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Simultaneous Equation Method for the Estimation of Caffeine and Pioglitazone HCL by UV-Visible Spectrophotometry

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ABSTRACT
One of the earliest instrumental techniques for analysis is UV-VIS spectroscopy. Many different types of materials Can be characterized using UV-Vis spectroscopy. The UV-Vis delivers details based on the degree of absorption Or transmittance of a varied wavelength of beam light and the various responses of samples. Radiant energy Absorption by materials can be quantitatively described using the general law known as Beer'slsmbart law. The UV-VIS Spectrometer is simple to use and handle. Both qualitative and quantitative analyses can make use of it. The metal And metal oxide nanoparticles are typically characterized using wavelengths between 200 to 700nm. It is quick, simple, and characterization Method. The composition and structure of the materials can be examined using the spectrum. TIt is uses in academia, business, medical labs, and chemical examination of environmental sampleThese newer chemometric procedures tend to be complex and Difficult to understand and implement and are successful under different and Conditions. In this study, we start from the very simple beginning and examine the factors that Can present difficulties with obtaining the correct results and observe how the system behaves So as to find a better and simpler chemometric procedure to perform mixture quantitative Analysis. We have used simulated and actual experimental data obtained from a UV-VIS Spectrophotometric measurement of caffeine and pioglitazone to conduct the study. Well understood And defined systems tend to give good results. Choices of a Common solvent were essential so various solvent ranges including 0.1 N HCL, And various concentrations ranges of various buffers were analyzed. Hence 0.1 N HCL was selected as a solvent for the Proposed method. Caffeine and Pioglitazone HCL showed maximum absorbance at 273 and 220 nm respectively. Both Drugs obey Beer Lambert's law in the concentration range of 3-18 µg/mL for Caffeine and Pioglitazone HCL respectively. . This method is also conducted with blank solution The method was quanti-Tatively evaluated in terms of linearity, precision, precision, and recovery. The method is simple, convenient And suitable for the analysis of Caffeine and Pioglitazone HCL in bulk drugs

I. INTRODUCTION

In modern analysis the complexity of analyzing samples with numerous unknown components presents a Major challenge In such cases Resolution of the components is often associated with cumbersome sample cleanup and Separation procedures. Some time there are risks associated with separation methods such as Loss of analytics ,contamination of sample, possibility of incomplete separationl, Simultaneous Multi-component analysis by UV-visible molecular absorption spectrophotometry are main purpose toDeveloped for the purpose of minimizing the cumbersome task of separating interferents and to Allow determination of an increasing number of analytes, consequently reducing analysis time And cost (4). Analytical Separation TechniquesThere are quite a number of separation techniques that can be employed in the Determination of the analytes of interest.

The use of traditional methods like extraction is quite Difficult because extraction techniques require large solvent consumption with accompanying High cost of disposal. The extraction time is long and generation of dirty extracts requires Tedious cleanup steps. Moreover, due to environmental concerns, there has been the need for The development of modern instrumental techniques such as the chromatographic separation Methods and spectroscopic methods that are able to perform simultaneous equation method for Analysis

.Spectroscopic Methods

In spectrometry, compounds or atoms are identified by their characteristic spectral Peaks and

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their concentrations are determined from the corresponding peak intensities using Some kinds of calibration methods. All organic compounds are capable of absorbing Electromagnetic radiation because all contain valence electrons that can undergo electronic Transitions. Promotion of electrons from low energy ground state orbital to higher energy Excited states orbital.

