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Review Article

ULTRAVIOLET SPECTROSCOPY AND ITS PHARMACEUTICAL APPLICATIONS- A BRIEF REVIEW

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ABSTRACT

Rapid and easy analytical methods are needed due to increasing number of multicomponent formulations, biotherapeutic products and samples of complex matrix in que. Number of Ultraviolet (UV) spectrophotometric methods used for these purpose. Different types of UV spectrometric methods developed on the basis of principle of additivity, absorbance difference, processing absorption spectra. The aim of this review is to present information on 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. A brief summary on theories, mathematical background and some applications of these methods are presented here.

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

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INTRODUCTION

In routine practice, analyst has to perform rapid analysis of multicomponent formulations, biotherapeutic products and samples of a complex matrix. Number of ultraviolet (UV) spectrophotometric methods used for these purpose. However, among all of these methods, UV spectrophotometry is favorite tool. The basic principle behind the UV spectroscopy is absorption of visible and UV radiation (200–400 nm) is associated with excitation of electrons, in both atoms and molecules, from lower to higher energy levels. Since the energy levels of matter are quantized, only light with the precise amount of energy can cause transitions from one level to another will be absorbed [1]. UV spectrophotometric methods based on principle of additivity and absorbance, recording and mathematical processing absorption spectra of standard solutions and sample solutions in same way or differently.

TYPES OF UV SPECTROSCOPIC ANALYTICAL TECHNIQUES

Following are the different UV spectroscopic analytical techniques: 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.

Simultaneous equation method

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

Consider

- The absorptivities of X at λ_1 and λ_2, a_{x1} and a_{x2}, respectively
- The absorptivities of Y at λ_1 and λ_2, a_{y1} and a_{y2}, respectively
- The absorbance of the dilute sample at λ_1 and λ_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 λ_1 $A_1 = a_{x1}bc_x + a_{y1}bc_y$ (1)

At λ_2 $A_2 = a_{x2}bc_x + a_{y2}bc_y$ (2)

If cell is 1 cm, b = 1 equation 2 become,

$c_y = (A - a_{x2}c_x)/a_{y2}$

Substituting value of cy in equation (1), thus

$a_{x1}bc_x = A - a_{y1}c_y$

$c_x = (A_2a_{y1} - A_1a_{y2})/(a_{x2}a_{y1} - a_{x1}a_{y2})$ (3)

Similarly for c_y

$c_y = (A_1a_{x2} - A_2a_{x1})/(a_{x2}a_{y1} - a_{x1}a_{y2})$ (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:

- λ_{max} of two-component should be reasonably dissimilar.
- Two-component should not interact chemically, thereby negating the initial assumption that the absorbance.

The additivity of the absorbance should always be confirmed in the development of a new application of this technique. Table 1 summarizes the application of simultaneous equation method for determination of binary mixture in the pharmaceutical dosage form and Table 2 summarizes the application of simultaneous equation method for determination of ternary mixture in the pharmaceutical dosage form.

Difference spectrophotometry

It is a spectrophotometric technique for quantitative determination of an analyte using an equimolar solution of the same analyte as a reference but in the different physicochemical environment, by virtue

of their differences in spectral properties [10]. In this spectroscopic technique, isolation of an analyte from another component of mixture or other UV active analyte present in mixture sample is achieved. Physicochemical conditions altered mainly involve changes in pH [11], temperature [12].

The requirement of technique is that the analyte under investigation must exist in different chemical forms having different absorbance values. The value is calculated in terms of absorbance difference (amplitude difference in maxima and minima) and plotted versus the concentration of solution examined. Both the selectivity and specificity of analytical method improves by difference spectroscopy, since difference spectra of dosage form overlapped on the pure drug without the presence of interfering peaks due to excipients involved in the dosage form. Table 3 summarizes the applications of difference spectroscopy to drug substance in dosage forms.

