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A Nover View on Validation of UV visible Spectros Copy

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ABSTRACT

UV-VIS Spectroscopy is the term used to test the various types of solvents and substances in an analysis.In particular, small-scale enterprises typically prefer spectroscopy, as the cost of equipment is lower andmaintenance issues are limited. The analysis approach is based on the calculation of the absorption ofmonochromaticlightinthenearultravioletdirection ofaspectrumbycolourlesscompounds(200400nm).The pharmaceutical analysis requires the necessary procedure for the determination of the "identity,intensity, consistency and purity of such compounds. Validation is the process of determining a method'sperformance features and limitations and defining the factors that which alter these characteristics and towhatextent.Thispaperspresentsasystematicanddetailedvalidationoforganiccompoundswiththemolarabsorptioncoefficientin UV-visibleregion.

KEYWORDS-ElectromagneticSpectrum,Ultraviolet,Visible,ElectronicTransition,Validation

I. INTRODUCTION OF ULTRA VIOLET/VISIBLE SPECTROS COPY

1.1. Spectroscopy

Spectroscopyisthestudyofthepropertiesofmatterthroughitsinteractionwithvarioustypesofradiation(mainlyelectromagneticradiation)oftheelectromagneticspectrum.

1.2. Spectrophotometry

Ultraviolet(UV)andVisible(VIS)spectrophotometry hasbecomethemethodofchoiceinmost laboratoriesconcernedwiththeidentificationandquantificationoforganic andinorganic compounds across a wide range of products and processes. Applied across research,quality,andmanufacturing,withcontinuingfocusonlifescienceandpharmaceuticalenvironments, they are equally as relevant in agriculture, animal husbandry and fishery,geologicalexploration,foodsafety,environmentalmonitoring,andmanymanufacturingindustries to nameafew.

Modern spectrophotometers are quick, accurate, and reliable. They require only smalldemands on the time and skills of the operator. However, the non-specialized end-user whowantstooptimizethefunctionsoftheirinstrument, andbeabletomonitoritsperformancewillbenefitfromtheappreciationoftheelementaryphysicallawsgoverningspectrophotometry,aswellasthebasicelementsofspectrophotometer design.

1.3. Ultraviolet-VisibleSpectroscopy

Ultraviolet and visible (UV-Vis) absorptionspectroscopyis the measurement oftheattenuationofabeamoflightafteritpassesthrough asampleorafterreflectionfromasamplesurface.Thevisiblespectrumrangesfrom400nmtoabout800nm.The colorweseedependsonwavelengthThecolorofasubstanceisdeterminedbywhichcolor(s)oflightitabsorbsandwhich color(s) it transmits or reflects (the complementary color(s)). Color is an importantproperty of a substance. The color of matter is related to its absorptivity or reflectivity. Thehumaneyesees thecomplementarycolor tothatwhichisabsorbed.

1.4. OrigenCharacteristicsofUV-VisibleSpectrum

UV-VIS spectrum results from the interaction of electromagnetic radiation in the UVVisible region with molecules, ions or complexes. It forms the basis of analysis of differentsubstancessuchas,inorganic,organicandbio

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molecules.Thesedeterminationsfindapplicationsinresearch,industry,clinicallaboratoriesandinthechemicalanalysisof

environmentalsamples. Itisthereforeimportantto learnaboutthe origin oftheUV-VISspectrumanditscharacteristics

1.5. Radiation andenergy

Radiation is a form of transmitted energy Electromagnetic radiation is so-named because ithas electric and magnetic fields that simultaneously oscillate in planes mutually perpendicularto each other and to the direction of propagation through space Electromagnetic radiation hasthedualnature:itsexhibitswavepropertiesandparticulateproperties.