Type of spectroscopic

- visabel spectrophotometer
- UV-VIS spectrophotometer
- Infrared spectrophotometer
- Fluorescence spectrophotometer
- Atomic absorption spectrophotomet

UV visible spectrophotometer •

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or Emitted when the molecules or atom UV s or ions of a sample move from one energy state to Another energy state. UV spectroscopy is a type of absorption spectroscopy in which light of the Ultra-violet region(200-400 nm) is absorbed by the molecule which results in the excitation of the ground state to higher energy

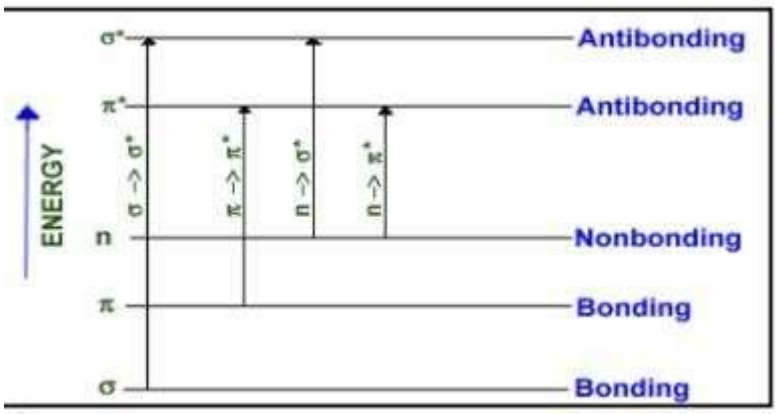
Electrons from the ground state to a higher energyThe basics ofspectrophotometeric techniques are that they measure the interaction of electromagnetic radiation with sample in quantized form.•

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorption or emitted when the molecules or atoms UV spectroscopy (200-400nm)

Ulraviolet-visible spectrophotometry has Extensively been used for quantitative determination of components present in a mixture This is largely because many molecules absorb radiation stronglyin this region. The low Cost and the simplicity in operating such instrumentation also add to the advantages of the UV-Visible spectrometry.

UV vis spectrophotometer contact electronic transactions like-

1)sigma-anti Sigma transaction
2)n -anti sigma transaction
3)pai -anti pai transaction



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4) n-anti pai transition

Principal:
The amount of light absorbed is directly proportional to the concentration of the solute in the Solution electrons from the ground state to a higher energy And thickness of the solutionunder.

Beers laws
It State that the absorption of monochromatic light is directlyproportional to concentration of solution

Lambert s law
When a ray of monochromatic light passes through absorbing medium it's intensity decreases potentially as the length of the absorbing medium increases
Absorbing is directly proportional to path length.

Beers Lambert LawIt
State that the amount of light absorbed is directly proportional to the concentration of the solute in the solution and thickness of the solution under analysis.
Absorbance is directly proportional to path length andconcentration of solut
A = -Log T = Log p°/T = ϵ b c

Instrumentation
The basic components of a spectrometer include: light source (UV and visible),Monochromator (wavelength selector),sample stage, and detector. A tungsten filament, continuous overUV region Is generally used as light source. Detector is usually a photodiode or CCD. Photodiodes go With to filter light of a particular wavelength, to be fed to the detector. WhileMonitoring The absorbance in UV spectrum, the visible lamp must be turned off, and vice-versaI

Part of spectroscopy

1. UV Source:
The power of radiating source should not vary in its operating wavelength range. Continuous UVSpectrum is produced by electrically exciting deuterium or hydrogen at low pressures. The mechanismFor generation of UV light includes creating an excited molecular species, that breaks into two atomicSpecies and a UV photon. The emission wavelengths of both deuterium and hydrogen lamps are in 160To 375 nm range. The material of the cuvettes needs to selected such that it does not absorb the lightIncident, because this will result in errors in obtained absorption spectrum. Thus, quartz is usually used

2. Visible Light Source:
Tungsten filament lamp is used as visible light source. This lamp can produce light in 350 to 2500 nmWavelength range. In a tungsten filament lamp, energy emitted is proportional to the Fourth power of the operating voltage. Thus, in order to get stable emission, a highly stable Voltage must be applied to the lamp. The stability of voltage is ensured by using electronic Voltage regulators or constant-voltageTransformers. Tungsten/halogen lamps include small Quantities of iodine embedded within a quartz'envelope', which also contains the tungsten Filament. The iodine reacts with gaseous tungsten, formedBy sublimation, and produces a Volatile compound WI2. As WI2 molecules hit the filament, theyDecompose, and redeposit Tungsten back on the filament.
The tungsten/halogen lamps usually haveLifetime twice to the Conventional tungsten filament lamp. Tungsten/halogen lamps are used in owing to their high efficiency, and their output extends to UV region as well.

