

MIXED HYDROTROPY APPROACH BASED MULTICOMPONENT ANALYSIS USING SIMULTANEOUS EQUATION FOR SELECTED DRUGS IN PHARMACEUTICAL DOSAGE FORMS

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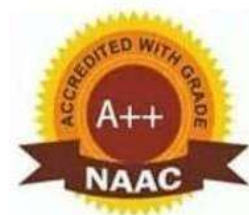
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Declaration

We do hereby declare that the work presented in the thesis entitled "**Mixed hydrotrophy approach based multicomponent analysis using simultaneous equation for selected drugs in pharmaceutical dosage forms**" submitted by us to the Sathyabama Institute of Science and Technology, Chennai-600119 for the award of the degree of **Bachelor of pharmacy (2020-2024)** by research is an original record of research work carried out by us under the supervision of our guide **Dr.N.Delhiraj M.Pharm., Ph.D. Professor, School of Pharmacy**. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associateship etc. In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

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ABSTRACT

A simple, accurate, novel, safe and precise method using simultaneous equation was developed for the simultaneous estimation of poorly water-soluble drugs, Paracetamol and caffeine anhydrous in tablet dosage form using 2M Sodium acetate and 8M Urea solution (50:50% W/V) as a mixed hydrotropic solution. Paracetamol and caffeine anhydrous show maximum absorbances at 245 nm and 273.5 nm respectively. Sodium acetate and Urea solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs. Paracetamol and caffeine anhydrous follows the Beer's law in the concentration range of 2-16 µg/ml and 1-10 µg/ml with correlation coefficient of 0.999 and 0.999. The optimized methods showed good reproducibility and recovery with ranging from 97.08±0.086 to 99.82±0.097 for Paracetamol and caffeine anhydrous respectively. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values therefore the method can be used for routine monitoring of Paracetamol and caffeine anhydrous in industry.

Keywords: Mixed hydrotrophy, Simultaneous equation, Paracetamol, caffeine anhydrous, Sodium acetate, Urea, Validation

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LIST OF ABBREVIATIONS

L	Liter
ml	Milliliters
μl	Microliters
G (or)g	Grams
Mg	Milligram
Nm	Nanometer
W/W	Weight by Weight
Avg	Average
λ _{max}	Lambda Max
UV	UltraViolet

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CHAPTER-1

INTRODUCTION

The science that deals with the identification and quantification of the components of material systems such as these is called analytical science. It is called that because the process of determining the level of any or all components in a material system is called analysis. It can involve both physical and chemical processes. If it involves chemical processes, it is called chemical analysis or, more broadly, analytical chemistry¹.

Analytical procedures can be classified in two ways: first, in terms of the goal of the analysis, and second, in terms of the nature of the method used. In terms of the goal of the analysis, classification can be based on whether the analysis is qualitative or quantitative.

Qualitative analysis, an analysis carried out to determine only the identity of a pure analyte, the identity of an analyte in a matrix, or the identity of several or all components of a mixture. **Quantitative analysis** is the analysis of a material (matrix of the analyte) for how much of one or more components are present. If a material is known by a particular name and an analysis is carried out to determine the level of that named substance in the material, the analysis is called an assay for that named substance.

Analysis procedures can be additionally classified into procedures that involve physical properties, wet chemical analysis procedures, and instrumental chemical analysis procedures.

- 1) **Analysis using physical properties** involves no chemical reactions and at times relatively simple devices to facilitate the measurement. Physical properties are especially useful for identification such as melting point, boiling point, specific gravity, viscosity, refractive index, solubility, particle size, in addition to characteristic absorption features and optical rotation play a vital role in characterization of pharmaceutical chemicals and drug substances.

2) **Wet chemical analysis techniques** are classical techniques usually involve chemical reactions or classical reaction. The two major subcategories of wet chemical analysis.

(a) **Gravimetric analysis** methods primarily utilize for the measurement of weight.

(b) **Titrimetric analysis** is also known as volumetric analysis, utilize solution chemistry where the volumes of solutions are prepared, measured and transferred and analyzed frequently.

Instrumental analysis can also involve chemical reactions, but it also involves modern sophisticated electronic instrumentation. Instrumental analysis techniques are fast and can offer a much greater scope and practicality to the analysis. In addition, instrumental methods are generally used to determine the minor constituents or constituents that are present in low levels, rather than the major constituents of a sample.

Instrumental methods are generally classified into two broad groups: Electrochemical and spectral (optical) methods.

Electrochemical methods of analysis involve the measurement of current, voltage or resistance in relation to the concentration of a certain species in solution. for example; Voltametry, Coulometry, Potentiometry, Conductometry.

Spectral methods are based on absorption and emission of radiation. Absorption: Ultraviolet (UV) -Visible spectrophotometry,
Emission: Fluorimetry

UVSPECTROMETRY

Spectroscopy in the ultraviolet (UV), visible (Vis), and near-infrared (NIR) range of the electromagnetic spectrum is frequently referred to as electronic spectroscopy because, when a substance is exposed to light, electrons are moved from low-energy to high-energy atomic or molecular orbitals. These electron transfer processes can occur in inorganic and organic molecules and transition metal ions. The primary foundation of spectroscopic methods is the detection of electromagnetic radiation's interactions with quantized matter at particular energies. The measurement and interpretation of electromagnetic radiation that is absorbed as molecules in a sample transition from one energy level to another, such as from ground state to excited state or from excited state to ground state, is known as spectrophotometry. ²

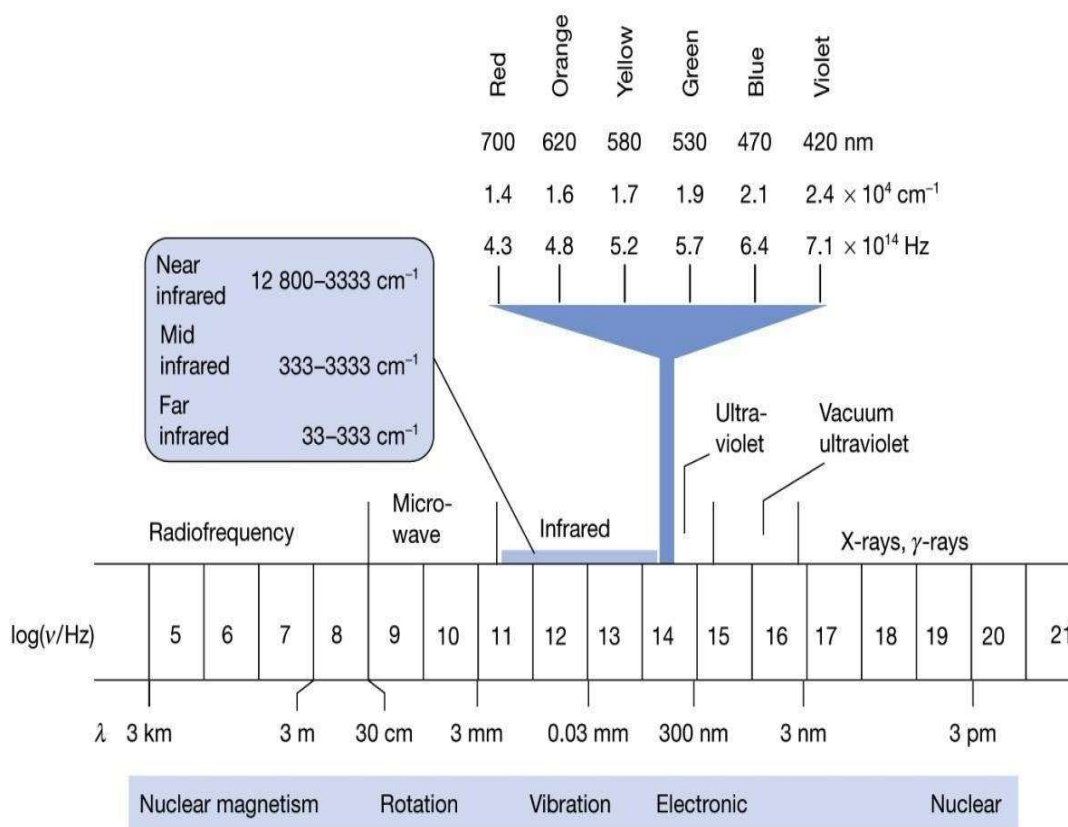


Fig1.1:ElectromagneticradiationRegion

INSTRUMENTATION

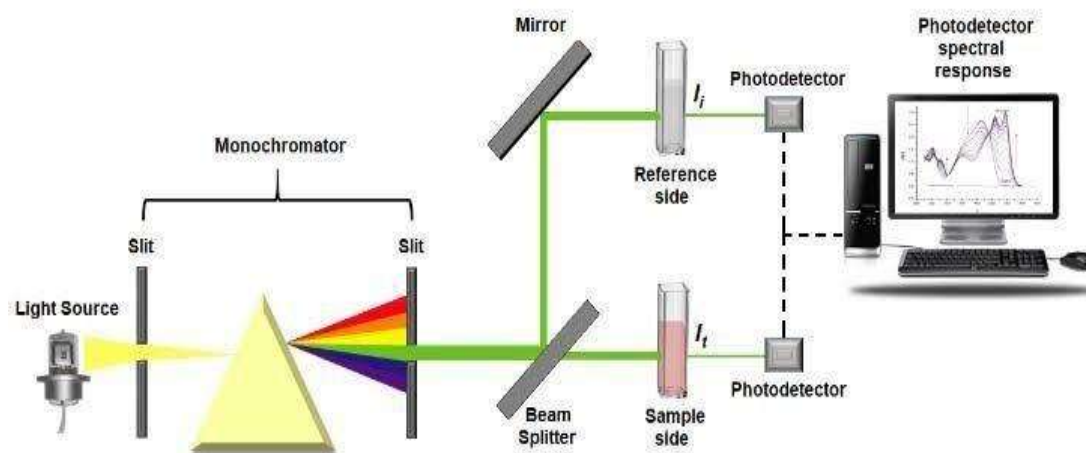


Fig1.2: Schematic representation of the dual-beam UV Spectrometer

(a) Light Source:

Tungsten-Halogen lamp: This lamp offers wavelength coverage from the near ultraviolet (300–320 nm) to the red end of the visible spectrum (750–800 nm).

Deuterium or hydrogen lamp: This is mostly used to measure far ultraviolet light to 200 nm

(b) Monochromators:

A monochromator's function is to create a beam of monochromatic (single wavelength) radiation from a variety of wavelengths. There are two fundamental techniques for choosing a wavelength: filters and a dispersing system.

Filters: Coloured glass or gelatine filters are the most basic kind of selection. Rarely do typical bandwidths exceed 30 to 40 nm

(c) Prisms

The spectrum is centered at the after-dispersion exit slit that may be moved around the beam and scanned to get the needed wavelength. In reality, the spectrum usually moves across the output slit when the prism is rotated. Typical monochromator prism-Mator is seen in the figure below. Instead of lenses, reflective components like mirrors are preferred in UV systems for both cost- and efficiency-related.

(d) Sample cells:

The sample supplied for spectrophotometric analysis may be in the solid, liquid, or gaseous condition. Cells made of optically flat fused glass may be used for measurements for the analysis of liquids and gases in the UV/visible range above 320 nm, but below 320 nm, more complex methods must be utilized.

(e) Detector:

Detectors used in UV/visible spectrophotometers can be called as photometric detectors. When a radiation is passed through a sample cell, part of it is being absorbed by the sample solution and the rest is being transmitted. This transmitted radiation falls on the detector and the intensity of absorbed radiation can be determined or displayed. In these detectors, the light energy is converted to electrical signal which can be read or recorded. The most commonly used detectors are

- 1) Barrier Layer cell or Photo Voltaic cell
- 2) Photo tubes or photo emissive cells and
- 3) Photo Multiplier tubes

(f) Recorder(or)display:

The provision of a signal (often an electrical voltage) that is proportionate to the absorption by a sample at a specific wavelength marks the fundamental function of a spectrophotometer. Clear, unambiguous digital readouts (LED or LCD) are preferred. and it could be connected to a microprocessor so that reading can be done in any desired words, such as directly in units of concentration.²²

MULTICOMPONENT ANALYSIS

Assay as a single component:

It is carried out on the basis where other components have a sufficiently small absorbance at the wavelength of measurement. It is satisfied only if the conc. of interfering substances, their absorptivity / path length of solution are sufficiently small that their absorbance can be ignored.⁵

Assay using absorbance corrected for interference:

If the identity, concentration and absorptivity of the absorbing interferents are known, it is possible to calculate their contribution to the total absorbance of a mixture. The concentration of the analyte is then calculated from the corrected absorbance (total absorbance – the absorbance of the interfering substances).⁴

Assay after solvent extraction of the sample:

If the interference from other interferents is large or its contribution to the total absorbance cannot be calculated, the separations by solvent extraction procedure have been opted. It is particularly for acidic or basic drugs where state of ionization determines their solvent partition. The choice of pH of the aqueous medium and of immiscible solvent effects the complete separation of interferents from analyte.^{4,5}

Simultaneous Equations:

If a sample contains two absorbing drugs (X & Y) each of which absorbs at the λ_{max} of the other (λ_1 & λ_2), it is possible to determine both drugs by simultaneous equation.

The information required

- (1) absorptivities of X at λ_1 & λ_2 , a_{X1} & a_{X2} respectively.
- (2) absorptivities of Y at λ_1 & λ_2 , a_{Y1} & a_{Y2} respectively.
- (3) absorbance of the diluted sample at λ_1 & λ_2 , A_1 & A_2 respectively.
- (4) concentration of X & Y, c_X & c_Y 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 , the absorbance of the mixture is the sum of the individual absorbance of x and

$$A_1 = a_{x1} b C_x + a_{y1} b C_y \dots\dots\dots (1)$$

$$A_2 = a_{x2} b C_x + a_{y2} b C_y \dots\dots\dots (2)$$

For measurements in 1 cm cells, $b=1$ cm. Rearrange Eq.

$$C_y = \frac{(A_2 - a_{x2} C_x)}{a_{y2}} \dots\dots\dots (3)$$

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

$$C_x = \frac{(A_2 a_{y1} - A_1 a_{y2})}{(a_{x2} a_{y1} - a_{x1} a_{y2})} \dots\dots\dots (4)$$

$$C_y = \frac{(A_1 a_{x2} - A_2 a_{x1})}{(a_{x2} a_{y1} - a_{x1} a_{y2})} \dots\dots\dots (5)$$

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

$$\frac{A_2/A_1}{a_{x2}/a_{x1}} \quad \text{and} \quad \frac{a_{y2}/a_{y1}}{A_2/A_1}$$

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 reasonably dissimilar and if the two components do not interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorbances.⁵

Difference Spectrophotometry:

The technique of difference spectrophotometry can significantly increase the selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferences. The absorbance difference (A) between two equimolar solutions of the analyte in various chemical forms with differing spectral properties is the key aspect of this approach. The criteria for using difference spectrophotometry in the presence of other absorbing substances are as follows:

1. Reproducible changes in the spectrum of the analyte may be introduced by the addition of one or more reagents.

2. The reagent has no effect on the absorbance of the interfering compounds.

The change of pH using aqueous solutions of acids, alkalis, or buffers is the simplest and most popular method for changing the spectral characteristics of the analyte⁵

Derivative Spectrophotometry:

By varying the sample's absorbance with respect to wavelength, derivative spectrophotometry transforms a normal spectrum (basic, zero-order spectrum) into its first, second, or higher derivative spectra. As it improves the detectability of minor spectral features, the differentiation of zero-order spectra can lead to the separation of overlapping signals, the elimination of background brought on by the presence of other compounds in a sample, the improvement of mixture resolution, and an increase in sensitivity and specificity.