1.6. Thenatureofelectromagneticradiationand spectralregions

Theelectromagneticspectrumiscomposedofalargerangeofwavelengthsandfrequencies (energies). It varies from the highly energetic gamma rays to the very low energyradio-waves. The entire range of radiation is commonly referred to as the electromagneticspectrum[1]

II. PRINCIPLEOFUV/VISIBLESPECTROSCOPY

Ultraviolet spectroscopy is concerned with the study of absorption ofUV radiation whichrange from 200nm to 400nm. Compounds which are coloured, absorbradiation from 400nm-800nm. But compounds which are colourless absorb radiation in Uv region. Inboth UV aswell as visible spectroscopy, only the valence electrons absorbthe energy, therebythemoleculeundergoestransition fromGroundstatetoexcitedstate.

2.1 Electronictransition

Theelectrontransitionthatresultinabsorptionofultravioletofvisibleradiationareσ-σ*,n-σ*, π-π* ,and n- π*.

1. σ-σ*

- σfromorbital isexcitedtocorrespondinganti-bondingorbitalσ*.
- Theenergyrequiredis largeforthistransition.
- The organic compounds in which all the valence shell electrons are involved in theformation of σ bond do not show absorption in normal uv region (200-400nm) Thistransitionisobserved withsaturated compounds.
- Theusualspectroscopictechniquecannotbeusedbelow200nm.

2. π-π*

- πelectroninabondingorbitalis excitedtocorrespondinganti-bondingorbitalπ*.
- Energyrequiredislesswhencomparedton-σ*.
- Compoundscontainingmultiplebondlikealkenes,alkynes,,nitriles,aromaticcompoundsetcundergo π-π* transition.
- Absorptionusuallyoccursintheordinaryuvspectrophotometer.
- Absorptionbandsinconjugatedalkenes(170-190nm)
- Absorptionbandsincarbonyls(180nm).

3. n-σ*

- Saturatedcompoundscontainingoneheteroatom withunsharedpairofelectrons(n)likeO,N,Sandhalogensarecapableofn-σ*transition.
- Thesetransactionrequirelessenergythanσ-σ*transition.
- Insaturatedalkylhalides,theenergyrequiredfortransitiondecreasewithincreaseinthesizeofhalogenatom(or decreasein electronegativity).

4. n-π*

- Anelectronfromnon-bondingorbitalis promotedtoanti-bondingπ*orbital.
- Compoundscontainingdoublebondsinvolvingheteroatoms(C=O,N=O)undergosuchtypeof transitions.
- Thistransitionrequireminimumenergyoutofalltransitionsandshowabsorptionbandatlonger wavelengtharound 300nm[1]

2.2. CHROMOPHORE AND RELATED TERMS1.Chromophore

Anyorganicfunctionalgroupthatexhibitcharacteristic absorptioninthevisibleorultraviolet region is called a chromophore. These are usually unsaturated groups containing nandπelectrons that conveniently undergo n to n- π* andπ-π* transitions. A compound thatcontains achromophoreis called chromogen.

Types ofChromophores

(a) IndependentChromophore

Whenasinglechromophoreissufficienttoimpartcolourtothechromogen,itiscalledanindependentchromophore.

(b) DependentChromophores

When more than one chromophore is required to produce colour in the chromogen it is

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calleddependentchromophore.Animportantfactabou tachromophoreisitmayormaynotimpart colour to the compound, but absorption of radiation takes place irrespective of whether coloris produced or not.For example , carbonyl group does not produce any colour in the UVregions,stillitisanimportantchromophore.

2.3. Auxochrome

It is a functional group that itself does not absorb in the UV region but when attached tothe chromophore, it shift the absorption maximum towards longer wavelength along with anincreasein theintensityofabsorption.

Types of Auxochrome(a)BathochromicGroups

The groups that deepen the colour of a chromogen and eventually cause shifting of theabsorptionmaximumtowardslongerwavelength arecalledbathochromic groups.

(b)Hypsochromicgroups

ThegroupsthatlightenordiminishthecolouroftheChromogenresultinginthedisplacementoftheabsorptionmaximumtowardsshorterwavelengtharecalledashypsochromicgroups.

2.4. Effect of solvent polarity on the various types of bandsK- band:

Due to conjugated enes & enones are affected differently by changing the polarity of thesolvent. K bands due to conjugated dienes are not affected by changing the polarity of thesolvent.whilethesebandsduetoenonesshowsared shiftbyincreasingthepolarityofsolvent.

