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Research Article

Method development and validation of simultaneous estimation of paracetamol & tramadol HCL in bulk and dosage form

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ABSTRACT

A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention or treatment of diseases in human beings or animals or for alternating any structure or function of the body of human being or animals. Pharmaceutical chemistry is a science that makes use of general laws of chemistry to study drugs i.e. their preparation, chemical natures, composition, structure, influence on an organism and studies the physical and chemical properties of drugs, the methods of quality control and the conditions of their storage etc. the family of drugs may be broadly classified as.

1. Pharmacodynamic agents.
2. Chemotherapeutic agents.

It is necessary to find the content of each drug either in pure or single, combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage form for treatment.

The scope of developing and validating analytical methods is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation.

INTRODUCTION

Method development of spectrophotometric methods

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal [1]. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Bouger-Lambert-Beer's law, which establishes the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with

solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer [2].

The important applications are

- Identification of many types of organic, inorganic molecules and ions.
- Quantitative determination of many biological, organic and inorganic species.
- Quantitative determination of mixtures of analytes
- Monitoring and identification of chromatographic of effluents.
- Determination of equilibrium constants.
- Determination of stoichiometry and chemical reactions.
- Monitoring of environmental and industrial process.
- Monitoring of reaction rates.
- Typical analysis times range from 2 to 30 min for sample

Pharmaceutical analysis simply means analysis of pharmaceuticals. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product [3]. The pharmaceutical industry is under increased scrutiny from the government and the public interested groups to contain costs and at consistently deliver to market safe, efficacious product that fulfill unmet medical needs [4].

The pharmaceutical analyst plays a major rule in assuring identity, safety, efficacy, purity, and quality of a drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. The commonly used tests of pharmaceutical analysis generally entail compendia testing method development, setting specifications, and method validation [5]. Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products. New methods are now being development with a great deal of consideration to worldwide harmonization [6]. As a result, new products can be assured to have comparable quality and can be brought to international markets faster [7].

Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities [8]. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (cGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility [9]. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs [10].

There are various spectrophotometric methods are available which can be used for the analysis of a combination samples. Following methods can be used [18].

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry
- Solvent extraction method
- AUC method
- HPLC method

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 1. 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 C_x and C_y 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

$\mathbf{A_1 = a_{x1}bC_x + a_{y1}bC_y}$ ..... (1)

At λ_2

$\mathbf{A_2 = a_{x2}bC_x + a_{y2}bC_y}$ ..... (2)

For measurements in 1 cm cells, b =1.

Rearrange equation **(2)**

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

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

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

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

Fig. 1: The overlain spectra of substance X and Y, showing the wavelength for the assay of X and Y in admixture by the method of simultaneous equation.

Criteria for obtaining maximum precision have been suggested by Glenn[12]. 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 $(a_{y2}/ay_1)/ (A_2/A_1)$

The criteria are that the ratios should lie outside the range 0.1- 2.0 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ_{max} of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically, thereby negating the initial assumption 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.

Simultaneous equation method using Matrices and Cramer's Rule can be explained as follows:

Consider a binary mixture of component X and Y for which the absorption spectra of individual components and mixture.

- 1 is the λ_{max} of component X
- 2 is the λ_{max} of component Y

The total absorbance of a solution at a given wavelength is equal to the sum of the absorbance of the individual components at the wavelength. Thus the absorbance of mixture at the wavelength 1 and 2 may be expressed as follows:

At λ_1

$\mathbf{A_1 = a_{x1}bC_x + a_{y1}bC_y}$ ..... (1)

At λ_2

$\mathbf{A_2 = a_{x2}bC_x + a_{y2}bC_y}$ ..... (2)

Such equation can be solved using matrices.

From equation (1) and (2),

$\mathbf{A_1 = k_{x1} C_x + k_{y1}C_y}$ ..... (3)

$\mathbf{A_2 = k_{x2} C_x + k_{y2}C_y}$ ..... (4)

Where k = a x b

Let A, be a column matrix with 'i' elements [i is the number of wavelength at which measurements are done; here two wavelength 1 and 2 are taken in to consideration, so i=2]. Let C, be a column matrix with 'j' elements [j, is the number of components, in this case X and Y are present, so j = 2]. Let k, be a matrix with i x j values so that it has number of rows equals to number of wavelength and number of columns equal to number of components (in this case it has two rows and two columns). Hence we have

A = k x C ..... (5)

$$\begin{vmatrix} A_1 \\ A_2 \end{vmatrix} = \begin{vmatrix} k_{x1} & k_{y1} \\ k_{x2} & k_{y2} \end{vmatrix} \times \begin{vmatrix} C_x \\ C_y \end{vmatrix}$$

Since the number of wavelength equal to number of components, the equation (5) has a unique solution.

$\mathbf{C = k^{-1} \times A}$ .....(6)

However, it will be faster to solve the equation (3) and (4) by means of cramer's rule. And unknown concentration Cj of component j is found by replacing' j column of matrix A. The determinant of the new matrix is divided by determinant of 'k' matrix.

$$C_x = \frac{\begin{vmatrix} A_1 & k_{y1} \\ A_2 & k_{y2} \end{vmatrix}}{\begin{vmatrix} k_{x1} & k_{y1} \\ k_{x2} & k_{y2} \end{vmatrix}}$$

$$\mathbf{C_x = (A_1 k_{y2} - A_2 k_{y1}) / (k_{x1} k_{y2} - k_{x2} k_{y1})} \quad \ldots\ldots\ldots\ldots (7)$$

$$C_y = \frac{\begin{vmatrix} k_{x1} & A_1 \\ k_{x2} & A_2 \end{vmatrix}}{\begin{vmatrix} k_{x1} & k_{y1} \\ k_{x2} & k_{y2} \end{vmatrix}}$$

$$\mathbf{C_y = (k_{x1} A_2 - k_{x2} A_1) / (k_{x1} k_{y2} - k_{x2} k_{y1})} \quad \ldots\ldots\ldots\ldots.. (8)$$

Therefore

$$\mathbf{C_x = (A_1 a_{y2} - A_2 a_{y1}) / (a_{x1} a_{y2} - a_{x2} a_{y1})} \quad \ldots\ldots\ldots\ldots\ldots(9)$$

$$\mathbf{C_y = (a_{x1} A_2 - a_{x2} A_1) / (a_{x1} a_{y2} - a_{x2} a_{y1})} \quad \ldots\ldots\ldots\ldots..(10)$$

In British Pharmacopoeia the assay of quinine-related alkaloids and Cinchonine related alkaloids are based on this technique. Drugs with large difference in the content in the combined dosage form have been estimated simultaneously by Simultaneous equation method by standard addition technique in which known amount of pure drug have been added to the sample drugs like,

- Estimation of Ibuprofen and Pseudoephedrine hydrochloride from tablets
- Estimation of Salbutamol and Theophylline from tablets

Q- Analysis (Absorbance Ratio Method)

Absorbance ratio 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 a constant value independent of concentration or pathlength. For example, two different dilution of the same substance give the same absorbance ratio A_1/A_2. In 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 λ_{max} of one of the component (λ_2) and the other being a wavelength of equal absorptivities of the two components (As shown in figure 2) i.e. an isoabsorptive point