In comparison to the original spectrum, derivative spectra provide a more distinctive profile; additional maxima and minima as well as locations where derivative spectra cross the X-axis appeared. Method upholds all of the classical spectrophotometry's laws, including the additivity law and the dependence of the derivative value on the analyte concentration. By measuring the amplitude of the derivative spectrum of the mixture at various wavelengths, these properties enable the identification of various components in a mixture. The observed amplitude is only proportional to the concentration of this analyte if the derivative peak height measurement of the analyte is carried out at the wavelengths at which the spectra of other components experience zeroing. The "zero-crossing technique" is the name given to this method of quantitative determination.⁵

Absorbance Ratio Spectrometry Method:

Consider a mixture of two compounds x and y. The absorption spectrum of the mixture "measured in 1 cm cell" is defined by the equation

$$A_M = a_x C_x + a_y C_y \dots \dots \dots (6)$$

Where; A_M is the absorbance of the mixture, a_x and a_y are the molar absorptivities, C_x and C_y are the concentrations of x and y, respectively. If the absorbance of the mixture is divided by the absorbance of a standard solution

of x (its absorbance $A_x^0 = a_x C_x^0$) the following equation results

$$\frac{A_M}{A_x^0} = \frac{C_x}{C_x^0} + \frac{A_y}{A_x^0} \dots \dots \dots (7)$$

$$\frac{C_x}{C_x^0}$$

The ratio is a constant value (Fig. 1) which can be eliminated by taking the difference in absorbance ratio amplitudes between two wavelengths λ_1 and λ_2 (peak to peak measurement)

$$\left[\frac{A_M}{A_x^0} \right]_{\lambda_1} - \left[\frac{A_M}{A_x^0} \right]_{\lambda_2} = \left[\frac{A_y}{A_x^0} \right]_{\lambda_1} - \left[\frac{A_y}{A_x^0} \right]_{\lambda_2} \dots \dots \dots (8)$$

Eq. (8) illustrates that the amplitude difference in the mixture absorbance ratio between two wavelengths λ_1 and λ_2 (termed; peak to peak, peak to trough, maximum to minimum measurement, or ratio difference spectrophotometric method) is equal to the same amplitude difference for compound y after cancelling the constant interference due to compound x. The concentration of compound y (C_y) is proportional to the peak-to-peak amplitudes of its absorbance spectra. A calibration graph is obtained by recording and storing the spectra of solutions of different concentrations of pure y, and the spectrum of a solution of pure x (the divisor x 0). The stored spectra of the solutions of pure y are divided by the standard spectrum of the divisor (x 0). In the generated ratio spectra, the peak-to-peak amplitudes between the selected wavelengths are measured and plotted against C_y to obtain the calibration graph. By using the calibration graph, the concentration of compound y in the mixture is determined after similar treatment for the mixture solution. The concentration of x in the mixture is determined by an analogous procedure.⁶

Method Using Derivative Ratio Spectra:

It is a brand-new method for figuring out the three chemicals in ternary mixes. This technique is based on the derivative of ratio spectrum, which is generated by dividing the ternary mixtures' absorption spectrums by a standard spectrum of a mixture of two of the three primary mixed components.

The calculation of the ratio spectra for resolving binary mixtures enables the utilization of the analytical signals' maximum wavelengths with multiple maxima and minima, allowing for the detection of active substances in the presence of excipients and other chemicals that might interfere with the assay.

Calculation of the first derivative will remove the constant value due to $\frac{C_X}{C_X^0}$ in Eq.9, so concentration of y can be easily determined without any

$$\frac{A_M}{A_X^0} = \frac{C_X}{C_X^0} + \frac{A_Y}{A_X^0} \quad \dots\dots\dots (9)$$

interferences from the drug x .

The difference between the two spectra $\frac{A_M}{A_X^0}$ and $\frac{A_Y}{A_X^0}$ is due to the constant interference value due to compound x $\left(\frac{C_X}{C_X^0}\right)$.

Elimination of such interference can be done by measurement of ratio spectra difference between two wavelengths or calculating derivative of the ratio spectra. Second derivative of the ratio spectra may be also used to improve linearity, mean % recoveries and decrease relative standard deviation. Spectra was modified for the determination of ternary mixtures using the derivative ratio spectra zero-crossing method. This method is realized by measurement of amplitudes at the zero crossing points in the derivative ratio spectra.⁷

Double Divisor Ratio Spectra Derivative Method:

The calculation of the ratio spectra for resolving binary mixtures is the foundation of this straightforward spectrophotometric technique created by Salinas et al. It enables the utilization of the analytical signals' maximum wavelengths with multiple maxima and minima, allowing for the detection of active substances in the presence of excipients and other chemicals that might interfere with the assay.

If a mixture of three compounds (x, y and z) is considered, if Beer's law is obeyed for all compounds over the whole wavelength range used and if the path length is 1 cm, the absorption spectrum of the ternary mixture at wavelength λ can be written in form of the equation.

$$A_m = a_x C_x + a_y C_y + a_z C_z \dots \dots \dots (10)$$

where A_m is the absorbance of the mixture,

a_x , a_y and a_z are the absorptivities of x, y and z, C_x , C_y and C_z are the concentrations of x, y and z, respectively.

A similar equation for two compounds in the same ternary mixture as in a standard binary mixture can be written as

$$A_m = a_x C_x^0 + a_y C_y^0 \dots \dots \dots (11)$$

If Eq. (10) is divided by Eq. (11) corresponding to the spectrum of a standard solution of two of the components in the ternary mixture, the ratio spectrum is obtained in the form of Eq.

$$\frac{A_m}{a_x C_x^0 + a_y C_y^0} = \frac{a_x C_x + a_y C_y}{a_x C_x^0 + a_y C_y^0} + \frac{a_z C_z}{a_x C_x^0 + a_y C_y^0} \dots \dots \dots (12)$$

The ratio of the sum of $(a_x C_x + a_y C_y)$ to the sum of $(a_x C_x^0 + a_y C_y^0)$ is equal to a constant (k) with respect to λ . If the above constant is replaced in Eq. (12), we obtain Eq. (13)

$$\frac{A_m}{a_x C_x^0 + a_y C_y^0} = k (\text{constant}) + \frac{a_z C_z}{a_x C_x^0 + a_y C_y^0} \dots \dots \dots (13)$$

However, if the standard concentrations of C_x^0 and C_y^0 or $C_x^0 \approx C_y^0$, we could write

$$a_x C_x^0 + a_y C_y^0 = C_x^0 (a_x + a_y) \dots \dots \dots (14)$$

When Eq. (14) is substituted into Eq. (13), Eq. (15) is obtained

$$\frac{A_m}{(a_x + a_y) C_x^0} = k (\text{constant}) + \frac{a_z C_z}{(a_x + a_y) C_x^0} \dots \dots \dots (15)$$

If the first derivative of Eq. (15) is taken, since the derivative of a constant is zero, Eq. (16) would be obtained ²

$$\frac{d}{d\lambda} \left[\frac{A_m}{(a_x + a_y) C_x^0} \right] = \frac{d}{d\lambda} \left[\frac{a_z}{(a_x + a_y)} \right] \frac{C_z}{C_x^0} \dots \dots \dots (16)$$

Eq. (16) is the mathematical foundation of multicomponent analysis which permits the determination of the concentration of each of the active compounds in solution without interference from the other components of the ternary system. In practice, Eq. (16) corresponding to the first derivative ratio spectrum of z is obtained by dividing the absorption spectrum of the ternary mixture of x, y and z by the standard spectrum of two of the compounds in the ternary mixture. Also, in Eq. (16), the derivative signal of the ratio spectrum of the ternary mixture is dependent only on the concentration values C_z and

C_x^0 , but is independent of the concentration values C_x and C_y in the ternary mixture. The concentrations of x and y are determined by analogous procedures.⁷

Successive Ratio-Derivative Spectra Method:

A new spectrophotometric technique was created for the simultaneous determination of ternary mixtures. The successive derivative ratio spectra is the name of this technique. The technique is based on the two-step successive derivative of ratio spectra. The process's mathematical justification is demonstrated. Both model data and experimental data were tested to determine whether the method was applicable.⁷

Q-Absorbance Ratio Method:

The simultaneous equations approach has been modified by the "also known as absorbance ratio method." This approach states that the ratio of absorbance at any material that complies with Beer's law has two wavelengths rule, which is a constant value unaffected by concentration also the path length. It is known as "Hufner's constant" or Q-value. The process makes use of the two wavelengths, one measurement of absorbance the maximum of one of the components (being 2) and the other being a wavelength where absorptivity is equal. the iso-absorptive point, which has components. It is possible to determine each component's concentration using algebraic equations where A is the sample's absorbance at the isoabsorptive

wavelength, C_x and C_y are the concentrations of x and y, respectively, A_1 and A_2 are the absorptivity of x and y, respectively.⁷

Isobestic/Isoabsorptive Point Method:

An isobestic point in spectroscopy is a particular wavelength at which two (or more) chemical species absorb light with equal efficiency. If both species are present in the same concentration, this wavelength on an isobestic plot corresponds to the point at which the absorption spectra of the two species cross. This wavelength corresponds to the molar absorptivities. According to this definition, an isobestic point is a wavelength where two or more species have extinction coefficients that are equal to one another.⁷

Absorptivity factor method:

If there is just a significant variation in the absorptivity of the two medications, preventing the occurrence of an iso-absorptive point, the absorptivity factor (modification of the classical iso-absorptive method) is used for the study of the binary mixture. Using any of the widely used spectrophotometric procedures, the concentration of drug y in a mixture of two medications x and y can be calculated; drug x can use the absorptivity factor approach to determine.⁷

Ratio subtraction method:

The ratio subtraction method is one of the spectrophotometric techniques that can be applied to the routine analysis without the necessity for derivatization and may be used to analyze two or more drug combinations simultaneously without the need for separation.

If a mixture of 2 medications, x and y, has overlapping spectra but y's spectrum is longer than x's, it is possible to determine x by reducing the mixture's spectrum by a specific y as a divisor concentration (y_0) after subtracting the old curve, multiply the new curve by y_0 . we'll discover the initial zero-order D_0X 's spectrum.⁷

Mean centering of the ratio spectra:

This technique is used to further enhance the selectivity and eliminate the drug overlap that exists in binary and ternary mixes. This gets rid of the signal-to-noise ratio is dependent on the derivative step and is enhanced.⁷

Absorption factor method:

This technique explains how to analyze a binary mixture in which the spectra of the two components, x and y, overlap. At the maximum of x, Y exhibits interference, however at a different wavelength (2), X exhibits no interference with Y. In the 200-300 nm wavelength range, there is a significant overlap between the absorption spectra of x and y. As a result, the absorption spectra of the standard solutions of y at various concentrations were captured between 200 and 400 nm. Calculated. Due to the absence of x's contribution at this wavelength, the absorbance of the combination (x + y) at 2 nm is equal to that of pure y.⁷

Multivariate chemometric methods:

In its broadest sense, chemometrics is the art of analyzing data using different numerical methods in order to uncover insightful information. Drug separation, acknowledges that measuring multiple things is often preferable. Combining non-selective signals first the use of a multivariate model or multivariate analysis. The simultaneous consideration of numerous variables. A multivariate measurement is one that includes A sample of interest is subjected to many measurements. Consequently, several variables' responses are measured. Each specimen. Multivariate techniques comprise numerous Methods for linear regression (MLR) and factor-based methods. It is incredibly convenient to arrange the data into matrices given the nature of the work. It is helpful to group the independent and dependent variables into, in particular, distinct matrices. If the spectroscopic method is used and the absorbance spectra of several known samples All of these spectra are measured, and composition compiled into a single matrix known as the absorbance The concentration values for each component of the Additionally, the sample's parts are put together to create the concentration matrix is a distinct matrix. In general, PLS approach uses data arranged as matrices of row vectors, whereas MLR and PCR techniques use data organized as matrices of column vectors.⁷

HYDROTROPY

Most of the drug molecules are lipophilic and possess low aqueous solubility. In 1916, the term 'Hydrotropy' was coined by scientist Carl A. Neuberg to describe anionic organic salts. He indicated that substances having the ability to make 'water-soluble' would be labeled as "hydrotropic substances". It is one of the methods in which aqueous solubility of poorly water-soluble drugs is improved by co-dissolving with other extremely water-soluble inert compounds. These agents are termed as hydrotropic agents or Hydrotropes. Sodium benzoate, Nicotinamide, sodium citrate, sodium acetate, and urea are few hydrotropic agents which are used to increase the solubility of a poorly water-soluble drug in an aqueous medium. Hydrotropic mixtures may also be used to improve the solubility of poorly water-soluble compounds by the synergistic or additive effect of combined hydrotropic solubilizers. Hydrotropes are capable of increasing solubility of a sparsely soluble organic molecule in water. It is a process that helps to improve the aqueous solubility of poorly soluble solutes by adding a second solution (hydrotrope). A hydrotropic molecule interacts with a less water-soluble molecule through weak van der Waals interactions such as π - π or attractive interaction between dipole and dipole. The presence of a large quantity of one solvent improves the solubility of another.

1.2.1 SOLUBILITY

Solubility refers to the ability of the solvent to dissolve a given substance, this is determined at equilibrium in terms of the average volume of solute dissolved in a solvent; the resulting solution is called a saturated solution.

The pharmacopeia mentions solubility in terms of several milliliters of solvent required to dissolve 1 g of solute. It is mentioned as a range if exact solubilities are not identified.

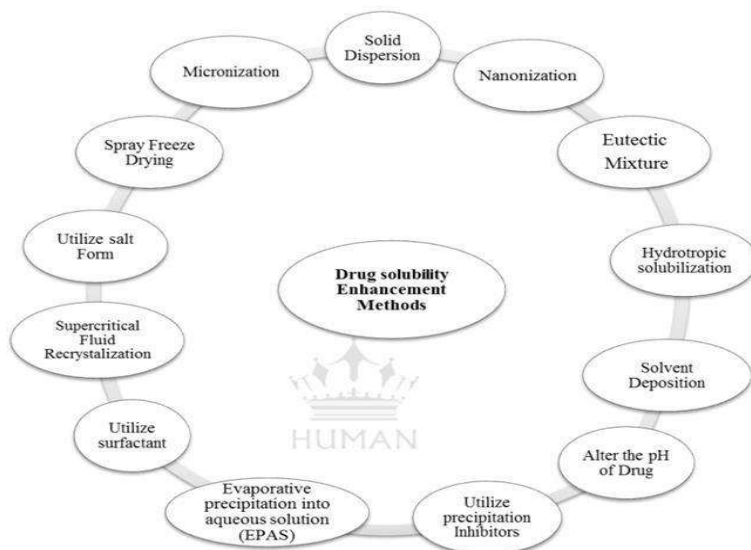


Fig1.3: Drug solubility enhancement methods

STRUCTURE OF HYDROTROPE:

Hydrotropes contain both hydrophobic and hydrophilic fractions. Hydrophobic-hydrophilic portion of the hydrotrope balance decides the efficiency of hydrotrope solubilization. The bigger the hydrophobic portion of an additive, the better the hydrotropic efficiency; the less important is the presence of the charge on the hydrophilic component. Hydrotropic agents may be liquid or solids, they may be anionic, cationic, or neutral, or anionic or inorganic, hydrotropic compounds are freely soluble in water, they enhance the aqueous solubility of organic substances by creating aggregations of stack forms. ⁸⁻¹²

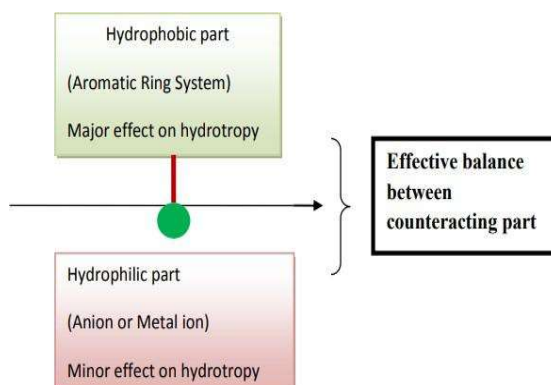


Fig1.4: Structure of Hydrotrope

MECHANISM OF HYDROTROPIC SOLUBILIZATION:

The conventional hydrotropic salts of Neuberg generally consist of two parts, an aromatic hydrophobic ring or ring system, an anionic group. The prerequisite for a hydrotropic substance is the anionic group responsible for bringing high solubility in the aqueous environment. The form of anion or metal ion has a slight influence.

