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Simultaneous Estimation of drugs through spectroscopic techniques: A tool for analytical investigation of formulations

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ABSTRACT: Simultaneous estimation plays a most important role in the pharmaceutical world. It is a time saving technique. Simultaneous estimation can be done by multiple techniques like chromatographic techniques (High Performance Liquid Chromatography, Thin Layer Chromatography and Gas Chromatography) and spectrophotometric techniques (Ultra Violet-Visible, Mass, Nuclear magnetic resonance, and Infrared Radiation). Different Ultra Violet-Visible spectrophotometric methods are used in simultaneous multicomponent analysis. This review is mainly focused on simultaneous equation methods, difference spectrophotometry, derivative spectrophotometry, absorbance ratio spectra, double divisor ratio spectra derivative method and isosbestic point method. These techniques provide high accuracy and precision. An overview of theories, history, advantage, disadvantage, and applications of these methods are discussed in this article.

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INTRODUCTION:

The advancement of pharmaceuticals and their examination has innovated the world to the moderate extent in health sciences. The method of drug molecule detection and pharmaceutical exploration or examination of the formulation delivers the safety and therapeutic effect to a high extent [1]. Accurate estimation of a system's state is intrinsically desirable and is critical for accurately forecasting its future states [2]. Several manipulations were performed on the raw overlapping spectral data to enable mixture resolution, for example, using different order derivatives and derivatives of the ratio spectrum and ratio subtraction techniques [3]. A new

method ratio difference has been developed having the advantages of less time consuming, cheap, specific and accurate which provides results up to high extent, minimal data processing and wider range of application [4,5].

When no region is often found free from overlapping spectra of two chromophores, it's still possible to plan a way to support measurements at two or more wavelengths. Two dissimilar chromophores have different powers of addition absorption at some or several points in their absorption spectra. If, therefore, measurements are made on an unknown solution at two wavelengths where the absorptivity of the 2 components are different, it's possible to line up two independent equations and solve them simultaneously for the two unknown concentrations.

Event history studies are commonly performed in many areas and in these simultaneous, two follow-up schemes are typically used. One is to follow all study subjects continuously and in consequence, complete data are observed for the underlying periodic event process. In contrast, the other is to observe all study subjects only at discrete time points and thus only incomplete data, which are often referred to as panel count data, are available for inference. Note that for the latter, one complicated factor is that both observation and follow-up times usually vary from subject to subject. Fields in which panel count data are common include demographic studies, epidemiological studies, medical periodic follow-up studies and tumorigenicity experiments [6,7].

Thus, the review highlights a simultaneous estimation spectrophotometric techniques and methods such as UV spectrophotometer, High performance liquid chromatography, Thin layer chromatography, Gas chromatography have wide application in assuring the quality and quantity of pharmaceutical products and these estimation techniques are simple, rapid, accurate, precise, selective, cost effective and hence can be used for simultaneous estimation of formulations.

SPECTROPHOTOMETRIC TECHNIQUES:

Spectrophotometric techniques are the necessary instrumental techniques used by pharmaceutical analysts. The fundamentals of spectrophotometric techniques are that they measure form. There are varied spectrophotometric techniques that are employed in the pharmaceutical world for the analysis of the API's and pharmaceutical ingredients in Fig 1.

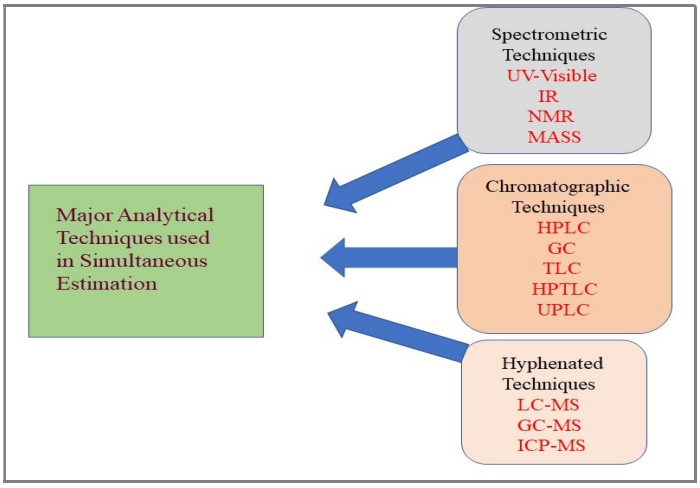

Fig 1. Different types of analytical techniques used in estimation of drugs.