3. Monochromators :
A monochromator is an optical device that transmits a mechanically selectable narrow band of Wavelengths of light orother radiation chosen from a wider range of wavelengths available at The input. The name is from the Greek roots mono-, "single", and chroma, "colour":
Monochromator source is used; before reaching sample, light is divided in two parts of similar intensityWith a half-mirror splitter. One part (or sample beam), travels via the cuvette having the solution ofMaterial to be examined in transparent solvent. Second beam, or reference beam, travels via similarCuvette having only solvent. Reference and sample solution containers have to be transparent towardsPassing beam

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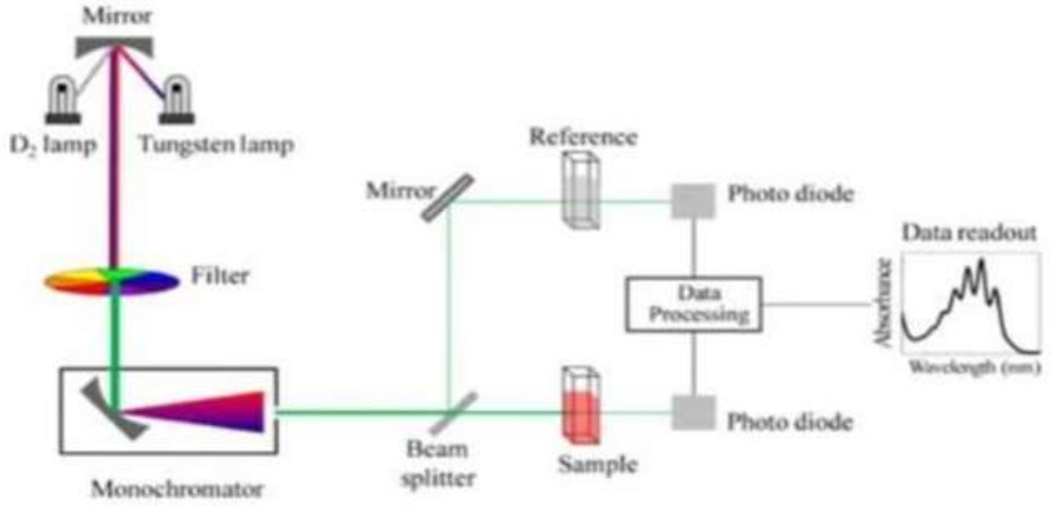


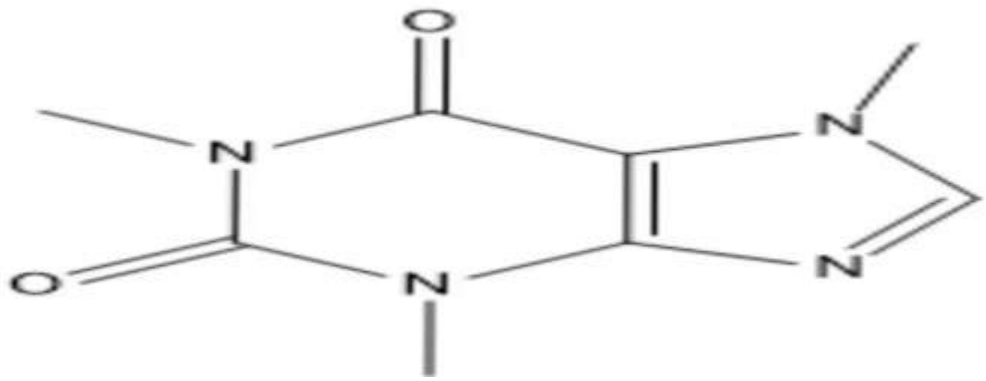
Detector,:

To detects intensity of light transmitted by cuvettes and sends this data to a meter to record anddisplay the values. Electronic detectors calculate and compare the intensities of light beams. SeveralUV−Vis spectrophotometers have two detectors – a phototube and a photomultiplier tube, and referenceAnd sample beams are monitored simultaneously. The photomultiplier tube is the extensively usedDetector in UV-Vis instruments. It includes a photoemissive cathode (electrons are emitted from theCathode when photons strike it), several dynodes (a dynode emits multiple electrons when one electronStrikes it) and an anode. The incident photon, after entering the tube, strikes the cathode. The cathodeThen emits multiple electrons, which are then accelerated towards the first dynode (whose potential is90V more positive thancathode). The electrons strike the first dynode, leading to the emission of severalElectrons for each incident electron. Theseelectrons are then accelerated towards the second dynode,To produce more electrons which are accelerated towardsdynode three and so on. All the electrons areEventually collected at

Application

- Quantitative and qualitative analysis
- °Detection of impurities from organic mixture.
- °Elucidation of structure and molecules
- °Forensic toxicology
- °Molecular Weight determination
- °Determination of metal contaminatio

Caffeine



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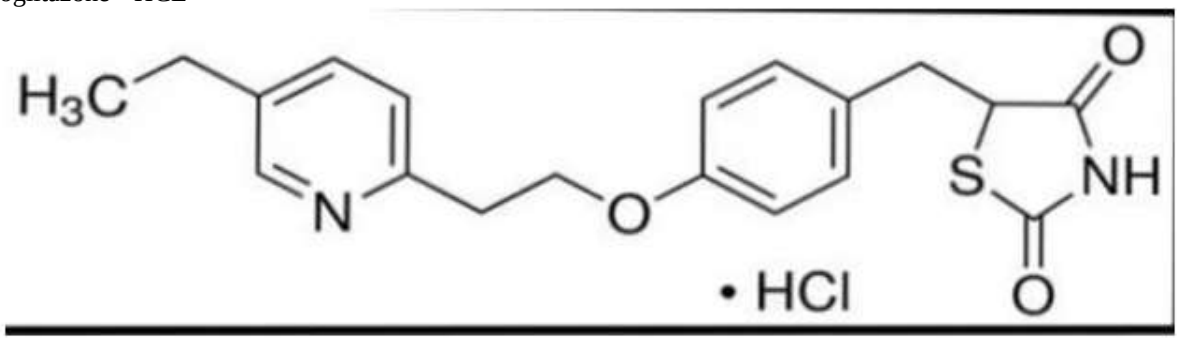
Caffeine (CAF)is chemically 1,3,7-Trimethylpurin-2,6-dione

- Molecular weight:356.4 g/mole

The chemical formula of caffeine is (C 8 H 10 N 4 O 2)

- Melting Point of Caffeine 235 °C
- Boiling Point of Caffeine178 °C
- Density 1.23 g/cm³USE OF CAFFEINE
- Caffeine is a stimulant, which means it increases activity in your brain and nervous system.
- It also increases the circulation of chemicals such as cortisoland adrenaline in the body.
- In Caffeine can prompt glowing, healthy skin by boosting skin circulation, and it has both antioxidant and anti-inflammatory effects
- small doses, caffeine can make you feel refreshed and focused

Pioglitazone HCL



Pioglitazone HCL (PIO) is chemically (RS)-5-(4-[2-(5-eth- Ylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2,4-dione .

- Chemical formula=C19H20N2O3S
- Molecular weight=365.44 g/mo
- lSolubility in water=Insoluble
- Melting Point= 183 – 184
- Density=(1.260g/cm cub) USES OF PIOGLITAZONE
- Pioglitazone is used with proper diet and exercise to treat highblood sugar levels caused by type 2 diabetes

• It may be used alone or with other medicines such as insulin, caffeine, metformin, or sulfonylurea agents.

