International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 933-940 www.ijprajournal.com ISSN: 2249-7781

A Review on combine study of UV spectroscopic and HPLC methods for simultaneous estimation

Diptee D. Marchande [1*], Shubhangi B. Sutar [1], Arati R. Rathod [1], Neha D. Desai [2], Sachinkumar V. Patil [2]

[1]*Department of Pharmaceutical Quality Assurance, Ashokrao Mane College of Pharmacy, Peth -Vadgaon, Dist-Kolhapur, Maharashtra, pin-416112, India*

[2]*Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Dist-Kolhapur, Maharashtra, pin-416112, India*

Date Of Submission: 01-06-2021 Date Of Acceptance: 14-06-2021

ABSTRACT: In pharmaceutical world, simultaneous estimation shows a vital desirability as it is very realistic. For multi component analysis,various ultra violet (UV) spectroscopic and chromatographic practices are used. Analysis of samples holding numerous components is a main task in modern analysis. In this estimation, spectroscopic and chromatographic techniques provide high grade of specificity and far along deliver high steps of assurance and selectivity. There are number of separation methods that can be use for identification purpose of the analytes of interest. Different UV spectrophotometric approaches are used in multicomponent study. This evaluation is primarily focused on simultaneous estimation techniques. Because of the some benefits like speed, specificity, accurateness, precision and comfort of modernization in this process, number of drugs in multi component dosage forms can be studied by high performance liquid chromatography (HPLC) method.This analysis also gives material regarding several platforms involved in growth and authentication of HPLC method. Method development and validation in HPLC shows main characters in new discovery, expansion and production of pharmaceutical drugs. These multi-component preparations are gaining attention due to better patient suitability, amplified effectiveness, multiple action, low side effects and quicker relief. And so, it is desired that these preparations meet all-inclusive morals related to their excellence, safety and ability. This can only be possible if diverse analytical practices are offered for their determination.

KEYWORDS: Simultaneous estimation, Spectroscopic methods, HPLC, Method development, Validation.

I. INTRODUCTION

Analytical chemistry is related to study of quantification, separation and chemical extracts identification of herbal and synthetic ingredients constituted byone or extra mixtures. Analytical chemistry is separated into two major classes, a qualitative assessment thatis identification of chemical extracts exists in the sample, whereas quantitative assessment is the mass of compound within the material(the sample)[1].In formulations, the analytical system plays the significant role in identification of physical as well as chemicalassets of the formulations [2].In currentage, marketplace is flooded with severaldosageforms. The present multicomponent preparations due to patient suitability, increasedeffectiveness, multipleact and fasterrelief are gaining attention. Therefore, it is desired that these preparations meet all values related to their quality, safety and efficacy and this can only be possible if they are analyzed by different approaches [3].Various analytical techniques can be applied for simultaneous estimation including; spectrophotometry,chromatography and electrophoresis. Spectrophotometric methods and high performance liquid chromatography (HPLC)process for simultaneous determination are highlighted in this review [4].

Ultra violet spectroscopic methods

Spectrophotometric methods areleading instrumental methods which are offeredtowardsdrug analyst. Measuring the interaction of an electromagnetic radiations with sample in quantized form are the basics of spectroscopic methods. There are number of spectrophotometricmethods which are use in pharmaceuticalsphere for the study of active pharmaceutical ingredients (API) and pharmaceutical constituents [2].

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1) Simultaneous equation method

If sample holdstwo absorbing drugs like x and yall of which absorbs at λ max of the further, it may likely to regulate equally both drugs by this technique. The data required is

a) The absorptivitiesat$\lambda_1$and $\lambda_2$of x, $ax_1$and ax_2correspondingly
b) The absorptivities at $\lambda_1$and $\lambda_2$of y,$ay_1$and ay_2correspondingly
c) Diluted samplesabsorbance at$\lambda_1$and λ_2, $A_1$and A_2individually

In sample,Let C_X and C_Y be concentration of drug x and y. Twocomparisons are built on the details that at λ_1, the absorbance of combination is the total of the separate ofxandy absorbance.