Rband:

Theabsorptionshiftstoshorterwavelength(blueshift) withincreasingpolarityofsolvent.

Bband:

The position as well as the intensity of the band is not shifted by increasing the polarityof the solvent. But the heterocyclic aromatic compound, a marked hyperchromic shift isobservedby increasing thepolarity ofthesolvent[2]

2.5. LAWS GOVERNING ABSORPTION OF RADIATIONLambert's Law:

When a monochromatic light passes through an absorbing medium at right angles to theplane of surface of mediums or solution, the rate of decrease in intensity with thickness ofmedium(b)is proportionalto the intensity of incidentlight.

Beer'sLaw :

BernardandBeer independently stated that ' The intensity ofincident light decreasesexponentiallyastheconcentrationofabsorbingmediumincreasearithmetically.ThisissimilartoLambert'slaw.

III. INSTRUMENTATION OF UV VISIBLE SPECTROSCOPYUV VISIBLE SPECTROMETER

ThespectrophotometersusedinUV-visiblespectroscopymeasuretheratiooftheintensity of light transmittedthrough a sample and the intensity of the incident light . Thecomponents of UV and visible spectrophotometer are identical except that they differ in theirradiation sources. The radiation source used in visible region is tungsten lamp whereas in UVregion deuterium lamp, hydrogen discharge lamp, mercury are and xenon discharge lamp arecommonlyused.

3.1. Sources ofRadiation

Anidealradiationsourceshouldhavethefollowingcharacteristics.

(a) Intensityoftheincidentlightshouldbehigh

(b) Itshouldemitcontinuousradiations

(c) Shouldbefreefromanyfluctuations

(d) Shouldnotshow exhaustiononcontinuous usage

HydrogenDischargeLamp

It is the most widely used UV radiation source. It consists of two electrodes enclosed ina glass tube with a quartz. The glass tube is filled with hydrogen gas under relatively highpressure. When a voltage is applied across the electrodes, the hydrogen molecules are excitedto higher energy level. While returning to the ground state the electrons emit UV radiations inthenearUVregion(180-350nm).Thehighpressureinsidethetubebringsaboutcollision

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betweenhydrogenmolecules.Thisresultsinpressurebroadeningandemissionofacontinuous(broadband)hydrogenspectruminsteadofasimplelinespectrum. Hydrogendischargelampsarestableand robust.

DeuteriumDischargeLamp

Thislampconsistsoftwoelectrodesenclosedwithinasilicaenvelopefilledwithdeuterium (D)underlowpressure.Whenahighvoltageisappliedacrosstheseelectrodes,itcauses

emission of deuterium lines. These lines under the influence of low pressure in the interior ofthelampbroadentogiveacontinuousspectruminthe nearUVregion.Theemissionintensityof deuterium discharge lamp is about 3-5 times the intensity of hydrogen discharge lamp. It ishighlyexpensiveand usedwhen highintensityisrequired.

Xenon DischargeLamp

Xenondischargelampisanelectricdischargelampwhichutilizesionizedxenongastoproduce an extremely,white light for short durations. It consists of a sealed tube made up ofglassorfedquartzwhichisfilledwithxenongasatapressureof10-30atmospheres.Theglasstubeiseitherstraightorhelical,circularorUshape.Additionally,itcontainsapairoftungstenmetalelectrodeswhichcarryelectricalcurrenttothegas.Theelectrodesprotrudethrougheachendofthetubeandareconnectedtoacapacitorwhichischargedatahighvoltage.ThexenonlampgeneratessignificantamountsofUVradiationandshowsspectrallinesintheUVregion.Its intensity is higher than the hydrogen discharge lamp. But, the UV radiations released by itcauseionizationoftheoxygenmolecules,whichis themajordrawback ofxenonlamp.

TungstenLamp

ItissimilartoanelectricbulbTungstenisthemostwidelyusedandmostsuitablematerialfor lamp filaments. It is used when polychromatic light (light with radiations of severalwavelengths)isrequired.Thelampconsistsofatungstenfilamentenclosedinavacuumbulb,whichisheatedelectricallyatahighertemperaturetoproducewhite light.Itrequiresapotentialof3-220volts.Itemits continuous radiations overwidewavelengthregion(350-2500mm).