Difference spectroscopy used in simultaneous determinations of many dosage forms. In case of binary mixtures determination, wavelength selected such that the contribution of each component is zero at the wavelength at which other components exhibited maximum absorbance. Table 4 summarizes some examples of pharmaceutical applications of difference spectroscopy involving binary mixtures.

Apart from pharmaceutical assay, difference spectroscopy also used in biopharmaceutical formulation development to characterize protein structure and to investigate the response of structure to the formulation composition. This application based on the fact that stable protein conformations provide high real-time physical stability and difference spectra used for characterizing and quantifying changes in protein structure [17].

Moschakis and Nikolaidis, done denaturation study of BSA, by investigating protein conformational changes induced either by heat. The spectra of the heat-treated samples were subtracted from that of the unheated protein solution. For the difference spectra of protein solutions in GdHCl or urea, 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) [12].

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 [18].

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) [19] and electrical method (analog resistance capacitance device) [18]. Later in

Table 1: Application of simultaneous equation method for determination of binary mixture in pharmaceutical dosage form

Drug	Spectroscopic condition (λmax and solvent)	Beer's law range μg\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]

Table 2: Application of simultaneous equation method for determination of ternary mixture in pharmaceutical dosage form

Drug	Spectroscopic condition λmax (solvent)	Beer's law range μg\ml	References
Tenofovir, efavirenz, and lamivudine	260 nm, 347 nm, 272 nm (methanol)	10–60 5–30 5–30	[6]
Amlodipine besylate, losartan potassium, and hydrochlorothiazide	236.5 nm, 254 nm, 271 nm (methanol)	5–25 10–50 5–25	[7]
Amlodipine besylate, valsartan, and hydrochlorothiazide	359 nm, 250 nm, 317 nm (methanol)	5–25 5–25 10–50	[8]
Quercetin, curcumin, and piperine	371.31 nm, 424.68 nm, 343.76 nm (methanol)	5–30 1–5 1–10	[9]

Table 3: Applications of difference spectroscopy to drug substance in dosage forms

Drug	Solvent conditions used	Wavelength of maxima and minima	References
Leflunomide	NaOH (0.1 M) and HCl (0.1 M)	293.5 nm and 261.5 nm	[13]
Tegaserod maleate	NaOH (0.1 M) and HCl (0.1 M)	226 nm and 256 nm	[14]

Table 4: Some examples of pharmaceutical applications of difference spectroscopy involving binary mixtures

Drug	Solvent conditions used	Wavelength of zero crossing	References
Pioglitazone and metformin	phosphate buffer (pH 9) and chloride buffer (pH 2)	228.1 nm and 228.2 nm	[15]
Olmesartan and hydrochlorothiazide	NaOH (0.1 M) and HCl (0.1 M)	257.8 nm and 240.2 nm	[16]

1974, new mathematical technique was introduced named as Golay-savitzky method [20] 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 [21].

Derivative spectroscopy offers following advantages [22,23]:

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)

$$\text{First order equation} \frac{dA}{d\lambda} = \frac{da}{d\lambda} bc \quad (6)$$

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

Derivative spectra magnify the information content from fundamental zero order spectra and are complicated comparatively. Table 5 summarizes the spectral feature of different order derivative spectra.

Measurement technique in derivative spectroscopy: Zero crossing technique and peak to through technique [20]. However for pharmaceutical analysis purpose zero crossing technique is most favorite tool. Zero crossing technique is based on the fact that in derivative spectra absorbance of one component shows no absorbance at such instance absorbance of sample is equal to that of other component in sample which can be used to find its concentration. Table 6 summarizes the applications of zero crossing technique to analysis of binary mixtures and Table 7 applications of zero crossing technique to analysis of ternary mixtures.

Apart from pharmaceutical assay derivative spectroscopy also finds its application in a clinical study such as quantitative assay of diazepam in human blood plasma without separation of the drug from the biological matrix [20].

Furthermore, derivative spectroscopy had been used for stability study purpose. Using the second derivative UV spectrophotometry, butamirate citrate, and formoterol fumarate were determined by measuring the peak amplitude at 260.4 and 261.8 nm, respectively, without any interference of their degradation products [33].