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

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

For measurement in 1cm cells, b=1cm, rearrange Equation(2),

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

Replacing for C_y in Equation (1) and after reorganizing,

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

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

Using directly above two equations,the componentxand component yconcentration in sample combination can be determine [4].

2) Absorption ratio method

According to this technique, theproportion at any dualwavelengths for a material of absorbance, which obeys Beer's law, whichisindependent constant value of concentration and the path length[9].This constant, is termed as "Hufner's Quotient" or Q value. The technique includes the absorbance capacity at two wavelength where one belongsto the λ max of one of constituents (λ_2) and second being wavelengthof two constituents of equivalentabsorptivity(λ_1), which is called as the iso-absorptive point.

Concentration of every component can be designed by using mathematical calculations,

$$C_x = (Q_m - Q_y / Q_x - Q_y) * A / a_1 (6)$$

$$C_y = (Q_m - Q_x / Q_y - Q_x) * A / a_2 (7)$$

Where,$C_x$and$C_y$are concentration of x and y respectively, A standsfor sample absorbance atiso-absorptivitywavelength and$a_1$and a_2 are for the absorptivities of xas well asy atiso absorptive wavelength[3].

Where,

$$Q_m = \frac{\text{Absorbance of sample solution at } \lambda_{max} \text{ of one of the components } (\lambda_2)}{\text{Absorbance of sample solution at isoabsorptive wavelength}}$$

$$Q_x = \frac{\text{Absorptivity of x at } \lambda_{max} \text{ of one of the components } (\lambda_2)}{\text{Absoptivity of x at isoabsorptive wavelength}}$$

$$Q_y = \frac{\text{Absorptivity of y at } \lambda_{max} \text{ of one of the components } (\lambda_2)}{\text{Absoptivity of y at isoabsorptive wavelength}}$$

3) Derivative spectroscopy

Derivative spectroscopy is innovative spectrophotometric method. It is established on derivative spectra which are produced from parent zero order. Derivative spectra yield a typical profile where delicate changes of gradient and curvatures in the normal spectrum are detected as distinctive bipolar functions. First derivative signifyies the gradient and use to detect hidden peaks.However,the second order and greater even order derivatives are potentially more valuable in analysis. Derivative spectrophotometry has initiate huge application in multicomponent samples analysis. The achieved derivative spectra produce a more typical profile in dissimilarity to the parent one which is new maxima and minima appeared and point where derivative spectra crosses the X-axis [5].

Advantages

1. It improves resolution allowing identification of analyte.
2. It removes baseline shift effect arising from apparatus.
3. It removes scattering properties present in turbid solution [3].

The drawback for this method is its low reproducibility which is caused by following reasons:

1. Dependence on instrumental parameters.
2. Non-robust properties of the derivatisation parameters.
3. Lack of homogeneous protocol of optimization the parameters of method[5].

4) Dual wavelength method

This methodenables analyzing a constituent in existence of an interfering component by measuring the variationamong absorbance of two points in the mixture spectrum [4].These method require two wavelengths in which one wavelength is generally selected such that one drug displays maximum absorbance whereasanother one shows significant absorbance[3].The expediency of this data processing is to compute the

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Volume 6, Issue 3 May - June 2021, pp: 933-940 www.ijprajournal.com ISSN: 2249-7781

unknownconcentration in a mixture containing both the component of interest and an interfering component done by the appliance of absorbance variance between two points on the combination spectra. This is directly associatedto concentrationof interest,where the interfering components shows similar absorbance at same time as components of interest is independent. The pre-requisite forthis method is hard which is selection of such two wavelengths, where one will show same absorbance and another one will shows variancesin the absorbance with concentration[6].

5) Area under curve method (AUC)

This method includes estimationof integrated value betweenabsorbance of two selected wavelength like λ_1 and $\lambda_2$on behalf offirst andlast curve section[7].At both selected wavelengths, this method is estimated which was from overlainspectrum of drugs. And their analysis was completedby Cramer's rule and matrix method. Consider twocomponents P and Qin mixture. Following informationis obtained from two spectra.