3.2. Wavelength SelectorsMonochromators

A monochromatoris a device which converts a polychromatic beam of light into amonochromaticbeam. Itconsistsofthefollowing parts.

1. EntranceSlit.Itdefinestheincomingbeamofpolychromaticlightintoanarrowbeamtoavoid aoverlappingmonochromaticimages.
2. Collimator1:Itcollimatesormakesparalleltheradiationscomingfromtheentranceslit.
3. PrismGrating:Itdispersestheradiationswithrespecttothecomponentwavelengths.
4. Collimator2:Itreformstheimages of theentranceslit
5. ExitSlit:Itselectsanarrowbandofdispersedspectrumforobservationbythedetector.

Thedifferenttypes ofmonochromators havebeendiscussedbelow.

PrismMonochromators

Theseareusuallymadeupofglass,quartzorfusedsilica. Theydispersethepolychromaticlight falling on the its component rainbow colors according to their wavelengths. They arecommonlyusedinPrismmonochromatorsgivenon-leardispersions.Prismmonochromatorsareof two types.

RefractiveType

LightfromtheradiationsourcepassesthroughtheentranceslitandfallsonthecollimatorThe rad are collimated and dispersed into component wavelengths by the prism. Collimator.reforms the images entrance slit into any one of the seven colors. The required wavelength isselectedbyeitherrotatingtheForbykeepingtheprism stationaryandmovingtheexitslit.

Reflectivetype(LittrowPrism)

Its working is similar to refractive prisms. It consists of a reflective surface on one sidesothatlightdoesnotontheprismontheotherside.Thedispersedradiationsarereflectedandcollectedonthesamesideonwhich theradiationsfromthesourcefalls[4]

GratingMonochromators

Gratings are made up of glass, quartz or alkyl halides like KBr and NaBr.Back surfaceof the gratings areCoated with aluminum to make them reflective. These are highly efficientfprismsinconvertingapolychromaticlightintomonochromaticlight.Theyconsistofdenselyarrangedparallellinesor grooves.

Gratingmonochromatorsareoftwotypes

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DiffractionGratings
Diffractiongratingisusedwhenpolychromaticlightist obeseparatedwithhighresolution. It works on the mechanism of reinforcement (strengthening). The incident rays arereinforced with those reflected, resulting in a radiation whose wavelength is expressed by theequation,
TransmissionGrating
Byrotatingthegratingandfixingtheentranceslitofmon ochromatororviceversa,thedesiredwavelengthatangl e(0)andorder(n)canbeselected.Intransmissiongratin g,theincidentanddiffractedrayslieon oppositesides[5]

3.3. Filters

Afilterisadevicewhichallowsonlythelightofrequired wavelengthtopassthroughandabsorbs the unwanted radiations either partially or completely. Analysis of different speciesrequires differentfilters whichareinterchangedaccordingto theneeds.offilters

3.4. SampleCells
Sample cells or cuvettes or sample holders are used to hold sample solutions. Their shape(rectangular or cylindrical)andmaterial of construction varies depending on the instrumentand the nature of the sample being analyzed. The path length (thickness or internal distance)of the cell is normally 1 cm, however cells with longer path lengths up to 10cm shorter Pathlengths of 1-2 mm are also available. Before taking the measurements, sample cells should bethoroughlycleaned toavoidanycontamination.Thelevelofthesamplesolut ionmustbeuptothe mark etched on its surface or above the lightbeam to avoid reflections from the uppersurfaceof theliquid[6].

3.5. Detectors
Detectorsarethedeviceswhichconvertlightenergyint oelectrical signals,thataredisplayed on the readout devices. After passing through the sample cell, a part of the radiationis absorbed by the sample and the remaining is transmitted. The radiation falls on the detectorwhichdeterminestheintensity of theradiation absorbedby thesample

PhotovoltaicCell
Itconsistsofaphotocathodewhichisathinmetalliclaye rcoatedwithgoldorsilver.Italsocontains a metal base (usually iron) which acts as anode. Between these two electrodes is asemiconductorlayer of selenium.