UV/VIS spectroscopy:

The analytical applications of the UV (Ultraviolet) spectroscopy are qualitatively and quantifiable. UV-visible spectroscopy is not an excess of required cause the simultaneous estimations and spectral overlapping. The by-product qualitatively analysis gives the improvement of specificity and sensitivity in pharmaceutical formulations. The ultraviolet visible spectrophotometry techniques are in the midst of foremost oft used techniques within the analysis which involves the measurement of the amount of ultraviolet and visible radiations absorbed by the pharmaceuticals in a solution. Various techniques have been utilized in simultaneous estimation by UV visible spectrum analysis such as Simultaneous Equation method, Absorbance quantitative relation method, Geometric correction method, Orthogonal polynomial method, Difference spectroscopy and Derivative spectroscopy. The advantages of the UV/VIS spectroscopy techniques are that they have low time and labour consumption. The precision and accuracy of analysts by using the ultraviolet-visible spectroscopy is achieved for a maximum limit [8,9].

Nuclear magnetic resonance spectroscopy:

Nuclear magnetic resonance (NMR) is a division of analytical chemistry and spectroscopy which deals with the frequency waves which transitions among magnetic energy levels of nuclei of a molecule. The magnetic energy levels square measure created by keeping the nuclei magnetic field. The first observation of NMR was observed and studied by Purcell and Bloch in 1945. Ethyl Alcohol was the primary compound which was studied and demonstrated by this system in 1951 [10,11].

Application of NMR:

- Food Chemistry: It is used for the authentication of the wine aging and identification of the fatty oil's constituents in food and other beverages.
- Clinical application: It is used for the identification and studies of metabolites in biological fluids *in vivo* or *in vitro* and used for diagnosis and helpful in treatment of diseases [9].
- Study of the Hydrogen Bonding: Hydrogen bonds within the metal chelates also as in organic compounds are often determined by this system. Hydrogen bonding leads to the decrease within the electron shield protons and signal is shifted towards the low field.

Infrared (IR)/ Fourier Transform Infrared (FTIR) spectroscopy:

Pharmaceutical quality control and quality assurance depends on observing the model and uniformity of the drug substance all over technique and inside the pharmaceutical product. This technique provides a spectrum containing sizable amount of absorption bands i.e. functional groups can be derived. The drugs estimated by FTIR are listed in Table1.

Vibrational chemical analysis techniques, together with Mid-infrared, near infrared and Raman, are planned as alternative various approaches. Infra-red is a crucial technique which provides sufficient information about the structure and its functional groups of a compound [12].

Application of IR spectroscopy:

Various pharmacopoeias like IP, BP and USP are unit accustomed establish Active Pharmaceutical Ingredients (API's) and to visualize the purity at short interval of the time that indirectly will increase the productivity e.g. Amylobarbitone, Betamethasone, Dexamethasone, Cyclophosphamide and Sulphalene [13].

Mass spectrometry:

Mass spectrum is an analytical technique which may provide information concerning the molecular structure of organic and inorganic compounds. It is often wanted to determine directly relative molecular mass as high as 4000. It is one of the few methods that can be used as a qualitative analytical tool to characterize different organic substances. With it, one can do analysis of mixtures (gases, or liquids, and in some cases solid) quantitatively. A mass spectrometer is also useful to investigate reaction mixture and in tracer work. It is also used in understanding kinetics and mechanisms of unimolecular decomposition reactions [14].

Advantage of mass spectrometry:

It is a highly sensitive and accurate technique. Small amount of sample is required from nanogram to microgram can be analysed. Resolution time is up to a high extent. Extremely specific because of fragmentation and helps in study of structure [15].

Disadvantage of mass spectrometry:

Sample recovery can't be achieved because of the damaging nature of the process. Very costly and required high maintenance. Introduction of samples is difficult due to small sample size.