The salts or additives that improve solubility in a solvent are referred to as "salt in" and those salts that decrease solubility, referred to as "salt out." Hydrotropism refers to the salting of highly soluble non-electrolytes of water. In the hydrophobic portion flip side planarity also plays a crucial role in the mechanism of hydrotropical solubilization. It is also related to complexation which involves the interaction of hydrotropic agents such as urea, Nicotinamide, sodium alginate, sodium benzoate, etc. with lipophilic drugs.

Hydrotropic molecular self-association and the combination of hydrotropic molecules with the solute enhance water solubility.

The following mechanisms are suggested to explain the mechanisms of hydrotropy.

1. Self-aggregation potential
2. Structure-breaker and structure-maker
3. Ability to form micelles like structure¹⁰⁻¹⁴

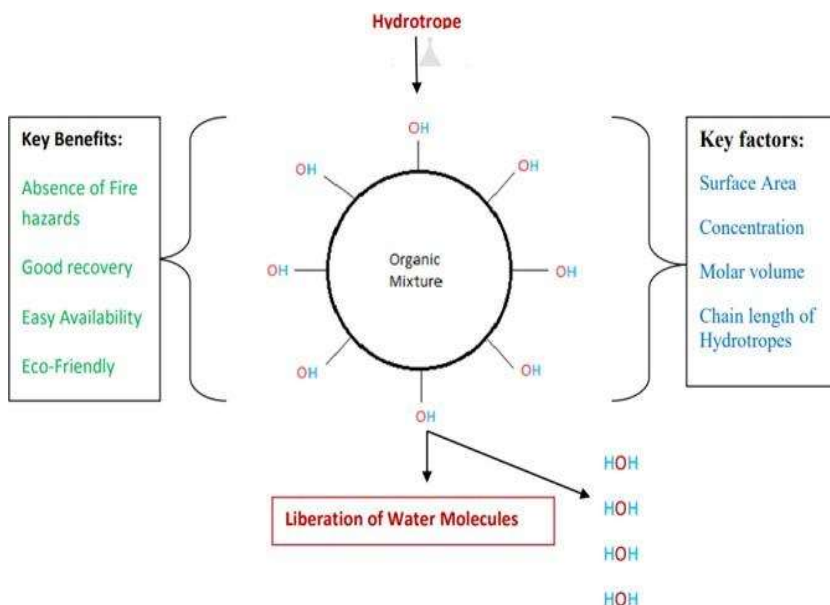


Figure 1.5: Hydrotropic Mechanism of Separation

CHARACTERISTIC FEATURES OF HYDROTROPES:

1. The hydrotrope system is absolutely water-soluble and virtually insoluble.
2. Hydrotropes are active at the surface and accumulate in aqueous solution due to its amphiphilic nature.
3. Hydrotrope solution is not exothermic. Hydrotropes are cheap, non-poisonous and non-reactive.
4. The solvent character of hydrotrope is highly selective, it is insensitive to the effects of temperature, and it is independent of pH.

ADVANTAGES OF HYDROTROPIC SOLUBILIZATION:

Hydrotropy involves simply mixing the drug in water with the hydrotrope. The solvent character is independent of pH, has high selectivity, and does not require emulsification; hence it is superior to miscibility, micellar solubilization, co-solvency, and salting in methods of solubilization. Solubilization doesn't require chemical modification of hydrophobic products, the use of organic solvents, or emulsion system preparation.^{15,16}

MIXED HYDROTROPIC SOLUBILIZATION:

In this technique, instead of using a single hydrotropic agent in high concentration, the combination of hydrotropic agents is used in lower concentrations, to increase the aqueous solubility of the poorly water-soluble drug.^{10,11,13}

ADVANTAGES OF MIXED HYDROTROPIC SOLUBILIZATION:

1. The high total concentration of hydrotropic agents required to achieve a small increase in solubility can be minimized by using a combination of hydrotropic agents at a lower concentration. The reduction of the concentration of individual hydrotropic agents to reduce side effect can reduce their toxicity.
2. It is a modern, quick, cost-effective, safe, reliable, and environmentally friendly method for the analysis of poorly water-soluble drugs (Titrimetric, Spectrophotometric, and Chromatographic).
3. It replaces the use of organic solvents and thus avoids problems associated with organic solvents such as residual toxicity, volatility error, contamination, and expense.
4. Mixed hydrotropy technology can be applied for designing a variety of dosage forms of poorly water-soluble drugs.

5. Application of hydrotropic solubilization (by controlled precipitation) in nanotechnology.
6. Usage of hydrotropical solubilization to extract active constituents from crude products.^{16,17}

APPLICATIONS:

- ❖ Preparation of dry syrups with poorly water-soluble medicines (for reconstitution).
- ❖ UV-Visible Spectrophotometric analysis quantitative estimates of poorly water-soluble drugs that restrict the use of organic solvents.
- ❖ Quantitative estimations of poorly water-soluble drugs by titrimetric analysis. Such as Ibuprofen, Flurbiprofen.¹⁰⁻¹⁶

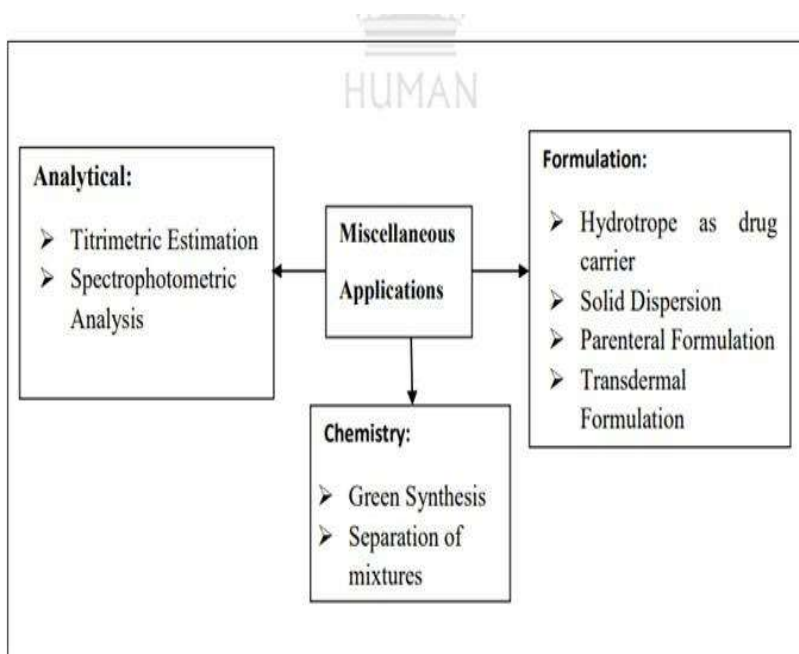


Figure 1.6: Application of Hydrotropy

VALIDATION

FDA defines validation as “establish the documented evidence which provides a high degree of assurance that a specific process will consistently produce a product of predetermined specifications and quality attributes” There are different types of validation;

- ❖ Equipment Validation
- ❖ Facility Validation including utilities.
- ❖ Process Validation
- ❖ Cleaning Validation
- ❖ Analytical Method Validation

Analytical method validation

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical testing of a pharmaceutical product is necessary to ensure its purity, stability, safety and efficacy. Analytical method validation is an integral part of the quality control system. Although a thorough validation cannot rule out all potential problems, the process of method development and validation should address the most common ones.¹⁸

Parameters

The validation of the assay procedure was carried out using the following parameters.

Specificity

The capacity to clearly evaluate the analyte in the presence of components that could be anticipated to be present is known as specificity.

When validating identifying tests, determining contaminants, and performing the assay, specificity should be investigated.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

$$\% \text{Recovery} = \frac{\text{Concentration found practically}}{\text{Concentration found theoretically}} \times 100$$

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

Repeatability expresses the precision under the same operating conditions. It is also termed as intra-assay precision. It is assessed by using a minimum of nine determinations for each tablet covering the specified range for the procedure (three concentrations / three replicates each).

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

$$\text{Standard Deviation} = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

$$\text{R.S.D (\%)} = \frac{\text{S.D}}{\bar{x}} \times 100$$

Σ = Sum of

$\bar{x}$ = Mean or arithmetic average ($\Sigma x/n$)

x = Individual observed value

$x - \bar{x}$ = Deviation of a value from the mean

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is the lowest concentration of the substance that the method can detect but not necessarily quantify. LOD simply indicates that the sample is below or above a certain level.

LOQ is the lowest concentration of the substance that can be estimated quantitatively with acceptable precision, accuracy and reliability by the proposed method. LOQ is determined by analysis of samples containing decreasing known quantity of the substance and determining the lowest level at which acceptable level of accuracy and precision is attained.

The limit of detection and limit of quantification were calculated based on the standard deviation of the response and the slope.

The limit of detection (LOD) may be expressed as

$$\text{LOD} = \frac{3.3\sigma}{S}$$

The limit of quantification (LOQ) may be expressed as

$$\text{LOQ} = \frac{10\sigma}{S}$$

Where σ = the standard deviation (SD) of peak areas of six injections of each drug.

S = the slope of the calibration curve

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was assessed by performing single measurement at several analyte concentrations. A minimum of five concentrations were recommended for linearity studies.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness

The robustness of an analytical technique is a measure of its ability to be unaffected by subtle but intentional changes to the method parameters and provides a clue as to its dependability under typical conditions.

Robustness evaluation depends on the kind of technique being studied and should be taken into account during the development phase.

System suitability testing

Testing for system compatibility is a crucial step in many analytical processes. The tests are predicated on the idea that the apparatus, electronics, analytical procedures, and sample material form an entire system.

CHAPTER-2

DRUGPROFILE

Introduction

The paracetamol/caffeine combination relieves pain in both acute mild migraine and episodic tension headache

Composition

Each uncoated tablet contains

Paracetamol	-	650mg
Caffeine anhydrous	-	50mg

Category

Paracetamol	-	non-opioid analgesic and antipyretic
Caffeine	-	Central Nervous Stimulant

Molecular formula

Paracetamol	-	$C_8H_9NO_2$
Caffeine	-	$C_8H_{10}N_4O_2$

Molecular weight

Paracetamol	-	151.165g/mol
Caffeine	-	194.194g/mol

Chemical Name

Paracetamol	-	N-(4-hydroxyphenyl)ethanamide
Caffeine	-	1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione

ChemicalStructure

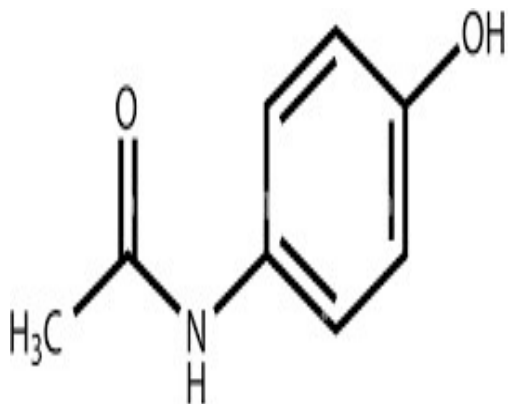


FIGURE 2.1:
STRUCTURE FOR PARACETAMOL

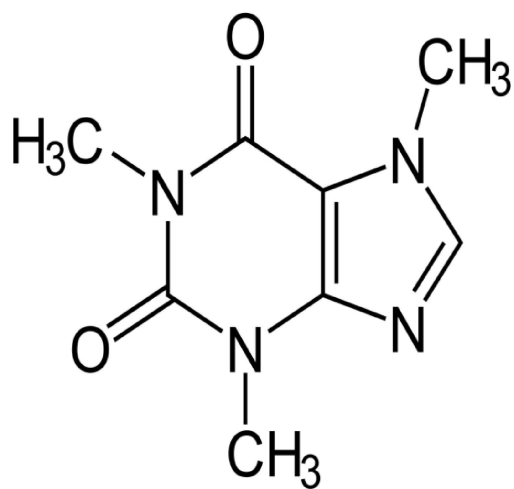


FIGURE 2.2
STRUCTURE FOR CAFFEINE

Description:

Paracetamol: White odourless crystalline powder

Caffeine: White odourless crystalline powder; has a bitter taste

Solubility:

Paracetamol: Soluble in water (1:70, 1:20 at 100°C), ethanol (1:7), acetone (1:13), chloroform (1:50), glycerol (1:40), methanol (1:10), propylene glycol (1:9) and solutions of alkali hydroxides; insoluble in diethyl ether.

Caffeine: Freely soluble in chloroform, sparingly soluble in water, in acetic anhydride and acetic acid, and slightly soluble in ethanol and in diethyl ether.

pH:

Paracetamol: A saturated aqueous solution has a pH of 6.

Caffeine: A saturated aqueous solution has a pH of 5.5-6.5.

Pharmacology for Paracetamol***Pharmacodynamics***

Paracetamol acts by inhibiting COX-1 and COX-2 enzymes and especially similar to selective COX-2 inhibitors. Paracetamol inhibits prostaglandin synthesis by reducing the active form of COX-1 and COX-2 enzymes. This occurs only when the concentration of arachidonic acid and peroxides is low.

Pharmacokinetics

After being taken by mouth, paracetamol is rapidly absorbed from the small intestine, while absorption from the stomach is negligible. Thus, the rate of absorption depends on stomach emptying. Food slows the stomach emptying and absorption, but the total amount absorbed stays the same. In the same subjects, the peak plasma concentration of paracetamol was reached after 20 minutes when fasting versus 90 minutes when fed. High carbohydrate (but not high protein or high fat) food decreases paracetamol peak plasma concentration by four times. Even in the fasting state, the rate of absorption of paracetamol is variable and depends on the formulation, with maximum plasma concentration being reached after 20 minutes to

1.5 hours. Paracetamol's bioavailability is dose-dependent: it increases from 63% for 500mg dose to 89% for 1000mg dose. Its plasma terminal elimination half-life is 1.9–2.5 hours, and volume of distribution is roughly 50 L. Protein binding is negligible, except under the conditions of overdose, when it may reach 15–21%. The concentration in serum after a typical dose of paracetamol usually peaks below 30 µg/mL (200 µmol/L). After 4 hours, the concentration is usually less than 10 µg/mL (66 µmol/L).¹⁹

Pharmacology for Caffeine

Pharmacodynamics

Caffeine acts by blocking binding of adenosine to the adenosine A1 receptor, which enhances release of the neurotransmitter acetylcholine. Caffeine has a three-dimensional structure similar to that of adenosine, which allows it to bind and block its receptors. Caffeine also increases cyclic AMP levels through nonselective inhibition of phosphodiesterase. In the absence of caffeine and when a person is awake and alert, little adenosine is present in CNS neurons. With a continued wakeful state, over time adenosine accumulates in the neuronal synapse, in turn binding to and activating adenosine receptors found on certain CNS neurons; when activated, these receptors produce a cellular response that ultimately increases drowsiness. When caffeine is consumed, it antagonizes adenosine receptors; in other words, caffeine prevents adenosine from activating the receptor by blocking the location on the receptor where adenosine binds to it. As a result, caffeine temporarily prevents or relieves drowsiness, and thus maintains or restores alertness.

Pharmacokinetics

Caffeine from coffee or other beverages is absorbed by the small intestine within 45 minutes of ingestion and distributed throughout all bodily tissues. Peak blood concentration is reached within 1–2 hours. It is eliminated by first-order kinetics. Caffeine's biological half-life is between 3 and 7 hours. The half-life is decreased by 30–50% in adult male smokers, approximately doubled in women taking oral contraceptives, and prolonged in the last trimester of pregnancy. In newborns the half-life can be 80 hours or more, dropping very rapidly with age, possibly to less than the adult value by age 6 months.²⁰

CHAPTER-3

REVIEW OF LITERATURE

Dinc E *et al.*, ²¹ have performed a study on **“The spectrophotometric multicomponent analysis of a ternary mixture of ascorbic acid, acetylsalicylic acid and paracetamol by the double divisor-ratio spectra derivative and ratio spectra-zero crossing methods”**. The double divisor-ratio spectra derivative and ratio spectra-zero crossing methods were used to analyse an effervescent tablet containing title compounds without chemical separation. Both methods yielded linear calibration graphs for three compounds, with both methods providing the best results.