3.6. SingleBeamUV-VISSpectrophotometers
In a single beam UV-visible spectrophotometer, light from the radiation source afterpassingthroughamonochromatorentersthesamp lecellcontainingthesamplesolution.Apartof the incident lightis absorbed by the sample while the remaining gets transmitted. Thetransmitted light strikes the detector and produces electrical signals. The signal produced bythe detector is directly proportional to the intensity of the light beam striking its surface. Theoutput is measured by a galvanometer and displayed on the readout device.The absorbance readings of both the standard and unknown solutions are recorded afteradjustingtheinstrumentto100%transmittancewi thablanksolutioneachtimethewavelengthis changed[7].

3.7. DoublebeamUV-VISSpectrophotometers
Double beam spectrophotometer allows direct measurement of the ratio of intensities ofsample and reference beams respectively. The design of a double beam spectrophotometer issimilar to single beam spectrophotometer except that it contains a beam splitter orChopper.In a double beam spectrophotometer, radiations from the radiation source are allowed to passthroughtheentranceslitintothemonochromaticM onochromatorselectstherequiredwavelengthoflight whichisthenpassedthroughtheexitslitandreceivedby arapidlyrotatingbeamsplitterorchopper.Beamsplitter isacirculardisconethirdofwhichisopaque,onethirdis transparent and the remaining is mirrored. The chopper splits the monochromatic beam oflight into two beams of equal intensities. One beamis passed through the sample cell and theother through the reference cell. After passing through the sample and reference cells, thetransmittedbeamsreachthedetectorsandproducea pulsatingcurrentwhichisproportionaltothe intensities of the incident lightand the transmitted light.The detectors are connected toan amplifier and readout device which gives the final result in absorbance or transmittance bycombiningtheoutput[8]

IV. VALIDATIONOFULTRAVIOLETSPECTROSCOPY

Itisdefinedasestablishingdocumentedevidencewhic hprovidesahighdegreeassurancethataspecificprocess willconsistentlyproduceaproductmeetingit'spredete rminedspecification&quality characteristics

3.8. Validationparameters

- Linearity
- Accuracy
- Precision
- Sensitivity

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- Range
- Selectivity
- Ruggedness

Linearity

Linearity is the ability of the method to produce test results that are proportional , eitherdirectly or by a well defined mathematical transformation, to the concentration of analyte insamples with in a given range.For UV – visible Measurements, the usual linear relationship isBeer'slaws,whichstatesthattheabsorbanceofasolut eisdirectlyproportionaltoconcentration. A linear calibration curve relating absorbance to concentration should have theform:

A =kc

Where,

AisabsorbanceCisconcentration
Kis thecalibrationfactor(theslopeofthecalibrationcurve).