Application of mass spectrometry [16]:

- Determination of molecular mass of the compounds: peak having highest m/e magnitude shows the molecular mass of the compound.
- Characterization of chemical compound: Determination of polymers and elucidation of polymer structure will be done by this system. The structure of the chemical compound will be distinguished on the basis of arrangement of atoms.
- Mass spectrometry is also used for the analysis of simple or complex proteomes using quantitative mass spectrometry [14].

CHROMATOGRAPHIC TECHNIQUE:

Chromatography is a little a replacement technique which was invented by M. Tsweet, a botanist in 1906 in Warsaw. In that year he was successful in elucidating chlorophyll, xanthophyll and numeric of other coloured substances from vegetable extracts throughout a column of carbonate. The calcium carbonate acts as an adsorbent and other various dissimilar substances get adsorbed to other extent and provides rise tow-coloured bands at different positions on the column. Tweet named this arrangement of coloured bands because the Chromatogram and thus the tactic as Chromatography after the Greek words chroma and graphs meaning "colour" and "writing" respectively. In the 1930's chromatography within the sort of thin layer chromatography and Ion Exchange chromatography was introduced as separation techniques. In 1941, Martin and Synge introduced Partition and chromatography. They introduced Gas chromatography in 1952. Chromatography is a non-devastative method from which multiple *constituents*

Table 1. Determination of pharmaceutical drugs by FTIR spectroscopy [11,12].

Sl. No.	Drug	Property Description	Type of IR
1	Acyclovir and lactose	Drug excipient compatibility	Mid FTIR
2	Bicifadine HCl	Characterization of polymorphs	Mid FTIR
3	DNA Prediction	Oligonucleotide of DNA	Far FTIR
4	Enalapril	Characterization of six salt forms	Mid FTIR
5	Indomethacin–saccharin	Characterization of co-crystals	Near & Mid FTIR
6	Omeprazole sodium	Characterization of API in salt form	Mid FTIR
7	Piroxicam monohydrate	Quantification during isothermal dehydration	Near FTIR
8	Sulfathiazole	Polymorph screening and Processing –induced transformation (PIT) screening	Near FTIR
9	Theophylline, and caffeine	Characterization of hydrate formation during wet Granulation	Near Mid FTIR
10	Troglitazone	Drug distribution in solid dispersion	Near FTIR

are generally derived and separated chromatography is the most vital single analytical technique used today and can most expectedly continue to be so far for the predictable future. It is the inspiration stone of molecular and pharmaceutical analytical chemistry and recently it's including atomic absorption and Mass spectroscopy that hold spread out his applications within the world pharmaceutical analysis. Different chromatographic techniques hold used as simultaneous estimation of API's or pharmaceutical dosage forms like Paper chromatography, Thin layer chromatography (TLC), Gas chromatography (GC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), Column chromatography and, Ultra-high-performance liquid chromatography (UPLC) [17-20].

High performance liquid chromatography (HPLC):

Chromatography is besides the highest typically utilized analytical technique in pharmaceutical analysis. The technique is used by chemists to separate and determine species in kind of organic, inorganic, and biological materials.

HPLC has been around for about 35 years and it is the main separation technique used. HPLC is one of the only methods of choice for analyzing various kinds of natural and artificial compounds [15].

Various sorts of HPLC's are used for simultaneous estimations e.g. normal phase high performance liquid chromatography, reversed phase high performance liquid chromatography, size exclusion high performance liquid chromatography, ion-exchange high performance liquid chromatography, bio-affinity high performance liquid chromatography. In the case of normal phase HPLC, the stationary phase is polar and the mobile phase is nonpolar.

Adsorption extends with increase in polarity, and therefore the interaction with polar analyte and polar stationary phase increases the elution time. Few drugs are enlisted in Table 2 which is simultaneously estimated by normal phase HPLC [17,18].

Thin layer chromatography:

In 1958, Stahl demonstrated the purpose and use of TLC in analysis, a way supported adsorption chromatography. Presently TLC is a crucial analytical tool for the qualitative and quantitative chemical analysis, of number of natural also as synthetic products.