- Pioglitazone works by helping your body use insulin betterIt affects lipid Metabolism through action at PPAR alphaPioglitazone and other similar medications For diabetes may cause or worsen heart failure (condition in which the heart is unable to pump Enough blood to the other parts of the body).

Literature survey publicized that certain UV ,HPLC ,HPTLC ,and LC-MS methods were reported For the estimation of these drugs individually or combined with Other drugs. On the other hand, Simultaneous equation was not reported for this new combination. Typically, the simultaneous equation Is used to estimate drug combinations that contain two or More Pharmaceuticals in the combined dosage form. Comparing this Method to other UV Technologies, the technical difficulties are Quite little. To ensure the safety and effectiveness of this Chosen Combination, an effort has been made to design an easy-to-use, Reproducible SE Approach. For the simultaneous determination Of CAF and PIO in pure and pharmaceutical Dosage forms, this Devised approach was fully validated and successfully

AIM

- To study Simultaneous equations method for the estimation ofcaffeine and pioglitazone HCl by UV visible spectrophotometry

Objective

Spectroscopy is the tool for study of atomic and molecular structure.It deals with interactions of electronic radiation with matter involving the measurement and interpretation of the extension of absorption of electro magnetic radiation molecules Caffeine increases intracellular concentrations of cy- Clicadenosine monophosphate (cAMP) by inhibiting phospho-Diesterase enzymes in skeletal muscle and adipose tissues .

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In persons with Type 2 diabetes, pioglitazone improves gly- Cemic management by increasing insulin sensitivity through its Activity at PPAR gamma 1 and PPAR gamma 2. It affects lipid Metabolism through action at PPAR alpha .

Plane of work
Simultaneous equations method for the estimation of caffeine and pioglitazone HCl by UV visible spectrophotometry

Project integration:
-Devlop project chart and project management plant
-Monitor and control project work
-Perform integrated project control Schedule management:
-Define spectrophotometry
-create validation and control the details of projects scope
-Estimate activity resources and time duration
-develop and control estimated schedule for the projectCost Management:
Estimate costs and determine budgetCosts

Selection of chemical :
By literature and Market survey online journal chemical and analytical abstract were stuided for fine chemicals
Market survey was carried to check the availability of theseschemical

Method Development:
Preparation of standard stock solutions

Simultaneous equations method development by selectingwavelength and optimization of run time
Validation of proposed method:
-system suitability parameters
-linearity and rang
-accuracy
-precision A)System precision

B)Method precision
C)Intermediate precision

Apparatus :
Weighing balance ,volumetric flask
Shimadzu 1650 UV-VIS double beam spectrophotometer with UV probe software was used. And it is made up of deuterium lamp, Monochromator ,
Detector,
Absorbance light Measurements were recorded with a pair of 1cmmatched quartz Cells
Chemical or Reagents
Caffeine in pure form Pioglitazone HCl HCl reagents used

Preparation of standard solution

Standard stock Solution of Caffeine (1000µg/mL) was prepared by dissolving 50mg of Caffeine in 30mL of 0.1N HCL. The resulting solution was sonicated for 10 minutes and the final volume was adJusted to 100mL with 0.1N HCL. From this standard stock solution and 1 mL was withdrawn and diluted to 10mL using the same Solvent to get a working standard solution of 10µg/mL.