Two equations are constructed as described for the method of simultaneous equation. Their treatment is somewhat different, however, and uses the relationship $a_{x1} = a_{y1}$ at λ_1. Assume b = 1cm

$$\mathbf{A_1 = a_{x1}C_x + a_{x1}C_y} \quad \ldots\ldots\ldots\ldots (1)$$

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

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

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

But $\mathbf{Fy = 1 - Fx}$

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

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

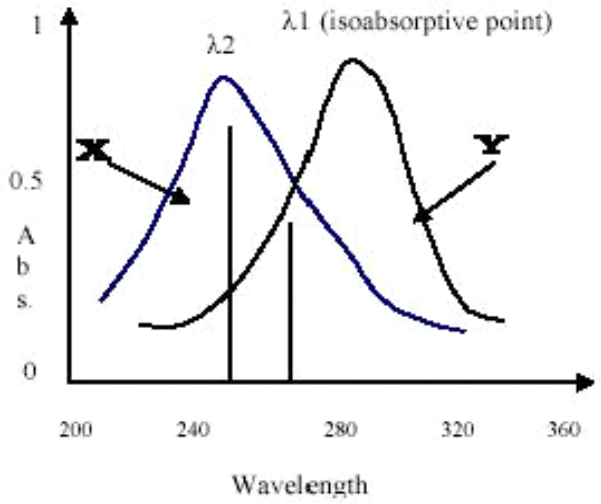


Fig. 2: Wavelength for the assay of substances X and Y in admixture by the method of absorbance ratio method.

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)

Above equation gives fraction, rather than the concentration of X in the mixture in terms of absorbance ratios. As these are independent of concentrations, only approximate, rather than accurate, dilutions of X and Y and the sample mixture are required to determine Q_X, Q_Y, and Q_M respectively.

For absolute concentration of X and Y, eq. (1) is rearranged

$A_1 = a_{x1} (C_x + C_Y)$

$C_x + C_y = A_1 / a_{x1}$

From equation (2)

$C_x / (C_x + C_y) = (Q_M - Q_Y) / (Q_X - Q_Y)$

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

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

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

Derivative Spectroscopy

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

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

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

The second derivative D^2 spectrum is a plot of the curvature of the D spectrum against wavelength or a plot of $d^2A/ d\lambda^2$ vs. λ. The maximum negative curvature in the D spectrum gives a minimum in the D^2 spectrum, and the maximum positive curvature in the D spectrum gives two small maxima called satellite bands in the D^2 spectrum. The wavelength of maximum slope and zero curvature in the D spectrum correspond with cross-over points in the D^2 spectrum. These spectral transformations confer two principal advantages on derivative spectrophotometry. Firstly, an eve order spectrum is of narrower spectral bandwidth than its fundamental spectrum. A

derivative spectrum therefore shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the λmax of the individual bands. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. All the amplitudes in the derivative spectrum are proportional to the concentration of the analyte, provided that Beer's law is obey by the fundamental spectrum.

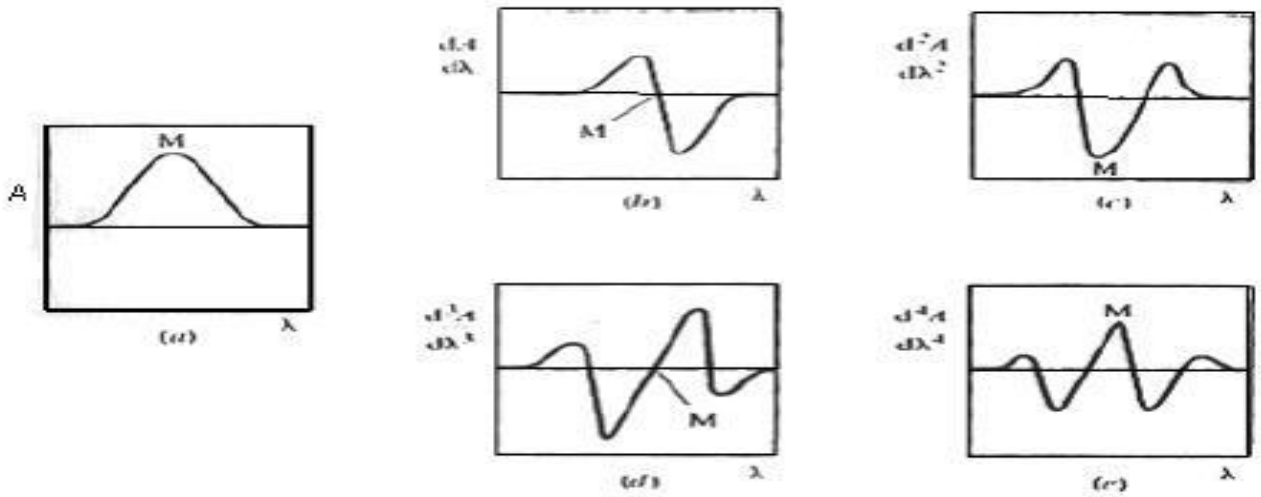


Fig. 3: (b) First, (c) Second, (d) Third and (e) fourth derivative Spectrum of (a) Gaussian peak.

The enhanced resolution and bandwidth discrimination increases with increasing derivative order. However, it is also found that the concomitant increase in electronic noise inherent in the generation of the higher order spectra, and the consequent reduction of the signal-to-noise ratio, place serious practical limitations on the higher order spectra. For quantitative purposes, second and fourth derivative spectra are the most frequently employed derivative orders.

Derivative spectra may be generated by any of three techniques. The earliest derivative spectra were obtained by modification of the optical system. Spectrophotometers with dual monochromator set a small wavelength interval (Δλ, typically 1-3nm) apart, or with the facility to oscillate the wavelength over a small range, are required. In either case the photodetector generates a signal with amplitude proportional to the slope of the spectrum over the wavelength interval. Instruments of this type are expensive and are essentially restricted to the recording of first derivative spectra only.

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

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

Derivative spectra obtained by RC modules are highly dependent on instrumental parameters, in particular the scan speed and the time constant. It is essential, therefore, to employ a standard solution of the analyte to calibrate the measured value the instrumental conditions selected.

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

Solvent Extraction Method

In solvent extraction method quantitation of individual drugs in combinations has been performed by separation of individual drugs based on 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 interference from the analyte by solvent extraction procedure. These are particularly appropriate for acidic or basic drugs whose state of ionization determines their solvent partitioning behavior. The judicious choice of pH of the aqueous medium may effect the complete separation of the interferences from the analyte, the concentration of which may be obtained by a simple measurement of absorbance of the extract containing the analyte.

VALIDATION

Defination

Validation is the documented act of demonstrating that a procedure, process, and activity will consistently lead to the expected results. It often includes the qualification of systems and equipment.

Types of Validation

- Process validatio
- Qualification validation
- Analytical validation
- Cleaning validation

Analytical method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical method validation characteristics

Typical analytical performance characteristics that should be considered in the validation of the types of procedures described in this chapter are listed below. Each validation characteristic is defined to ensure consistency in usage of terminology and interpretation:

- Accuracy
- Precision
- Repeatability
- Intermediate precision
- Specificity
- Detection limit
- Quantitation limit
- Linearity
- Range
- Robustness

Accuracy

It is an analytical procedure as the closeness of agreement between the values that are accepted either as conventional true values or an accepted reference value and the value found. For drug substance, accuracy may be defined by the application of the analytical procedure to an analyte of known purity (e.g., a reference standard). For the drug product, accuracy will be determined by application of the analytical procedure to synthetic mixtures of the drug product components to which known amounts of analyte have been added within the range of the procedure. The ICH document also recommends assessing a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations/three replicates).

