

**REVIEW ON VARIOUS PHARMACEUTICAL APPLICATIONS OF ULTRAVIOLET SPECTROSCOPY**

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**ABSTRACT**

Due to the growing amount of multicomponent formulations, biotherapeutic medicines, and complicated matrix samples in queue, quick and simple analytical methods are required. For these purposes, a variety of ultraviolet (UV) spectrophotometric approaches are used. On the basis of the principle of additivity, absorbance difference, and processing absorption spectra, many UV spectrometric methods have been created. This review will cover simultaneous equation method, difference spectrophotometry, derivative spectrophotometry, absorbance ratio spectra, derivative ratio spectra, successive ratio - derivative spectra, Q-absorbance ratio method, absorptivity factor method, dual wavelength method, absorption factor method, multivariate chemometric methods, and isosbestic point method.

**KEYWORDS:** Ultraviolet spectroscopy, Simultaneous equation method, Derivative spectrophotometry, Derivative ratio spectra, Isosbestic point method, Multivariate chemometric methods.

**INTRODUCTION**

In everyday practise, analysts must analyse multicomponent formulations, biotherapeutic medicines, and complicated matrix materials quickly. For these purposes, a variety of ultraviolet (UV) spectrophotometric approaches are used. Among all of these techniques, UV spectrophotometry is the most popular. The absorption of visible and UV radiation (200–400 nm) is connected with the excitation of electrons in both atoms and molecules from lower to higher energy levels, according to the basic concept of UV spectroscopy. Because matter's energy levels are quantized, only light with precisely the right quantity of energy may drive transitions from one level to the next.<sup>[1]</sup> UV spectrophotometric approaches supported the principles of additivity and absorbance, which involve recording and mathematically processing the absorption spectra of normal and sample solutions within the same or other ways.

**Types Of UV Spectroscopic Analytical Techniques**

The many UV spectroscopic analysis procedures are as follows: Simultaneous equation method, difference spectrophotometry, derivative spectrophotometry, absorbance ratio spectra, derivative ratio spectra, successive ratio - derivative spectra, Q-absorbance ratio method, absorptivity factor method, dual wavelength method, absorption factor method, multivariate chemometric methods, and isosbestic are some of the absorption factor methods.

**Simultaneous Equation Method**

Simultaneous equation method is useful to determine drugs which absorb at the  $\lambda_{max}$  of other in the binary or ternary mixture.

Consider

The absorptivities of X at  $\lambda_1$  and  $\lambda_2$ ,  $ax_1$  and  $ax_2$ , respectively.

The absorptivities of Y at  $\lambda_1$  and  $\lambda_2$ ,  $ay_1$  and  $ay_2$ , respectively.

The absorbance of the dilute sample at  $\lambda_1$  and  $\lambda_2$ ,  $A_1$  and  $A_2$ , respectively X, have concentration  $c_x$  and Y have concentration  $c_y$  in dilute sample.

According to the fact, the concentration of mixture is the sum of the individual concentrations of X and Y. So, at

$$\lambda_1 A_1 = ax_1bcx + ay_1bcy \quad \dots (1)$$

$$\text{At } \lambda_2 A_2 = ax_2bcx + ay_2bcy \quad \dots (2)$$

If cell is 1 cm,  $b = 1$  equation 2 become,  $c_y = (A_2 - ax_2cx)/ay_2$

Substituting value of  $c_y$  in equation (1), thus  $ax_1bcx = A_1 - ay_1cy$

$$c_x = (A_2 ay_1 - A_1 ay_2)/(ax_2ay_1 - ax_1ay_2) \quad \dots (3)$$

imilarly for  $c_y$

$$c_y = (A_1 ax_2 - A_2 ax_1)/(ax_2ay_1 - ax_1ay_2) \quad \dots (4)$$

“Glenn” have been suggested criteria for obtaining maximum precision, based on absorbance ratio that place limit on the relative concentration of the component of the mixture. The criteria for that ratio should lie outside

the range 0.1–2 for precise determination of X and Y, respectively.