$AUC^P \lambda_1 - \lambda_2$: Thisis for component P at wavelength$\lambda_1 - \lambda_2$

$AUC^P \lambda_3 - \lambda_4$:This is for component P at wavelength$\lambda_3 - \lambda_4$

$AUC^Q \lambda_1 - \lambda_2$:This is for component Q at wavelength$\lambda_1 - \lambda_2$

$AUC^Q \lambda_3 - \lambda_4$:This is for component Q at wavelength$\lambda_3 - \lambda_4$

At similar wavelength range, the overall area under the curve at particular wavelength series is equal to quantityof the single[3].

6) Difference spectroscopy

This technique is forquantitative purpose of analyte of the similar analyte using an equimolar solution as reference but in a different physicochemical situation. In this method, isolationof analyte from another constituent of combination or other UV active analyte existing in mixture, sample is achieved. Physicochemical environments altered changes in pH and heat. The necessity of this technique is only the sample under study exists in different organic forms having unlike absorbance standards. Thistechnique is frequently used in estimation o f differentdosage forms[8].The selectivity and precision of sample containing interferents might be enhanced by the difference spectrophotometry method. Calculated value is absorbancedifferenceamongtwo samemolar solutions inunusual chemical formof analyte which display different spectra characteristics.The principles for applying this method:

1-Reproducible variations may be presented by the adding together one orextra chemicals.

2-Interferingsubstances absorbance is not changed by those chemicals.

A substance whose range or spectrum is unchanged by change of pH may be determined by this method[9].

7) Geometric correction method

A quantity of calculated trial have been established which reduces the background unrelated absorption that might be existing in sample of biological source. The speciality inthis process is the three point's geometric processwhich might be useful if the unrelated absorption is linear on three selected wavelength[9].

8) Orthogonal polynomial method

Orthogonal polynomial function technique is a mathematical approachuse for elimination of irrelevant absorption. This method supports thevariations in the modeof spectra in a componentmixture. The absorption rangecan represent in word of orthogonal function then influence to the coefficient of givendegree builton the formof spectrum and concentration. Though it is a potential method for analysis of multicomponentsamples, the method includes complex calculations to choose the correct combination of degree of polynomial, number of point in spectrum, interval among the point and optimization of these parameters[10].

HPLC Method

HPLC is finestprocess of choice for analyzing various natural and synthetic mixtures. Types of HPLC's used for simultaneous assessment are likenormal phase,reverse phase,sizeexclusion,ion exchange and bio-affinity HPLC[2].HPLC is the accurate analytical method use for quantitativeand also for qualitativestudy of drug product and its stability[11].Analytical method is significant part in the growth of pharmaceuticals. These approaches areuse to make sure the uniqueness, purity, potency, & performance of products.The goal line behind H PLC isto split and quantify the active drug,all synthetic material,impurities and degradants.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 933-940 www.ijprajournal.com ISSN: 2249-7781

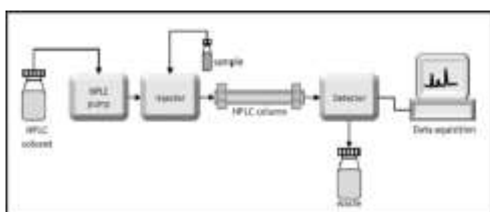

Figure 1. Flow Diagram of HPLC

Method development steps of HPLC are :

1. Drug molecules physicochemical assets.
2. HPLC conditions set up.
3. Sample solution preparation
4. Method Optimization
5. Method validation[12].

Phase of HPLC

1) Normal phase

Thispoint involve mobile phase asnon-polarand stationaryphase generally as polar solvents. In this phase mostly depending on polarity, analyte is separated. Absorption strength and the communication amongthe polar analyte plusstationary partrise elution time. Therefore, this mode is not mostly use for pharmaceutical application as most of drugs are naturally polar[13].