Astandard stock solution of Pioglitazone HCL (1000µg/mL) was prepared by dissolving 50mg of Pioglitazone HCL in 30mL of 0.1N HCL. The resulting solution was sonicated for 10 minutes and the final volume was adjusted to 100mL with 0.1N HCL. Thissolution was further diluted to get a working Standard solution of10µg/m

Simultaneous Equations Method was Development

Working Solutions of both drugs were scanned in the UV range of 200–400nm. The overlay spectra of both drugs were recorded. From overlain spectra, wavelengths 273nm (of CAF) and220 nm of PIoglitazonewere selected for analysis of both drugs using

Suppose it may be possible to determine both Drugs by the technique of from method or simultaneous equation Method fivestandard solutions having concentrations of 3,6,9,15 and 18µg/mL for CAF and 3,6,9,15 and 18µg/mL for PIO were Prepared in 0.1N HCL and their correspondingabsorbance was Measured at 273 nm and 220nm.

The concentration of drugs A for (CAF) and B for (PIO)
In sample solutions were determined by the SE method using thefollowing formula:
A1 = aA1 b CA + aB1 b CB equation 1A2 = Ax2 b CA + aB2 b CB equation 2
Where CA and CB are the concentration of CAF and PIO, A1 and A2 are the absorbance of sample solution at 273 nm and 220nm,
Respectively, aA1 and aA2 are absorptivities of CAF at 273nm

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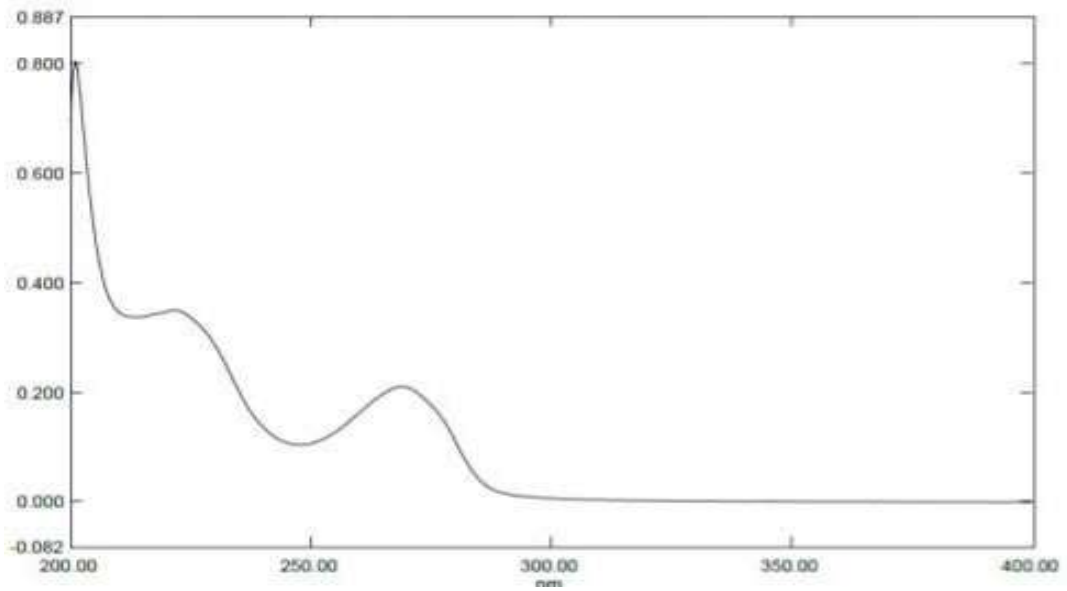
And 220 nm, aB1 and aB2 are absorptivities of PIO at 273 nmand 220nm, respectively
The absorptivity value of CAF and PIO from each solution was Calculated using the following formula and the results were pre-