Erk N *et al.*, ²² have done research on **“Simultaneous determination of paracetamol and methocarbamol in tablets by ratio spectra derivative spectrophotometry and LC ”**. The study uses ratio spectra derivative spectrophotometry and high-performance liquid chromatography (HPLC) to determine paracetamol and methocarbamol in pharmaceutical tablets. The spectrophotometric method detects PAR and MET with detection limits of 0.097 and 0.079 microg/ml, respectively, with reasonable repeatability.

Subramanian G *et al.*, ²³ have done research on **“Simultaneous reverse phase HPLC estimation of paracetamol and rofecoxib in tablets. Indian journal of pharmaceutical sciences”**. A reverse phase HPLC method has been developed for estimating paracetamol and rofecoxib in tablets, using a Hypersil C18 column and validated for linear, precise, accurate, and selective analysis.

Bhavsar AS *et al.*, ²⁴ have worked on **“RP-HPLC Estimation of Paracetamol and Valdecoxib in Combined Dosage Form”**. A reverse phase liquid chromatography method was developed for estimating paracetamol and valdecoxib in tablet formulation, validated for linearity, accuracy, and precision, with linearity in 25-150 µg/ml and 1-6 µg/ml ranges.

Gandhimathi M *et al.*,²⁵ performed a study on **“High Performance Thin Layer Chromatographic Method for Simultaneous Estimation of Paracetamol and Valdecoxib in Tablet Dosage Form”**. A new high-performance thin layer chromatographic method has been developed for estimating paracetamol and valdecoxib simultaneously from a combined dosage form. The method uses a pre-coated silica gel 60 GF 254 TLC plate and chloroform: isopropyl alcohol: glacial acetic acid as mobile phases.

Khoshayand MR *et al.*,²⁶ have worked on **“Simultaneous spectrophotometric determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric methods”**. The study introduces UV spectrophotometry methods for determining paracetamol, ibuprofen, and caffeine in pharmaceuticals, including GA-PLS and PC-ANN, which are simple, rapid, and suitable for drug quality control in pharmaceutical formulations and capsules.

Nikam AD *et al.*,²⁷ have done research on **“Estimation of paracetamol and aceclofenac in tablet formulation by ratio spectra derivative spectroscopy. Indian journal of pharmaceutical sciences”**. A new method for estimating paracetamol and aceclofenac in combined tablet dosage form has been developed using ratio derivative spectrophotometry. The method, based on the amplitude of the first derivative of ratio spectra at 256 nm and 268 nm, demonstrated good linearity, accuracy, and reproducibility.

Shirkhedkar AA *et al.*,²⁸ have worked on **“Simultaneous Determination of Paracetamol and Piroxicam in Tablets by Thin Layer Chromatography Combined with Densitometry”**. A new densitometric method has been developed and validated for determining Paracetamol and Etodolac in tablet dosage form, using a linear calibration curve and meeting ICH guidelines for pharmaceutical formulation analysis.

Pawar UD *et al.*,²⁹ have done research on **“Simultaneous determination of aceclofenac, paracetamol and chlorzoxazone by HPLC in tablet dose form”**. A reversed-phase liquid chromatographic method has been developed to determine paracetamol, aceclofenac, and chlorzoxazone in their ternary mixtures

in commercial pharmaceutical preparation. The method is validated and linear, with recovery values ranging from 100.7-101.4%.

Chitlange S *et al.*,³⁰ performed a study on **“Development of stability indicating HPTLC method for simultaneous estimation of paracetamol and dexibuprofen”**. The study presents a HPTLC method for analyzing paracetamol and dexibuprofen in bulk and pharmaceutical dosage form. The method uses silica gel 60F 254 plate and n-hexane: ethyl acetate: glacial acetic acid, with linearity in 10-50 µg/band range for paracetamol and 6-30 µg/band for dexibuprofen.

Gondalia R *et al.*,³¹ have worked on **“Development and validation of spectrophotometric methods for simultaneous estimation of ibuprofen and paracetamol in soft gelatin capsule by simultaneous equation method”**. A spectrophotometric method has been developed for simultaneous estimation of Ibuprofen and Paracetamol in soft gelatin capsule dosage form. The method uses two wavelengths and follows Beer's law in concentration ranges of 4-14 µg/mL and 2-12 µg/mL, with statistical and recovery studies validated results.

Patel DJ *et al.*,³² have done research on **“Simultaneous determination of paracetamol and lornoxicam in tablets by thin layer chromatography combined with densitometry”**. A new HPTLC method has been developed to determine paracetamol and lornoxicam in tablets, using ethyl acetate, methanol, toluene, and glacial acetic acid as mobile phases. The method was validated in accordance with ICH guidelines.

Patil PP *et al.*,³³ have done research on **“Simultaneous Estimation of Aceclofenac and Paracetamol in Bulk and Combined Tablet Dosage Form and From Biological Fluid by Planar Chromatography”**. A high-performance thin-layer chromatographic method has been developed for simultaneous determination of Aceclofenac and Paracetamol in tablet dosage form and biological fluids, with successful recovery and compliance with ICH guidelines.

Vichare V *et al.*,³⁴ have performed a study on **“Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation”**. Two UV spectrophotometric methods have been developed for

estimating pharmaceutical dosages of Paracetamol and Caffeine. Method A uses a simultaneous equation method, while Method B uses a Q-absorbance equation. Both methods show linearity in concentration ranges.

Anuradha SJ *et al.*,³⁵ have worked on **“Simultaneous determination of paracetamol and lornoxicam by RP-HPLC in bulk and tablet formulation”**. The study developed a RP-HPLC method for determining paracetamol and lornoxicam without separation, using a Kromasil C8 column and methanol: phosphate buffer mobile phase. The method was rapid, precise, and accurate, making it suitable for bulk and pharmaceutical formulation analysis.

Badgujar MA *et al.*,³⁶ have performed a study on **“Simultaneous determination of paracetamol and mefenamic acid in tablet dosage form by high performance liquid chromatography”**. A reversed phase HPLC method was developed to determine Paracetamol and Mefenamic acid in tablets using an Inertsil ODS 3V C18 column and UV detection. The method showed acceptable repeatability and intermediate precision.

Balan P *et al.*,³⁷ have performed a study on **“Simultaneous Estimation of Dexibuprofen and Paracetamol by UV Spectroscopic Method in Bulk and Tablet Formulation”**. The study introduces a simple, accurate, and cost-effective method for estimating Dexibuprofen and Paracetamol in bulk and dosage form, utilizing ultraviolet absorbance maxima and Beer's Lambert law, and confirmed in commercial tablet preparations.

Induri M *et al.*,³⁸ have worked on **“Simultaneous quantification of paracetamol and meloxicam in tablets by high performance liquid chromatography”**. The study developed a rapid, inexpensive RP-HPLC method for estimating paracetamol and meloxicam in tablets. Validated for linearity, accuracy, precision, robustness, and application, it was found to be accurate, precise, and robust, making it suitable for routine quality control.

Gandhi SV *et al.*,³⁹ have worked on **“Simultaneous HPTLC determination of nabumetone and paracetamol in combined tablet dosage form”**. A new HPTLC method has been developed and validated for determining nabumetone and paracetamol in tablet dosage form. Tested on Merck aluminium plates, the

method showed linearity, accuracy, precision, and robustness, making it suitable for pharmaceutical formulation analysis.

Kondawar MS *et al.*,⁴⁰ have worked on **“UV spectrophotometric estimation of paracetamol and lornoxicam in bulk drug and tablet dosage form using multiwavelength method”**. A new UV/VIS spectrophotometric method has been developed to determine Paracetamol and Lornoxicam in bulk and tablet formulations. The method demonstrated linearity over concentration ranges and repeatability in intraday and interday studies, with successful tablet analysis results.

Murtaza G *et al.*,⁴¹ have worked on **“Development of a UV-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets”**. A UV Spectrophotometric method has been developed for estimating meloxicam and paracetamol dosages. The method uses equation, absorbance ratio, and correction methods, and follows Beer-Lambert's law. The accuracy is near 100%, allowing for routine use.

Nikhade RD *et al.*,⁴² have done research on **“Simultaneous estimation of paracetamol, thiocolchicoside and aceclofenac by UV spectrophotometer using multicomponent mode method”**. A multicomponent mode analysis method has been developed to determine Paracetamol, Thiocolchicoside, and Aceclofenac in tablet formulations using wavelengths of 249nm, 258nm, and 276nm, with linearity between 5-25 µg/mL for PAR, 1-5 µg/mL for THC, and 1-5 µg/mL for ACE.

Rao JR *et al.*,⁴³ have done research on **“Development and validation of HPLC method for simultaneous quantitation of Paracetamol and Dexketoprofen Trometamol in bulk drug and formulation”**. A RP-HPLC method was developed and validated for determining paracetamol and dexketoprofen trometamol in bulk drug and tablet dosage form. The method used Thermo Hypersil ODS–C18 stationary phase and methanol: ammonium acetate buffer mobile phase.

Rao JR *et al.*,⁴⁴ have prepared a study on **“Simultaneous HPTLC Determination of Paracetamol and Dexketoprofen Trometamol in**

pharmaceutical dosage form". A fast, precise, accurate, and robust HPTLC method was developed for simultaneous determination of paracetamol and dextetoprofen trometamol in tablet dosage form. The method used Silica gel G60 F 254 plates as a stationary phase and toluene: ethyl acetate: acetic acid as a mobile phase.

Sinha S *et al.*,⁴⁵ have done research on "**Validated simultaneous multicomponent spectrophotometric determination of paracetamol, aceclofenac and tizanidine in tablets**". A spectrophotometric method has been developed to estimate paracetamol, aceclofenac, and tizanidine from tablet dosage form using methanol as a solvent. The method measures absorbances at three wavelengths, offering rapid, specific, precise, and accurate analysis for bulk and combined dosage forms.

Chandra P *et al.*,⁴⁶ have performed a study on "**Application of HPLC for the simultaneous determination of aceclofenac, paracetamol and tramadol hydrochloride in pharmaceutical dosage form**". A reversed-phase liquid chromatographic method has been developed for estimating pharmaceutical dosages of aceclofenac, paracetamol, and tramadol hydrochloride using a HiQ-Sil™ HS C18 column and methanol mobile phase, demonstrating linearity, precision, robustness, and accuracy.

Hajian R *et al.*,⁴⁷ have worked on "**The spectrophotometric multicomponent analysis of a ternary mixture of ibuprofen, caffeine and paracetamol by the combination of double divisor-ratio spectra derivative and H-point standard addition method**". A new spectrophotometric method has been developed for analyzing a ternary mixture containing paracetamol, ibuprofen, and caffeine without prior separation. The H-point standard addition method and double divisor ratio spectra derivative method were successfully applied to Novafen capsule analysis.

Joshi R *et al.*,⁴⁸ have performed a study on "**Simultaneous estimation of paracetamol, chlorzoxazone and ibuprofen by validated spectrophotometric methods**". Shimadzu UV 1700 was used for drug quantitation, measuring absorbance at 287.2 nm and 295.2 nm for paracetamol, chlorzoxazone, and

ibuprofen respectively. Beer's law limits were found, and validation studies showed statistical significance.

Kumar Talluri MV *et al.*,⁴⁹ have prepared a study on **“Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for simultaneous determination of paracetamol and lornoxicam in pharmaceutical dosage forms”**. A high-performance liquid chromatographic and UV spectrophotometric method was developed to determine paracetamol and lornoxicam in pharmaceutical formulations, demonstrating good linearity, accuracy, and precision, with UV spectroscopy being an inexpensive, reliable, and less time-consuming alternative.

Manoj Kumar Jet *et al.*,⁵⁰ have worked on **“Validated UV-spectrophotometric and RP-HPLC method for the simultaneous estimation of paracetamol and chlorzoxazone in tablet dosage form”**. Two methods are described for determining Paracetamol and Chlorzoxazone in binary mixtures: UV-spectrophotometric determination using methanol absorbance and HPLC separation, both validated according to ICH guidelines, and linearity obtained in concentration ranges of 100-500 µg.mL⁻¹ for Paracetamol and 50-250 for Chlorzoxazone.

More SJ *et al.*,⁵¹ have worked on **“Application of HPLC for the simultaneous determination of paracetamol, chlorzoxazone, and nimesulide in pharmaceutical dosage form”**. A reversed-phase liquid chromatographic method has been developed to determine pharmaceutical dosages of paracetamol, chlorzoxazone, and nimesulide. The method, validated for linearity, precision, robustness, limit of detection, limit of quantitation, specificity, and accuracy, is suitable for routine quality control analysis.

Kulkarni VG *et al.*,⁵² have done research on **“High Performance Thin Layer Chromatographic Analysis of Paracetamol and Etodolac in Combined Tablet Dosage Form”**. The study uses HPTLC-densitometry and D1 spectrophotometry to determine etodolac and paracetamol in binary mixtures, addressing toxicity issues and validating analytical methods for bulk powder and pharmaceutical formulations.

KumarTalluriMV *et al.*,⁵³ have worked on **“DevelopmentandvalidationofRP-HPLC and ultraviolet spectrophotometric methods of analysis for simultaneous determination of paracetamol and lornoxicam in pharmaceutical dosage forms”**. A high-performance liquid chromatographicand UV spectrophotometric method was developed to determine paracetamoland lornoxicam in pharmaceutical formulations, demonstrating good linearity, accuracy, and precision, with UV spectroscopy being an inexpensive, reliable, and less time-consuming alternative.

Singh KB *et al.*,⁵⁴ have done research on **“A validated RP-HPLC method for the simultaneous estimation of paracetamol and naproxen in tablet formulation”**. The study presents a validated reverse-phase HPLC method for estimating paracetamol and naproxen in bulk and tablet formulations. The method, using an Eclipse XDB C18 column and water: acetonitrile mobile phase, achieved a mean recovery of 99.72% and 100.88%, making it useful for quality control.

Sivasubramanian L *et al.*,⁵⁵ have performed a study on **“HPTLC for the Simultaneous Determination of Paracetamol and Lornoxicam in Pharmaceutical Dosage Form”**. The study presents a method for determining Paracetamol and Lornoxicam in a binary mixture using HPTLC separation and densitometric measurements, successfully applied to pharmaceuticalformulations and validated for precision, robustness, recovery, and detection limits.

Tsvetkova B *et al.*,⁵⁶ have worked on **“Validated LC method for simultaneous analysis of paracetamol and caffeine in model tablet formulation”**. The paper presents a high-performance liquid chromatographic analytical procedure for determining paracetamol and caffeine in tablet formulations. Validated for specificity, linearity, precision, accuracy, LOD, LOQ, and robustness, it was successfully applied for identification, purity, and assay tests.

Khan IU *et al.*,⁵⁷ have performed a study on **“Simultaneous determination of piroxicam and paracetamol in pharmaceutical formulations using stability indicating HPLC method”**. The study presents a liquid chromatographic method

for detecting piroxicam and paracetamol in bulk medications and pharmaceutical formulations. The method achieved separations in 6 minutes and was validated using ICH recommendations, indicating complete separation from stress-induced degradation products.

Aktaş AH *et al.*,⁵⁸ have done research on **“Spectrophotometric simultaneous determination of caffeine and paracetamol in commercial pharmaceutical by principal component regression, partial least squares and artificial neural networks chemometric methods”**. The study utilized three multivariate calibration-prediction techniques for spectrometric multicomponent analysis of a drug containing paracetamol and caffeine, demonstrating their potential for routine drug analysis and quality control.

Haritha P *et al.*,⁵⁹ performed a study on **“Method Development and Validation for Simultaneous Determination of Paracetamol and Ketoprofen BY RP-HPLC Technique”**. The study presents a precise RP-HPLC technique for determining pharmaceutical dosages of Paracetamol and Ketoprofen, validated for specificity, linearity, range, precision, accuracy, and robustness. The method has a 97%-104% recovery rate, with all validation parameters within the acceptance range.