Accuracy is usually reported as percent recovery by the assay (using the proposed analytical procedure) of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. The range for the accuracy limit should be within the linear range.

Typical accuracy of the recovery of the drug substance is expected to be about 99 – 101%. Typical accuracy of the recovery of the drug product is expected to be about 98 – 102%. Values of accuracy of recovery data beyond this range need to be investigated as appropriate. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method.

Accuracy is best reported as percentage bias, which is calculated from the expression

$$\%Bias = \frac{(\text{measured value} - \text{true value})}{\text{true value}} \times 100$$

Since for real samples the true value is not known, an approximation is obtained based on spiking drug – free matrix to a nominal concentration. The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentrations of the analytes in the spiked drug – free matrix sampler.

Calibration

Calibration is the most important step in bioactive compound analysis. A good precision and accuracy can only be obtained when a good calibration procedure is adopted. In the spectrophotometric methods, the concentration of a sample cannot be measured directly, but is determined using another physical measuring quantity 'y' (absorbance of a solution). An unambiguous empirical or theoretical relationship can be shown between this quantity and the concentration of an analyte. The calibration between y = g (x) is directly useful and yields by inversion of the analytical calculation function.

The calibration function can be obtained by fitting an adequate mathematical model through the experimental data. The most convenient calibration function is linear, goes through the origin and is applicable over a wide dynamic range. In practice, however, many deviations from the ideal calibration line may occur. For the majority of analytical techniques the analyst uses the calibration equation.

Y = a + bx.

In calibration, univariate regression is applied, which means that all observations are dependent upon a single variable X.

Standard deviation of slope (Sb)

The standard deviation of slope is proportional to standard error of estimate and inversely proportional to the range and square root of the number of data points.

$$Sb = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{(n-2)}} \sqrt{\frac{1}{\sum_{i=1}^{n}(x_i - \bar{x}_i)^2}}$$

Where Xi is the arithmetic mean of Xi values.

Standard deviation of intercept, (Sa)

Intercept values of least squares fits of data are often to evaluate additive errors between or among different methods.

$$Sa = \sqrt{\frac{\sum_{i=1}^{n}\left(y - \hat{y}_i\right)^2}{(n-2)}} \sqrt{\frac{1}{\sum_{i=1}^{n}(x_i - \bar{x}_i)^2}} \sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n}}$$

Where Xi denote the arithmetic mean of xi, values.

Correlation Coefficient, (r)

The correlation coefficient r (x,y) is more useful to express the relationship of the chosen scales. To obtain a correlation coefficient the covariance is divided by the product of the standard deviation of x and y.

$$r = \frac{\left[\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})\right] / (n-1)}{\left[\sum_{i=1}^{n}(x_i - \bar{x})^2 (y_i - \bar{y})^2\right] / (n-1)^2}$$

Linearity and sensitivity of the method

Knowledge of the sensitivity of the color is important and the following terms are commonly employed for expressing sensitivity. According to Bouger- Lambert – Beer's law, log intensity of incident radiations

$$A = \text{Log}\frac{\text{Intensity of incident light}}{\text{Intensity of transmitted light}} = \text{ct}$$

The absorbance (A) is proportional to the concentration (c) of the absorbing species, if absorptivity () and thickness of the medium (t) are constant. When c is in moles per liter, the constant is called molar absorptivity. Beer's law limits and max values are expressed as µg ml-^{1} and mole-^{1} cm-^{1} respectively.

Sandell's sensitivity refers to the number of µg of the drug to be determining, converted to the colored product, which in a column solution of cross section 1cm2 shows an absorbance of 0.001 (expressed as µg cm-^{2}).

Recovery

The absolute recovery of analytical method is measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard, which has not been subjected to sample pre-treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample. It is best established by comparing the responses of extracted samples at low, medium and high concentrations in replicates of at least 6 with those non-extracted standards, which represent 100 % recovery.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions. Precision is usually investigated at three levels: repeatability, intermediate precision, and reproducibility for simple formulation it is important that precision be determined using authentic homogeneous samples. A justification will be required if a homogeneous sample is not possible and artificially prepared samples or sample solutions are used.

Repeatability

Repeatability is a measure of the precision under the same operating conditions over a short interval of time, that is, under normal operating conditions of the analytical method with the same equipment. It is sometimes referred to as intra - assay precision. The ICH recommends that repeatability be assessed using a minimum of nine determinations covering the specified range for the procedure (e.g., three concentrations/ three replicates as in the accuracy experiment) or using a minimum of six determinations at 100% of the test concentration. Reporting of the standard deviation, relative standard deviation (coefficient of variation), and confidence interval is required. The assay values are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result. Table 1 provides an example set of repeatability data.

Example for Repeatability Data

Replicate	Percentage of Labeled Clime
•	100.6
•	102.1
•	100.5
•	99.4
•	101.4
•	101.1
Mean	100.9
Percentage relative standard Deviation (%RSD)	0.90

Intermediate precision

Intermediate precision is defi ned as the variation within the same laboratory. The extent to which intermediate precision needs to be established depends on the circumstances under which the procedure is intended to be used. Typical parameters that are investigated include day - to - day variation, analyst variation, and equipment variation. Depending on the extent of the study, the use of experimental design is encouraged. Experimental design will minimize the number of experiments that need to be performed. It is important to note that ICH allows exemption from doing intermediate precision when reproducibility is proven. It is expected that the intermediate precision should show variability that is in the same range or less than repeatability variation. ICH recommends the reporting of standard deviation, relative standard deviation (coefficient of variation), and confidence interval of the data table.

Reproducibility

Reproducibility measures the precision between laboratories. This parameter is considered in the standardization of an analytical procedure (e.g., inclusion of procedures in pharmacopeias and method transfer between different laboratories).

To validate this characteristic, similar studies need to be performed at different laboratories using the same homogeneous sample lot and the same experimental design. In the case of method transfer between two laboratories, different approaches may be taken to achieve the successful transfer of the procedure. The most common approach is the direct - method transfer from the originating laboratory to the receiving laboratory. The originating laboratory is defined as the laboratory that has developed and validated the analytical method or a laboratory that has previously been certified to perform the procedure and will participate in the method transfer studies. The receiving laboratory is defined as the laboratory to which the analytical procedure will be transferred and that will participate in the method transfer studies. In the direct - method transfer, it is recommended that a protocol be initiated with details of the experiments to be performed and acceptance criteria (in terms of the difference between the means of the two laboratories) for passing the method transfer.

Specificity

Selectivity and specificity are often used interchangeably. However, there are debates over the use of specificity over selectivity and some authorities, for example, the International Union of Pure and Applied Chemistry (IUPAC), have preferred the term selectivity reserving specificity for those procedures that are completely selective. For pharmaceutical application, the above definition of ICH will be used. For identity test, compounds of closely related structures which are likely to be present should be discriminated from each other. This could be confirmed by obtaining positive results (by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. Furthermore, the identification test may be applied to material structurally similar or closely related to the analyte to confirm that a positive response is not obtained.

The choice of such potentially interfering materials should be based on sound scientific c judgment with a consideration of the interferences that could occur.

