – 6.0 µg/ml for Escitalopram oxalate and Etizolam respectively.

3. Target range: It is that concentration which is 80%, 100% and 120% of the target concentration. These were equal to 28 µg/ml, 35.0 µg/ml and 42.0 µg/ml for Escitalopram oxalate and 2.8 µg/ml, 3.5 µg/ml and 4.2 µg/ml for Etizolam.

4. Target concentration: It is defined as the concentration, which is equal to the midpoint of linearity range. Values of target concentration are given in **table 18**

Table 12: Values of target concentration

S. No.	Drug	Concentration level µg/ml		Target concentration (Lower + Higher) /2 µg/ml
		Lower	Higher	
1.	Escitalopram oxalate	10.0	60.0	35.0
2.	Etizolam	1.0	6.0	3.5
3.	Mixture	$\frac{\text{T.C.(ESC)+T.C.(ETI)}}{2}$		$\frac{35.0 + 3.5}{2} = 19.25$

6.4 Limit of Detection & Limit of Quantitation :

Limit of detection is the lowest amount of analyte that can be detected but not quantitated as an exact value and Limit of quantitation is the lowest amount of analyte that can be quantitatively determined in a sample with suitable precision and accuracy.

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The results are shown in **table 13**

Table 13: Value of LOD & LOQ

S. No.	(x) Slope ESC	(x) Slope ETI	(y) Intercept ESC	(y) Intercept ETI	L.O.D.	L.O.Q
1	0.0168	0.1029	-0.0274	0.0056	ESC	ESC
2	0.0171	0.1021	-0.0263	0.0061	0.259	0.786
3	0.0165	0.1026	-0.0259	0.0043		
4	0.0169	0.1024	-0.0297	0.0198	ETI	ETI
5	0.0177	0.1027	-0.0269	0.0095	0.194	0.589
6	0.0187	0.1023	-0.0278	0.0133		
Mean	0.0172	0.1025				
S.D.			0.001352	0.005898		

***** Intercept is to considered as S.D. for calculation

The Limit of detection (LOD) was found to be 0.259 µg/ml and 0.194 µg/ml and Limit of quantitation (LOQ) was found to be 0.786 µg/ml and 0.589 µg/ml for Escitalopram oxalate and Etizolam respectively which represents that sensitivity of the method is high.

6.5 Accuracy

Accuracy of analytical method expresses the closeness of agreement between the value which is expected either as conventional true value or an accepted reference value and value found. The results of analysis, obtained in three groups containing three replicate experiments with API and different tablet dosage forms, had good agreement with labeled amount of the drug.

Method: To prepare a stock solution 01 ml of the prepared solution (1000 µg/ml) was taken and transferred to 10 ml volumetric flask, volume was made up to the mark with phosphate buffer (6.8) to get desired stock solution (100 µg/ml of ESC). Further 2.0 ml of preanalyzed stock solution was taken and , 0.56, 1.1 and 1.66 ml of standard solution of API mixture (30µg/ml ESC and 3 µg/ml ETI) was added and volume was made up to the mark with phosphate buffer (6.8) as a solvent. This method meant for recovery of mixture of Escitalopram oxalate and Etizolam by spiking with pure standard of A.P.I. mixture solution.

The recovery study results are shown in **table 14**

Table 14: Data showing recovery study for Escitalopram oxalate and Etizolam

Mix	WL. (nm)	Conc. of tablet sol^{n} (ppm) ESC:ETI	Std. added (ppm)	Abs.	Amt. found (mg)	% recovery	Mean Recovery ± S.D.	% R.S.D.
1	238.0	30.10	8.0	0.592	38.02	99.79	**Escitalopram**	
	248.6	3.06	0.8	0.396	3.79	98.18	99.46	0.363
	238.0	30.20	8.0	0.601	38.03	98.52	±	
	248.6	3.02`	0.8	0.391	3.79	99.21	0.371	
	238.0	30.08	8.0	0.599	38.49	100.07		
	248.6	2.99	0.8	0.408	3.68	100.02		
2	238.0	30.31	10	0.671	40.27	99.90		
	248.6	2.89	1	0.438	3.91	100.06		
	238.0	30.09	10	0.680	40.04	99.87	**Etizolam**	
	248.6	2.94	1	0.441	3.95	100.17	99.96	0.431
	238.0	30.09	10	0.675	40.02	99.82	±	
	248.6	3.06	1	0.435	4.07	100.08	0.434	
3	238.0	30.20	12	0.709	42.50	100.08		
	248.6	2.89	1.2	0.481	3.95	99.33		
	238.0	30.03	12	0.701	42.52	99.43		
	248.6	2.96	1.2	0.490	4.14	100.4		
	238.0	30.18	12	0.699	42.52	100.5		
	248.6	3.08	1.2	0.494	4.13	100.39		