Karbhari PA *et al.*,⁶⁰ have performed a study on **“RP-LC gradient elution method for simultaneous determination of related substances of Zaltoprofen and Paracetamol and application for drug excipient compatibility study”**. A gradient mode reverse phase liquid chromatography method has been developed for quantitative determination of impurities, related substances, and degradation products of Zaltoprofen and Paracetamol, with validation for selectivity, linearity, accuracy, precision, and robustness.

Karunakaran K *et al.*,⁶¹ have done research on **“A validated stability-indicating RP-HPLC method for paracetamol and lornoxicam: Application to pharmaceutical dosage forms”**. A new method for determining paracetamol and lornoxicam simultaneously using reversed phase HPLC has been developed, achieving linear response in concentration ranges of 125-375 µg/mL and 2-6 µg/mL, with a correlation coefficient of over 0.999, and a stable accuracy.

Khatal LD *et al.*,⁶² have done research on **“Validated HPLC method for simultaneous quantitation of paracetamol, diclofenac potassium and famotidine in bulk drug and formulation”**. A new HPTLC method has been developed and validated for analyzing paracetamol, diclofenac potassium, and famotidine in tablet formulations. The system uses aluminium plates, silica gel 60 F 254, and a mobile phase of toluene-acetone-methanol-formic acid.

Shah DA *et al.*,⁶³ have performed a study on **“Development and validation of a liquid chromatographic method for estimation of dicyclomine hydrochloride, mefenamic acid and paracetamol in tablets”**. A liquid chromatographic method was developed to quantitatively determine dicyclomine hydrochloride, mefenamic acid, and paracetamol in their combined dosage form. The method demonstrated linearity in concentration ranges and was successfully used for quantitative analysis of commercially available dosage forms.

Sharma A *et al.*,⁶⁴ have performed a study on **“Development of Simultaneous Estimation Method for Paracetamol and Chlorzoxazone in Marketed Formulation by Validated UV-Spectrophotometric and RP-HPLC Method”**. A UV spectrophotometric and reverse phase HPLC method have been developed for simultaneous estimation of paracetamol and chlorzoxazone from pharmaceutical formulations. The primary method uses the simultaneous equation method, while the secondary method uses reverse phase HPLC separation. Both methods are validated according to ICH guidelines.

Abdel Aleem EA *et al.*,⁶⁵ have done research on **“HPTLC and RP-HPLC methods for simultaneous determination of Paracetamol and Pamabrom in presence of their potential impurities”**. Two chromatographic methods were developed to determine potential impurities in Paracetamol and Pamabrom, including P-aminophenol and Theophylline. HPTLC and RP-HPLC were successfully applied for determining impurities in pharmaceutical formulations, with no significant difference in results, indicating potential for routine quality control analysis.

Mahajan K S *et al.*,⁶⁶ have worked on **“Development of HPTLC method for simultaneous estimation of paracetamol and flupirtin maleate in their**

combined tablet dosage form". A high-performance thin layer chromatographic method has been developed to estimate paracetamol and flupirtine maleate in tablet dosage form. Validated for linearity, specificity, accuracy, precision, limit of detection, and limit of quantitation, the method showed a linear relationship.

Patel Twinkle K *et al.*,⁶⁷ have done research on **"Development and validation of high-performance thin layer chromatography for estimation of mefenamic acid, paracetamol and dicyclomine HCl in tablet dosage form"**. A high-performance thin layer chromatographic method was used to analyze mefenamic acid, paracetamol, and dicyclomine HCl in tablet dosage form. The method tested for UV absorbance at 254 nm, but Dicyclomine HCl was not detected. The linear response was observed in the 100-700 ng/spot range.

Babu GR *et al.*,⁶⁸ have performed a study on **"Bioanalytical Method Development and Validation for Simultaneous Estimation of Paracetamol and Cefixime by using RP-HPLC in Rabbit Plasma"**. A new bioanalytical RP-HPLC method was developed to estimate Paracetamol and Cefixime in rabbit plasma using Cefaclor as an internal standard, with a retention time of 3.618min for Cefaclor, 4.608min for Paracetamol, and 5.914min for Cefixime.

Glavanović S *et al.*,⁶⁹ have worked on **"Simultaneous quantitative determination of paracetamol and tramadol in tablet formulation using UV spectrophotometry and chemometric methods"**. UV spectrophotometric methods were used to quantitatively determine paracetamol and tramadol in paracetamol-tramadol tablets. The results were compared with the validated UHPLC method, and good agreement was found between the two, with GA-PLS results being more reliable for tramadol.

Mali A *et al.*,⁷⁰ have worked on **"Simultaneous Determination of Paracetamol and Domperidone in Pharmaceutical Dosage Form by First Order Derivative UV Spectrophotometry"**. The study employs a first-order derivative spectrophotometric method to determine tablet dosages of Paracetamol and Domperidone, revealing a linear relationship between the two substances at 262 nm and 297 nm, respectively.

Sharma H *et al.*, ⁷¹ have performed a study on **“Validated RP-HPLC method for simultaneous estimation of paracetamol, pamabrom and dicyclomine, hydrochloride in bulk and pharmaceutical dosage form”**. A rapid Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) and UV-spectrophotometry method was developed for determining Paracetamol, Pamabrom, and Dicyclomine hydrochloride. The method achieved chromatographic separation within minutes and was accurate, 98-102%, making it suitable for pharmaceutical formulation analysis.

Surekha K *et al.*, ⁷² have done research on **“Simultaneous Estimation and Quantification of three main ingredients, Aceclofenac, Thiocolchicoside & Paracetamol in Finished Dosage Form by using Reversed Phase High Performance Liquid Chromatography”**. A high-pressure liquid chromatographic method has been developed for estimating Paracetamol, Aceclofenac, and Thiocolchicoside from pharmaceutical formulations. Validated for precision, robustness, and recovery, it is repeatable and selective.

Elkhoudary MM *et al.*, ⁷³ have done research on **“Resolution and quantification challenge of modern chemometric models in the determination of anti-migraine tablets containing ergotamine, caffeine, acetaminophen, and metoclopramide”**. The study compares five multivariate models for determining unique mixtures of ergotamine, metoclopramide, caffeine, and paracetamol in pharmaceutical formulations. Linear learning machine methods outperform SRCLS, MCR-ALS, and PLS.

Escobar SC *et al.*, ⁷⁴ have performed a study on **“Optimization and Validation of a Simple and Fast RPHPLC Method for Simultaneous Determination of Acetaminophen and Caffeine in Tablets”**. The study developed a high-performance liquid chromatographic method for determining acetaminophen and caffeine in tablets without an internal standard. The method demonstrated linearity, precision, and accuracy, with recovery percentages of 99.53% and 99.12%, and demonstrated reproducibility through an interlaboratory assay.

Navneet Kumar U *et al.*, ⁷⁵ have worked on **“Development and Validation of a New Chromatographic Method for the Simultaneous Estimation of**

Serratiopeptidase, Aceclofenac and Paracetamol by RP-HPLC". The study developed and validated a new chromatographic method for simultaneous estimation of Serratiopeptidase, Aceclofenac, and Paracetamol using RP-HPLC. The method was validated for system suitability, linearity, accuracy, precision, specificity, LOD, LOQ, and robustness.

Zivkovic A *et al.*,⁷⁶ have worked on **"Quantitative analysis of multicomponent mixtures of over-the-counter pain killer drugs by low-field NMR spectroscopy"**. A student lab successfully analyzed pain relief drugs using a compact 1H NMR spectrometer, revealing its potential in life sciences by analyzing over 50 self-made sample mixtures and marketed tablet formulations.

Shanker K *et al.*,⁷⁷ have done research on **"Simultaneous determination of Etodolac and paracetamol in bulk drugs and pharmaceutical formulation by HPTLC-densitometric and UV-derivative spectrophotometry methods"**. The study uses HPTLC-densitometry and D1 spectrophotometry to determine etodolac and paracetamol in binary mixtures, addressing the issue of etodolac's pain reduction and paracetamol's limited solubility due to nephrotoxic metabolite P-aminophenol.

Aktaş A *et al.*,⁷⁸ have done research on **"Spectrophotometric Determination of Paracetamol, Propyphenazone and Caffeine in Tablets by Multivariate Calibration Approach"**. The study uses UV spectrophotometry to determine Paracetamol, Propyphenazone, and Caffeine in drug samples. It uses principal component regression and partial least squares regression for chemometric analysis, and tests synthetic solutions for prediction ability, suggesting their use for standard investigation and quality control.

Elbagary RI *et al.*,⁷⁹ have done a study on **"Development and Validation of RP-HPLC and TLC Methods for Simultaneous Estimation of Paracetamol, Thiocolchicoside and Aceclofenac in Bulk and Combined Pharmaceutical Dosage Form"**. Two methods have been developed to determine ternary mixtures containing paracetamol, thiocolchicoside, and aceclofenac in bulk powder and combined dosage form without prior separation. The first uses RP-HPLC for isocratic elution, while the second uses TLC separation and

densitometric measurements. Both methods were successfully applied for pharmaceutical preparation analysis.

Uddin MN *et al.*,⁸⁰ have done research on **“Chemometrics assisted spectrophotometric method for simultaneous determination of paracetamol and caffeine in pharmaceutical formulations”**. The study compares PLSR and ANN techniques for assaying paracetamol and caffeine in pharmaceutical formulations using spectrophotometric data. Both methods were successful, with ANN showing better prediction efficiency. The drugs contained 75-86% paracetamol and 77-92% caffeine.

El-Yazbi AF *et al.*,⁸¹ have worked on **“Validated specific HPLC-DAD method for simultaneous estimation of paracetamol and chlorzoxazone in the presence of five of their degradation products and toxic impurities”**. The study presents a reliable HPLC method using diode array detection (DAD) for estimating paracetamol and chlorzoxazone in the presence of degradation products and toxic impurities. The method was validated for linearity, range, precision, accuracy, specificity, robustness, detection, and quantitation limits, and was successfully applied for capsule dosage analysis.

Vu Dang H *et al.*,⁸² have done research on **“RP-HPLC and UV spectrophotometric analysis of paracetamol, ibuprofen, and caffeine in solid pharmaceutical dosage forms by derivative, fourier, and wavelet transforms: a comparison study”**. The study utilized signal-transforming algorithms for UV spectrophotometric analysis of paracetamol, ibuprofen, and caffeine in ternary mixtures. The spectrophotometric methods showed linearity and accuracy in assay and dissolution tests. An isocratic RP-HPLC analysis was developed and validated, suggesting possible interchange between UV spectrophotometric and HPLC methods for routine drug analysis.

Alnuwaiser MA *et al.*,⁸³ have done research on **“Utilisation of both UV and GC-MS spectroscopy to track the estimation of caffeine and acetaminophen in a number of paracetamol analgesic drugs”**. A study using UV-Vis and GC-MS spectrometers found acetaminophen and caffeine disappearing from a caffeine

standard sample and both in a gaseous state, possibly due to strong intermolecular interaction in the liquid state.

Ibrahim H *et al.*, ⁸⁴ have done research on **“Simultaneous determination of paracetamol, propyphenazone and caffeine in presence of paracetamol impurities using dual-mode gradient HPLC and TLC densitometry methods”**. Two chromatographic methods were developed to determine the analgesic and antipyretic drug combination of paracetamol, propyphenazone, and caffeine in the presence of PC impurities. The dual-mode gradient high-performance liquid chromatography method and thin-layer chromatography densitometry method were validated and proven accurate.

Kokilambigai KS *et al.*, ⁸⁵ have done research on **“Utilization of green analytical chemistry principles for the simultaneous estimation of paracetamol, aceclofenac and thiocolchicoside by UV spectrophotometry”**. The paper presents a green UV spectrophotometric method for determining paracetamol, aceclofenac, and thiocolchicoside using green analytical chemistry principles, evaluating its greenness profile using metrics like National Environmental Methods Index and Analytical Eco-scale.

Abdel Rahman MA *et al.*, ⁸⁶ have worked on **“Validated HPLC–PDA methodology utilised for simultaneous determination of Etoricoxib and Paracetamol in the presence of Paracetamol toxic impurities”**. A selective HPLC method was used to separate Etoricoxib, Paracetamol, and toxic impurities from PCM, a commonly used analgesic and antipyretic medication in COVID-19 supportive treatment, suitable for routine analysis in quality control laboratories.

Arage A *et al.*, ⁸⁷ have performed a study on **“High performance thin layer chromatography (HPTLC) method development and validation for the simultaneous determination of paracetamol, caffeine, chlorpheniramine and phenylephrine in tablet formulation”**. A thin layer chromatographic method was developed and validated for analyzing paracetamol, caffeine, phenylephrine, and chlorpheniramine in tablets. The method was validated for precision, accuracy, and recovery, and successfully applied to two marketed tablets.

Chandratrey A *et al.*,⁸⁸ have prepared a study **“Simultaneous spectrophotometric estimation and validation of three component tablet formulation containing paracetamol, nimesulide and tizanidine”**. The study evaluated the predictive power of various chemometric methods on UV spectra and first-to-fourth order derivative spectra in pharmaceutical formulations containing paracetamol, propyphenazone, and caffeine, using principal component regression and partial least squares, and analyzing multicomponent mixtures.

Saminathan Jet *al.*,⁸⁹ have prepared a study on **“Validated HPTLC Method for Simultaneous Quantitation of Aceclofenac, Paracetamol and Thiocolchicoside in Bulk drug and Pharmaceutical Formulation”**. A high-performance thin layer chromatographic method was developed for simultaneous quantification of aceclofenac, paracetamol, and thiocolchicoside in bulk and tablet dosage form, validated for linearity, accuracy, precision, robustness, and specificity.

CHAPTER-4

AIM AND OBJECTIVE OF THE WORK

From the literature survey conducted, it was found that there are analytical methods reported for Paracetamol and Caffeine anhydrous either individually or in combination with other drugs by Spectrophotometry, HPLC, Electrochemical and Thin layer chromatographic methods. Since both the drugs are poorly soluble, use of hydrotropic concept to improve solubility of drugs and also applied for the estimation of Paracetamol and Caffeine in combined pharmaceutical dosage forms. So, the present work is aimed at development of the spectrophotometric method for the simultaneous estimation of Paracetamol and Caffeine in tablets using Simultaneous Equation Method. The above objective was accomplished by the following

- ❖ Establishment of parameters
- ❖ Assay Procedure
- ❖ Validation of the method
 - Accuracy
 - Precision-Repeatability

CHAPTER-5

EXPERIMENTALWORK

In this study, Combination tablets containing Paracetamol and Caffeine were subjected to the following methods of analysis for quantitative estimation using reference standards (Paracetamol and Caffeine anhydrous).

MATERIALS

Instrumentation

UV–Visible Spectrophotometer–Shimadzu, UV–1900i series, matched with quartz cells corresponding to 1 cm path length

Solvent Used:

Diluent (Mixed Solution of 2M Sodium Acetate: 8M Urea) (50; 50% W/V)

Preparation of 2M Sodium Acetate: 272 g of sodium acetate dissolved in sufficient volume of water to make a final volume of 1000 ml.

Preparation of 8M Urea: 480 g of urea dissolved in sufficient volume of water to make a final volume of 1000 ml.

Reference Standards:

Paracetamol

Supplier: LOBACHEMIE Private Limited, Mumbai
Caffeine anhydrous

Supplier: Sisco Research Laboratories Private Limited, Mumbai

Tablet brands used

CROCIN (650 mg of Paracetamol and 50 mg of Caffeine) Batch. No:
EP23044 Manufacturing. Date: OCTOBER 2023, Expiry. Date: SEPTEMBER 2025,
Manufacturer: Encore Healthcare Private Limited, Aurangabad.

ESTABLISHMENT OF PARAMETERS

In developing a quantitative method for determining an unknown concentration of analyte by absorption spectrophotometry, certain parameters have to be established.

The most important are

- i. Absorption maximum ($\lambda_{\max}$)
- ii. Beer's law concentration range.