#### Condition to Fulfill This Criteria

- $\lambda_{\max}$  of two-component should be reasonably dissimilar.
- The absorbance of a two-component system should not interact chemically, contradicting the initial assumption. In the creation of a novel application of this technology, the absorbance additivity should always be validated. Table 1 summarises the use of the simultaneous equation method to determine a binary mixture in a pharmaceutical dosage form, as well as Table 2 shows how the simultaneous equation approach was used to determine a ternary mixture in a medicinal dose form.

#### Difference Spectrophotometry

It is a spectrophotometric approach for quantitative determination of an analyte employing an equimolar solution of the same analyte as a reference but in a different physicochemical environment.<sup>[10]</sup> Isolation of an analyte from another component of the mixture or another UV active analyte present in the mixture sample is accomplished using this spectroscopic approach. Changes in Ph,<sup>[11]</sup> and temperature,<sup>[12]</sup> are the most common physicochemical conditions that have been altered. The approach requires that the analyte under examination exist in many chemical forms with varying absorbance values. The value is plotted against the concentration of the solution studied in terms of absorbance difference (amplitude difference in maxima

and minima). Because difference spectra of dosage forms overlapped on the pure drug without the existence of interference peaks due to excipients in the dosage form, difference spectroscopy increases both the selectivity and specificity of analytical methods. The uses of difference spectroscopy to medication material in dosage forms are summarised in Table 2. Simultaneous detection using difference spectroscopy. In the case of binary mixes, the wavelength should be chosen so that each component's contribution is zero at the wavelength where the other components' absorbance is highest. Table 3 lists a few instances of pharmaceutical difference spectroscopy applications utilising binary mixes. Aside from pharmacological assays, difference spectroscopy is also utilised in biopharmaceutical formulation development to assess protein structure and explore how structure responds to formulation composition. This application is founded on the fact that stable protein conformations provide excellent physical stability in real time, and differential spectra are utilised to characterise and measure change in measure structure.<sup>[17]</sup> Moschakis and Nikolaidis investigated protein structural changes produced by heat or cold in a denaturation study of BSA. The heat-treated samples' spectra were subtracted from the unheated protein solution's. Protein solutions in GdHCl or urea have different spectra. the spectrum of 0.2% (w/w) BSA solutions in GdHCl (or urea) against the solvent (GdHCl or urea solution of the same molarity) in the reference cuvette was subtracted from the spectrum of the protein solution against double distilled water in the reference cuvette (untreated protein solution).<sup>[12]</sup>

Drug	Spectroscopic condition ( $\lambda_{\max}$ and solvent)	Beer's law range $\mu\text{g/ml}$	Reference
Rabeprazole sodium and levosulpiride	284 nm, 232 nm methanol	1–20 and 1–20	[2]
Ofloxacin and ornidazole	240.6 nm, 279.4 nm methanol	20–40 and 16–32	[3]
Norfloxacin and tinidazole	273 nm, 319 nm methanol	2.5–20 and 5–40	[4]
Paracetamol and diclofenac sodium	247 nm, 276 nm water	5–35 and 5–40	[5]

#### Derivative Spectrophotometry

Derivative spectroscopy, as per name indicates, involves derivative of absorbance of zero order or simple absorption spectrum with respect to wavelength. Derivative spectroscopy follows principle additivity, and absorbance is also dependence on concentration.<sup>[18]</sup> Nowadays derivative spectra obtained directly from spectrophotometers enabled with advanced software such as UV-probe. These software eliminate the need for additional mathematical process or changes in instrumental parameters. Previously derivative spectra were generated by optical method (wavelength modulation technique)<sup>[19]</sup> and electrical method (analog resistance capacitance device).<sup>[18]</sup> Later in 1974, new mathematical technique was introduced named as Golaysavitzky method.<sup>[20]</sup> which became commercially popular and part of software now. Derivative spectroscopy used to analyze wide variety and complex origin such as pharmaceutical dosage forms, inorganic

samples with metal content biological samples, and samples of food content.<sup>[21]</sup> Derivative spectroscopy offers following advantages.<sup>[22,23]</sup>

1. Resolve overlapping peaks of complex samples such as ternary mixture
2. Improve spectral quality by eliminating baseline shift and scattering
3. Direct UV-analysis of samples of complex origin without any chemical pre-treatment of sample of biological origin
4. Allows analysis at lower sample content impurity profiling. From analytical method point of view, both sensitivity and selectivity of analytical method is improved.