The TLC technique is a very important tool in analysis of Alkaloids, Glycosides and Isoprenoids constituents. A TLC sample is spotted on the plate with a micropipette and the chromatogram is developed by placing the bottom of the plate or strip.

Table 2. Simultaneous estimation of drugs by normal phase HPLC& RPHPLC.

Sl. No.	Drugs	Category	Mobile phase	Type of HPLC	Refe-rences
1	Aceclofenac and Paracetamol	NSAIDs	Methanol and water (70:30) v/v	RP-HPLC	16
2	Benzoyl peroxide, Benzoic Acid	Pregnancy category C.	Methanol: water (65:35) v/v	Normal	17
3	Cefixime and Cloxacillin	Antibiotics	Phosphate buffer: ACN: Methanol (80:17:3) v/v	RP-HPLC	18
4	Drotaverine hydrochloride and Omeprazole	Pregnancy	n-heptane: Dichloromethane: Methanolic Ammonia (5%): Methanol (50:25:1:4) v/v/v	Normal	19
5	Metoprolol and Hydrochlorothiazide	Pregnancy	Di-sodium hydrogen phosphate: MeOH: ACN (525:225:250) v/v	RP-HPLC	20
6	Tocopherols and Tocotrienols	Vitamin	Hexane: 1,4-dioxane (95.5:4.5) v/v	Normal	21

The solvent is connected with the occurrence of capillary work, and accordingly sample constituents transfer the plate at various rates, numbering on their solubility and their degree of affinity towards solvent. The spots will generally move at a certain fraction of the rate at which the solvent moves, and they are characterized by the R_f value [19,20].

$R_f = D_{st}/D_{sv}$ .....(1)

Where, D_{st} and D_{sv} are distance travelled by solute and solvent.

TLC plays important role within the analysis of various groups of drugs, essential constituents of food. TLC provides the identification of drugs simultaneously in various pharmaceutical dosage forms [21].

Various amino acids which are detected by TLC are Alanine, Asparagine, Leucine, Glycine, Valine, Threonine, Isoleucine, Serine, Aspartic acid, Asparagine, Glutamic acid, Glutamine, Lysine, Histidine, Arginine, Phenylalanine, Tyrosine, Tryptophan, Hydroxy Proline, Cysteine, cystine, Proline and Methionine TLC [22].

Gas chromatography:

Gas chromatography is a technique accustomed for separation of mixtures into single entities by a process which lies on the redistributing of constituent between a stationary phase or supporting material within the form of a liquid-solid or combination of both and gaseous mobile phase. Mechanism of the GC is predicated on adsorption, mass distribution or size exclusion [23].

Application of GC in bio analysis:

- Use of GC within the bio analysis of the drugs within the plasma and its metabolism increase the efficacy of this sort of chromatography.
- Determination of Valproic acid within the plasma are often determined by this method.
- Quantification of bupivacaine in plasma [24].
- Measurement of isoprene solubility in water, human blood and plasma [25].
- Simultaneous determination of three, 4-Dihyroxyphenylglycol, Catecholamines and three, 4-Dihydroxyphenylalanine in plasma [26].

SIMULTANEOUS ESTIMATION METHOD:

Combination drug products engage time-honored and important role in therapeutics. When rationally formulated, fixed-combination drugs may produce greater convenience, lower cost and sometimes greater efficacy and safety [27,28].

Simultaneous equation method:

Sample with two absorbing drugs (x and y) every of that absorbs at the λ_{max} of the opposite, it should be attainable to work with both the drugs by the technique of simultaneous equation (mainly Verret's method) providing suitable conditions (Fig 2).

The information required is the specific absorption coefficient of x at λ1 and λ2, ax1 and ax2 respectively; the specific absorption coefficient of **y** at λ1 and λ2, ay1 and ay2 respectively and the absorbance of the diluted samples at λ1 and λ2, A1 and A2 respectively.

Let Cx and Cy be the concentration of **x** and **y** severally within the diluted samples. Two equations are constructed based upon the very fact that at λ1, the absorbance of the mixture is that the sum of the individual absorbance of x and y.