Table 15: Data showing accuracy study for Escitalopram oxalate and Etizolam

A.P.I. name ESC:ETI 10 : 1	Spiking concentration (ppm) 10 :1	Mean % Recovery	% R.S.D.
Escitalopram oxalate (ESC)	8, 10 and 12	99.46 ± 0.371	0.363
Etizolam (ETI)	0.8, 1.0 and 1.2	99.96 ± 0.434	0.431

The results obtained for the accuracy study (Recovery method) from three sample studies (n = 3) are as given in table 21. It was concluded that the mean of the % recovery was within the range of 98-102% and % R.S.D. was less than 2%.

6.3 Development and Simultaneous estimation of Escitalopram oxalate and Etizolam as API and in combination in tablet dosage form by first order derivative UV Spectrophotometric method:

6.3.1 Preparation of standard stock solution of Escitalopram oxalate:

Accurately weighed Escitalopram oxalate (10 mg) was transferred to 100 ml volumetric flask, dissolved and sonicated in methanol and made-up the volume to 100 ml with methanol as solvent. The final solution contained 100 ppm of Escitalopram oxalate.

6.3.2 Preparation of standard stock solution of Escitalopram oxalate and Etizolam:

Accurately weighed Etizolam(10 mg) was transferred to 100 ml volumetric flask, dissolved and sonicated in methanol and made-up the volume to 100 ml with methanol as solvent. The final solution contained 100 ppm of Escitalopram oxalate and Etizolam.

6.3.3 Selection of Analytical wavelength for Escitalopram oxalate:

Standard solutions of Escitalopram oxalate in the concentration range of 10 to 60 ppm were obtained by transferring 1, 2, 3, 4, 5 and 6 ml of Escitalopram oxalate stock solution (100 ppm) to a series of six volumetric flasks of 10 ml. The volume in each volumetric flask was made-up to 10 ml with methanol as solvent. The absorption spectra of all prepared dilutions of Escitalopram oxalate were recorded in the range of 200-400 nm. All zero order spectra (D^0) of Escitalopram oxalate were transformed to corresponding first order derivative spectra (D^1) using delta lambda 4.0 and scaling factor 1.0. The overlain first order derivative spectra of Escitalopram oxalate is shown in **figure 9**.

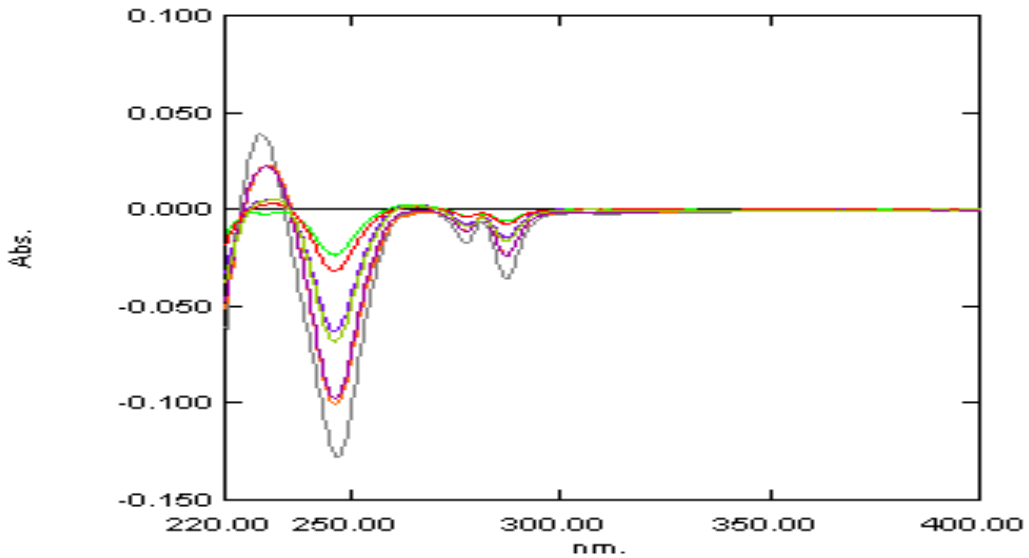


Figure 9: Overlain First Order Derivative Spectra of Escitalopram oxalate (10-60 ppm)