Zero order equation  $A = abc$  ..... (5)

First order equation  $dA/d\lambda = da/d\lambda.bc$  ..... (6)

$n^{\text{th}}$  order equation  $dnA/d\lambda^n = dna/d\lambda^n.bc$  ..... (7)

Subordinate spectra amplify the data content from central zero request spectra and are convoluted relatively. Table 5 sums up the otherworldly component of various request subordinate spectra. Estimation method in subsidiary spectroscopy: Zero getting procedure and top to through strategy.<sup>[20]</sup> Anyway for drug examination reason zero intersection procedure is most loved device. Zero intersection method depends on the way that in subsidiary spectra absorbance of one segment shows no absorbance at such case absorbance of test is equivalent to that of other part in example which can be utilized to discover its fixation. Table 6 sums up the utilizations of zero intersection procedure to investigation of parallel combinations and Table 7 uses of zero intersection strategy to examination of ternary blends. Aside from drug measure subsidiary spectroscopy likewise discovers its application in a clinical report, for example, quantitative test of diazepam in human blood plasma without detachment of the medication from the natural

grid.<sup>[20]</sup> Besides, subsidiary spectroscopy had been utilized for steadiness study reason. Utilizing the second subsidiary UV spectrophotometry, butamirate citrate, and formoterol fumarate were controlled by estimating the pinnacle plentifulness at 260.4 and 261.8 nm, separately, with no obstruction of their debasement items.<sup>[33]</sup> With derivatization of spectra, signal-to-commotion proportion increments. Besides, reproducibility acquired with subordinate spectroscopy is extremely low. Anotov *et al.* revealed technique for bit by bit channel strategy to work on the sign to commotion proportion.<sup>[34]</sup> Brown *et al.* announced strategy for subsidiary reprocessing where float clamor decrease accomplished for multivariate otherworldly information.- Aside from previously mentioned philosophy, barely any variations of subsidiary spectroscopy were accounted for worldwide. Wavelet change procedure utilized effectively for subordinate spectroscopy as well.

**Table 4: Spectral feature of different order derivative spectra.**

Derivative order	Spectral feature
First order	First order derivative spectra start and finish at zero. It also passes through zero at the same wavelength as $\lambda_{max}$ of the absorbance band of zero order spectra, at an inflection point. Either side of this point possesses positive and negative bands with maxima and minima
Second order	Main extreme minima appear at $\lambda_{max}$ of the absorbance band, along with two positive satellite bands on its either side
Third order	Similar to first order derivative spectra it also possesses inflection point at $\lambda_{max}$ of the absorbance band of zero order spectra. Along with positive and negative satellite bands on either side of minima and maxima, respectively
Fourth order	Main extreme maxima appear at $\lambda_{max}$ of the absorbance band, along with two negative satellite bands on its either side

**Table 5: Applications of zero crossing technique to analysis of binary mixtures.**

Drug	Order of dvt spectra	Wavelength of zero crossing	References
Imipenem and cilastatin	1	243 and 300 nm	[24]
Gatifloxacin and prednisolone	1	348 and 263 nm	[25]
Ofloxacin and ornidazole	1	278 and 293.6 nm	[26]
Ezetimibe and lovastatin	1	265.20 and 245.4 nm	[27]
Ofloxacin and cefixime	1	282.8 and 318.6 nm	[28]

### Ratio Derivative Spectroscopy

Spectrophotometric assurance of at least two mixtures in a similar example without starter division is sought after. In past decade proportion subordinate spectroscopy arose as great instrument to fill this need which depended on work of Salinas *et al.*, where they fostered a spectrophotometric technique dependent on the utilization of the principal subsidiary of the proportion spectra for settling twofold combinations when the spectra of the parts are covered. It allows the utilization of the frequency of the greatest worth of logical signs with a few pinnacles and box, which allows the assurance of an analyte within the sight of different mixtures and excipients which might actually meddle in the investigation.<sup>[30]</sup>

The method involves following steps

- Recording mixture spectra of samples under investigation.
- Dividing the mixture spectra by a standard divisor spectrum.
- The concentration of one of the components in the mixture is directly determined by peak-to-peak/peak-to-trough measurements in the resulting ratio spectra. The method does away with the derivative step and does not necessitate the search for zero-crossing locations or any complex mathematical or chemometric data processing.

$$\lambda_1 A_M = \lambda_1 E_A C_A + \lambda_1 E_B C_B \quad \dots \dots \dots (8)$$

$\lambda_1 A_M$  - absorbance of mixture.