$A1 = a_{x1}\ b\ C_x + a_{y1}\ b\ C_y$ .....(2)

$A2 = a_{x2}\ b\ Cx + a_{y2}\ b\ C_y$ .....(3)

For measurements in 1 cm cells, b = 1 cm. Rearrange equation 3,

$C_y = (A_2 – a_{x2} C_x)/a_{y2}$ .....(4)

Substituting for Cy in equation 2 and rearranging gives,

$C_x = (A_2 a_{y1} – A_1 a_{y2})/ (a_{x2}a_{y1} – a_{x1}a_{y2})$ ……(5)

Cy = (A1 ax2 – A2 ax1)/ $(a_{x2}a_{y1} – a_{x1}a_{y2})$ …(6)

Criteria for getting maximum preciseness, 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 $A_2/A_1/a_{x2}/a_{x1}$ and Ay2 should lie outside the range 0.1 to 2.0 for the precise determination of **y** and **x** respectively.

Simultaneous equation method was developed for simultaneous determination of many mixtures, e.g. atenolol and indapamide [28] and dexibuprofen and paracetamol [29-32].

Derivative spectrophotometry (DS):

It is one of the most highly developed spectrophotometric techniques. The origin of derivative spectrophotometry is connected with appearance of spectrophotometers enabling recording of derivative spectra. The derivative spectrophotometry involves the change of normal spectrum to its first, second or higher derivative spectrum. The derivative spectra are frequently procured by optical, electronic and mathematical plan. In optical technique there's wave length modulation wherever the wavelength of incident lightweight is quickly if the derivative spectrum is expressed as absorbance (A) as function of wavelength (λ), the derivative spectra are Zero order: A = f (λ), First order: $dA/d\lambda = f'(\lambda)$ and Second order: $d^2A/d\lambda^2 = f''(\lambda)$ [33,34].

The derivative spectra are employed to get the better differences among spectra to resolve the overlapping bands in qualitative analysis and to reduce the outcome of scattering matrix [35,36].

The strong positive and negative bands with maximum and minimum at same wavelength of an absorption band as inflection point in absorbance band governs the odd (first and second) derivative spectrum whereas the robust positive and negative bands with minimum or most at same wavelength as λ scope of absorbance band governs the even (second and fourth) spinoff spectrum [37,38].

Number of bands = derivative order +1 …… (7)

The deserves of spinoff chemical analysis square measure to extend the resolution allowing identification of analyte with shut λ_{max}, to say no the baseline shift arising from instrument or sample handling and diminish the scattering result so useful for analyte gift in opaque answer [31]. Examples of drug estimated by first order UV spectroscopy are listed in Table 3 [39,40].

Absorbance ratio spectra 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 [41].

$A_M = a_x C_x + a_y C_y$ ……(8)

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 $A^0_x = a_x C^0_x$), the following equation results.

$AM/A^0x = Cx/C^0x + Ay/A^0x$ ……(9)

The ratio Cx/C^0x is a constant value (Fig 3) which can be eliminated by taking the difference in absorbance ratio amplitudes between two wavelengths λ1 and λ.

$[A_M/A^0x]\,\lambda_1 – [A_M/A^0x]\,\lambda_2 = [Ay/A^0x]\,\lambda_1 – [Ay/A^0x]\,\lambda_2$ …(10)

Equation 9 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 distinction spectrophotometric method) is capable a similar amplitude distinction for compound y when cancelling the constant interference because of compound x. The concentration of compound y (C_y) is proportional to the height to peak amplitudes of its absorbance spectra. A standardization graph is obtained by recording and storing the spectra of solutions of various concentrations of pure y, and the spectrum of a solution of pure x (the divisor x_0). The keep spectra of the solutions of pure y area unit divided by the quality spectrum of the divisor (x_0) (Fig 3). In the generated magnitude relation spectra, the peak to peak amplitudes between the chosen wavelengths area unit measured and aforethought against C_y to get the standardization graph. By mistreatment the standardization graph, the concentration of compound y within the mixture is set when similar treatment for the mixture resolution.