Preparation of standard stock solution

The standard stock solutions of both Paracetamol and Caffeine were prepared separately by dissolving 100mg of Paracetamol and 100mg of Caffeine in 100ml standard flasks separately using diluent as the solvent to give a concentration of 1000 $\mu\text{g/ml}$ respectively. These solutions were then used for establishing the following parameters.

Absorption Maximum ($\lambda_{\max}$)

The stock solutions were suitably diluted with diluent so as to contain 10 $\mu\text{g/ml}$ of Paracetamol and 10 $\mu\text{g/ml}$ of Caffeine respectively. These solutions were then scanned in the UV region between 400 – 200 nm and found that Paracetamol exhibited ($\lambda_{\max}$) at 245 nm and Caffeine exhibited ($\lambda_{\max}$) at 273.5 nm.

Beer's law concentration Range

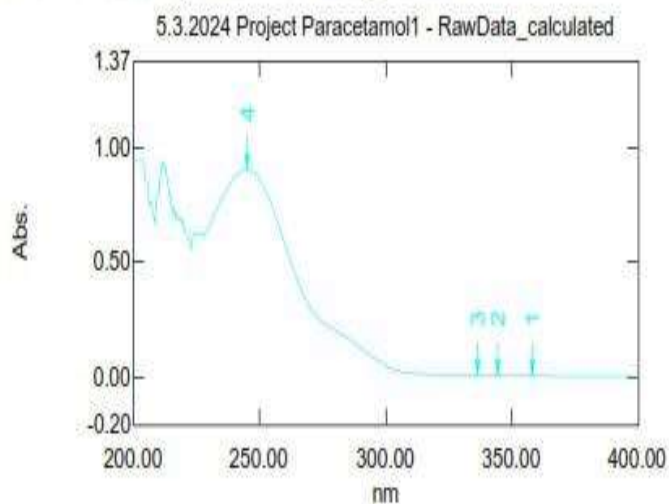
The stock solutions were suitably diluted with diluent to get concentrations ranging from 2– 16 $\mu\text{g/ml}$ for Paracetamol and 1–10 $\mu\text{g/ml}$ for Caffeine respectively. These solutions were measured at their respective absorption maxima ($\lambda_{\max}$) points. The calibration curve was determined for each of the drug independently by plotting the absorbance values against concentrations. From the graphs, it was found that Paracetamol obeys Beer's law between 2–16 $\mu\text{g/ml}$ and Caffeine between 1–10 $\mu\text{g/ml}$. The regression analysis was carried out for the calibration graphs to find out correlation coefficient, y-intercept and slope of the regression line which estimates the degree of linearity.

FIGURE 5.1
UV SPECTRUM FOR PARACETAMOL

Spectrum Peak Pick

Print Date : 03/05/2024 02:40:46 PM

File Name: 5.3.2024 Project Paracetamol1 - RawData_calculated



[Summary]

Data Information		Software Information	
Data is:	Transformed Data	Software Name:	LabSolutions UV-Vis
Data Set Name:	RawData_calculated	Version:	1.10
Sample Name:	Paracetamol	Instrument Information	
Sample ID:	P	Instrument Name:	UV
Option:		Instrument Type:	UV-1900
Analyst:	Prawin	Model (S/N):	UV1900I (A12535780067)
Date/Time:	03/05/2024 02:40:37 PM		
Comments:			
Parameter File Name:	C:\UVVis-Data\Parameter\SCAN.vspn		
Report File Name:			

[Peak Pick Table]

Threshold: 0.001

Number of Points: 4

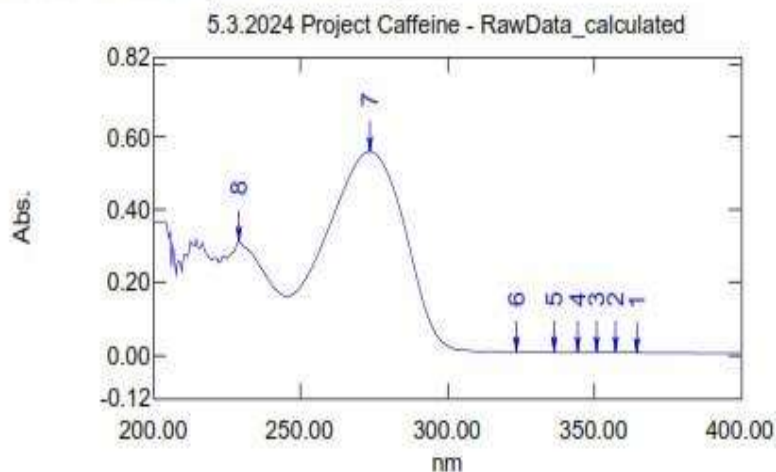
No.	P/V	Wavelength nm.	Abs.	Description
1	1	356.0	0.010	
2	1	344.5	0.009	
3	1	336.5	0.010	
4	1	245.0	0.904	

FIGURE 5.2
UV SPECTRUM FOR CAFFEINE ANHYDROUS

Spectrum Peak Pick

Print Date : 03/05/2024 02:34:35 PM

File Name: 5.3.2024 Project Caffeine - RawData_calculated



[Summary]

Data Information	Transformed Data	Software Information
Data is:	RawData_calculated	Software Name: LabSolutions UV-Vis
Data Set Name:	Caffeine	Version: 1.10
Sample Name:	C	Instrument Information
Sample ID:		Instrument Name: UV
Option:		Instrument Type: UV-1900
Analyst:	Prawin	Model (S/N): UV1900i (A12535750067)
Date/Time:	03/05/2024 02:34:05 PM	
Comments:		
Parameter File Name:	C:\UVVis-Data\Parameter\SCAN.vspn	
Report File Name:		

[Peak Pick Table]

Threshold: 0.001

Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1		364.5	0.008	
2		357.5	0.010	
3		351.0	0.009	
4		344.5	0.009	
5		336.5	0.009	
6		323.5	0.011	
7		273.5	0.562	
8		229.0	0.314	

TABLE5.1
LINEARITYDATAFORPARACETAMOL

Concentration($\mu\text{g/ml}$)	Absorbance
2	0.3646
4	0.5321
6	0.708
8	0.889
10	1.0569
12	1.2203
14	1.3769
16	1.5636

FIGURE5.3
LINEARITYDATAFORPARACETAMOL

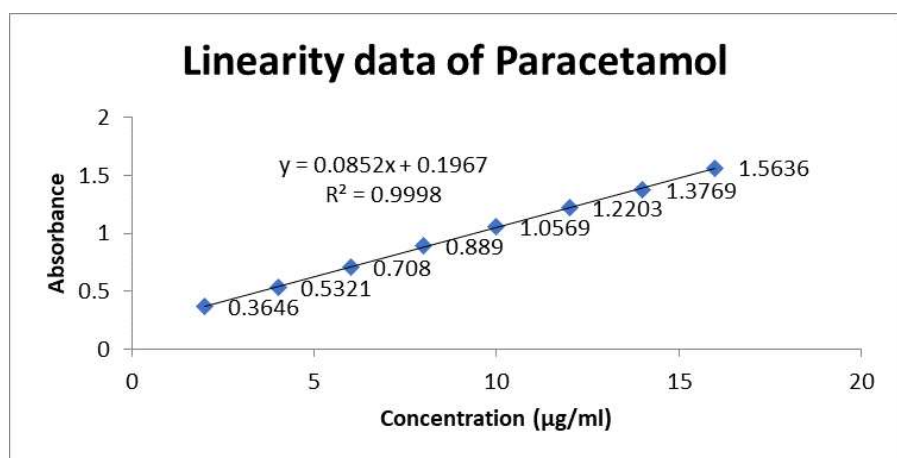


TABLE 5.2
ANALYTICAL PERFORMANCE PARAMETERS OF PARACETAMOL

Parameters	Paracetamol
Linear Dynamic Range	2-16($\mu\text{g/ml}$)
Correlation coefficient(r)	0.99984
Slope(m)	0.085249
Intercept (c)	0.196686
Standard deviation of slope(Sb)	0.000621
Standard deviation of intercept(Sa)	0.006276
Standard error of estimation(Se)	0.008054
F distribution	18822.19
Degrees of freedom	6
Residual sum of squares	0.000389
Regression sum of squares	1.220916

TABLE5.3
LINEARITYDATAFORCAFFEINEANHYDROUS

Concentration($\mu\text{g/ml}$)	Absorbance
1	0.1884
2	0.2403
3	0.2941
4	0.3522
5	0.3975
6	0.4496
7	0.5031
8	0.5508
9	0.6139
10	0.6698

FIGURE5.4
LINEARITYDATAFORCAFFEINEANHYDROUS

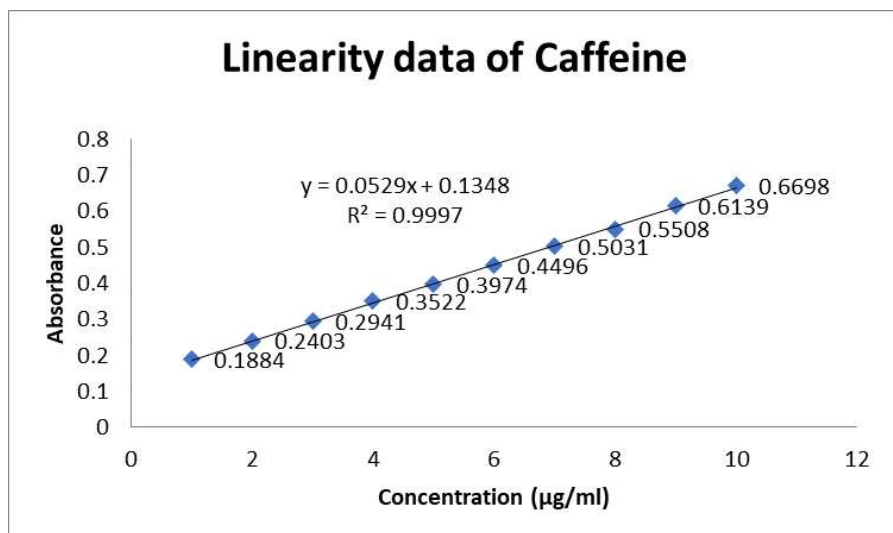
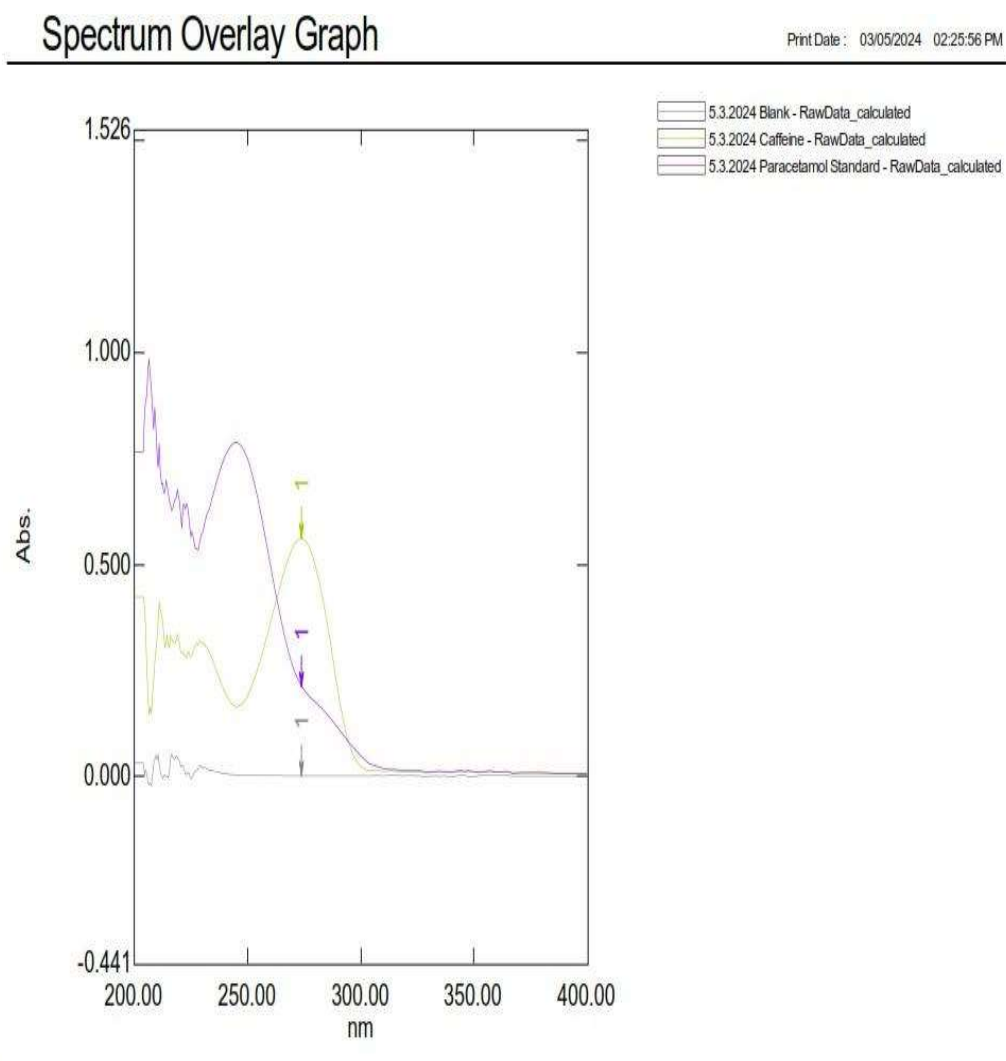


TABLE 5.4
ANALYTICAL PERFORMANCE PARAMETERS OF CAFFEINE

Parameters	Caffeine
Linear Dynamic Range	1–10 µg/ml
Correlation coefficient (r)	0.999373
Slope (m)	0.052947
Intercept (c)	0.134753
Standard deviation of slope (S _b)	0.000469
Standard deviation of intercept (S _a)	0.00291
Standard error of estimation (S _e)	0.00426
F distribution	12745.19
Degrees of freedom	8
Residual sum of squares	0.000145
Regression sum of squares	0.231276

FIGURE 5.5
OVERLAYGRAPH



SIMULTANEOUS EQUATION METHOD

Procedure

Preparation of standard solution

The stock solutions of Paracetamol and Caffeine were suitably diluted with diluent to give dilutions in the range of 2 – 16 µg/ml of Paracetamol and 1 – 10 µg/ml of Caffeine respectively. These dilutions were scanned on a spectrophotometer in the wavelength range of 400 – 200 nm and the two wavelengths selected for measuring down the absorbances are 245 and 273.5 nm. Absorptivity values of both the drugs were calculated from the mean of six independent determinations by the following formula.

$$\text{Absorptivity (a)} = \frac{A}{bc}$$

Where A - absorbance
 b - Pathlength
 c - Concentration in gms/ 100ml

Assay of tablet formulation

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of powder equivalent to about 50 mg of Paracetamol was transferred to a 50 ml standard flask. The contents of the flask were mixed with diluent and shaken so as to dissolve the active ingredients and then made up to the volume with the same solvent. The solution was filtered and the filtrate was further diluted with diluent to give a final drug concentration of 10 µg/ml of Paracetamol. Absorbance values of sample solution were recorded at 245 nm and 273.5 nm as A₁ and A₂ respectively and concentration of the two drugs in sample were determined using the following equations.

$$\text{Concentration of X (C}_x\text{)} = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2}$$

$$\text{Concentration of Y (C}_y\text{)} = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2}$$

The amount of drug present in the tablet formulation was calculated as follows:

$$\text{Amount of drug present} = \frac{\text{Concentration} \times \text{Average weight}}{\text{Sampled dilutions}}$$

Recovery Studies

To study the accuracy of the established method, recovery tests were accomplished using standard addition method at 10, 20 and 30% levels and the percentage recovery from the spiked solution was determined.

Precision

Precision confirmed by repeatability study. Repeatability study was done over a short interval of time and under the same operating conditions. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variance was calculated. The results of statistical evaluation are given in Table

CHAPTER-6

RESULTS AND DISCUSSION

The UV spectra of Paracetamol and Caffeine are presented in Fig.1 and 2 respectively. The absorption maxima ($\lambda_{\max}$) were observed at 245 nm and 277 nm for Paracetamol and Caffeine.