The specificity for an assay and impurity tests should be approached from two angles:

When Impurities Are Available

The specificity of an assay method is determined by comparing test results from an analysis of sample containing the impurities, degradation products, or placebo ingredients with those obtained from an analysis of samples without the impurities, degradation products, or placebo ingredients. For a stability - indicating assay method, degradation peaks need to be resolved from the drug substance. However, these impurities do not need to be resolved from each other. For the impurity test, the determination should be established by spiking drug substance or drug product with the appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Representative chromatograms should be used.

If Impurities Are Not Available

Specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well - characterized procedure or other validated analytical procedure (orthogonal method).

This should include samples stored under relevant stress conditions (light, heat, humidity, acid/base hydrolysis and oxidation). For the assay method, the two results should be compared; for impurity tests, the impurity profiles should be compared. Peak homogeneity tests should be performed using PDA or mass spectrometry to show that the analyte chromatographic peak is not attributable to more than one component. Figure 2 illustrates the selectivity of a method to resolve known degradation peaks from the parent peak.

Detection limit

The detection limit (DL) is a characteristic for the limit test only. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection is usually expressed as the concentration of the analyte in the sample, for example, percentage, parts per million (ppm), or parts per billion (ppb). There are several approaches to establish the DL. Visual evaluation may be used for non instrumental (e.g., solution color) and instrumental methods. In this case, the DL is determined by the analysis of a series of samples with known concentrations and establishing the minimum level at which the analyte can be reliably detected. Presentation of relevant chromatograms or other relevant data is sufficient for justification of the DL.

For instrumental procedures that exhibit background noise, it is common to compare measured signals from samples with known low concentrations of analyte with those of the blank samples. The minimum concentration at which the analyte can reliably be detected is established using an acceptable signal - to - noise ratio of 2: 1 or 3: 1. Presentation of relevant chromatograms is sufficient for justification of The DL.

Another approach estimates the DL from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the *y* intercepts of the regression lines done in the range of the DL. This estimate will need to be subsequently validated by the independent analysis of a suitable number of samples near or at the DL:

$$DL = \frac{3\sigma}{S}\frac{3\sigma}{S}$$

Where σ is the standard deviation of the response and *S* is the slope of the calibration curve

Quantitation limit

The quantitation Limit (QL) is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. QL is defined as the concentration of related substance in the sample that will give a signal - to - noise ratio of 10: 1. The QL of a method is affected by both the detector sensitivity and the accuracy of sample preparation at the low concentration of the impurities. In practice, QL should be lower than the corresponding ICH report limit. ICH recommends three approaches to the

estimation of QL. The first approach is to evaluate it by visual evaluation and may be used for non instrumental methods and instrumental methods. QL is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantitated with acceptable accuracy and precision.

The second approach determines the signal - to - noise ratio by comparing measured signals from samples with known low concentrations of anlayte with those of blank samples. QL is the minimum concentration at which the analyte can be reliably quantified at the signal - to - noise ratio of 10: 1.

The third approach estimates QL by the equation. The slope *S* may be estimated from the calibration curve of the analyte. The value of may be estimated by (1) calculating the standard deviation of the responses obtained from the measurement of the analytical background response of an appropriate number of blank samples or (2) calculating the residual standard deviation of the regression line from the calibration curve using samples containing the analyte in the range of the QL.

Whatever approach is applied, the QL should be subsequently validated by the analysis of a suitable number of samples prepared at the QL and determining the precision and accuracy at this level.

Linearity

It is an analytical procedure as the ability (within a given range) to obtain test results of variable data (e.g., absorbance and area under the curve) which are directly proportional to the concentration (amount of analyte) in the sample. The data variables that can be used for quantitation of the analyte are the peak areas, peak heights, or the ratio of peak areas (heights) of analyte to the internal standard peak. Quantitation of the analyte depends on it obeying Beer's law for the spectroscopic method over a concentration range. Therefore, the working sample concentration and samples tested for accuracy should be in the linear range. There are two general approaches for determining the linearity of the method. The first approach is to weigh different amounts of standard directly to prepare linearity solutions at different concentrations. However, it is not suitable to prepare solution at very low concentration, as the weighing error will be relatively high.

Another approach is to prepare a stock solution of high concentration. Linearity is then demonstrated directly by dilution of the standard stock solution. This is more popular and the recommended approach. Linearity is best evaluated by visual inspection of a plot of the signals as a function of analyte concentration. Subsequently, the variable data are generally used to calculate a regression line by the least - squares method. At least five concentration levels should be used. Under normal circumstances, linearity is acceptable with a coefficient of determination (r 2) of 0.997. The slope, residual sum of squares, and y intercept should also be reported as required by ICH.

The slope of the regression line will provide an idea of the sensitivity of the regression, and hence the method that is being validated. The y intercept will provide an estimate of the variability of the method. For example, the ratios percent of the y intercept with the variable data at nominal concentration are sometimes used to estimate the method variability.

For the determination of potency assay of a drug substance or a drug product, the usual range of linearity should be 20% of the target or nominal concentration. For the determination of content uniformity, it should be 30% of the target or nominal concentration. Figure 3 illustrates the linearity of a sample set of data.

Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally expressed in the same units as test results (e.g., percent, parts per million) obtained by the analytical procedure.

For the assay of drug substance or finished drug product, it is normally recommended to have a range of 80 – 120% of the nominal concentration.

For content uniformity, a normal range would cover 70 – 130% of the nominal concentration, unless a wider and more appropriate range (e.g., metered - dose inhalers) is justified.

For dissolution testing, a normal range is ± ± 20% over the specified range. If the acceptance

criterion for a controlled - release product covers a region from 20% after 1 hr, and up to 90% after 24 hr, the validated range would be 0 – 110% of the label claim. In this case, the lowest appropriate quantifiable concentration of analyte will be used as the lowest limit as 0% is not appropriate.

Robustness

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

The evaluation of robustness is normally considered during the development phase and depends on the type of procedure under study. Experimental design (e.g., fractional factorial design or Plackett – Burman design) is common and useful to investigate multiple parameters simultaneously. The result will help to identify critical parameters that will affect the performance of the method.

When the results are affected by some critical experimental parameters, a precautionary statement should be included in the analytical procedure to ensure that this parameter is tightly controlled between experiments.

Ruggedness

Method Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

PROCESS OF ANALYTICAL METHOD VALIDATION

The typical process that is followed in an analytical method validation is chronologically listed below:

1. Planning and deciding on the method validation experiments
2. Writing and approval of method validation protocol
3. Execution of the method validation protocol
4. Analysis of the method validation data
5. Reporting the analytical method validation
6. Finalizing the analytical method procedure

The method validation experiments should be well planned and laid out to ensure efficient use of time and resources during execution of the method validation. The best way to ensure a well - planned validation study is to write a method validation protocol that will be reviewed and signed by the appropriate person (e.g., laboratory Management and quality assurance).

The validation parameters that will be evaluated will depend on the type of method to be validated. Analytical methods that are commonly validated can be classified into three main categories: identification, testing for impurities, and assay. Table 3 lists the ICH recommendations for each of these methods.

Execution of the method validation protocol should be carefully planned to optimize the resources and time required to complete the full validation study. For example, in the validation of an assay method, linearity and accuracy may be validated at the same time as both experiments can use the same standard solutions. A normal validation protocol should contain the following contents at a minimum:

a. Objective of the protocol
b. Validation parameters that will be evaluated
c. Acceptance criteria for all the validation parameters evaluated
d. Details of the experiments to be performed
e. Draft analytical procedure

The data from the method validation data should be analyzed as the data are obtained and processed to ensure a smooth flow of information. If an experimental error is detected, it should be resolved as soon as possible to reduce any impact it may have on later experiments. Analysis of the data includes visual examination of the numerical values of the data and chromatograms followed by statistical treatment of the data if required.