2) Reverse phase

It is widespread type for analytical as well as preparative separation of compound. In this approach non-polar stationary phase hydrophobic packing containing octyl functional assemblyfused with silica gel, where polar group eluted first and non –polar retain for longer period[14].Low molecular weight sample is essentially capturedusing RP-HPLC[13].

3) Size exclusion

In Size-exclusion, components are divided on basis of their size and also on molecular weights.Basically it is used when compounds in mixture have molecular mass difference of at least 10 %. In this method small molecules last for long time than large ones which elute first [14].

4) Ion-exchange HPLC

It is built on exchange equilibria among ions on high molecular mass solid and ions in solution. Some natural ion exchangers like zeolite and clays are been identified and used for number of decades[15].

5) Bio Affinity HPLC

Bio affinity is a category of absorption chromatography. It is built on the superior ability of biologically active elements to bind exactly and reversibly complementary materials. They are usually called ligands [16].

Steps in Method Development

1) Drug molecules physicochemical assets

Polarity helps an analyst tomake a decision aboutthe solvent and mobile phase composition .The solubility of molecule is always based on polarity. Polar and nonpolar liquid should not combine.Diluents are selected generally on fact of the solubility.pH and pKa alwaysplays asignificant role in drug constituents. The acidity or else basicity of materialare defined mostly by pH unit. Sharp peaks are detected by selecting suitable pH in favor of ionizable analytes. Aqueous solution acidityis calculated by the absorption of $[H_3O^+]$ ions. Therefore, theabsorption or concentration of hydrogen ions is indicated by pH of solutions[11].

2) HPLC conditions set up

A buffer is apartlyneutralisedacid. Salts similar to Sodium citrate or lactate are generally used to partlyneutralise the acid[17].

1. Buffer selection

Buffer selection is generally governed by desired pH (Table 1). Table 1 Shows HPLC buffers with pKa values and useful pH range.

Broad consideration for buffer selection are :

1. Phosphate remains more soluble in methanol/water.
2. Some of the salt buffers are mostly hygroscopic.
3. Ammonium salt is normally extra soluble in organic mobile phases.
4. Microbial growth rapidly occurs in buffered mobile phases that have no organic modifier.
5. Organic buffers supposed to be use at pH superior than 7.
6. Ammonium bicarbonate bufferare generally stable for24 -48 hours.
7. Buffers must be always filtered through 0.2-µm filter.
8. Mobile phases aresupposed to be degassed[14].

Table 1: pka and pH for HPLC buffers

Buffer	pKa	Useful pH Range
Ammonium acetate	4.8 9.2	3.8-5.8 8.2-10.2
Ammonium for mate	3.8	2.8-4.8

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 933-940 www.ijprajournal.com ISSN: 2249-7781

	9.2	8.2-10.2
Ammonium hydroxide or ammonia	9.2	8.2-10.2
KH2PO4 or K2PO4	7.2	6.2-8.2
KH2PO4/ phosphoric acid	2.1	1.1-3.1
Potassium Acetate/ acetic acid	4.8	3.8-5.8
Potassium format or formic acid	3.8	2.8-4.8
Trifluoroacetic acid	<2	1.5-2.5
Tri-K-Citrate/hydrochloric acid 1	3.1	2.1-4.1
Tri-K-Citrate/hydrochloric acid 2	4.7	3.7-5.7
Tri-K-Citrate/hydrochloric acid 3	5.4	4.4-6.4

2. Buffer concentration

Generally, for small particles buffer concentration up to 10-50 mM is suitable.U sually, buffer should not be high than 50 %organic.Phosphoric acid plusits potassium or sodium salts are ordinary buffers for reversed-phase HPLC[12].

3. Selection of detector

Detectors are mostvital part wherechoice of detector based on the chemical character of analytes,detection limit , potential interference, availability andrate of detector[13].Few commercialdetectors are ultraviolet detectors, fluorescence detectors, refractive index, mass spectrometry detectorsand electrochemical detectors[11].UV-Visible detector is adaptableand offersthe sensitivity. Photodiode Array offers superior optical detection designed for preparative HPLC or liquid chromatography .Refractive Index offer goodsensitivity; constancy which creates this detector the supreme solution for study of components, Multi-Wavelength Fluorescence Detector offers immensecompassion and selectivity[18].