With derivatization of spectra, signal-to-noise ratio increases. Furthermore, reproducibility obtained with derivative spectroscopy is very low. Anatov *et al*. reported method of step-by-step-filter method to improve the signal to noise ratio [34]. Brown *et al*. reported method of derivative reprocessing where drift noise reduction achieved for multivariate spectral data [35]. Apart from above-mentioned methodology, few variants of derivative spectroscopy were reported globally. Wavelet transformation technique employed successfully for derivative spectroscopy too [36].

Ratio derivative spectroscopy

Spectrophotometric determination of two or more compounds in the same sample without preliminary separation is in demand. In past decade ratio derivative spectroscopy emerged as good tool to serve this purpose which was based on work of Salinas *et al*., where they developed a spectrophotometric method based on the use of the first derivative of the ratio spectra for resolving binary mixtures when the spectra of the components are overlapped. It permits the use of the wavelength of the highest value of analytical signals with several peaks and trough, which permits the determination of an analyte in the presence of other compounds and excipients which could possibly interfere in the analysis [37].

The method involves following steps

- Recording mixture spectra of samples under investigation.
- Dividing the mixture spectra by a standard divisor spectrum.
- Followed by peak-to-peak/peak-to-trough measurement in the produced ratio spectra, which directly gives the concentration of one of the component in mixture.

The method eliminates the derivative step and does not require searching for zero-crossing points or any sophisticated mathematical or chemometric treatment of data.

$$_{\lambda 1}A_M = {}_{\lambda 1}E_A C_A + {}_{\lambda 1}E_B C_B \quad (8)$$

Table 5: Spectral feature of different order derivative spectra [18]

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 λ_{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 λ_{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 λ_{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 λ_{max} of the absorbance band, along with two negative satellite bands on its either side

Table 6: 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]
Rosuvastatin Ca and fenofibrate	1	243 and 224 nm	[29]
Salbutamol sulfate and ketotifen fumarate	1	257 and 278 nm	[30]

$_{\lambda 1}A_M$ -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 λ1 whose concentration is C°_A, then equation becomes,

$$\frac{\lambda 1 A_M}{\lambda 1 E_A C R_A} = \frac{C_A}{CR_A} + \frac{\lambda 1 E_B C_B}{\lambda 1 E_A C R_A} \quad (9)$$

This equation can be simplified to

$$\frac{\lambda 1 A_A}{\lambda 1 E_A} = \frac{C_A + \lambda 1 E_B C_B}{\lambda 1 E_A}$$

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 [42]. 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:

$$A_m = \alpha_x C_x + \alpha_y C_y + \alpha_z C_z \quad (10)$$

Where A_m is the total absorbance of the mixture, α_x, α_y, and α_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 α_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 αZ should not be used in the divisor):

Table 7: 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, bromhexine hydrochloride, and etofylline	1,1,1	273 nm, 323 nm and 279 nm	[32]

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

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 obtainedin the form of equation (12):

$$dB/d\lambda = d/d\lambda\ (\alpha_x C_x/\alpha_z) + d/d\lambda\ (\alpha_y C_y/\alpha_z) \quad (12)$$

Dividing equation (6) by d/dλ (αY/αZ), 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/dλ)(αY/αZ) should not be used in the divisor):

$$D = (dB/d\lambda)/d/d\lambda\ (\alpha Y/\alpha Z) = d/d\lambda[\alpha XCX/\alpha Z]/d/d\lambda\ (\alpha Y/\alpha Z) + CY \quad (13)$$

If the first derivative of equation (13) is taken since the derivative of a constant (C_Y) is zero,

Equation (14) would be obtained:

$$dD/d\lambda = d/d\lambda\ \{[d/d\lambda(\alpha XCX/\alpha Z)]/[(d/d\lambda(\alpha Y/\alpha Z)]\} \quad (14)$$

Equation (14) is the mathematical foundation of multicomponent analysis that permits the determination of the concentration of each of the active drugs in the solution (X in this equation) without interference from the other drugs of the ternary system (Y and Z in these equations). As equation (14) shows, there is a linear relationship between the amount of dD/dλ and the concentration of X in the solution. A calibration curve could be constructed by plotting dD/dλ against the concentration of X in the standard solutions of X or in the standard ternary mixtures.