6.3.4 Selection of Analytical wavelength for Etizolam:

Standard solutions of Etizolam in concentration range of 1 to 6 ppm were obtained by transferring 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml of Etizolam stock solution (100 ppm) to a series of six volumetric flasks of 10 ml. The volume in each volumetric flask was made-up to 10 ml with methanol as solvent. The absorption spectra of all prepared dilutions of Etizolam were recorded in the range of 200-400 nm. All zero order spectra (D^0) of Etizolam were transformed to corresponding first order derivative spectra (D^1) using delta lambda 5.0 and scaling factor 1.0. Overlain first order derivative spectra of Etizolam is shown in **figure 10**

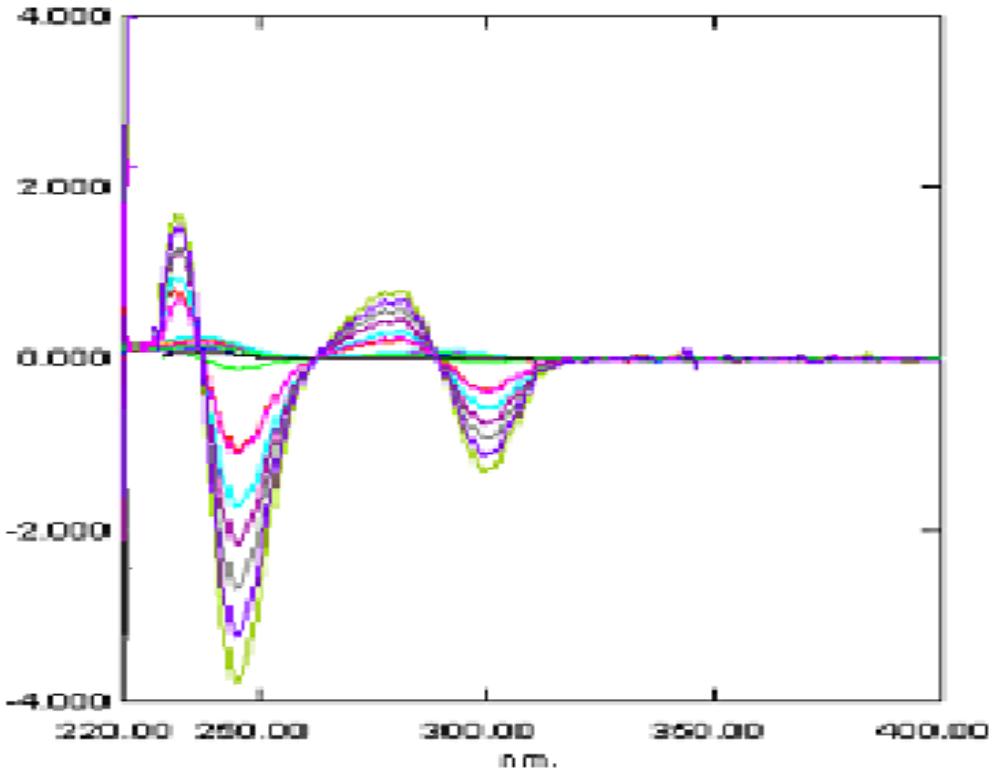


Figure 10: Overlain First Order Derivative Spectra of Etizolam

Overlain zero order spectra of Escitalopram oxalate and Etizolamis shown in **figure 11**.

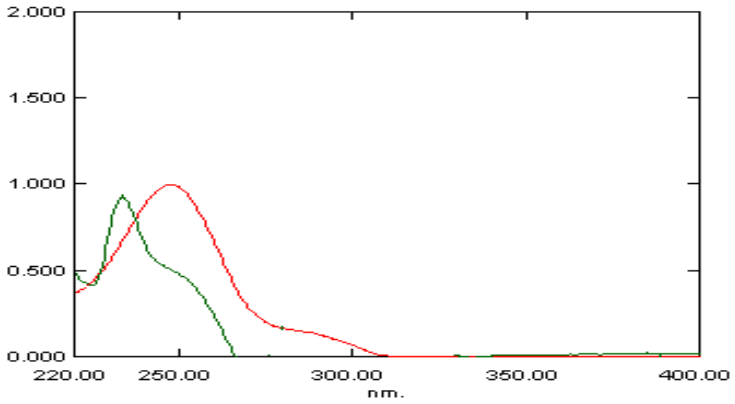


Figure 11: Overlain Zero Order Spectra of Escitalopram oxalate and Etizolam

Overlain first order derivative spectra of Escitalopram oxalate (10,20,30,40,50 and 60 ppm) and Etizolam(1,2,3,4,5 and 6 ppm) is shown in **figure 12**.

