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DEVELOPMENT AND VALIDATION OF HPLC AND UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE PHARMACEUTICAL DOSAGE FORM AND BIOLOGICAL FLUID –REVIEW

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ABSTRACT
Many methods are emerging for the method development and validation of pharmaceutical dosage form and biological fluid out of which High performance liquid chromatography method and UV & Visible method are used today. HPLC is a powerful analytical technique which is able to detect, separate and quantify the drug. A number of chromatographic parameter were analysed to optimize the method like sample pretreatment, choosing mobile phase, column, detector selection. This review article helps to understand nature of the drug moiety thereby method development is possible.

KEYWORDS: Method development, validation, instrumentation.

INTRODUCTION

Analytical method development and validation play important role in the discovery, development and manufacture of pharmaceuticals. Drug 'analysis' means identification, characterization and determination of drugs, drug assay refers to determination of drugs in mixtures such as dosage forms and biological fluids.

1. Analysis of drugs in bulk and dosage form

Bulk drugs are obtained by chemical synthesis, biosynthesis, isolation from plants or animals or biotechnological source. The elevation of dosage forms varies with type of dosage form. It includes physical appearance, strength, content uniformity, active ingredient etc. various components present in dosage forms, including the presence of additives, impurities and multiple drug entities such as vitamins present are some of the challenges encountered during the development.

2. Analysis of drugs in biological fluid

The drugs with narrow therapeutic indices require bioanalytical methods to estimate from biological fluids and to study the pharmacokinetic parameters during therapy. Complexity of body fluids and tissues, protein binding, drug analogues and metabolites are the interference in estimation of drugs from biological samples. The contamination of samples, drug stability and adsorption of drugs on the surfaces are the additional challenges.

Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and biological samples are spectrophotometer and chromatographic methods have gained the significance in recent years.

Sample pretreatment option

Obtain representative samples-------proper storage condition till use-----preliminary sample processing------ weighing and volumetric dilution -----processing method-----particulates removal by means of filtration, solid-phase extraction and centrifugation----Sample extraction ----Derivatization.[1]

Method optimization

Method development in pharmaceutical analysis plays a very important role in the new drug design and development, to ensure quality control of drug and to study stability till expiry. Analytical method development should be performed to the extent that is sufficient for its intended purpose. It is essential to know the molecular structure of the analyte during the method development process, as this will facilitate the identification and rectification of potential degradation impurities.

Modern analytical instrumentation, like high performance liquid chromatography with versatile detectors, allows simplified procedures to be employed. This enables verification of analysis procedure accurately and consistently will deliver a reliable

measurement of an active ingredient in a compounded preparation.[2] Successful completion of method development using preferred instrument, the method will be validated. Documentation starts at the very beginning of the development process, a system for full documentation of the development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database. Using the information in the literatures and prints, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analytes and samples. Development of a new HPLC method involves selection of best mobile phase, efficiency detector, column, stationary phase are to be considered.

I. Chromatographic methods

The technique chromatography was originally developed by the Russian botanist M.S. Tswett in 1903.[3] The word chromatography is derived from Greek word 'Chroma' meaning 'color' and 'graphein' meaning to write.[4] Chromatography is probably the most powerful analytical technique available its power arises from its capacity to determine quantitatively many individual components present in mixture by single analytical procedure. Two mutually immiscible phases are brought into a mobile phase is carried along through a column containing a distributed stationary phase. Species in the sample undergo repeated interactions between the mobile phase and the stationary phase, the sample components are gradually separated into bands in the mobile phase. The least retarded component emerges first, the most strongly retained components elutes last. Among other chromatographic method HPLC is used widely.

High Performance Liquid Chromatography

The excellent and most wonderful technique of High Performance Liquid Chromatography is an outcome of various theories and instrumentation. In late 1960s and 1970s HPLC is one of several chromatographic methods for the separation, identification, quantification and purity of an individual components of chemical mixtures. HPLC is applied for the determination of related substances, foreign substances, purity of drug, identification, drug assay and stability studies.[5] HPLC method involves sample preparation, selection of best mobile phase, column, efficiency detector, stationary phase are to be considered.