Obedience to Beer's law was confirmed by the linearity of the calibration curve of Paracetamol and Caffeine which are represented in Fig.3 and 4. Paracetamol showed linearity in the concentration range of 2-16 $\mu\text{g/ml}$ and Caffeine showed linearity in the concentration range of 1-10 $\mu\text{g/ml}$. The data regarding calibration curves are given in Tables 3 and 5. The overlain spectra for the linearity of Paracetamol and Caffeine are represented in Fig.5. Absorptivity values for Paracetamol and Caffeine were calculated and are presented in Table 7.

The quantitative estimation was carried out on tablet formulations by taking a concentration of 10 $\mu\text{g/ml}$ of Paracetamol. The data regarding the quantitative estimation is given in Table 8. The overlain spectra of standard Paracetamol and Caffeine are represented in Fig.7 and 8. The spectrum of sample formulation is represented in Fig.9. Both the brands of tablet formulations show percentage purity values ranging from 98.50 – 99.51% w/w for Paracetamol and 100.05 – 100.94% w/w for Caffeine.

The validation of the proposed simultaneous equation method was further confirmed by recovery studies. The recovery data is given in Table 10. The percentage recovery values vary from 99.26 to 100.12% w/w for Paracetamol and 99.38 to 100.11% w/w for Caffeine. This serves as a good index of accuracy and reproducing of the study. The relative standard deviation values are below 2% indicating the precision of the methodology.

TABLE 6.1
ABSORPTIVITY VALUES FOR PARACETAMOL AND CAFFEINE

Concentration (μg/ml)		Absorptivity at 245 nm		Absorptivity at 273.5 nm	
Paracetamol	Caffeine anhydrous	Paracetamol	Caffeine anhydrous	Paracetamol	Caffeine anhydrous
13	1	710	173	126	412

TABLE 6.2
QUANTITATIVE ESTIMATION

Tablet Sample	Label claim (mg / tablet)	*Amount present (mg / tablet)	*Percentage label claim (% w/w)
Paracetamol	650	649.01	99.51
Caffeine	50	50.47	100.94

*Each value is a mean of six readings.

TABLE 6.3
RECOVERY STUDIES

Tablet Sample	Amount present in the pre analysed sample($\mu\text{g/ml}$)	Percentage level of standard added	Spiked sample 1	Spiked sample 2	Spiked sample 3	Percentage Recovery (%w/w)
Caffeine	1.09	10	0.2550	0.2622	0.2516	99.6
		20	0.3395	0.3402	0.3429	100.26
		30	0.4050	0.4046	0.4108	99.43
Paracetamol	10.21	10	0.4115	0.4182	0.4084	99.80
		20	0.5064	0.5101	0.5133	99.62
		30	0.5889	0.5852	0.5935	99.38

TABLE 6.4
PRECISION STUDIES

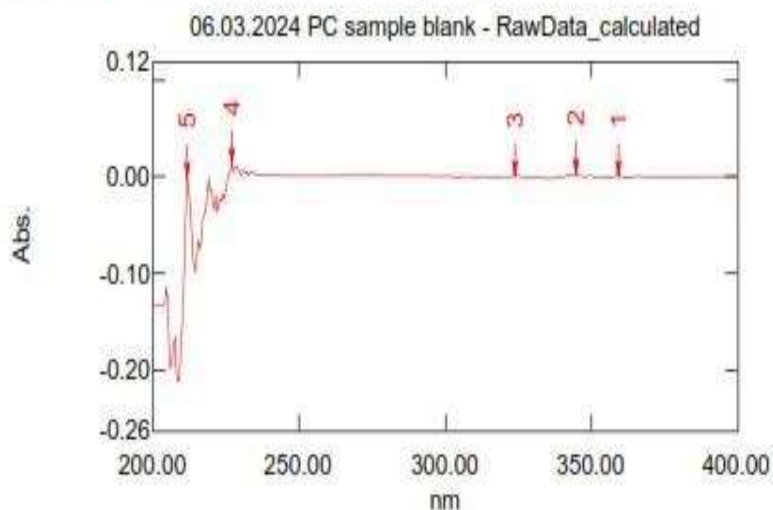
SampleNo	Paracetamol	Caffeineanhydrous
1	0.3526	0.8994
2	0.3587	0.9058
3	0.3591	0.9058
4	0.3596	0.9062
5	0.3583	0.9047
6	0.3574	0.9042
%RSD	0.718	0.281

FIGURE6.1
UVSPECTRUMFORBLANK

Spectrum Peak Pick

Print Date : 03/06/2024 02:54:09 PM

File Name: 06.03.2024 PC sample blank - RawData_calculated



[Summary]

Data Information		Software Information	
Data is:	Transformed Data	Software Name:	LabSolutions UV-Vis
Data Set Name:	RawData_calculated	Version:	1.10
Sample Name:	Paracetamol and Caffeine	Instrument Information	
Sample ID:	PC		
Option:			
Analyst:	Kannan		
Date/Time:	03/06/2024 02:53:01 PM	Instrument Name:	UV
Comments:		Instrument Type:	UV-1900
Parameter File Name:	C:\UVVis-Data\Parameter\SCAN.vspn	Model (S/N):	UV1900I (A12535750067)
Report File Name:			

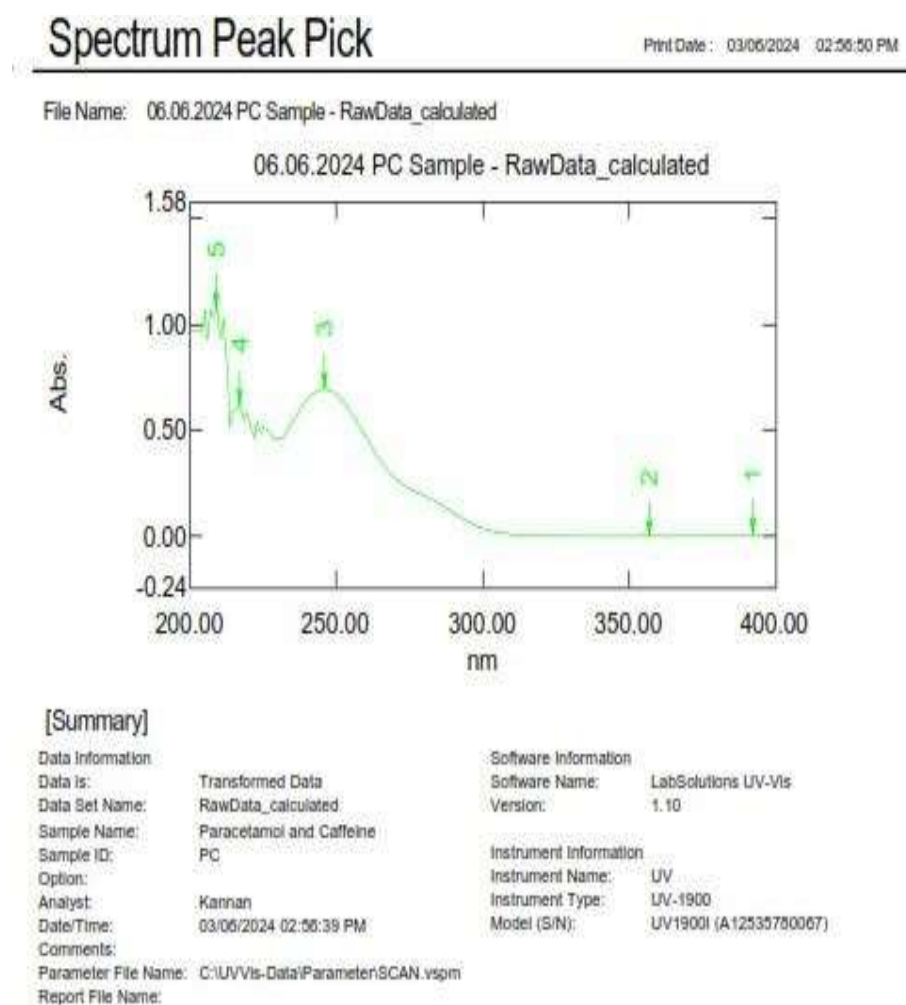
[Peak Pick Table]

Threshold: 0.001

Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1		359.5	0.001	
2		345.0	0.002	
3		324.0	0.001	
4		227.0	0.014	
5		211.5	-0.001	

FIGURE6.2
UVSPECTRUMFORSAMPLE



[Peak Pick Table]

Threshold: 0.001

Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1	1	392.5	0.005	
2	1	357.0	0.005	
3	1	246.0	0.693	
4	1	217.0	0.616	
5	1	209.0	1.076	

CHAPTER-7

SUMMARY AND CONCLUSION

Analytical methods play a vital role in new drug development, preformulation and formulation studies, stability studies, quality control testing and quality assurance programmes. So, analysts are always in search of developing rapid and accurate new methods of analysis that are able to exist in routine analytical work. The present analytical work comprises of simple, precise, rapid, sensitive and accurate methods for the simultaneous estimation of Paracetamol and Caffeine in combined dosage form.

The simultaneous equation method is based on the fact that the absorbance of the mixture is the sum of the individual absorbance of the two components. Paracetamol and Caffeine show $\lambda_{\max}$ at 245 nm and 273.5 nm respectively in mixed Hydrotropy solution which acts as diluent. Beer's law concentration range for Paracetamol and Caffeine are found to be 2–16 $\mu\text{g/ml}$ and 1–10 $\mu\text{g/ml}$. The method involves solving the simultaneous equations based on the measurement of absorbances at two wavelengths 245 nm and 273.5 nm.

The percentage purity for the selected formulation was found to be 99.51% w/w for Paracetamol and 100.94% w/w for Caffeine respectively. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2% indicating the accuracy and precision of the method. The percentage recoveries vary from 99.37 – 100.20% w/w for Paracetamol and 99.51 – 100.59% w/w for Caffeine.

The results obtained shows developed method of analysis are simple, specific, accurate and precise and can be employed in the routine analytical work, since the RSD values are less than 2%. But for economical reasons, spectrophotometric methods can be employed for regular quantitative works.

CHAPTER-8

REFERENCES

1. ChristianGD, Analytical chemistry, Sixth edition, 2003:1-11.
2. Kaur.H, Spectroscopy, Third edition, 2006:175-232.
3. GurudeepR. Chatwal, ShamK. Anand, Instrumental methods of chemical analysis, Fifth edition, 2002:2.149-2.184
4. RaviShankarS, Textbook of Pharmaceutical Analysis, Third Edition, 2006, :18-6-18-13.
5. SharmaBK. Instrumental methods of chemical analysis. Twenty-eight edition, 2012:286-300.
6. BecketAH, StenlakeJB, Practical Pharmaceutical Chemistry, Fourth edition-Part Two, 1988:281-300.
7. Kamal AH, El-Malla SF, Hammad SF. A review on UV spectrophotometric methods for simultaneous multicomponent analysis. Eur J Pharm Med Res. 2016 Jan 27;3(2): 348-3
8. Kapadiya N., Singhvi I., Mehta K., Karwani G., and Dhruvo J. Sen. 'Hydrotropy: A Promising Tool for Solubility Enhancement: A Review'. International Journal of Drug Development & Research. 2011(April-June), 3(2): 26-33
9. Ghogare D., Patil S. 'Hydrotropic solubilization tool for Eco-friendly analysis'. International Journal of Pharmacy and Pharmaceutical Research. 2018 (February); 11(3): 300-322.
10. Md. Ali Sajid, Choudhary V. 'Solubility Enhancement Methods with Importance of Hydrotropy'. Journal of Drug Delivery & Therapeutics; 2012, 2(6):96-101.
11. Gawai M. N., Aher S.S., Saudagar R. B. 'Mix Hydrotropy Technique for Solubility Enhancement - A Review'. International Journal of Institutional Pharmacy and Life Sciences. 2015 (September-October) 5(5): 442-452.

12. Patil M.S., Shirkhedkar A.A. 'A Review - Solubility Enhancement of Poorly Water-Soluble Drugs'. 'International Journal of Advances in Pharmacy and Biotechnology'. 2016.2(1): 10-24.
13. Sreejith K.R., Rajagopal P.L., Jyolsna K.P., Sharbina M.K. 'A review on analytical methods based on hydrotropic phenomenon'. European Journal of Pharmaceutical and medical research 2015, 2(5):1293-1319.
14. Dhapte V., Mehta P. 'Advances in hydrotropic solutions: An updated view'. St. Petersburg Polytechnic University Journal: Physics And Mathematics 1.(2015):424-435.
15. Kulkarni N.S., Ghule S.B., Dhole S.N. 'A Review on Hydrotropic Solubilization for Poorly Water-Soluble Drugs: Analytical Application and Formulation Development'. Research Journal of Pharmacy and Technology. 2019;12(7): 3157-3162
16. Arjaria P., Goyal M., Jain S. 'Review Article Hydrotropic Solubilization'. International Journal of Pharmaceutical and Phytopharmacological Research. 2013; 3 (1): 17-23
17. Gowardhane A.P., Kadam N.V., and Dutta S. Review on Enhancement of Solubilization Process. American Journal of Drug Discovery and Development. 2014;4 (2): 134-152.
18. ICH Guidelines. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.
19. Fairbrother J.E. Acetaminophen. Anal. Profiles Drug Subst. 1974;3:2-109.
20. Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev. 1992 May-Aug;17(2):139-70.
21. Dinç E. The Spectrophotometric multicomponent analysis of a ternary mixture of ascorbic acid, acetylsalicylic acid and paracetamol by the double divisor-ratio spectra derivative and ratio

spectra-zero crossing methods. *Talanta*. 1999 May 10;48(5):1145-57.

22. ErkN, ÖzkanY, BanoğluER, ÖzkanSA, ŞentürkZ. Simultaneous determination of paracetamol and methocarbamol in tablets by ratio spectra derivative spectrophotometry and LC. *Journal of pharmaceutical and biomedical analysis*. 2001 Jan 1;24(3):469-75.
23. Subramanian G, Shetty R, Agarwal S, Pandey S, Udupa N. Simultaneous reverse phase HPLC estimation of paracetamol and rofecoxib in tablets. *Indian journal of pharmaceutical sciences*. 2005;67(2):247-9.
24. Bhavsar AS, Talele GS, Fursule RA, Surana SJ. RP-HPLC Estimation of Paracetamol and Valdecoxib in Combined Dosage Form. *Indian journal of pharmaceutical sciences*. 2006 Sep 1;68(5).
25. Gandhimathi M, Ravi TK, Shukla N, Sowmiya G. High Performance Thin Layer Chromatographic Method for Simultaneous Estimation of Paracetamol and Valdecoxib in Tablet Dosage Form. *Indian journal of pharmaceutical sciences*. 2007 Jan 1;69(1).
26. Khoshayand MR, Abdollahi H, Shariatpanahi M, Saadatfard A, Mohammadi A. Simultaneous spectrophotometric determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric methods. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2008 Aug 1;70(3):491-9.
27. Nikam AD, Pawar SS, Gandhi SV. Estimation of paracetamol and aceclofenac in tablet formulation by ratio spectra derivative spectroscopy. *Indian journal of pharmaceutical sciences*. 2008 Sep;70(5):635.
28. Shirkhedkar AA, Shaikh AM, Surana SJ. Simultaneous Determination of Paracetamol and Piroxicam in Tablets by Thin Layer Chromatography Combined with Densitometry. *Eurasian Journal of Analytical Chemistry*. 2008 Jul 1;3(2).
29. Pawar UD, Naik AV, Sulebhavikar AV, Datar TA, Mangaonkar K. Simultaneous determination of aceclofenac, paracetamol and

chlorzoxazone by HPLC in tablet dose form. Journal of Chemistry. 2009 Jan 1; 6:289-94.