The concentration of x in the mixture is determined by an analogous procedure. Ratio spectra method was developed for the simultaneous determination of many binary mixtures e.g. emtricitabine and tenofovir [42], diclofenac and pantoprazole [43] and ternary mixtures, e.g. omeprazole, tinidazole and clarithromycin [44].

Table 3. Simultaneous estimation of combination drugs by first-order derivative UV spectrophotometer.

Category	Drug Combination	Wavelength (nm)	References
Sympathomimetic	Adrenaline and Noradrenaline	394 and 342	32
Diuretics	Amiloride and Furosemide	241.4 and 343.6	33
Tricyclic antidepressants & antipsychotic	Amitriptyline and Chlorpromazine Hydrochloride	254 and 260	34
Penicillin & mucolytic	Amoxicillin and Bromhexine HCl	278.8 and 326.2	35
NSAID	Analgen and Adamon	600 and 310.5	36
NSAID	Analgen and Hyoscine N-butyl bromide	291.8 and 219.8	37
Beta blockers & CCB	Atenolol and Nifedipine	276 and 340	38
Antibiotic	Cephalothin and Cefoxitin	235 and 236.7	39
ACE inhibitor & diuretics	Cilazapril and Hydrochlorothiazide	242.8 and 282.8	40

ACE – Angiotensin Converting Enzyme and CCB - calcium channel blockers.

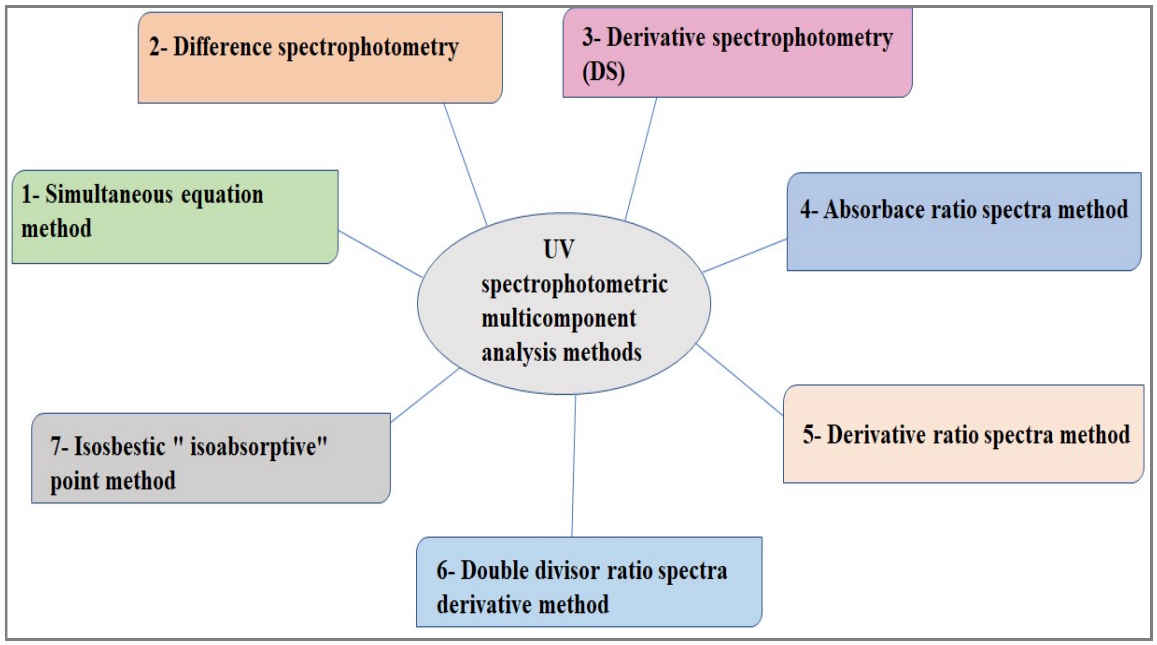

Fig 2. Different UV spectrophotometric multicomponent analysis methods.