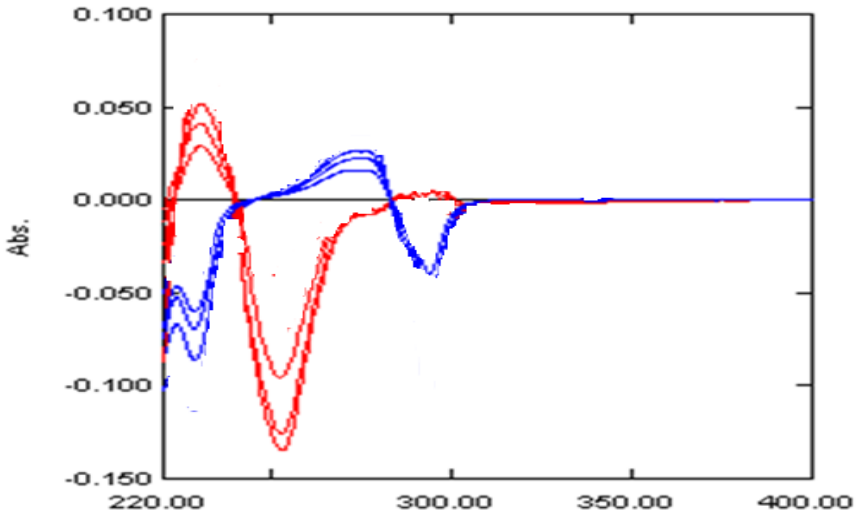


Figure 12: Overlain First Order Derivative Spectra of Escitalopram oxalate and Etizolam

Derivative conditions: First-order derivative spectra of Escitalopram oxalate and Etizolam were overlapped. The overlain D^1 spectra of Escitalopram oxalate and Etizolam different concentrations revealed that at 238.0 nm, the derivative response for Escitalopram oxalate was zero and Etizolam possesses significant D^1 absorbance. At 245.6 nm, the derivative response for Escitalopram oxalate was zero and Etizolam possesses significant D^1 absorbance. Considering these facts, wavelength 243.5.0 nm and 245.6 nm were selected for the estimation of Escitalopram oxalate and Etizolam respectively. Second and third-order derivative spectra of the drugs were not tested because the first-order spectra give satisfactory Zero Crossing Point (ZCP) and good quantitative determination of both the drugs without any interference. The zero crossing point (ZCP) of Escitalopram oxalate was found to be 243.5 nm and ZCP of Etizolam was found to be 245.6 nm. Characteristic wavelengths (zero-crossing points) for Escitalopram oxalate and Etizolam were confirmed by varying the concentrations of both drugs.

7. Summary

In the present work, U.V. spectrophotometric method for simultaneous estimation of Escitalopram oxalate and Etizolam in tablet dosage form has been developed. The proposed method was found to be precise, accurate and do not suffer from any interference due to common excipients.

The summary of optical and regression parameters of UV spectrophotometric method are shown in **table 16**.

Table 16: Optical parameters & regression characteristics of Escitalopram oxalate and Etizolam

Parameters	Escitalopram oxalate		Etizolam	
	243.4 nm	248.6 nm	243.4 nm	248.6 nm
Beer's law limit (µg/ml)	10-60	10-60	1-6	1-6
Molar absorptivity (l mole^{-1}cm^{-1})	0.765 x 10^4	4.057 x 10^4	0.429 x 10^4	0.735 x 10^4
Sandell's sensitivity (mg/cm^2/.001 absorbance unit)	0.054117	0.102045	0.07551	0.04653
Regression equation (y= mx + c)				
slope (m)	0.0156	0.0088	0.1073	0.0157
intercept (c)	0.0269	0.0091	0.0196	0.0523
Correlation coefficient (r^2)	0.9996	0.9999	0.9993	0.9997

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