$\lambda_1 E_A$  and  $\lambda_1 E_B$  - molar absorptivities of A and B.

$C_A$  and  $C_B$  - concentration of A and B in mixture.

Above equation (8) is divided by the absorbance of a standard solution of A at  $\lambda_1$  whose concentration is  $C_A^\circ$ , then equation becomes,  
 $\lambda_1 A_{M/\lambda_1} E_A C_{RA} = C_A / C_{RA} + \lambda_1 E_B C_B / \lambda_1 E_A C_{RA} \dots\dots\dots(9)$

This equation can be simplified to  
 $\lambda_1 A_{A/\lambda_1} E_A = C_A + \lambda_1 E_B C_B / \lambda_1 E_A$

By plotting  $\lambda_1 A_{M/\lambda_1} E_A$  as a function of  $E_B/E_A$ , a straight line is obtained. The intercept of the straight line provides the value of  $C_A$ , and the slope of the straight line is  $C_B$ . To obtain the ratio  $E_B/E_A$  at each wavelength, the absorption spectra of equimolar standard solutions of B and A are measured, and the absorbance ratio at each wavelength is calculated. Table 8 summarizes the application of ratio derivative spectroscopy to pharmaceutical dosage forms

**Successive Ratio Derivative Spectra Method**

This method is used for determination of drugs in the ternary mixture without information of ratio of drugs concentration in the mixture. Consider a mixture of three drugs X, Y, and Z following Beer's law is obeyed in the whole wavelength range used and by considering the path length as 1 cm, the total absorbance of the ternary mixture at each wavelength can be written as:  $A_m = \alpha_x C_x + \alpha_y C_y + \alpha_z C_z \dots\dots\dots(10)$

Where  $A_m$  is the total absorbance of the mixture,  $\alpha_x$ ,  $\alpha_y$ , and  $\alpha_z$  are the absorptivity values of X, Y and Z and  $C_x$ ,  $C_y$ , and  $C_z$  are the concentrations of X, Y, and Z, respectively. If equation 10 is divided by  $\alpha_z$  corresponding to the spectrum of a standard solution of Z in ternary mixture, the first ratio spectrum is obtained in the form of equation (11) (for possibility of dividing operation, the zero values of  $\alpha Z$  should not be used in the divisor):

$B = A_m / \alpha_z = \alpha_x C_x / \alpha_z + \alpha_y C_y / \alpha_z + C_z \dots\dots\dots (11)$

If the first derivative of equation (11) is taken since the derivative of a constant ( $C_z$ ) is zero, first derivative ratio spectra would be obtained in the form of equation (12):  
 $dB/d\lambda = d/d\lambda (\alpha_x C_x / \alpha_z) + d/d\lambda (\alpha_y C_y / \alpha_z) \dots\dots\dots (12)$

Dividing equation (6) by  $d/d\lambda (\alpha Y / \alpha Z)$ , corresponding to the derivative of the ratio of the spectra of the standard solutions of Y and Z, the second ratio spectrum is obtained as equation (13) (for possibility of dividing operation, the zero values of  $(d/d\lambda)(\alpha Y / \alpha Z)$  should not be used in the divisor):  
 $D = (dB/d\lambda) / d/d\lambda (\alpha Y / \alpha Z) = d/d\lambda [\alpha X C_X / \alpha Z] / d/d\lambda (\alpha Y / \alpha Z) + C_Y \dots\dots\dots(13)$

If the first derivative of equation (13) is taken since the derivative of a constant ( $C_Y$ ) is zero, Equation (14) would be obtained:

$dD/d\lambda = d/d\lambda \{ [d/d\lambda (\alpha X C_X / \alpha Z)] / [d/d\lambda (\alpha Y / \alpha Z)] \} \dots\dots\dots (14)$