Type of analytical procedure characteristics	identification	Testing for impurities		Assay –dissolution (measurement only)- $\frac{\text{content}}{\text{potency}}$
		quantitation	Limit	$\frac{\text{content}}{\text{potency}}$
Accuracy	–	+	–	+
Precision	–			
Repeatability	–	+	–	+
Intermediate precision	+	+[a]	–	+[a]
Specificity	–	+	+	+
Detection limit	–	–[c]	+	–
Quantitation limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

Upon completion of all the experiments, all the data will be compiled into a detailed validation report that will conclude the success or failure of the validation exercise. Depending on the company's strategy a summary of the validation data may also be generated. Successful execution of the validation will lead to a final analytical procedure that can be used by the laboratory to support future analytical work for the drug substance or drug product.

INFORMATION REQUIRED IN ANALYTICAL PROCEDURE

The minimal information that should be included in final analytical procedure areas follows:

a. Rationale of the analytical procedure and description of the capability of the method. Revision of analytical procedure should include the advantages offered by the new revision.
b. Proposed analytical procedure. This section should contain a complete description of the analytical procedure in sufficient detail to enable another analytical scientist to replicate it. The write - up should include all important operational parameters and specific instructions, for example, preparation of reagents, system suitability tests, precautions, and explicit formulas for calculation of the test results.
c. List of permitted impurities and its levels in an impurity assay.
d. Validation data. Either a detailed set or summary set of validation data is included
e. Revision history.
f. Signature of author, reviewer, management, and quality assurance.

PHASE - APPROPRIATE METHOD VALIDATION

The original intent of the cGMPs was to describe standards and activities designed to ensure the strength, identity, safety, purity, and quality of pharmaceutical products introduced into commerce. However, the GMPs are silent on explicit guidances for the development phase of pharmaceuticals in several areas.

Regulatory bodies recognize that knowledge of the drug product and its analytical methods will evolve through the course of development. This is stated explicitly in ICH Q7A: Changes are expected during development, and every change in product, specifications, or test procedures should be recorded adequately. It is therefore reasonable to expect that changes in testing, processing, packaging, and so on, will occur as more is learned about the molecule. However, even with the changes, the need for ensuring the safety of subjects in clinical testing should not be compromised.

According to the ICH guidance, the objective of method validation is to demonstrate that analytical procedures "are suitable for their intended purpose." Therefore the method's purpose should be linked to the clinical studies and the pharmaceutical purpose of the product being studied.

The purpose in the early phase of drug development is to deliver a known dose that is bioavailable for clinical studies. As product development continues, increasing emphasis is placed on identifying a stable, robust formulation from which multiple, bioequivalent lots can be manufactured and ultimately scaled up, transferred, and controlled for commercial manufacture.

The development and validation of analytical methods should follow a similar progression. The purpose of analytical methods in early stages of development is to ensure potency, to understand the impurity and degradation product profile, and to help understand key drug characteristics. As development continues, the method should be stability indicating and capable of measuring the effect of key manufacturing parameters to ensure consistency of the drug substance and drug product.

Analytical methods used to determine purity and potency of an experimental API that is very early in development will need a less rigorous method validation exercise than would be required for a quality control laboratory method at the manufacturing site. An early phase project may have only a limited number of lots to be tested and the testing may be performed in only one laboratory by a limited number of analysts. The ability of the laboratory to "control" the method and its use is relatively high, particularly if laboratory leadership is clear in its expectations for the performance of the work.

The environment in which a method is used changes significantly when the method is transferred to a quality control laboratory at the manufacturing site. The method may be replicated in several laboratories, multiple analysts may use it, and the method may be one of many methods used in the laboratory daily. The developing laboratory must therefore be aware of the needs of the receiving laboratories, for example, quality control laboratory, and regulatory expectations for the successful validation of a method to be used in support of a commercial product.

Note that the postponement of intermediate precision is aligned with previous discussion that the use of early phase analytical method resides mainly in one laboratory and is used only by a very limited number of analysts. Each individual company's phased method validation procedures and processes will vary, but the overall philosophy is the same. The extent of and expectations from early phase method validation are lower than the requirements in the later stages of development. The validation exercise becomes larger and more detailed and collects a larger body of data to ensure that the method is robust and appropriate for use at the commercial site.

However, certain fundamental concepts of cGMPs must be applied regardless of the details of the phased appropriate method validation strategy used. Examples are (1) proper documentation, (2) change control, (3) deviations, (4) equipment and utilities qualification, and (5) proper training.

A detailed method validation report may not be necessary until submission of the final market application. However, summary reports should be available to facilitate efficient data retrieval and fulfill requests from regulatory agencies for the information when required.

METHOD VERIFICATION

The U.S. Food and Drug Administration (FDA) regulation 21 CFR 211.194(a)(2) specifically states that users of analytical methods in the U.S. Pharmacopeia/National Formulary (USP/NF) are not required to validate the accuracy and reliability of these methods but merely verify their suitability under actual conditions of use. U Range Defined in linearity Defined in linearity Robustness Solution stability Solution stability has issued guidance for verification in general chapter 122. This proposal provides general information to laboratories on the verification of compendial procedures that are being performed for the first time to yield acceptable results utilizing the laboratories' personnel, equipment, and reagents.

Verification consists of assessing selected analytical validation characteristics described earlier to generate appropriate, relevant data rather than repeating the validation process for commercial products. The guidance in this general chapter is applicable to applications such as titrations, chromatographic procedures (related compounds, assay, and limit tests), and spectroscopic tests. However, general tests (e.g., water, heavy metals, residue on ignition) do not typically require verification.

Validation and verification requirement for uv/hplc assay of final dosage forms

Performance characteristics	Validation	verification
Accuracy	Yes	May be
Precision	Yes	Yes
Specificity	Yes	Yes
Limit of detection	No	No
Limit of quantification	No	No
Linearity	Yes	No
Range	Yes	No

Table 5: summarizes the comparison of the validation requirements with the verification requirements of the HPLC assay of an example fi nal dosage form. ICH requires the validation of accuracy, precision, specificity, linearity, and range. Generally, verification will only require a minimal of precision and specificity validation.

METHOD REVALIDATION

There are various circumstances under which a method needs to be revalidated. Some of the common situations are described below:

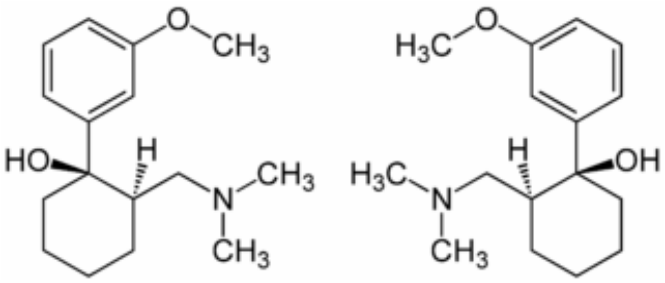
1. During the optimization of the drug substance synthetic process, significant changes were introduced into the process. To ensure that the analytical method will still be able to analyze the potentially different profile of the API, revalidation may be necessary.
2. If a new impurity is found that makes the method deficient in its specificity, this method will need to be modified or redeveloped and revalidated to ensure that it will be able to perform its intended function.
3. A change in the excipient composition may change the product impurity profile. This change may make the method deficient in its specificity for the assay or impurity tests and may require redevelopment and revalidation.
4. Changes in equipment or suppliers of critical supplies of the API or final drug product will have the potential to change their degradation profile and may require the method to be redeveloped and revalidated.