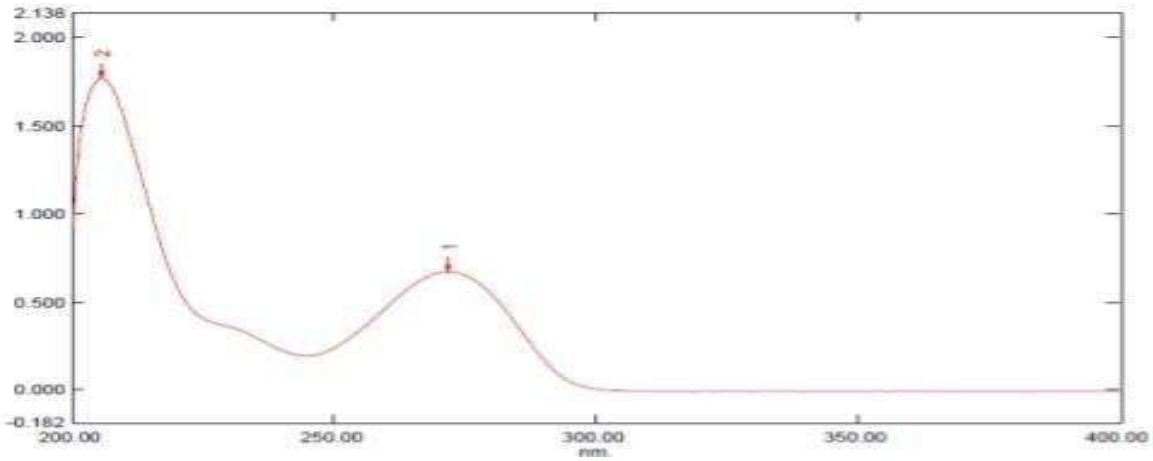
Develop method according to ICH guidelines
Evaluation of Method1)Specificity

The specificity of the method was measuring the abSorbance of CAF and PIO individually at 273nm and 220nm Against the blank and and their absorbance
Was compared with the blank and . No change was observed at273nm and 220nm

It shows that the Method is specific.



Specifying of pioglitazone



Specifying of caffeine

2) Linearity

Relation coefficient values 0.887for CAF and 0.734for PIO. Results show that good correlation exists between the con-Centration of the sample and their absorbanc.

3) Stability:

The stability of the standard and sample solutions Was checked for two days at normal temperature and the absorbance was measured on each day. The amount of drug present in The sample solution was calculated and the results

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confirmed that The sample solution is stable for two days without any degrada- Tion at normal temperature

Application of Developed Method to Marketed Dosage

Take 15 tablets were weighed and flattened into powder. Powder weight equivalent to 150 mg of CAF and 15 0mg of PIO

Was transferred into a 100mL volumetric flask. 50mL of solvent (0.1N HCl) was added and sonicated for 20 minutes. Then the Final volume was diluted up to the mark with the solvent (0.1N HCl) and filtered. 2mL of the above filtrate was transferred into A 25mL volumetric flask, and the final volume was adjusted up

To the mark with the same solvent to get sample solution. The Absorbance of the resulting solution was measured at 273 and 220nm and the amount of CAF and PIO present in each tabletWas found to be 143 mg and 153 mg, respectively

Result:

Parameter	Caffeine	Pioglitazone HCL
Wavelength	273 nm	220 nm
Equation	y = 0.0478x + 0.0247	y = 0.0415x + 0.025
Slope	0.0478	0.0415
Intercept	0.0247	0.025
Correlation Coefficient (R2)	0.9993	0.9991
Range	3-18	3-18

Precision:

The precision of an analytical procedure ex-Presses the closeness of agreement (degree of scatter) between A series of measurements obtained from multiple samplings of The same homogeneous sample under the prescribed conditions. Precision can be considered at three levels: repeatability, interMediate precision and reproducibilitnc

methods was hindered by unresolved peaks throughout the wavelength range selected, i.e200to 700nm.Components with more structured features were also studied. It was found that the method of solving Vu disable spectroscopy and simultaneous equations method were capable of giving good results in most instances for components with greater structural features and varying degree of overlap

II. CONCLUSION

The developed simultaneous equation method is simple, , andaccurate for any bulk dosage forms.

Analysis proved that the method Was repeatable and selective for the simultaneous estimation of CAF and PIO in pure and pharmaceutical dosage forms without Any interference from the excipients. simultaneous quantitative determination of the complexes in their mixtures using conventional spectrophotometric

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