HPLC has numerous advantages like

- Simultaneous Analysis
- High Resolution
- High Sensitivity
- Good repeatability
- Small sample size
- Moderate analysis condition.
- Easy to fractionate the sample and purify.

1. Sample preparation

Sample preparation is an essential part of analytical cycle in HPLC. Aim of sample preparation is to provide a reproducible and homogenous solution suitable for injection into column. It should be 1. Free from possible interference 2. should not damage the column 3. Should be compatible with proposed HPLC method 4. Solvent used for preparation of the sample should be compatible with mobile phase and should not significantly affect the retention and resolution of the analyte.[6]

Type of samples and extraction procedure:

Solids

1. Solid-liquid extraction
2. Soxhlet extraction
3. Homogenization
4. Blending
5. Sonication
6. Dissolution
7. Accelerated solvent extraction
8. Extraction with microwave
9. Supercritical fluid extraction

Liquids

1. Solid-phase extraction
2. Liquid-Liquid extraction
3. Dilution
4. Filtration
5. Evaporation
6. Distillation
7. Concentration
8. Lyophilization
9. Centrifugation
10. Sedimentation

Samples

The samples for testing must be representative of the production lot.

Bulk drug

Solvent or combination of solvents must be selected so that analyte is soluble and the solvents are compatible.

Tablets

It involves grinding; there is possibility of physical separation of active ingredients fromother tablet matrix, particularly of active ingredients to the total mass of the dosage form.

Liquid Dosage form

Ointment, creams

Suspended in acetone or mixture of chloroform – methanol or THF-methanol.

Suspension/lotions

Either directly diluted and filtered or partitioned between water and immiscible solvents such as chloroform.

Syrups/elixirs
Samples are directly diluted with water or water miscible solvents such as methanol or require pH adjustments followed by extraction with an organic solvent.

Gels
Dissolved in 0.001N Hydrochloric acid or dissolved in alcohol or partitioned between solution of various buffers and chloroform.

Suppositories
Diluted with 0.001N Hydrochloric acid and extracted with chloroform.

Aerosols
Entire contents are removed by boiling and diluted with isopropanol –acetic acid (1000:1) and filtered.[7]

2. The mobile phase
Variation in sample retention for optimum separation is achieved exclusively by changes in the composition of the mobile phase. The eluting power of the mobile phase is determined by its interaction with its stationary phase, rention time. Initially a solvent can be selected by matching the relative polarity of the solvent to that of the sample components. A solvent is chosen to match the most popular group in the sample. Alcohol for the hydroxyl group and ketones or acetates for the carbonyl group can be chosen. If sample elutes too rapidly then a weaker solvent is substituted. If the sample does not elute in respected time then a solvent with high polarity is selected. Two solvents whose solvent strength parameters are known blended together in various proportions.[8]

Buffer strength & pH, buffer concentration, mobile phase polarity, viscosity, absorbance and additives are some of the parameters taken into consideration for optimizing method development. In reverse phase chromatography buffer is used as a mobile phase. Phosphate buffers prepared using salts like potassium dihydrogen phosphate, potassium hydrogen phosphate, sodium dihydrogen phosphate, sodium hydrogen phosphate etc. acetate buffers- ammonium acetate, sodium acetate etc. Acetic acid buffers prepared using acetic acid. Phosphoric acid buffers are prepared using phosphoric acid.

PH is defined as the negative logarithm to base 10 of the concentration of the hydrogen ion. $pH=-\log_{10}[H_3O^+]$. selection of proper pH for Ionizableanalytes leads to symmetrical and sharp peaks. the acidity of an aqueous solution is determined by the concentration of $[H_3O^+]$ions. The pH of a solution indicates the concentration of hydrogen ions cane be indicated as [H+] or its solvated form in as $[H_3O^+]$ whose value lies between 0 and 14.