Equation (14) is the numerical establishment of multicomponent investigation that allows the assurance of the centralization of every one of the dynamic medications in the arrangement (X in this condition) without obstruction from different medications of the ternary framework (Y and Z in these conditions). As condition (14) shows, there is a direct connection between the measure of  $dD/d\lambda$  and the grouping of X in the arrangement. An alignment bend could be developed by plotting  $dD/d\lambda$  against the centralization of X in the standard arrangements of X or in the standard ternary blends. For greater affectability, the measure of  $dD/d\lambda$  relating to most extreme or least frequency ought to be estimated. Alignment diagrams for Y and Z could be additionally built as portrayed for X. Abdelrahman and Abdelaleem applied progressive proportion spectra technique to drug ternary combinations including isopropamide iodide, trifluoperazine hydrochloride, and trifluoperazine oxidative degradate.

**Table 6: Applications of zero crossing technique to analysis of ternary mixtures.**

Drug	Order of dvt spectra	Wavelength of zero crossing	References
Amiloride, hydrochlorothiazide	1,3,1	365 nm, 265 nm and 385 nm	[31]
salbutamol sulfate, bromohexine hydrochloride and etofylline.	1,1,1	273 nm, 323 nm and 279 nm	[32]

**Table 7: Application of ratio derivative spectroscopy to pharmaceutical dosage forms**

Drug	Wavelength of determinations	References
Rabeprazole sodium and itopride hydrochloride	231 nm (rabeprazole sodium) and 260 nm (itopride hydrochloride)	[38] [39]
Naphazoline and antazoline Paracetamol and aceclofenac	227.2 nm (naphazoline) and 235 nm (antazoline) 256 nm (paracetamol) and 268 nm (aceclofenac)	[38]
Salbutamol sulfate, bromhexine hydrochloride, and etofylline	247.8 nm (salbutamol sulfate) 248.6 nm (bromhexine hydrochloride), 276.8 nm (etofylline)	[32]
Diflucortolone valerate and isoconazole nitrate	241.1 nm (diflucortolone valerate) and 279.8 nm (isoconazole nitrate)	[40]

**Q-ABSORBANCE RATIO METHOD**

The strategy is material just when brews law is followed for a given blend of the medication. This strategy depends on the way that the proportion of absorbance at any two frequencies for a substance, which submits to Beer's law, is a consistent worth free of the focus and way length. This consistent is named as "Hufner's Quotient" or Q-esteem. The Q-absorbance condition framed utilizing the absorptivity esteems at two frequencies utilized thusly, one being the  $\lambda_{max}$  of one of the segments and the other being a frequency of isoabsorptive point.<sup>[43,44]</sup> Table 9 sums up the uses of Q absorbance proportion strategy. The absorbance and absorptivity esteems at the specific frequencies were determined and subbed in the accompanying condition; to get the focus

$$C_X = (Q_m - Q_y) \times A / (Q_X - Q_Y) \times a_{X1}$$

$$C_Y = (A / a_{X1}) - C_X$$

$$Q_m = A_2 / A_1$$

$A_1$  is absorbance of sample at isoabsorptive point,  $A_2$  is absorbance of sample at  $\lambda_{max}$  of one of the two components  $a_{X1}$  and  $a_{X2}$  represent absorptivities of X at  $\lambda_{X1}$  and  $\lambda_{X2}$  and  $a_{Y1}$  and  $a_{Y2}$  denote absorptivities of Y at  $\lambda_{Y1}$  and  $\lambda_{Y2}$ , respectively,  $C_X$  and  $C_Y$  are the concentrations of X and Y, respectively

**ABSORPTIVITY FACTOR METHOD**

This method is a modification of classical absorption method. For implementing this method of spectroscopic analysis following conditions must be fulfilled.<sup>[34]</sup>

1. This method is applicable to binary mixture
2. There should be larger difference in between absorptivity of both drugs
3. There should not be isoabsorptive point.