METHODOLOGY

Drug profile

Drug name: TRAMADOL

Structure



Systemic (IUPAC) name

(1*R*, 2R)-*rel*-2-[(dimethylamino) methyl]-1(3methoxyphenyl) cyclohexanol

Molecular formula

263.4 gm/mol

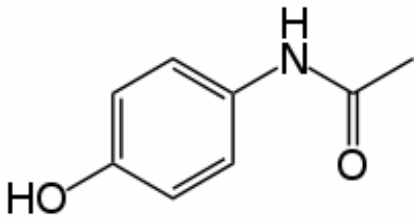
Empirical formula

C16**H**25**NO**2

Category

Analgesic.

Structure



Systemic (IUPAC) name

N-(4-hydroxyphenyl) acetamide.

Molecular formula

151.169 gm/mol

Empirical formula

C8**H**9**NO**2

Category

Analgesic and anti pyretic

DRUG PROFILE

Drug name: PARACETAMOL

EXPERIMENTAL METHODS

Method development and optimization

Scanning and determination of maximum wavelength ($_{max}$)

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, any one of the concentration among the prepared solutions of the drugs (10µg/ml) in distilled water were scanned using spectrophotometer within the wavelength region of 200 – 380 nm against blank. The resulting spectra were shown in fig and the absorption curve showed characteristic absorption maxima at 245 nm for Paracetamol and 275 nm for Tramadol hydrochloride.

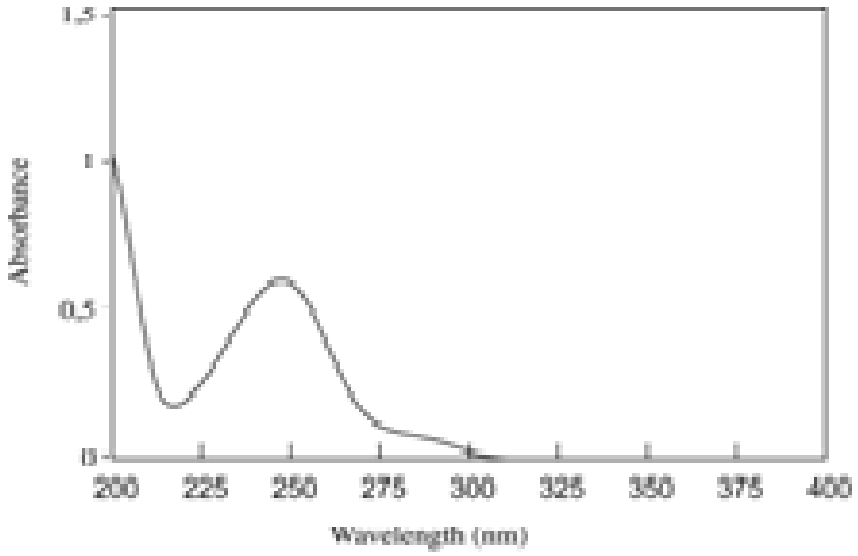


Fig: 4.1 UV Spectrum of Paracetamol

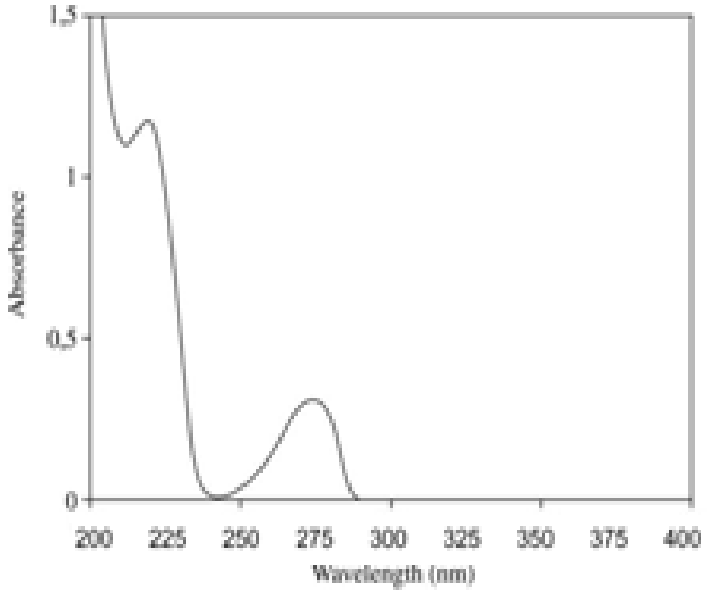
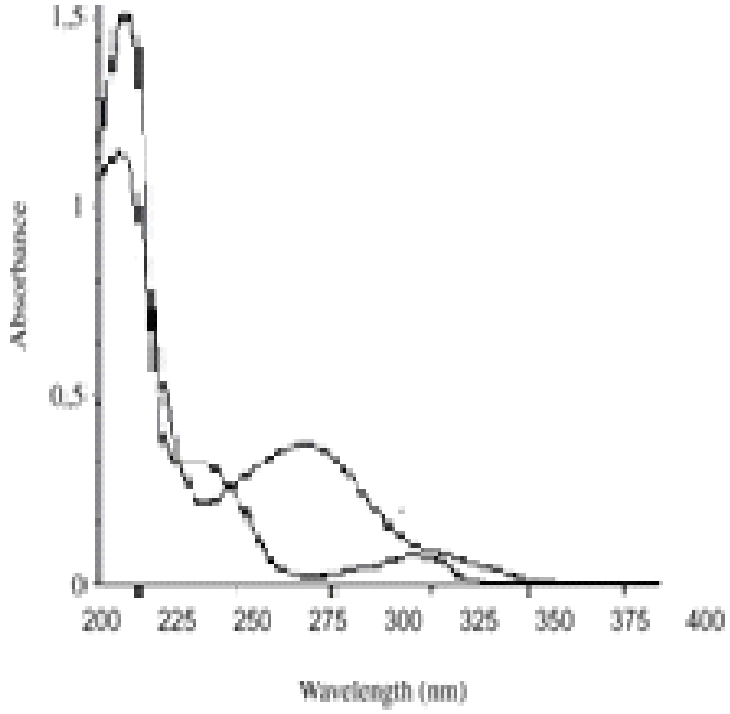


Fig: 4.1 UV Spectrum of Tramadol hydrochloride



UV overlain Spectrum of Paracetamol and Tramadol hydrochloride

Preparation of standard stock solutions

Standard stock solution was prepared by dissolving 25 mg of each drug in 25 ml of distilled water to get concentration of 1mg/ml (1000 µg/ml) solutions

Preparation of working standard solutions and construction of standard graph

The prepared stock solutions were further diluted with distilled water to get working standard solutions of 5 g/ml to 30 g/ml for Tramadol hydrochloride and 1 g/ml to 5 g/ml for Paracetamol to construct Beer's law plot for pure drug, different

aliquots of Tramadol hydrochloride and Paracetamol were taken and diluted to 10 ml with distilled water. The absorbance was measured maximum at 245 nm for Paracetamol and 275 nm for Tramadol hydrochloride against blank. The results were shown in table 4.1. The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Fig