The detectors are considered to include certain properties like:

1. They should be non-reactive to sample.
2. They should be non-destructive to sample.
3. Consistentand reproducible detection.
4. Should include good sensitivity[19].

4. Column selection

Column selection significant step in HPLC.Theexpansion of a ruggedness and reproducible process is impossible without stable and high performance of column . Column size, silica properties and bonded stationary phase uniqueness arethe majorones[19].Stationary phase is introduce to matrix by reacting chloro silane with hydroxyl set. Generally, the expected history of stationary phase hasmaximumoutcome on capacity aspect, selectivity, effectiveness and elution. There are more than few types of matrices like silica, polymers and alumina. Silica is mostlyused matrixwhere silicamatrix is generally tough, easily derivatized and does not be inclined to condense under pressure. Only shortcoming of silica is, it will dissolveonly above pH 7. To create a stationary phase, free silanols are reactingwith chlorosilane to commence the non-polar surface. The left over free silanols can interrelate with analytes which cause peak tailing[17].

5. Mobile Phaseselection.

Mobile phase compositions always acta significant function in RP-HPLC separation. Acetonitrile, methanol and tetrahydrofuran are normallyuse solvents in RP-HPLC. Mixture containing water and acetonitrile is the finest choice for mobile phase . Selection isbasically built on the ionogenic environment and the hydrophobicnature ofcompoundsin the mixture. Mobile phase will protonate free silanols on column and decrease peak tailing. Acidic analytes with suitably low pH in buffer will be remaining uncharged with increasing retention. On the other side, neutral compounds at high pH will more retained and acidic ionized compound will elute first. The most employed buffer is phosphate buffer. Phosphate buffers pH can be effortlesslyadjusted by usingbasic phosphate salts. However, phosphatesalts must be filtered with 0.22µm filter paper to remove insoluble particles . Methanol and Acetonitrile are most broadly employed organic modifiers. This is dangerousbelongingfor RP-chromatography ascolumn elution is monitorby UV detectors. Acetonitrile is use exclusivelywhile separating peptides[21].

Isocratic and Gradient Separations

Isocratic mode holdstable eluent work; which means balancecircumstances and the real velocity moving during columnis stable. As peakability is low,the component willretainoncolumn results in wider resultant peak. Gradient mode considerably increasesthe separation control mainly due to raise of the apparent efficiency means reduceof peak width.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 933-940 www.ijprajournal.com ISSN: 2249-7781

Peak width depends on rapidityof eluent composition variation. Whether a gradient or isocraticmodewould be essential, an initial gradient run is carry out and the proportionamong the total gradient moment and the variationin gradient time among first and last component are calculated. The estimate ratio is<0.25, then isocratic would be adequate. Only if ratio is >0.25, gradient would be adequate[20].

3) Sample solution preparation

The motive behind sample preparation is sample aliquot thatiscomparatively free of obstructions,will not harm columnand well-matched with the proposed HPLC process that issamplewill dissolve in mobile phase without disturbing sample retention or resolution. Sample preparation start at the tip of gathering;extend to sample inoculationonto column. All these operations willstructureavitalpiece in sample preparationplus have a seriousresulton validation parameters. The sample supposed to bedissolve in mobile phase. Butif sample does not dissolve due to reliability or solubility problemthen formic acid, acetic acid or saltscan be added to increase solubility[22].

4) Method Optimization

The condition in experiment mustbe optimized to acquire desired separation and sensitivity after receiving proper separations. Mobile phase optimization has parameters at all times considered as much as easier and convenient than stationary part. Primary control factor of liquid chromatography methods are the different part of mobile phase determining acidity, solvent strength, gradient, flow rate,temperature,sample amount, injection volume and diluenttypes. This is use to locate the balance among resolution and analysis time after satisfying selectivity has been achieved. The parameters involved contains column size, columnprotective material,particle size and flow speed, which may be alteredwith nodisturbing capacity factor or selectivity[23].