For more sensitivity, the amount of dD/dλ corresponding to maximum or minimum wavelength should be measured. Calibration graphs for Y and Z could be also constructed as described for X.

Abdelrahman and Abdelaleem applied successive ratio spectra method to pharmaceutical ternary mixtures including isopropamide iodide, trifluoperazine hydrochloride, and trifluoperazine oxidative degradate [42].

Q-absorbance ratio method

The method is applicable only when beers law is followed for a given combination of the drug. This method is based on the fact that the ratio of absorbance at any two wavelengths for a substance, which obeys Beer's law, is a constant value independent of the concentration and path length. This constant is termed as "Hufner's Quotient" or Q-value. The Q-absorbance equation formed using the absorptivity values at two wavelengths used as such, one being the λ_{max} of one of the components and the other being a wavelength of isoabsorptive point [43,44]. Table 9 summarizes the applications of Q absorbance ratio method.

The absorbance and absorptivity values at the particular wavelengths were calculated and substituted in the following equation; to obtain the concentration.

Table 8: 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]
Naphazoline and antazoline	227.2 nm (naphazoline) and 235 nm (antazoline)	[39]
Paracetamol and aceclofenac	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]
Gabapentin, methylcobalamin and alpha lipoic acid	731.10 nm (gabapentin), 768.53 nm (methylcobalamin), and 242.21 nm (alpha lipoic acid)	[41]

Table 9: Illustrates the applications of Q absorbance ratio method

Drug	Wavelength	References
Carvedilol and hydrochlorothiazide	241 (λ_{max} of carvedilol) and 229.2 (isoabsorptive point)	[43]
Atenolol and ivabradine	276 (λ_{max} of atenolol) and 286.40 (isoabsorptive point)	[44]
Propranolol and flunarizine	253 (λ_{max} of flunarizine) and 272.8 (isoabsorptive point)	[45]
Prednisolone and 5-aminosalicylic acid	302 (λ_{max} of 5-ASA) and 283 (isoabsorptive point)	[46]
Naproxen and paracetamol	257 (λ_{max} of paracetamol) and 234 (isoabsorptive point)	[47]
Cefixime and moxifloxacin	293.6 (λ_{max} of moxifloxacin) and 276 (isoabsorptive point)	[48]
Difluprednate and gatifloxacin	241 (λ_{max} Difluprednate) and 236 (isoabsorptive point)	[49]

Table 10: 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]

$C_X = (Q_m-Q_y)\times A/(Q_X-Q_Y)\times ax_1$

$C_Y = (A/ax_1)-C_X$

$Qm = A_2/A_1$

A_1 is absorbance of sample at isoabsorptive point, A_2 is absorbance of sample at λ_{max} of one of the two components ax1 and ax2 represent absorptivities of X at λ_x1 and λ_x 2 and ay1 and ay2 denote absorptivities of Y at λ_y1 and λ_x2, respectively, CX and CY 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 [50,51].

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

In contrast to isoabsorptive point method, crossing of spectra do not occur at same concentration, however, it may occur at different drug concentrations. At such crossing point in absorptivity factor method, absorptivity is equal to the inverse ratio of concentrations used. The ratio found is known as absorptivity as absorptivity factor (F), and the crossing point is known as absorptivity factor point [50].