Crossing of spectra does not occur at the same concentration as the isoabsorptive point method, but it can occur at various drug concentrations. Absorptivity equals the inverse ratio of concentrations utilised at such a crossing point in the absorptivity factor technique. The ratio discovered is known as absorptivity, and the crossing point is known as absorptivity factor (F).

$$A_x = a_x b c_x \text{ and } A_y = a_y b c_y$$

At crossing point of equal absorptivity having different drug concentrations

$$A_x = A_y$$

$$a_x b c_x = a_y b c_y$$

$$a_x c_x = a_y c_y$$

$$a_x / a_y = c_y / c_x = F$$

$$a_x / a_y = F \dots\dots\dots (15)$$

$$A_m = A_x + A_y = a_x b c_x + a_y b c_y$$

Where  $b = 1$ .

$$A_m = a_x c_x + a_y c_y \quad a_x = F a_y$$

$$\dots\dots\dots (16)$$

$$A_m = F a_y c_x + a_y c_y = a_y (F c_x + c_y)$$

Similarly,  $A_m = a_x (F c_y + c_x)$

Concentration of y drug can be determined using linear regression equation between its concentration and absorbance at its wavelength of maximum absorption where interference due to other drugs is null. Later from the concentration of y the concentration of x can be determined using following equation.<sup>[4-35]</sup>

$$A_m = a_y (F c_x + c_y) = a_x (F c_y + c_x)$$

$$a_y (F c_x + c_y) = a_x (F c_y + c_x) \quad c_x = [(F c_x + c_y) - c_y] / F$$

$$\dots\dots\dots (17)$$

Table 10 summarizes the applications of absorptivity factor method application to following drugs in pharmaceutical dosage forms.

**Table 8: Absorptivity factor method application to following drugs in pharmaceutical dosage forms.**

Drug	Wavelength used for analysis of mixture	References
Salmeterol xinafoate and fluticasone propionate	227.8 nm	[50]
Sodium cromoglicate and fluorometholone	241 nm	[51]

**MULTIVARIATE CHEMOMETRIC METHOD**

Mathematical procedures are used to process analytical data. It can also be defined as taking several measurements on the same sample in order to correlate physical qualities with analytical results. Chemometric approaches suggest that it is generally preferable to measure a large number of nonselective signals before combining them in a multivariate model that considers many variables at the same time.

Multivariate methods include:

1. Multiple linear regression (MLR) methods
  - a. Classical least squares or (K-matrix)
  - b. Inverse least squares or (P-matrix)
2. Factor-based methods
  - a. Principal component regression (PCR)
  - b. Partial least squares (PLS).

On account of spectroscopy, if the absorbance spectra of various examples of realized structure are estimated, every one of these spectra are amassed into one lattice called the absorbance grid. While in focus framework, all fixation esteems for the segments of the example are gathered. As a rule, MLR and PCR methods utilize information coordinated as grids of segment vectors, while PLS procedure utilizes information coordinated as lattices of line vectors.<sup>[34]</sup> The information of networks are coordinated into sets; every absorbance grid is combined with its relating fixation lattice. The pair of networks contains an informational index. Informational indexes have various names relying upon their starting point and reason.<sup>[35]</sup> Preparing set is an informational index containing estimations on a bunch of known examples. It is utilized to foster the alignment which is applied to anticipate the convergences of obscure examples. Preparing set ought to contain every normal

segment, length the fixation scopes of intrigue and contain commonly autonomous examples.<sup>[34]</sup> Approval set is an extra dataset containing autonomous estimations on examples that are free from the examples used to make the preparation set. Approval set is utilized to test the legitimacy of the adjustment created with the preparation set. The created adjustment is utilized to foresee the centralizations of the segments in the

approval tests. Then, at that point, these anticipated focuses are contrasted with the genuine fixations. The absorbance grid containing the unknown(s) spectra along with the relating result framework containing the anticipated fixations involve an obscure set. Table 9 sums up drug utilizations of multivariate chemometric strategy to parallel combinations of medications in consolidated measurement structure.