5) Method Validation

Validation isatechnique which is create by laboratory study thataperformance of the process meets the necessities for the proposed analytical function. Performance characteristics will be stated in term of analytical parameters[24]. The purpose of validation is always to produce proof that this method will do what it is supposed to do accurately, reliably and consistently[1].

Accuracy

Accuracy represents closeness of an agreement among the valuesrecognized asa true value / else knownas reference value. N o measurement procedure ispractically ideal, therefore true value cannot be exactlyknown in any particular measurement. By analyzing a sample with known concentration the known true value can be estimated [25].

Precision

Precision represents closeness of agreementamong a chain of measurement obtained from various sampling of similaruniform sample below prescribed conditions.It might be calculated at three stages as

a) Repeatability
b) Intermediate precision
c) Reproducibility[26].

Repeatability is the precision in the similar situations over a small interval of a time. Itis also labelled as intra-assay precision. Intermediate precision statesin laboratories variations: different days, unlike analyst,different equipments. Reproducibility is the precision obtained by analysis between laboratories [25].The precision can also determine by intraday and interday precision. In intraday, the similarconcentration of samples was injected six times in the identical day while in interday, injecting six solutions of similar concentration for six dissimilar days in a week[24].

Linearity

Linearity is ability of analytical process to gain a certain response that is directly relevant to amount of analyte in sample. If method is linear then the test outcome are well-defined mathematical conversion relative to concentration within a specified range. Linearity is basically articulated as the confidence limit around the angle of regression line[27].

Robustness

Robustness is describe as the assess of ability of analytical technique to remain modest by minor but deliberate differences in process parameters(e.g. pH,mobile phase,heat and instrumental setting) and also delivers asign of its reliability. Evaluation of robustness is an organized procedure of varying the parameters and determining the effect onthe technique by monitoring system suitability[11].

Range

Range is define as limit between upper and lesser concentration in sample,with the concentrations for those whose appropriatepoint of accuracy, precision and linearity has been recognized that an analytical process has an appropriate rank of accuracy, precisionplus linearity[28].

Detection Limit andQuantitation Limit

Detection limit is an lowest quantity of an analyte which is not necessarily quantified as atrue value but is able todetect in sample. Quantitation limit is the lowermostquantity of analyte which will be capable to be determining quantitatively with an appropriateprecisionas well asaccuracy. Quantitation limit is a factor of quantitative assay for stages of compounds in the sample mediums[29].

Specificity

Itis an ability to estimate the analyte in the existence of constituents like impurities and degradation products. Specificity measures only the desired constituent without interfering from other species that might be present, separation is not essentially required [12].

Systemsuitability

System suitability an essential part of systematic procedures. The examination is builton idea that the apparatus, electronics, systematic operations and sample to be analyzerepresenta vital system that is able to be evaluated[29].These factors can be measure practically to deliver a quantitative suitability report such as separation, resolution, capacity factor, theoretical plates, tailing factorand standard deviation [27].

II. CONCLUSION

From the over study, it has been concluded that the analytical techniques can be used productively for the estimation of drug combinations. The compensation at the back of performing the simultaneous estimation is quick, easy, less time consuming, precise and sensitive for research purpose where no new technique of estimation and analysis has been reported yet.Hence, simultaneous evaluation using a variety of analytical technique is greatly expensive for the future requirements in pharmaceutical world. A general and very simple approach for the HPLC process development designed for the separation of compounds is discussed. Therefore, more development and advancement is needed in these technique which will be cooperative to analytical and bio analytical researchers in developing strategies for new analytical method and high productivity outcome in the laboratories.

ACKNOWLEGDMENT

The Authors are grateful to Ashokrao Mane College of pharmacy, peth vadgaon for providing all the facilities.

CONFLICT OF INTEREST

There is no any conflict of interest by authors to declare regarding this investigation.

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