$A_x = a_xbc_x$ and $A_y = a_ybc_y$

At crossing point of equal absorptivity having different drug concentrations

$A_x = A_y$

$a_xbc_x = a_ybc_y$

$a_xc_x = a_yc_y$

$a_{x/}a_y = c_{y/}c_x = F$

$a_{x/}a_y=F$ (15)

$A_m = A_x+A_y = a_xbc_x+a_ybc_y$

Where b = 1

$A_m = a_xc_x+a_yc_y$

$a_x = Fa_y$ (16)

$A_m = Fa_yc_x+a_yc_y = a_y (Fc_x+c_y)$

Similarly, $A_m = a_x (Fc_y+c_x)$

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 [4-35].

$A_m = a_y (Fc_x+c_y) = a_x (Fc_y+c_x)$

$a_y (Fc_x+c_y) = a_x (Fc_y+c_x)$

$c_x = [(Fc_x+c_y)-c_y]/F$ (17)

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

Absorption factor method

Absorption factor method is another spectroscopic method applicable to the analysis of binary mixtures. In those cases where the overlapped spectra observed found, interferences at absorbance maxima of one component observed while no interference observed at absorbance maxima of another compound [51].

Consider a mixture of x and y having a wavelength of maxima at λ_x and λ_y. Y shows interference at λ_x but x do not shows interference at λ_y. In this method different standard concentration of Y such as a, b, c, and d are scanned in the range of 200–400 nm.

The average value of absorbance factor was calculated using the following equation:

$(A_{Y1}\lambda_{Y1}/A_{Y2}\lambda_{Y2})_a+(A_{Y1}\lambda_{Y1}/A_{Y2}\lambda_{Y2})_b+(A_{Y1}\lambda_{Y1}/A_{Y2}\lambda_{Y2})_c+(A_{Y1}\lambda_{Y1}/A_{Y2}\lambda_{Y2})_d = (A_{Y1}\lambda_{Y1}/A_{Y2}\lambda_{Y2})_{avg}$ (18)

$(A_{Y1}\lambda_{Y1}/A_{Y2}\lambda_{Y2})_{avg}$ is the average value of absorbance factor.

Since at λ_y only y shows absorbance at this wavelength concentration of y can be determined. From this concentration of x can be obtained using the following formula:

$A_x\lambda_x = A_{(X+Y)}\lambda_1-A_{(X+Y)}\lambda_2*(A_{Y1}\lambda_{Y1}/A_{Y2}\lambda_{Y2})_{avg}$ (19)

The above equation is foundation of absorption factor method.

Table 11 summarizes pharmaceutical applications of absorption factor method to binary mixtures of drugs in combined dosage form.

MULTIVARIATE CHEMOMETRIC METHOD

It is the processing of analytical data by mathematical techniques. It can also be defined as multiple measurements on the same sample due to

Table 11: Pharmaceutical applications of absorption factor method to binary mixtures of drugs in combined dosage form

Drug	Wavelength 1 (both drugs shows absorbance) (nm)	Wavelength 2 (one drugs shows absorbance) (nm)	References
Sodium cromoglicate and fluorometholone	241	325	[51]
Ramipril and olmesartan medoxomil	210	256	[29]
Perindopril erbumine and amlodipine besylate	215	237	[52]

this correlation of physical properties to analytical data can be done. According to chemometric methods, it is often better to measure many nonselective signal and later combining them in a multivariate model where multiple variable considered simultaneously [53].

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).

In the case of spectroscopy, if the absorbance spectra of a number of samples of known composition are measured, all these spectra are assembled into one matrix called the absorbance matrix. While in concentration matrix, all concentration values for the components of the sample are assembled.

In general, MLR and PCR techniques employ data organized as matrices of column vectors, while PLS technique employs data organized as matrices of row vectors [53].

The data of matrices are organized into pairs; each absorbance matrix is paired with its corresponding concentration matrix. The pair of matrices comprises a data set. Data sets have different names depending on their origin and purpose [53].

Training set is a data set containing measurements on a set of known samples. It is used to develop the calibration which is applied to predict the concentrations of unknown samples. Training set should contain all expected components, span the concentration ranges of interest and contain mutually independent samples [53].