**Table 9: Pharmaceutical applications of multivariate chemometric method to binary mixtures of drugs in combined dosage form.**

Drug	Multivariate chemometric method	References
Levodopa and benserazide	PLS	[54]
Cypermethrin and tetramethrin	PLS	[55]
Moexipril and hydrochlorothiazide	PLS, PCR	[56]

**ISOSBESTIC POINT METHOD**

This method can be utilized just if the spectra of similar centralization of the two examined drugs cross at a point called isosbestic or isoabsorptivity point. At the isosbestic point, the two medications have equivalent absorptivities, and their blend goes about as a solitary segment and gives a similar absorbance as unadulterated medication. The absorbance esteem at the isosbestic point (Aiso) was resolved, and the complete grouping of the two medications was determined. Since the centralization of one of them in this blend can be estimated A straight relationship was acquired between the absorbance esteems and the comparing drug focuses. Consider you have a combination of two medications x and y. The absorbance of each medication can be determined at any frequency ( $\lambda$ ) from the condition  $A = abc$  Therefore, for drug x:

$$A = a_x C_x + a_y C_y \dots\dots\dots(21)$$

$$A = a_x (C_x + C_y) \dots\dots\dots (22)$$

Where A is the absorbance of their mixture at isosbestic point and is the concentrations of drugs x and y in the mixture, respectively, and CTM is the concentration of their mixture. Therefore, we can conclude that  $A = ax CTM$  (23) Thus, having the total concentration of both drugs, if the concentration of one of them can be determined separately by any other method, the concentration of the second drug can be calculated by subtraction. This method applied for the analysis of the ternary mixture of chloramphenicol, dexamethasone sodium phosphate (DXM) and tetryzoline hydrochloride in eye drops using other spectroscopic method (DS), the concentration of the other could be calculated by subtraction.

$Ax = a_x bC_x$  and For drug y:  $Ay = a_y bC_y$  Where  $a_x$  and  $a_y$  are the absorbances of x and y, respectively;  $C_x$  and  $C_y$  are the concentrations of x and y, respectively; and are the absorbtivities when the path length (b) is 1 cm, and concentration is 1 g/100 mL for x and y, respectively.

**Advantages of UV Spectroscopy over Other Analytical Techniques**

Furthermore, UV spectrophotometer is a highly simple instrument which makes it easier to couple with other analytical instrument such as RP-HPLC. Apart from this one UV spectrophotometric method had been conveniently adopted to develop a new better analytical method. Such method transfer and instrument compatibility are facilitated with UV spectroscopy only. (Table 10)

If  $C_x = C_y$ , and  $a_x = a_y$ , this  $\lambda$  is called the isosbestic point, and At this  $\lambda A_x = A_y \dots\dots\dots (20)$

For a mixture of both drugs, the absorbance at this  $\lambda$  can be calculated from the equation

**Table 10: Advantages of ultraviolet spectroscopy over other analytical techniques.**

Parameter	Ultraviolet spectroscopy	Chromatography	Thermal technique
Instrumentation	Easy	Complex High	Complex High
Interference in analysis	Less	(instrumental, physicochemical)	(instrumental, physicochemical)

**CONCLUSION**

Number of multicomponent details, biopharmaceutical items and tests of complex lattice and natural beginning are available on the lookout for which changed insightful strategies can be applied including spectrophotometry, chromatography, and electrophoresis yet UV spectrophotometric techniques for assurance of medications are simpler, less expensive, straightforward,

and fast. Since most analytes of interest are went with in their dose structures by different builds engrossing in a similar ghostly district, old style UV unearthly estimations couldn't be utilized for their assurance. Consequently, the entirety of the above techniques can be utilized by their tendency. Among the UV spectroscopic strategies relying upon the idea of investigation specific strategy can be chosen. Strategy,

for example, concurrent and subsidiary spectroscopy can be utilized to break down both twofold and tertiary blend, where, for example, for settling intently retaining tops subsidiaries spectroscopy worthwhile while synchronous spectroscopy is better as far as its straightforwardness. Moreover, variations dependent on subsidiary spectroscopy like proportion subordinate spectroscopy, progressive proportion subsidiary spectroscopy offer more benefits as far as dispensing with synthetic impedances. High selectivity and explicitness can be acquired for examining equimolar arrangements of analyte in UV dynamic lattice utilizing contrast spectroscopy, can be applied to bi single medication just as parallel blend examination. Contingent upon the absorbance points of medications in paired blend ingestion factor technique, absorptivity factor strategy and q-absorbance proportion strategies are utilized. Aside from that UV noticeable spectroscopy offer more benefits as far as strength, less investigating, physicochemical obstructions when contrasted with other modern instruments like chromatographic and warm strategies. Henceforth, UV spectrophotometry is the most ideal choice for an expert for examination in the drug business

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