Thus testing for linearity in effects tests how well our theoretical model (Beer's law) fitstheactualmeasurements.Theoretically,anabsorba nceofonlyonestandardofknownconcentration is required in order to calibrate for quantification. The measured absorbancevalue divided by the concentration gives the slope. A number of instrumental and sampleparameterscancausedeviationsfromBeer'sla w,andsignificantquantitativeerrorscanresultifthecali brationcurveisnotaccuratelycharacterize.However,b ecausethesedeviationsarewavelength can minimize their influence on results. To construct a calibration curve, thespectraofasetofatleastthreestandardanalytesoluti onsshouldbemeasured.Theconcentrationsofthestand ardsolutionsshouldbrackettheexpectedconcentration rangeofthesamples for analysis. Ideally, all measured standard values would lie on a straight line, but inpractice the values always exhibit some scatter. A statistical method must be applied to findthe best fit of the calibration curve to the data and, in a second step, to determine which typeof calibration curve gives the best fit. Thestatistical method most often used is linearregression, which is also known as the least squares method. To compare two calibrationcurves, a measure ofthe goodness of the fit of the standards to line is required.
Severalstatisticalvalues,includingcorrelationcoeffici ent,standarderrorofregression,anduncertaintycanbeu sedtoobtainthismeasurement.Ofthese,thecorrelation coefficientisthemostpopular.This value always lies between +1 and -1. A value of +1 indicates a perfect linear relationshipbetweenabsorbanceandconcentration,wi thAincreasing.Avalueof-1alsoindicatesaperfectlinearrelationship,butwithAd ecreasing(whichcanoccurifderivativedataisused).Av alueof0indicatesthatthereisnocorrelationbetweenabs orbanceandconcentration.Todeterminethe best wavelength or combination of wavelengths the spectra of a set of pure standards witha wide range of concentrations are measured. A linear calibration curve is applied to eachwavelength, and the chosen statistic for the assessment of linearity is calculated. For quickestevaluation, a graphical plot of the linearity statistic versus wavelength is useful linearity.
Theabovefiguredepictsthespectraoffouryellowdyest andardsandthecorrespondingcorrelationcoefficient spectrum for a simple linear calibration curve. The best calibration in terms oflinearity is achieved at the point at which the correlation coefficient approaches unity. In thisexample,thewavelengthofmaximumabsorbance(414nm)isnotidenticaltothewavelength(402nm) that gives the best linearity. Typically, correlation coefficient values of better than0.999can beexpected[9].

ACCURACY

Accuracy of a method is the degree of agreement betweenan individual test resultgenerated by themethod andthetruevalue.

DefinitionoftermsThetermsaccuracyandprecisionar eusedthroughoutthisprimer,butnotinterchangeably. It is therefore important to clearly understand the difference between them.As an analogy,thebelow figureshows theperformanceofamarksmanon ariflerange.

- In(a),theshotsareneitheraccuratenorprecise.

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- In(b),theshotsareprecisebutinaccurate;themarksmanisperformingwell,butaconsistentbiasisevident.
- In(c),theshotsareaccuratebutimprecise:theaverageoftheshotswouldlieinthecenterofthetarget, buttheindividualshotsdeviatesignificantly
- In(d),theshotsarebothaccurateandprecise.

To determine which wavelength or wavelengths give the best accuracy, the spectra of a set ofstandards are measured and calibration curves constructed at all wavelengths, as describedabove.Asampleofknownconcentrationisthenrequired.Thissampleisideallyoneforwhichthe concentration of the analyte has been determined using a different technique. However, ifsuchasampleis notavailable,asyntheticsamplecontainingaknownweightofthesample isprepared. The spectrum of the sample is measured, and quantification is performed at allwavelengthsoverthemeasuredwavelengthrange.Thequantitativeresultsateachwavelengthare then compared with the known value. A graphical plot of the quantitative results versuswavelengthenablesquickevaluation.Theanalyticalwavelengthwouldhavebeensetat414nmusingtraditionalmethods,thewavelengthorwavelengthsthatgivethebestaccuracylieintheregion of 400nm. Because noise may bias the accuracy of any individual measurement, it ispreferable to perform a series of measurements on the sample and then calculate the average.Thismethod reduces thecontributionof noiseto errors in accuracy.

PRECISION

Precision of a method is the degree of agreement among individual test results when theprocedure is applied repeatedly to multiple samplings. A statistical value is required in orderto determine precision. Standard deviation, percent relative standard deviation (obtained bydividing the standard deviation by the average value and multiplying by 100), and confidenceintervalarethemostpopulartoolsforassessingtheprecisionorrepeatabilityofasetofvalues.To determine the precision of a method, a set of typically 10-20 samples with the sameconcentration are prepared. These samples are then measured, and the amount of analyte iscalculated. The standard deviation of the results is a measure of the precision. If the desiredlevel of precision is not achieved, noise reduction techniques such as wavelength averaging,time averaging, and internal referencing should be used to improve the value . The sametechniques can be appliedin multicomponentanalyses[10]