Degassing of mobile phase is done to remove the air bubbles and dissolved oxygen in the mobile phase. The mass of gas dissolved in a given volume of solvent is directly proportional to the partial pressure of that particular gas in the vapour phase of the solvent. If the pressure of a particular gas is reduced over the surface of a solvent, the amount of that gas in solution will be reduced. Solvent degassing methods are of four types they are: Sparing, heat, vaccum and Sonication. There are some lists of mobile phase used based on the type of compound and modes of chromatography are.

Mode	Solvent type used	Type of compound used
Reversed Phase	Water/Buffer, ACN, Methanol	Neutral or non-ionized compounds which can be dissolved in water/ organic mixtures.
Ion-pair	Water/Buffer, ACN, Methanol	Ionic or Ionizable compounds
Normal Phase	Organic solvents	Mixtures of isomers and compounds not soluble in Organic/ Water mixtures.
Ion exchange	Water/Buffer	Inorganic ions, proteins, nucleic acids, organic acids.
Size exclusion	Water, Tetrahydrofuran, chloroform	High molecular weight compounds.

The number of experiments in which the mobile phase combination used is

1) Phosphate buffer and methanol
Phosphate is more soluble than in methanol/water combination. A number of experiments are done on this mobile phase combination.

1. Phosphate buffer and methanol (30:70v/v) is used for the stability indicating HPLC method for simultaneous estimation of Chlorozoxazone, diclofenac potassium and paracetamol in bulk and pharmaceutical dosage form.[9]
2. 0.03 MPhosphate buffer with pH 7.5and methanol (20: 80%v/v) use for the Analysis of Azithromycin and Its RelatedCompounds by RP-HPLC with UV Detection.[10]
3. 0.2M phosphate buffer PH 7.0 and methanol (30: 70%v/v) is used for the Development and Validation of Glibenclamide in Nanoemulsion Formulation by using RP-HPLC.[11]

2) Phosphate buffer and Acetonitrile
10mM Phosphate buffer and Acetonitrile (60:40%v/v) is used for the study of Stability indicating RP-HPLC method for analysis of Sitagliptin in the bulk drug and its pharmaceuticaldosage forms.[12]

1. 0.1N Phosphate buffer and Acetonitrile (50:50%v/v) is used for the simultaneous estimation of Nebivolol and Hydrochlorothiazide by HPLC.[13]
2. Phosphate buffer and Acetonitrile (55:45%v/v) is used for the simultaneous estimation of Ramipril,

Telmisartan and Hydrochlorothiazide by RP-HPLC.[14]

3. 0.025MPhosphate buffer and Acetonitrile (60:40%v/v) is used for the simultaneous estimation of Hydrochlorothiazide and Losartan Potassium by RP-HPLC.[15]
4. Acetonitrile: 50mM potassium dihydrogen phosphate buffer (pH 3.0, 70:30%v/v) is used for the simultaneous estimation of CefpodoximeProxetil and Clavulanic acid by RP-HPLC.[16]
5. 0.01M 5.5 pH phosphate buffer: Acetonitrile (60:40%v/v) and pH adjust to 5.5 with phosphoric acid for the estimation of Duloxetine Hydrochloride RP-HPLC.[17]
6. Phosphate buffer (pH 6.60) and acetonitrile (40:60 v/v) for the estimation of Chloroquine HPLC Methods.[18]

3) Methanol, water and glacial acetic acid

1. Methanol, water and glacial acetic acid (60:40:0.25v/v)is used for the estimation of Nalidixic acid and Metronidazole in tablet dosage form by simultaneous reverse phase HPLC.[19]
2. Methanol–water–aceticacid (20:75:5v/v/v) is used for the Simultaneous Determination of Caffeine, Theobromineand Theophylline by High-Performance Liquid Chromatography.[20]