Preparation of test solution

20 tablets were taken & weighed, reduced to fine powder. Accurately weighed about tablet powder containing 100mg & 16mg of Paracetamol & Tramadol hydrochloride respectively, transferred into 100ml volumetric flask. Then weighed accurately about 84mg of Tramadol hydrochloride pure drug & add transferred to the same volumetric flask dissolved & diluted with distilled water & make up the volume to the mark with water to obtain the solution which contains 100mg/ml of each drug. From this solution 0.2ml is transferred into a 10ml volumetric flask, diluted & make up the volume with distilled water & taken the absorbance

Preparation of mixed standard solution

25mg of each of the drugs were taken into a 25ml volumetric flask and this was dissolved with a suitable quantity of methanol & volume made up to the mark with methanol. Thus a concentration of 1mg/ml of each of the drug was obtained. From this solution 1, 2, 3 mcg/ml solution were prepared, absorbance was taken

Application of standard addition method for analysis:

Marketed formulation containing Paracetamol 500mg & Tramadol hydrochloride equivalent to 80mg. For analysis of drugs paracetamo 100mg & Tramadol hydrochloride equivalent to 16mg were weighed & finely powdered. For analysis of drug, a standard addition method was used. An accurately weighed 84mg of drug of pure Tramadol hydrochloride was added to finely powdered samples to bring the concentration of Tramadol hydrochloride in linearity range with this addition, the ratio of Paracetamol & Tramadol hydrochloride in the samples was brought to 1:1. This stock solution containing 100mcg/ml of each drug .From these solutions prepare the 2 mcg/ml concentration, which is in the beers law limit of both drugs. By simultaneous equation method, the analysis of both Paracetamol & Tramadol hydrochloride drugs was carried by measuring absorbance of sample at 245 nm (λ1), & 275 nm (λ2)

Application of simultaneous equation for analysis

Application is considered Paracetamol as x & Tramadol hydrochloride as y. Molar absorptivities of Paracetamol and Tramadol hydrochloride are taken as ax1, ax2& ay1, ay2 respectively at wave length λ1 &λ2. A1 & A2 are the absorbance of unknown or sample at λ1 &λ2 respectively.

Sample	Absorbance 245nm	Absorptivity (a)	Absorbance 275nm	Absorptivity (a)
Paracetamol	1.870	935 (ax1)	2.261	1108 (ax2)
Tramadol hydrochloride	1.180	590 (ay1)	0.615	307.5 (ay2)
Unknown	3.0 (A1)		2.5 (A2)	

Assay of dosage form

Prepared test solution was scanned and absorption maxima was found at 245 nm for Paracetamol and 275 nm for Tramadol hydrochloride. The absorbance values are taken as A1 & A2. For the determination the absorptivities, the following expression is used

A=act

[A=absorbance, a=molar absorptivity, c=concentration, t=thickness of medium]

VALIDATION AND RESULTS

Linearity

Various aliquots of mixed std. solution from 10-100 mg of both Paracetamol and Tramadol hydrochloride are prepared & scanned at their respective absorbance maxima. A graph is plotted by taking conc. on x-axis & absorbance on y-axis. The concentrations are found to be linear within the range of 2-30mg/ml & 5- 60mg/ml for Paracetamol and Tramadol hydrochloride respectively. The coefficient of correlation was found to be 0.993 for both Paracetamol and Tramadol hydrochloride respectively.

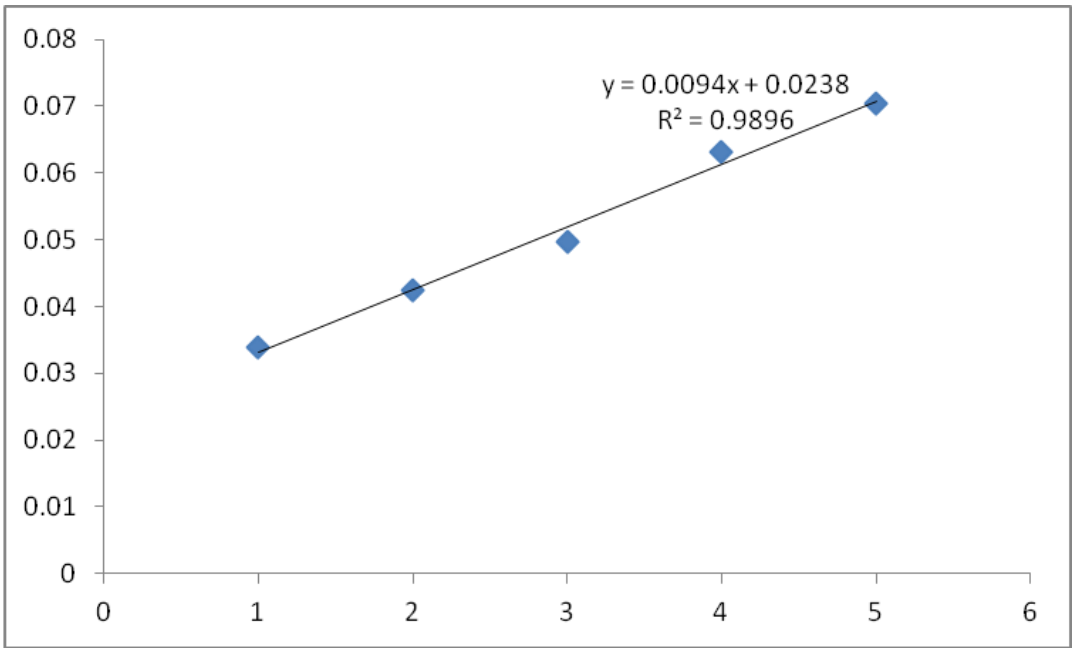
Paracetamol (245nm)

Concentration (µg/ml)	Absorbance	Statistical data
1	0.034	
2	0.0424	Range: 1-5µg/ml
3	0.0497	$R^2 = 0.9896$
4	0.0632	y = 0.0094x + 0.0238
5	0.0705	

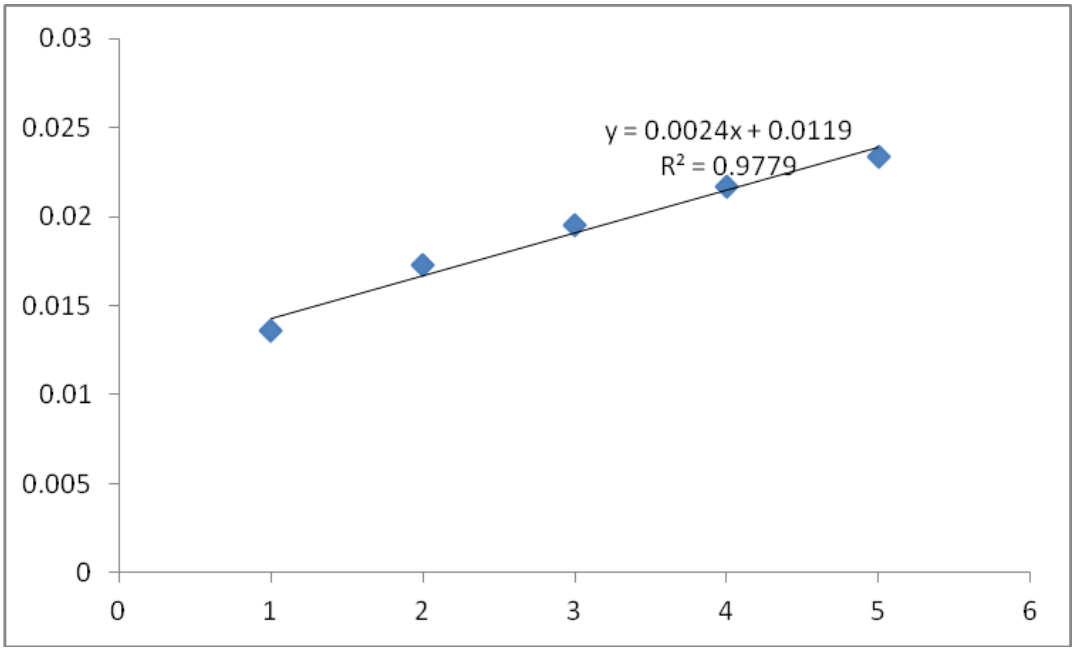
Tramadol hydrochloride (275nm)