Validation set is an additional dataset containing independent measurements on samples that are independent from the samples used to create the training set. Validation set is used to test the validity of the calibration developed with the training set. The developed calibration is used to predict the concentrations of the components in the validation samples. Then, these predicted concentrations are compared to the actual concentrations [53].

The absorbance matrix containing the unknown(s) spectra together with the corresponding result matrix containing the predicted concentrations comprise an unknown set.

Table 12 summarizes pharmaceutical applications of multivariate chemometric method to binary mixtures of drugs in combined dosage form.

Isosbestic point method

This technique can be used only if the spectra of the same concentration of the two studied drugs cross at a point called isosbestic or isoabsorptivity point. At the isosbestic point, both drugs have equal absorptivities, and their mixture acts as a single component and gives the same absorbance as pure drug. The absorbance value at the isosbestic point (Aiso) was determined, and the total concentration of both drugs was calculated. Since the concentration of one of them in this mixture can be measured using other spectroscopic method (DS), the concentration of the other could be calculated by subtraction.

Table 12: 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]

A linear correlation was obtained between the absorbance values and the corresponding drug concentrations. Consider you have a mixture of two drugs x and y. The absorbance of each drug can be calculated at any wavelength (λ) from the equation

$A = abc$

Therefore, for drug x: $A_x = a_x bC_x$ and

For drug y: $A_y = a_y bC_y$

Where a_x and a_y are the absorbances of x and y, respectively; Cx and Cy 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.

If $C_x = C_y$, and $a_x = a_y$, this λ is called the isosbestic point, and

At this λ $A_x = A_y$ (20)

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

$A = a_x Cx + a_y Cy$ (21)

$A = a_x (Cx+Cy)$ (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 C_{TM} is the concentration of their mixture.

Therefore, we can conclude that

$A = a_x C_{TM}$ (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 [57].

This method applied for the analysis of the ternary mixture of chloramphenicol, dexamethasone sodium phosphate (DXM) and tetryzoline hydrochloride in eye drops [58].

Advantages of UV spectroscopy over other analytical techniques (Table 13)

Furthermore, UV spectrophotometer is a highly simple instrument which makes it easier to couple with other analytical instrument such

Table 13: Advantages of ultraviolet spectroscopy over other analytical techniques

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

as RP-HPLC [59]. Apart from this one UV spectrophotometric method had been conveniently adopted to develop a new better analytical method [60]. Such method transfer and instrument compatibility are facilitated with UV spectroscopy only.

CONCLUSION

Number of multicomponent formulations, biopharmaceutical products and samples of complex matrix and biological origin are present in the market for which different analytical techniques can be applied including spectrophotometry, chromatography, and electrophoresis but UV spectrophotometric methods for determination of drugs are easier, cheaper, simple, and rapid. Since most analytes of interest are accompanied in their dosage forms by other compounds absorbing in the same spectral region, classical UV spectral measurements could not be used for their determination. Hence, all of the above methods can be used according to their nature. Among the UV spectroscopic techniques depending on the nature of analysis particular method can be selected. Method such as simultaneous and derivative spectroscopy can be used to analyze both binary and tertiary mixture, where such as, for resolving closely absorbing peaks derivatives spectroscopy advantageous while simultaneous spectroscopy is better in terms of its simplicity. Furthermore, variants based on derivative spectroscopy such as ratio derivative spectroscopy, successive ratio derivative spectroscopy offer more advantages in terms of eliminating chemical interferences. High selectivity and specificity can be obtained for analyzing equimolar solutions of analyte in UV active matrix using difference spectroscopy, can be applied to bi single drug as well as binary mixture analysis. Depending on the absorbance points of drugs in binary mixture absorption factor method, absorptivity factor method and q-absorbance ratio methods are used. Apart from that UV visible spectroscopy offer more advantages in terms of robustness, less troubleshooting, physicochemical interferences as compared to other sophisticated instruments such as chromatographic and thermal techniques. Hence, UV spectrophotometry is the best option for an analyst for analysis in the pharmaceutical industry.

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