4) Water and Methanol

1. Water: methanol (pH adjusted to 3.4 using orthophosphoric acid) is used for the simultaneous estimation of aspirin, isosorbide 5-mononitrate by RP-HPLC.[21]
2. Methanol: water (80: 20, v/v) and precise methods were developed and validated for the determination of fluvastatin sodium (Flu) in pure form, in presence of its acid degradation and in pharmaceutical formulations.[22]

5) Phosphate buffer, Acetonitrile and Methanol

1. 0.025M potassium dihydrogen phosphate pH 2.5: acetonitrile: methanol (40:55:5) used in RP-HPLC method development and validation for estimation of Voglibose in bulk and tabletdosage forms.[23]

2. 0.02M potassium dihydrogen phosphate buffer solution: methanol: acetonitrile (60:30:10v/v) used for the estimation Atenolol in tablets before and after expiry period by RP-HPLC.[24]

6) Acetonitrile, water and methanol

1. Acetonitrile, water and methanol in the ratio of 25:70:05is used for the quantitative estimation of Azathioprine in pure drug and its formulations RP-HPLC.[25]

3. Column selection

Columnis referred to as the heart of HPLC separation process. Stable high performance column is essential requisite for rugged and reproducible method. The components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent called stationary phase. The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte. Many different types of columns are available, filled with sorbents varying in particle size and in the nature of their surface. The use of smaller particle size packing materials requires the use of higher operational pressure as called "backpressure" and improves chromatographic resolution i.e. the degree of separation between consecutive analytes emerging from the column.[26] There are five prominent causes responsible for the deterioration of HPLC column.

- Loss of bonded phase
- Dissolution of column surface/packing
- Binding to column packing
- Pressure increase
- Column channeling.[27]

The liquid phase is covalently bonded to the supporting material which is silica or a silicone polymer. The silicone polymer bonded phases have the advantage not being eluted by the developing solvent and are chemically, hydrolytically and thermally stable. After the derivatization of a column with the desired stationary phase, the column is further reacted with chlorotrimethylsilane to end cap the remaining free silanols and improve the column efficiency. The commonly used column are Si (silica), C_1 (trimethyl), C_2 (Dimethyl), C_3 (propyl), C_4 (butyl), C_8 (octyl), C_{18} (octyldecyl), base deactivated silane(C_{18}) BDS phenyl, cyanopropyl (CN), nitro, amine. Commonly used ion pairing columns are C_3 (propyl), C_4 (butyl), C_5 (pentyl). This column is generally stable to hydrolysis than column with longer alkyl chains. Most widely used C_{18} (octadecyl) tend to be the most retentive for non-polar analyte. Examples are Zorbax SB-C_{18}, YMC-packODS and Luna C_{18}. Phenyl compounds are commonly used to resolve aromatic compounds examples are Zorbax SB-Phenyl, YMC-Pack Phenyl and Luna Phenyl-Hexyl. Nitro columns are polar and can be used for both normal and reverse used to increase the retention of –polar analyte. Examples are Zorbax SB-CN, Luna-CN, and YMC-Pack CN.

1. Validation of a stability indicating reverse-phase HPLC (RP-HPLC) method for the analysis of oseltamivir active pharmaceutical ingredient (API) utilizes Kromasil C_{18}, 5 µm, 250 mm×4.6 mm i.d. column at ambient temperature.[28]
2. RP-HPLC method development and validation for estimation of Voglibose in bulk and tablet dosage forms was carried out by RP-18e, Hibar RT column (250×4.6mm).[29]

4. Detector selection

The detector is to monitor the mobile-phase coming out of the column, which in turn emits electrical signals that are directly proportional to the characteristics either of the solute or the mobile phase. The advantage of using UV or Visible detector has high sensitivity, relative robust to temperature and flow rate change and compatible with gradient elution. Only compounds with UV or Visible absorption could be detected is the only disadvantage in using UV or Visible detector. Fluorescence detector has high sensitivity, selectivity and compatible with gradient elution. Disadvantage of using Fluorescence detector is greatly affected by solvent, pH, temperature, viscosity, ionic strength and dissolved gas. Refractive index detector responds to nearly all solutes unaffected by flow rate but not sensitive and could not be used with gradient elution. Conductivity detector responds to ionic compounds and suitable for ion chromatography and high sensitivity for low concentration range. Evaporative light scattering detector is called Universal detector because most compounds can be detected and compatible with gradient elution.[30] The detector is used based on the type of the compound to be detected they are.