Concentration (µg/ml)	Absorbance	Statistical data
1	0.0136	
2	0.0173	Range: 1- 5 µg/ml
3	0.0195	$R^2 = 0.9779$
4	0.0217	y = 0.0024x + 0.0119
5	0.0234	

Linearity curve for paracetamol



Linearity curve for tramadol hydrochloride



Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples within the linearity range and added to the pre analysed formulation of concentration 2µg/ml and from that %recovery values were calculated.

Preparation of samples

Mixed standard solutions of the drugs were prepared having concentration of 1000 µg/ml each. From this solution 0.02ml was pipetted and transferred into 10ml volumetric flask and was diluted and volume was made to mark with distilled water. This solution was marked as 100%, 50% and 150% solutions were prepared by pipetting out 0.1ml and 0.3ml of the stock solution into the 10ml volumetric flask and diluting and make up to the mark with distilled water and absorbances were noted

	Concentration (g/ml)		*%Recovery of*		
Sample ID	***Pure drug***	***Formulation***	***pure drug***	***Statistical Analysis***	
S1 : 50 %	*1*	*2*	*101.78*	*Mean*	*101.51*
S2 : 50 %	*1*	*2*	*101.24*	*SD*	*0.27*
S3 : 50 %	*1*	*2*	*101.51*	*% RSD*	*0.266*
S4 : 100 %	*2*	*2*	*98.13*	*Mean*	*98.143*
S5 : 100 %	*2*	*2*	*97.87*	*SD*	*0.2802*
S6 : 100 %	*2*	*2*	*98.43*	*% RSD*	*0.2855*
S7 : 150 %	*3*	*2*	*99.11*	*Mean*	*99.34*
S8 : 150 %	*3*	*2*	*99.52*	*SD*	*0.2095*
S9 : 150 %	*3*	*2*	*99.39*	*% RSD*	*0.2109*

Precision

The precision of the proposed method was ascertained by actual determination of 6 replicates of fixed concentrations of the drugs. Within the Beer's range and finding out he absorbance by the proposed method. From the absorbance mean, standard deviation 4% RSD were calculated. Different parameters included for precision study repeatability, intraday and interday precision.

Precision data showing reproducibility

Concentration (2 µg/ml)	PARACETAMOL (245)		TRAMADOL HYDROCHLORIDE (275)	
	Absorbance	Statistical data	Absorbance	Statistical data
1	0.0464		0.0136	
2	0.0466		0.0140	
3	0.0471	M: 0.0468	0.0145	M: 0.0146
4	0.0475	SD: 0.0188	0.0148	SD: 0.0071
5	0.0482	%RSD: 1.966	0.0150	%RSD: 2.02
6	0.0486		0.0155	

Intraday precision

Concentration (2 µg/ml)	PARACETAMOL (245)		TRAMADOL HYDROCHLORIDE (275)	
	Absorbance- 1	Absorbance- 2	Absorbance- 1	Absorbance- 2
1	0.0464	0.0464	0.0136	0.0136
2	0.0466	0.0466	0.0140	0.0140
3	0.0471	0.0471	0.0145	0.0145
4	0.0475	0.0475	0.0148	0.0148
5	0.0482	0.0482	0.0150	0.0150
6	0.0486	0.0486	0.0155	0.0155
Average	0.0458	0.0458	0.0142	0.0144
% RSD	1.966	2.037	2.02	1.955

Interday precision

Concentration (2 µg/ml)	PARACETAMOL (245)		TRAMADOL HYDROCHLORIDE (275)	
	Day- 1	Day- 2	Day- 1	Day- 2
1	0.0464	0.0465	0.0134	0.0132
2	0.0466	0.0466	0.0141	0.0140
3	0.0471	0.0472	0.0144	0.0143
4	0.0475	0.0473	0.0148	0.0146
5	0.0482	0.0474	0.0147	0.0146
6	0.0486	0.0486	0.0144	0.0142
Average	0.0474	0.0471	0.0142	0.0144
% RSD	1.966	1.71	2.02	1.955

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. These conditions included different analysis and different instruments etc. in this method different analyst were considered. The data was subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD was taken

Concentration (2 µg/ml)	PARACETAMOL (245)		TRAMADOL HYDROCHLORIDE (275)	
	Analyst- 1	Analyst- 2	Analyst- 1	Analyst- 2
1	0.0464	0.0464	0.0136	0.0136
2	0.0466	0.0466	0.0140	0.0140
3	0.0471	0.0471	0.0145	0.0145
4	0.0475	0.0475	0.0148	0.0148
5	0.0482	0.0482	0.0150	0.0150
6	0.0486	0.0486	0.0155	0.0155
Avg	0.0478	0.0469	0.0158	0.0151
% RSD	1.966	1.059	2.02	2.086

Robustness

Robustness of the method was studied by deliberate variations of the analytical parameters such as solvent composition, PH, temperature .the data was then subjected to statistical ana lysis and the results are expressed in mean, standard deviation and % RSD.

Concentration (2 µg/ml)	PARACETAMOL (245)		TRAMADOL HYDROCHLORIDE (275)	
	Room temp	30 ^{0}C	Room temp	30 ^{0}C
1	0.0464	0.0464	0.0134	0.0136
2	0.0466	0.0465	0.0141	0.0140
3	0.0471	0.0471	0.0145	0.0145
4	0.0475	0.0474	0.0148	0.0142
5	0.0482	0.0480	0.0150	0.0150
6	0.0486	0.0487	0.0152	0.0153
Avg	0.0475	0.0474	0.0148	0.0147
% RSD	1.966	0.02	2.02	1.77

SUMMARY OF RESULTS

Validation parameter	PARACETAMOL (245)	TRAMADOL HYDROCHLORIDE (275)
Linearity/ range	Range- 2-30mcg/ ml R^2 0.9924	Range- 2-50 mcg/ml R^2 0.995
Accuracy/ % recovery	99.4% - 101.2%	99.5% - 100.4%
Precision	%RSD 1.96	%RSD 2.00
Ruggedness	%RSD 1.5	%RSD 2.02
Robustness	%RSD 0.21	%RSD 1.77

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Paracetamol and Tramadol hydrochloride in bulk and dosage forms. The method is simple and economical. The sample recoveries in all formulations were in good agreement within the limit. Hence, this method can be easily and conveniently adopted for routine analysis of simultaneous estimation of Tramadol hydrochloride and Paracetamol in tablet dosage form can also be used for routine analysis.

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