SENSITIVITY

Sensitivity refers to the response obtained for a given amount of analyte and is oftendenotedbytwoanalyticalfactors:thelimitofdetection(LOD)andthelimitofquantification (LOQ).TheLODisthelowestconcentrationofanalytethatcanbedetectedbutnotnecessarilyquantified in sample matrices. In general, the LOD is the point at which the signal from theanalyte is equal to three times the noise in the measurement. Measurements results from somespectrophotometerliststandarddeviationsbasedonthenoiseinthemeasurement.TheLODisapproximately three times the standard deviation. The LOQ is the lowest concentration ofanalyte that can be determined with acceptable precision and accuracy (which depend on theobjectives for the analysis) must be defined. The tools described above then can be used todetermine the acceptable limits. It is often assumed that the wavelength with maximumabsorbance will givethe best sensitivity. However, because instrumental noisecan varysignificantly with wavelength, this is not necessarily the case . A better way to determine thewavelength or wavelengths of optimum sensitivity is to measure the spectrum of a sample oflow concentration several times. The average and percent relative standard deviation of themeasured values at each wavelength are then calculated. The wavelength with the lowestpercent relative standard deviation likely will yield the best sensitivity. The above figureillustrates this technique for a yellow dye sample. Although wavelengths between 400 and450nmgiveexcellentsensitivity, thebestsensitivityisobtainedat220nm[11]

RANGE

Range is the interval between (and including) the upper and lower levels of analyte thathavebeencalculatedwiththerequiredprecision,accuracy,andlinearity.Therangeisdetermined by first analyzing samples that contain varying concentrations of the analyte andthen using the tools described above to calculate the linearity, precision, and accuracy of theresults.

SELECTIVITY

Selectivityistheabilityofamethodtoquantify accuratelyandspecificallytheanalyteoranalytesinthe presenceofothercompounds.Thepresenceofanyother compoundthatabsorbsat the wavelength used to

quantify the analyte will result in quantitative error. These othercompoundsmaybesynthesisprecursors,knownimpurities,excipients,ordegradationproductsin the sample matrix. If the type of interferent is known , the method developer can examinethe spectra of the analyte and of the Interferent to select a wavelength does not suppresssufficiently the effect of the Interferent , an appropriate correction technique such as use of anisoabsorbancewavelengthWhen a known interfering component with a known spectrum ispresent, the error introduced by this component at the analytical wavelength for the targetanalytecanbeeliminatedbyselectingareference wavelengthatwhichtheinterfering compoundexhibitsthesameabsorbanceasitdoesattheanalyticalwavelength.Theabsorbanceat this reference wavelength is subtracted from the absorbance at the analytical wavelength.The residual absorbance is the true absorbance of the analyte. This technique is less reliablewhen the spectra of the analyteof the interferent are highly similar. Moreover, it can correctforonlyoneinterferent.Iftheidentityand/orspectraofpossibleinterferentsareunknown,anempirical approach almost identical to that described above may be used to determine whichwavelengthor wavelengthsgivethebestaccuracy.

RUGGEDNESS

Ruggedness is y degree of reproducibility of test results obtained by analyzingthe samesampleunderavarietyofnormaltestconditions.Themethodshouldnotbeaffectedbychangesin time or place. The reproducibility of the method should be established under variousconditions, for example with different elapsed assay times. The ruggedness of an analyticalmethodisdeterminedbyanalyzingsubsamplesofahomogeneoussampleindifferentlaboratoriesandondifferentinstruments.Thesetestsshouldbeperformedbydifferentanalysisunderoperationalandenvironmentalconditionsthatmayvarybutthatfallwithinthespecifiedparameters for the method. The degree of reproducibility of the results is then calculated as afunction of the assay variables. This value can be compared with the precision of the methodundernormalconditionsto obtain ameasureof itsruggedness[12]