Detector	Type of compound can be detected
UV-Visible &Photodiode array	Compounds with chromophores, such as aromatic rings or multiple alternating double bonds.
Fluorescence detector	Fluorescent compounds, usually with fused rings or highly conjugated planer system.
Conductivitydetector	Charged compounds, such as inorganic ions and organic acid.
Electrochemical detector	For easily oxidized compounds like quinines or amines
RefractiveIndex detector & Evaporative light scattering detector	Compounds that do not show characteristics usable by the other detectors, eg.polymers, saccharides.

i. UV-Visible detector

1. Development and Validation of HPLC Methods for Quantitative Determination of Chloroquine and Amodiaquine in Pharmaceutical Formulations using sodium benzoate as internal standard (IS) with UV detection at 242 nm.[31]
2. The estimation Atenolol in tablets before and after expiry period by RP-HPLC using UV detection at 275nm.[32]
3. RP-HPLC method was developed and validated for the determination of an anti-thrombocythemic agentanagrelide in pure and pharmaceutical formulationsusing UV-visible detection at 260nm.[33]

ii. Fluorescence detector

1. A reverse-phase HPLC and fluorescence detection280/340 nm method formeasurement of 5-hydroxytryptamine (serotonin) in Planaria.[34]
2. A highly sensitive and selective high performance liquid chromatographic fluorescence detection method has been developed and validated for the quantification of rivastigmine in rat plasma and brain Fluorimetric detection was used and excitation and emission wavelengths were of 220 and 293 nm, respective.[35]
3. Development and validation of a rapid HPLC-fluorescence detector200 nm/λem 300 nm method for simultaneous determination of venlafaxine and its major metabolites in human plasma.[36]

2. Spectroscopic methods

The sample is made to interact with a wide spectrum of wavelengths in a given zone of electromagnetic radiation giving rise to a collection of measurement signals as a function of wavelength is termed as a spectrumand terminology used is called spectroscopic. It is the most powerful tool available for the study of atomic and molecular structure and is used in that analysis of a wide range of samples.[37]

Ultra Violet and Visible spectroscopy

Ultra violet absorption spectra arises from transition of electron or electrons within a molecule or an ion from lower to a higher electronic energy level and the emission spectra arises from the higher electronic energy level tolower energy level. The UV & Visible region extends from 200 to 780nm. Ultraviolet and visible spectroscopic methods are based on the type of chromophores/functional group present in the drug moiety. Multiple component system are analysed by means of spectral isolation and are being used routinely, they are Simultaneous equation method, Geometric correction method, Absorbance method, Orthogonal polynomial method, Derivative Spectrophotometric method, Difference Spectrophotometric method and chemical derivatization method.[38]

1. Simultaneous equation method

Sample contains two absorbing drugs (A and B) each of which absorbs at the λmax of the other (λ_1 and λ_2), it is possible to determine both the drugs by the technique of simultaneous equation provided that certain rules apply:

The values required are:

(i) The absorptivities of A at λ_1 and λ_2, ax_1 and ax_2 respectively

(ii) The absorptivities of B at λ_1 and λ_2, ay_1 and ay_2 respectively

(iii) The absorbance of the diluted sample at λ_1 and λ_2, X_1 and X_2 respectively.

Cx and Cy be the concentrations of A and B respectively in the diluted sample.