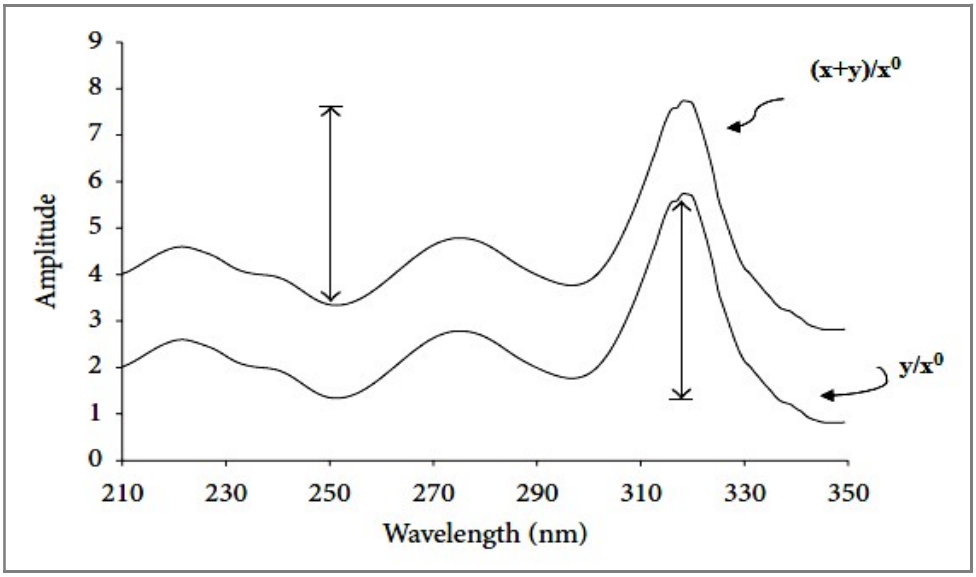

Fig 3. Magnitude relation spectra of a typical resolution of y and a mixture solution (x and y) containing a similar concentration of y, using x0 as a divisor [42].

Derivative ratio spectra method:

This simple spectrophotometric methodology, developed by Salinas, *et al*., [41] is predicated on the derivation of the quantitative relation spectra for resolution binary mixtures. It permits the utilization of the wavelength of highest price of analytical signals with many maxima and minima, that offer a chance for the determination of active compounds in the presence of other compounds and excipients which could possibly interfere in the assay [43,45].

Calculation of the first derivative will remove the constant value due to Cx/C^0x in equation 10, thus concentration of *y* is of tens imply determined with none interferences from the drug *x*.

$A_M/A^0x = Cx/C^0x – Ay/A^0x$ ……(11)

The difference between the two spectra AM/A0x and Ay/A^0x (Fig 3) is due to the constant interference value due to compound x (Cx/C^0x). Elimination of such interference are often done by activity of quantitative relation spectra distinction between two wavelengths or scheming by product of the quantitative relation spectra [43].

Second derivative of the ratio spectra may be additionally accustomed improve one-dimensionality, mean percentage recoveries and decrease relative standard deviation [46]. Derivative ratio spectra were modified for the determination of ternary mixtures using the derivative ratio spectra zero-crossing method. This methodology is realised by activity of amplitudes at the zero-crossing points within the by-product quantitative relation spectra [47].

Isosbestic "isoabsorptive" point method:

Errum and Tipnis developed the isosbestic point method. This technique is often used as long as the spectra of an equivalent concentration of the two studied drugs cross at some extent called isosbestic or is absorptivity point.

At the isosbestic point both drugs have equal absorptivity and their mixture acts as a one component and provides an equivalent absorbance as pure drug. This theory can be confirmed experimentally by recording the absorbance spectra of a certain concentration of the two drugs and the absorbance spectra of a binary mixture containing the same concentration. The absorbance value at the isosbestic points (Aiso) decided, and therefore the total concentration of both drugs was calculated (Fig 4).