30. Chitlange SS, Soni R. Development of stability indicating HPTLC method for simultaneous estimation of paracetamol and dexibuprofen. J. Chem. Pharm. Sci. 2010;3(1):1-4.
31. Gondalia R, Mashru R, Savaliya P. Development and validation of spectrophotometric methods for simultaneous estimation of ibuprofen and paracetamol in soft gelatin capsule by simultaneous equation method. International Journal of ChemTech Research. 2010 Oct;2(4):1881-5.
32. Patel DJ, Patel VP. Simultaneous determination of paracetamol and lornoxicam in tablets by thin layer chromatography combined with densitometry. Int J Chem Tech Res. 2010;2(4):1929-32.
33. Patil PP, Deshpande MM, Kasture VS, Gosavi SA. Simultaneous Estimation of Aceclofenac and Paracetamol in Bulk and Combined Tablet Dosage Form and From Biological Fluid by Planar Chromatography. Research Journal of Pharmacy and Technology. 2010;3(4):1194-9.
34. Vichare V, Mujgond P, Tambe V, Dhole SN. Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation. International Journal of PharmTech Research. 2010;2(4):2512-6.
35. Anuradha SJ, Savita SY, Rao JR. Simultaneous determination of paracetamol and lornoxicam by RP-HPLC in bulk and tablet formulation. Int J Comp Pharm. 2011; 9:1-4.
36. Badgujar MA, Mangaonkar KV. Simultaneous determination of paracetamol and mefenamic acid in tablet dosage form by high performance liquid chromatography. J. Chem. Pharm. Res. 2011;3(4):893-8.
37. Balan P, Nimila IC, Movva VR, Prasanna ML, Rajasekar S. Simultaneous Estimation of Dexibuprofen and Paracetamol by UV

Spectroscopic Method in Bulk and Tablet Formulation. *Inventi Impact: Pharm Ana & Qual Assur.* 2011 Apr 15.

- 38.** Induri M, Mantripragada BR, Yejella RP, Kunda PR, Arugula M, Boddu R. Simultaneous quantification of paracetamol and meloxicam in tablets by high performance liquid chromatography. *Tropical Journal of Pharmaceutical Research.* 2011;10(4):475-81.
- 39.** Gandhi SV, Ranher SS, Deshpande PB, Shah DK. Simultaneous HPTLC determination of nabumetone and paracetamol in combined tablet dosage form. *Journal of the Brazilian Chemical Society.* 2011; 22:1068-72.
- 40.** Kondawar MS, Shah RR, Waghmare JJ, Shah ND, Malusare MK. UV spectrophotometric estimation of paracetamol and lornoxicam in bulk drug and tablet dosage form using multiwavelength method. *International Journal of PharmTech Research.* 2011;3(3):1603-8.
- 41.** Murtaza G, Khan SA, Shabbir A, Mahmood A, Asad MH, Farzana K, Malik NS, Hussain I. Development of a UV-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets. *Scientific research and Essays.* 2011 Jan 18;6(2):417-21.
- 42.** Nikhade RD, Thakur AD, Choudhari SB, Chandewar AV. Simultaneous estimation of paracetamol, thiocolchicoside and aceclofenac by UV spectrophotometer using multicomponent mode method. *Journal of pharmacy research.* 2011 Jul;4(7):2297-9.
- 43.** Rao JR, Mulla T, Yadav S, Bharekar V. Development and validation of HPLC method for simultaneous quantitation of Paracetamol and Dexketoprofen Trometamol in bulk drug and formulation. *Pharmacie Globale (IJCP).* 2011;7(9).
- 44.** Rao JR, Mulla TS, Bharekar VV, Yadav SS, Rajput MP. Simultaneous HPTLC Determination of Paracetamol and Dexketoprofen trometamol in pharmaceutical dosage form. *Der Pharma Chemica.* 2011;3(3):32-8.

45. Sinha S, Rajput MS. Validated simultaneous multicomponent spectrophotometric determination of paracetamol, aceclofenac and tizanidine in tablets. *Int. J. Chemtech Res.* 2011; 3:963-6.
46. Chandra P, Rathore AS, Lohidasan S, Mahadik KR. Application of HPLC for the simultaneous determination of aceclofenac, paracetamol and tramadol hydrochloride in pharmaceutical dosage form. *Scientia pharmaceutical.* 2012 Jun;80(2):337-52.
47. Hajian R, Afshari N. The spectrophotometric multicomponent analysis of a ternary mixture of ibuprofen, caffeine and paracetamol by the combination of double divisor-ratio spectra derivative and H-point standard addition method. *Journal of Chemistry.* 2012 Jan 15; 9:1153-64.
48. Joshi R, Pawar N, Sawant R, Gaikwad P. Simultaneous estimation of paracetamol, chlorzoxazone and ibuprofen by validated spectrophotometric methods. *Analytical Chemistry Letters.* 2012 Jan 1;2(2):118-24.
49. Kumar Talluri MV, Bairwa MK, Theja Dugga HH, Srinivas R. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for simultaneous determination of paracetamol and lornoxicam in pharmaceutical dosage forms. *Journal of liquid chromatography & related technologies.* 2012 Jan 1;35(1):129-40.
50. Manoj Kumar J, Narayan UL. validated UV-spectrophotometric and RP-HPLC method for the simultaneous estimation of paracetamol and chlorzoxazone in tablet dosage form Indira Gandhi Institute of Pharmaceutical Sciences. 2012
51. More SJ, Tandulwadkar SS, Nikam AR, Rathore AS, Sathiyarayanan L, Mahadik KR. Application of HPLC for the simultaneous determination of paracetamol, chlorzoxazone, and nimesulide in pharmaceutical dosage form. *International Scholarly Research Notices.* 2012;2012.

52. Kulkarni VG, Gandhi SV, Deshpande PB, Divekar P. High Performance Thin Layer Chromatographic Analysis of Paracetamol and Etodolac in Combined Tablet Dosage Form. *Journal of Chemical and Pharmaceutical Research*. 2012;4(3):1750-5.
53. Kumar Talluri MV, Bairwa MK, Theja Dugga HH, Srinivas R. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for simultaneous determination of paracetamol and lornoxicam in pharmaceutical dosage forms. *Journal of liquid chromatography & related technologies*. 2012 Jan 1;35(1):129-40.
54. Singh KB, Waikar SB, Padmane SP. A validated RP-HPLC method for the simultaneous estimation of paracetamol and naproxen in tablet formulation. *International Journal of Pharmaceutical Sciences and Research*. 2012 Nov 1;3(11):4270.
55. Sivasubramanian L, Tintu T, Lakshmi KS. HPTLC for the Simultaneous Determination of Paracetamol and Lornoxicam in Pharmaceutical Dosage Form. *Asian Journal of Research in Chemistry*. 2012;5(8):1025-8.
56. Tsvetkova B, Kostova B, Pencheval, Zlatkov A, Rachev D, Peikov P. Validated LC method for simultaneous analysis of paracetamol and caffeine in model tablet formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2012;4(4):680-84.
57. Khan IU, Ashfaq M, Razzaq SN, Mariam I. Simultaneous determination of piroxicam and paracetamol in pharmaceutical formulations using stability indicating HPLC method. *Journal of Liquid Chromatography & Related Technologies*. 2013 Apr 1;36(10):1437-50.
58. Aktaş AH, Şahin HO. Spectrophotometric Determination of Paracetamol, Propyphenazone and Caffeine in Tablets by Multivariate Calibration Approach. *Anal chem Indian j*. 2019;19(1):143.

59. Haritha P, Rao BS, Sunandamma Y. Method Development and Validation for Simultaneous Determination Of Paracetamol And Ketoprofen by RP–HPLC Technique. *Int J Appl Biol Pharm.* 2014;110-22.
60. Karbhari PA, Joshi SJ, Bhoir SI. RP-LC gradient elution method for simultaneous determination of related substances of Zaltoprofen and Paracetamol and application for drug excipient compatibility study. *International Journal of Pharmaceutical Sciences and Research.* 2014; 6:698-703.
61. Karunakaran K, Navaneethan G, Pitchaimuthu KE. A validated stability-indicating RP-HPLC method for paracetamol and lornoxicam: Application to pharmaceutical dosage forms. *Chemical Industry and Chemical Engineering Quarterly.* 2014;20(1):109-14.
62. Khatal LD, Rajage AD, More HN, Dhaneshwar SR, Polawar AR. Validated HPLC method for simultaneous quantitation of paracetamol, diclofenac potassium and famotidine in bulk drug and formulation. *International Journal of Pharmaceutics and Drug Analysis.* 2014 Aug 21;2(8):633-40.
63. Shah DA, Rana JP, Chhalotiya UK, Baldania SL, Bhatt KK. Development and validation of a liquid chromatographic method for estimation of dicyclomine hydrochloride, mefenamic acid and paracetamol in tablets. *Indian journal of pharmaceutical sciences.* 2014 Nov;76(6):529.
64. Sharma A, Gupta MK, Jain NK, Rajput A, Ahmed A, Khan AA. Development of Simultaneous Estimation Method for Paracetamol and Chlorzoxazone in Marketed Formulation by Validated UV-Spectrophotometric and RP-HPLC Method. *pain.* 2014;1:3.
65. Abdel Aleem EA, Naguib IA, Hassan ES, Ali NW. HPTLC and RP-HPLC methods for simultaneous determination of Paracetamol and Pamabrom in presence of their potential impurities. *Journal of pharmaceutical and biomedical analysis.* 2015 Oct 10; 114:22-7.

66. Mahajan KS, Chauhan RS, Shah SA, Shah DR. Development of HPTLC method for simultaneous estimation of paracetamol and flupirtine maleate in their combined tablet dosage form. *J. Pharm. Appl. Sci.* 2015;2(1):1-7.
67. Patel Twinkle K, Meshram Dhananjay B. Development and validation of high-performance thin layer chromatography for estimation of mefenamic acid, paracetamol and dicyclomine HCl in tablet dosage form. *International Journal of Pharmaceutical Sciences and Drug Research.* 2015;7(4):361-4.
68. Babu GR, Rao AL, Rao JV. Bioanalytical Method Development and Validation for Simultaneous Estimation of Paracetamol and Cefixime by using RP-HPLC in Rabbit Plasma. *Oriental Journal of Chemistry.* 2016 Mar 1;32(1):701-7.
69. Glavanović S, Glavanović M, Tomišić V. Simultaneous quantitative determination of paracetamol and tramadol in tablet formulation using UV spectrophotometry and chemometric methods. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 2016 Mar 15; 157:258-64.
70. Mali A, Kolekar S, Panachery J, Tamboli A. Simultaneous Determination of Paracetamol and Domperidone in Pharmaceutical Dosage Form by First Order Derivative UV Spectrophotometry. *Asian Journal of Pharmaceutical Research.* 2016 Jan;6(1):22-6.
71. Sharma H, Vishakha K, Kumar KV, Bhatta HP. Validated RP-HPLC method for simultaneous estimation of paracetamol, paracetamol and dicyclomine hydrochloride in bulk and pharmaceutical dosage form. *Int J Pharm Sci Res.* 2016 Jan 1;7(1):316-24.
72. Surekha K, Rama L, Sanket G, Sanjay P, Sandip S. Simultaneous Estimation and Quantification of three main ingredients, Aceclofenac, Thiocolchicoside & Paracetamol in Finished Dosage Form by using Reversed Phase High Performance Liquid Chromatography. *Int. J. Pharm. Sci. Rev. Res.* 2016;38(2):29-34.

73. Elkhoudary MM, Salam RA, Hadad GM. Resolution and quantification challenge of modern chemometric models in the determination of anti-migraine tablets containing ergotamine, caffeine, acetaminophen, and metoclopramide. *Rsc advances*. 2017;7(34):20936-46.
74. Escobar SC, Cubides LR, Pérez C. Optimization and Validation of a Simple and Fast RPHPLC Method for Simultaneous Determination of Acetaminophen and Caffeine in Tablets. *Indian Journal of Pharmaceutical Sciences*. 2017 Sep 1;79(5).
75. Navneet Kumar U, Afroze A, Pradeepti C, Shailendra K, Naik KK. Development and Validation of a New Chromatographic Method for the Simultaneous Estimation of Serratiopeptidase, Aceclofenac and Paracetamol by RP-HPLC. *Pharm Anal Chem*. 2017; 3:122.
76. Zivkovic A, Bandolik JJ, Skerhut AJ, Coesfeld C, Prascevic M, Zivkovic L, Stark H. Quantitative analysis of multicomponent mixtures of over-the-counter pain killer drugs by low-field NMR spectroscopy. *Journal of Chemical Education*. 2017 Jan 10;94(1):121-5.
77. Shanker K, Kuna SK, Purra S. Simultaneous determination of Etodolac and paracetamol in bulk drugs and pharmaceutical formulation by HPTLC-densitometric and UV-derivative spectrophotometry methods. *IJPSR*. 2017 May 1;8(5):2056-63.
78. Aktaş AH, Şahin HO. Spectrophotometric Determination of Paracetamol, Propyphenazone and Caffeine in Tablets by Multivariate Calibration Approach. *Anal chem Indian j*. 2019;19(1):143.
79. Elbagary RI, Elkady EF, Ibrahim MM, Goubran SM. Development and Validation of RP-HPLC and TLC Methods for Simultaneous Estimation of Paracetamol, Thiocolchicoside and Aceclofenac in Bulk and Combined Pharmaceutical Dosage Form. *Indo American J F Pharmaceutical Research*. 2019;9(09):520-36.

80. Uddin MN, Mondol A, Karim MM, Jahan RA, Rana AA. Chemometrics assisted spectrophotometric method for simultaneous determination of paracetamol and caffeine in pharmaceutical formulations. *Bangladesh Journal of Scientific and Industrial Research*. 2019 Aug 19;54(3):215-22.
81. El-Yazbi AF, Guirguis KM, Bedair MM, Belal TS. Validated specific HPLC-DAD method for simultaneous estimation of paracetamol and chlorzoxazone in the presence of five of their degradation products and toxic impurities. *Drug Development and Industrial Pharmacy*. 2020 Nov 1;46(11):1853-61.
82. Vu Dang H, Truong Thi Thu H, Dong Thi Ha L, Nguyen Mai H. RP-HPLC and UV spectrophotometric analysis of paracetamol, ibuprofen, and caffeine in solid pharmaceutical dosage forms by derivative, fourier, and wavelet transforms: a comparison study. *Journal of analytical methods in chemistry*. 2020 Feb 8;2020.
83. Alnuwaiser MA. Utilization of both UV and GC-MS spectroscopy to track the estimation of caffeine and acetaminophen in a number of paracetamol analgesic drugs. *Multi-Knowledge Electronic Comprehensive Journal for Education & Science Publications (MECSJ)*. 2021 Feb 1(40).
84. Ibrahim H, Hamdy AM, Merey HA, Saad AS. Simultaneous determination of paracetamol, propyphenazone and caffeine in presence of paracetamol impurities using dual-mode gradient HPLC and TLC densitometry methods. *Journal of Chromatographic Science*. 2021 Feb;59(2):140-7.
85. Kokilambigai KS, Lakshmi KS. Utilization of green analytical chemistry principles for the simultaneous estimation of paracetamol, aceclofenac and thiocolchicoside by UV spectrophotometry. *Green Chemistry Letters and Reviews*. 2021 Jan 2;14(1):99-107.
86. Abdel Rahman MA, Elghobashy MR, Zaazaa HE, Atty SA, El-Mosallamy SS. Validated HPLC-PDA methodology utilized for simultaneous determination of Etoricoxib and Paracetamol in the

presence of Paracetamol toxic impurities. BMC chemistry. 2022Dec 2;16(1):108.

- 87.**Arage A, Layloff T, Hymete A, Ashenef A. High performance thin layer chromatography(HPTLC) method development and validation for the simultaneous determination of paracetamol, caffeine, chlorpheniramine and phenylephrine in tablet formulation. Acta Chromatographica. 2023 Apr 25;35(2):170-8.
- 88.**Chandratrey A, Sharma R. Simultaneous spectrophotometric estimation and validation of three component tablet formulation containing paracetamol, nimesulide and tizanidine.
- 89.**Saminathan J, Sivakalai S, Vetrichelvan T. Validated HPTLC Method for Simultaneous Quantitation of Aceclofenac, Paracetamol and Thiocolchicoside in Bulk drug and Pharmaceutical Formulation.