Two equations are constructed based upon the fact that λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbances of A and B.[39]

$\frac{X_2/X_1}{Ax_2/ax_1}$ and $\frac{ay_2/ay_1}{X_2/X_1}$

1. Method employed for solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 265 and 257 nm, λ max for aspirin and paracetamol, respectively. The calibration curve was linear for both drugs in a concentration range of 2 to 64 µg/ml.[40]
2. First method based on solving of simultaneous equation using 228 nm (λmax of Gliclazide) and 234 nm (λmax of Metformine hydrochloride) as two analytical wavelengths for both drugs in mixture of Water and Methanol (60:40) solvent. Second method based on an equation of area calculation of curve at two wavelength resion (233 to 223nm and 239 to 229 nm). Linearity was observed in the concentration range of 2-24 µg/ml for Gliclazide and 2-14 µg/ml for Metformine hydrochloride.[41]
3. The method employed simultaneous equation method for analysis usingmethanol as a solvent. The two wavelengths 229.5 nm and 237 nm were selected for estimation of Glibenclamide and Metformin HCl respectively. Linearity was observed in the concentration range of 3-15 µg/ml and 2-10 µg/ml for Glibenclamide and Metformin HCl respectively.[42]
4. The drug obeyed the Beer's law [for rosuvastatin concentration range 1-10µg/ml and for fenofibrate concentration range 2-20µg/ml] and showed good correlation.[43]
5. The Method A employs estimation of drugs by simultaneous equation method using 250.0 and 238.0nm i.e. λmax values of Valsartan and Amlodipine respectively. Method B employs the estimation of drugs by Absorption Correction method (ACM) at 360.0 i.e. λmax values of one drug and 236.0 nm an isobestic wavelength. Valsartan and Amlodipine individually and in mixture follow Beer's law over the concentration range 5-30 µg/mL at all the selected wavelengths.[44]
6. For simultaneous equation method measurement of absorbance at two wavelengths, 210 nm and 238 nm, λ max of Ramipril and Amlodipine respectively. Beer's law obeyed in concentration range of 15–35 µg/mL and 5–25 µg/mL for Ramipril and Amlodipine respectively.[45]
7. The λmax values for Amlodipine Besylate and Lisinopril Dihydrate in the solvent medium were found to be 256 nm and 216 nm respectively. The systems obey Beer's law in the range of 10.0 to 70.0 mg/ml and 4.0 to 40.0 mg/ml with correlation coefficient of 0.9994 and 0.9996 for Amlodipine Besylate and Lisinopril Dihydrate respectively.[46]

2. **Absorbance ratio method**

In quantitative assay, two components are measured at two wavelengths. One is beingλmax of one of the components (λ1) and the other being a wavelength of equal absorptivities of the two components (λ2). Two equations are constructed for the method of simultaneous equation.

1. Cinnarizine and Dimenhydrinate show an isoabsorptive point at 267 nm in methanol. The second wavelength used is 252 nm, which is the λ-max of Cinnarizine in methanol. The linearity was obtained in the concentration range of 2-20 µg/ml for Cinnarizine and Dimenhydrinate.[47]
2. Prednisolone and 5-Amino Salicylic Acid shows their iso-absorptivepoint at 283 nm in ethanol and 0.1N HCl respectively. The second wavelength used is 302 nm which is the λmax of 5- Amino Salicylic Acid in 0.1N HCl. The linearity was obtained in the concentration range of 1-10 µg/ml for prednisolone and 5-Amino Salicylic Acid.[48]
3. In UV-Spectrophotometric method, estimation of Cilnidipine and Metoprolol succinate was carried out at 240 nm and 224 nm by Q-Absorbance ratio method. Absorbance uses the ratio of absorbance at two selected wavelengths, one which is an Iso-absorptive point and other being the λmax of one of the two components. From the overlay spectra of two drugs, it is evident that Cilnidipine and Metoprolol succinate show an Iso-absorptive point at 231 nm. The second wavelength used is 224 nm, which is λmax of Metoprolol succinate.[49]
4. The method involved Q-absorption ratio analysis using two wavelengths, with one being the of lamivudine (272 nm) and the other being the isoabsorptive point of both drugs (246 nm). Beer's law was obeyed in the concentration range between 5 and 30 µg/mL for both lamivudine and isoniazid.[50]
5. The first UV spectrophotometric method was a determination using the simultaneous equation method at 239 nm and 255 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 245 nm (isobestic point) and at 255 nm the maximum absorption of olmesartan medoxomil.[51]
6. In Method-I (Q-absorbance absorbance ratio) involves formation of Q-absorbance equation at two wavelengths i.e. 272.8 nm (isoabsorptive point) and 253 nm (λ max of). While, in Method-II (Multicomponent Mode of Analysis) involves the measurement of absorbance at two wavelengths i.e. 289 nm, λ max of PRH and 253 nm, λ max of Flunarizine Dihydrochloride.[52]