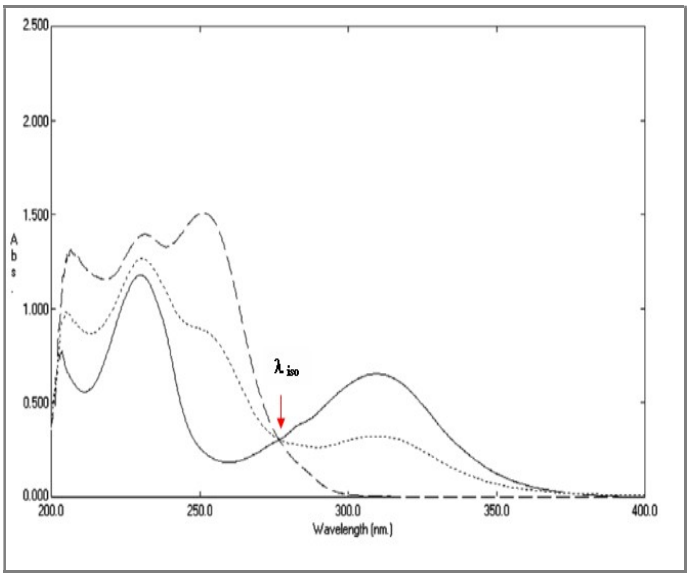

Fig 4. Zero order absorption spectra of 20 µg/ml of metronidazole, 20 µg/ml diloxanide furoate and (1:1) mixture containing 10 µg/ml of each using methanol as a blank [47].

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. A linear correlation was obtained between the absorbance values and the corresponding drug concentrations.

For example, In the mix of two drugs x and y. The absorbance of each drug can be calculated at any wavelength (λ) from the equation,

$A = A^{1\%}_{1cm}\, b\, c$……(12)

Therefore, for drug ***x***:

$A_x = A_x^{1\%}{}_{1cm}\, b\, c_x$ ……(13)

For drug ***y***:

$A_y = A_y^{1\%}{}_{1cm}\, b\, c_y$ ……(14)

Where *Ax* and *Ay* are the absorbance of ***x*** and ***y***, respectively; *Cx* and *Cy* are the concentrations of ***x*** and ***y***, respectively; and $A_x^{1\%}{}_{1cm}$ and $A_y^{1\%}{}_{1cm}$ are the absorbtivities when the trail length (b) is 1 cm and concentration is 1 g/100 ml for ***x*** and ***y***, respectively. If *Cx*= *C*y, and *Ax*= *A*y, this λ is named the isosbestic point, and at this λ

$A_{x,}^{1\%}{}_{1cm} = A_{y,}^{1\%}{}_{1cm}$ …… …(15)

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

$A_M = A_x^{1\%}{}_{1cm}\, cXm + A_y^{1\%}{}_{1cm}\, c_{yM}$ …… (16)

$A_M = A_x^{1\%}{}_{1cm}\,(c_{Xm} + c_{yM}) = A_x^{1\%}{}_{1cm}\,(c_{TM})$ ……(17)

Where *AM* is the absorbance of their mixture at isosbestic point and c_{Xm} and c_{yM} are the concentrations of

drugs ***x*** and ***y*** in the mixture, respectively, and CTM is the concentration of their mixture.

Therefore, we can conclude that,

$(c_{Xm} + c_{yM}) = (c_{TM})$ ……(18)

Thus, having the entire concentration of both drugs, if the concentration of 1 of them are often determined separately by the other method, the concentration of the second drug can be calculated by subtraction [48]. This method has was successfully applied for the simultaneous determination of several binary mixture, e.g., metronidazole and diloxanide furoate, [49] ezetimibe and atorvastatin, [50] and sitagliptin and metformin.

CONCLUSION:

From the above studies, it has been concluded that spectrophotometric techniques and simultaneous estimation methods can be used successfully for the determination of drugs either individually or in combinations. These drugs are recommended as medicines for treatment of various diseases. The advantages of simultaneous estimation method are fast, simple, less time consuming, accurate and sensitive for research purpose where no new method of estimation and analysis has been reported yet. This review also represents the history of simultaneous, applications of Fourier transform infrared spectroscopy, Nuclear magnetic resonance, Mass and the advancement of chromatographic and spectroscopic techniques. Hence, the simultaneous estimation of chemical entities using various analytical techniques are very much valuable for the future needs in pharmaceutical as well as other fields of investigation.

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