V. APPLICATION OF UV-VISIBLES SPECTROS COPY

5.1(I)SpectrophotometricTitration-SingleComponentandMulticomponentAnalysis

Photometric or spectrophotometric titration signifies the equivalence point provided theconditionthatanalyte,thereagentorthetitrationproductabsorbsradiantenergy,theequivalence point of the titration is given out by the absorbing indicator which shows thechangeinabsorbance.Agraphisplottedbytakingabsorbance(Lambda)onY-axisandvolumeofthetitrantonX-axis.Mostofthephotometrictitrationsdemonstratetwolinearpointshavingdifferent slopes. One of the slopes occur early in the titration and the other one appears afterthe equivalence point . The linear portions of the curve are extrapolated and inter sected to getthe end point. At a fixed absorbance, the titration provides the end points. Moreover the endpoints are determined by conversion of the linear segment curve to a sigmoid-shaped curvewiththehelpofaderivative.Thetitrationcurveswithlinearportionsareobtainedprovidedthe absorbingspeciesobeyBeer'slawMoreovercorrectionsareperformedonabsorbancesforvolumechanges.

5.2. (II) Qualitative Analysis(a)DeterminationofPurity

Impuritiespresentinthesamplecanbedetectedfromthe absorptionspectrumbyconsideringthefollowingfactors.

(I)Presenceofadditionalpeaks inthespectrum

(ii) Enhancedpeakintensities

(iii) Bymeasuringtheabsorbanceofthesampleatspecificwavelength.

Asubstancecanbepurifiedbycontinuingthepurificationprocessuntiltheabsorptionbandoftheimpurity disappearscompletely.

(b)Identification oftheCompound

Compounds containing longpair ofelectrons or conjugated double bonds absorb UVradiationsandgivecharacteristicabsorptionspectrum.Theunknowncompoundcanbeidentifiedbycomparingitsabsorptionspectrumwiththatofthestandard.Aromaticcompoundsandconjugatedalkenes can becharacterizedusing UV absorptionspectroscopy[13].

5.3. (III)QuantitativeAnalysis

QuantitativeanalysisbyUV-visiblespectroscopyhelpstodeterminetheconcentrationandamount of drug present in a given sample solution as well as its percentage purity. A numberof metallic elements, ions and functional groups can be estimated by coupling them with asuitablechromogenicreagent,whichabsorbedvisible light..

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5.4. Cis-transIsomerism

In this type of isomerism, the compounds have different spatial arrangement of atoms ofgroupswithrespecttothecentralatomsordoublebondorring.UVspectroscopydifferentiatesCisandtransisomers.Trans-isomerexhibitsabsorbanceatalongerwavelengththan cis-isomer. Cis and trans -isomers are interconvertible . The conversion causes change inlambdamaxandcolourintensity.Conversionofcis-isomertotransformsresultsinbathochromic shift and hyperchromic effect. Conversion of trans -isomerto cisform resultsin hypsochromic shift and hypochromic effect. For example, the cis-isomer of vitamin D2,giveslambdamaxat265nmwhileitstrans-isomerexhibitsA at294 nm.

5.5. Conjugation

Conjugation can occur between two or more carbons containing double or triple bonds andalso in carbon - oxygen double bond. Conjugation helps in determining the presence of anaromaticring,thenumberandpositionofthesubstituentspresentonthecarbonofconjugatedsystem.Conjugationcausesshiftingoflambdamaxtowardslongerwavelength,asthenumberofdoublebondsincreases.

5.6. Alkyl Substitution:

Thenumber of alkyl substituents in an organic compound can be determined byUV –visiblespectroscopy.Substitutionofalkylgroupinacompoundcausesbathochromicshifti.e.,lambdamax shifttowardslongerwavelength[14]

VI. CONCLUSION

UV-VisibleSpectroscopyisbasedonafirmtheoreticalbasis,moreselective,efficient,fastand reproducible analytical methods can be developed. In general terms, there are two majormeasurementtechniques;howmuchanalyteisin thesample(quantitativeanalysis)andwhichanalyte is in the sample (qualitative analysis). An area under curve method is "the area undertwopointsonthemixturespectraisdirectlyproportionaltotheconcentrationofthecompoundof interest" particularly suitable for the compounds where there is no sharp peak or broadspectra are obtained The pharmaceutical analysis by UV-Visible Spectroscopy comprises theproceduresnecessarytodeterminethe"identity,strength,qualityandpurity"ofcompounds.

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