3. **Geometric correction method**

1. A three point geometrical correction method was used for quantification of Metaxalonefrom biological samples (egg albumin). The method was

developed at three different wavelengths 260nm, 270.80nm, 282 nm for Metaxalone by using egg albumin as biological media. The proposed method showed linear relationship at three wavelengths in the range of 30-180 µg/ml with regression coefficient of 0.994, 0.995, 0.993.[53]

2. The first UV derivative spectrophotometric method was a determination using the simultaneous equation method at 239.0 and 256.0 nm over the concentration range 15 and 15 µg/ml for amlodipine besylate, Olmesartan Medoxomil, respectively.[54]

4. Orthogonal polynomial method

1. UV spectra of 6 µg/mL solution of Cefixime in distilled water and 6 µg/mL solution of Moxifloxacin in distilled water were recorded separately between 200 nm and 400 nm. for estimation of Cefiximewas 6 point quadratic polynomial covering the wavelength range from 221.80 to 251.80 nm. The wavelength range from 304 to 314 nm. Orthogonal polynomial function method for Moxifloxacin.[55]

2. Mesalazine and Prednisolone were found to have absorbance maxima at 320 and 246 nm respectively in phosphate buffer (pH 7.4). Boththese drugs obeyed Beer's law in the concentration range of 2-20 µg/ ml. The high values of correlation coefficients (r2) indicated good linearity of calibration curve for both the drugs. Sandell's sensitivity µg/cm2/0.001/abs unit of Mesalazine and Prednisolone.[56]

3. Pioglitazone was freely soluble in Methanol and 0.1 N NaOH. 0.1 N NaOH was choosen as a solvent. The drug has maximum absorbance at 231.5 nm. The optical characteristic of drug was found to be Beer's law limits 15- 65 µg/mL, Correlation coefficient is 0.9983.[57]

5. Derivative Spectrophotometric method

Derivative Spectrophotometric method is one of the most extensively employed technique in which zero-order spectrum are transformed into derivative spectrum to eliminate interference of background or matrix.

1. 250 nm of Lacosamide for first order derivative spectra was selected for the analysis in which linearity range of 5-50 µg/ml. Straight line equations were obtained from mean of five sets and the calibration curves. The Correlation Coefficients (r2) for Lacosamide was found to be 0.9980.[58]

2. The first, second and third order derivative spectra are characterized by a few peaks.Absorbance and derivative absorbance values of the spectra at 205 nm (A), 213 nm (D1), 219 nm (D2) and 223 nm (D3) were measured for the determination and evaluation of benazepril hydrochloridestability.[59]

3. The quantitative determination of ezetimibewas carried at 233 nm and the linearity range was found to be 6-16 µg/ml. For the first, second and third derivative spectrophotometric methods the drug was determined at 259.5 nm, 269 nm and 248 nm with the linearity ranges 4-14 µg/ml, 4-14 µg/ml and 4-16 µg/ml.[60]

4. The first-order derivative spectra were obtained for estradiol valerateat N = 5, $\Delta\lambda$ = 4.0 nm and determinations were made at 270 nm. The method showed specificity and linearity in the concentration range of 0.20 to 0.40 mg/mL.[61]

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