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Methods Development and Validation for the Estimation of Pioglitazone HCl in Bulk and Formulations by UV Spectroscopy and FTIR

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of spectroscopic analysis, particularly UV spectrophotometer, is a simple and essential technique for bulk drug estimation, formulation studies, and compatibility assessments of drugs with various excipients. In the pharmaceutical industry, various analytical instruments, including Fourier transform infrared spectroscopy (FTIR), are employed for investigating drug-excipient interactions that can impact the stability of active pharmaceutical ingredients. This study aimed to develop a UV spectrophotometric method for the analysis of Pioglitazone hydrochloride in phosphate buffer (pH 7.4) and methanolic solution, assessing its linearity and compliance with Beer's Law. Furthermore, we aimed to use FTIR to characterize potential interactions between Pioglitazone and common

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pharmaceutical excipients, such as Guar Gum, Chitosan, and Sodium Alginate. Standard solutions of Pioglitazone were prepared in phosphate buffer (pH 7.4) and methanol. UV spectrophotometer was conducted to determine the maximum absorption wavelength. Calibration curves were constructed to evaluate linearity and adherence to Beer's Law. FTIR analyses were performed to investigate drug-excipient interactions by examining the functional groups. In phosphate buffer (pH 7.4), the maximum absorption wavelength for Pioglitazone hydrochloride was 268 nm. The calibration curve for Pioglitazone in phosphate buffer (pH 7.4) demonstrated linearity in the concentration range of 1–20 µg/ml, with a correlation coefficient of 0.998. In methanol, the maximum absorption wavelength for Pioglitazone hydrochloride was found to be 272 nm. The calibration curve in methanol exhibited linearity in the range of 1–20 µg/ml, with a correlation coefficient of 0.999. FTIR analysis revealed potential drug-excipient interactions, particularly in the case of Guar Gum, Chitosan, and Sodium Alginate, suggesting the formation of stable hydrogen bonds. The developed UV spectrophotometric method for Pioglitazone analysis is a reliable, cost-effective, and reproducible approach, making it a valuable tool for drug development and quality control. Additionally, the FTIR characterization confirmed interactions between Pioglitazone and common pharmaceutical excipients, enhancing our understanding of formulation compatibility.

Keywords: Pioglitazone; UV spectrophotometer; drug-excipient interactions; FTIR; pharmaceutical analysis; Beer's Law.

1. INTRODUCTION

Spectrophotometer, particularly in the ultraviolet (UV) range, is a fundamental and versatile analytical technique extensively employed in the pharmaceutical industry. It provides a straightforward and reliable means for the estimation of bulk drugs, the analysis of drug samples, and the investigation of drug interactions with various excipients. In the realm of pharmaceutical development, ensuring the stability, bioavailability, and manufacturability of solid dosage forms hinges on a comprehensive understanding of drug-excipient interactions during preformulation studies [1-5]. The validation of analytical methods for drug analysis and the assessment of compatibility between active pharmaceutical ingredients (APIs) and excipients are pivotal steps in the pharmaceutical development process. These processes are essential to guarantee the safety, efficacy, and quality of pharmaceutical formulations. In this context, analytical techniques such as UV spectrophotometry and Fourier transform infrared spectroscopy (FTIR) play crucial roles [6-8]. This study focuses on the analysis of Pioglitazone hydrochloride, a Class-II Biopharmaceutics Classification System (BCS) medication known for its low solubility in water but significant solubility in organic solvents. Pioglitazone, a 2,4-thiazolidinedione derivative, finds application as an oral hypoglycemic medication and belongs to the thiazolidinedione class. Typically, it is prescribed as a monotherapy or in combination with other antidiabetic agents when necessary. Understanding the physicochemical properties and interactions of Pioglitazone with common pharmaceutical excipients is crucial for the formulation of stable and effective drug products [9-10]. The methodology employed in this study encompasses the development of a UV spectrophotometric method for Pioglitazone analysis in both phosphate buffer (pH 7.4) and methanolic solutions. The study aims to establish the maximum absorption wavelengths, linearity, and adherence to Beer's Law for Pioglitazone in these solvents. Moreover, FTIR analyses are conducted to investigate potential interactions between Pioglitazone and excipients such as Guar Gum, Chitosan, and Sodium Alginate, which are commonly used in pharmaceutical formulations [11-12]. By providing a robust analytical method for Pioglitazone analysis and insights into drug-excipient interactions, this study enhances the understanding of Pioglitazone's behavior during formulation development. These findings are vital for ensuring the quality, efficacy, and safety of Pioglitazone-containing pharmaceutical products, ultimately benefiting patients with diabetes and related conditions. The subsequent sections of this paper will detail the materials and methods used in this study, present the results and discussions, and conclude with a summary of the key findings and their implications for pharmaceutical development [13-15].

2. MATERIALS AND METHODS

2.1 Materials

Pioglitazone HCl for the purpose of this research was obtained from Sun Pharma Pvt Ltd., which is

located in Baddi in the state of Himachal Pradesh. All of the trials were carried out with water that had been distilled twice. Sodium Alginate LV, Ultra-Pure, manufactured by Sisco Research Laboratories Pvt. Ltd. in Maharashtra, India. Chitosan was produced by Central Drug House (P) Ltd. in Delhi. Guar Gum and Endosperm Powder are both products of the Central Drug House (P) Ltd. in Delhi. The other substances were all put to use in an analytical capacity throughout the process. The Shimadzu UV/Vis double beam spectrophotometer (Pharma Spec- 1700) that was used to obtain all of the absorbance readings was equipped with 1 cm matched quartz cells. The Thermo-Scientific Nicole 6700 FTIR spectrometer is a well-known model of FTIR equipment. It was manufactured in the United States.

2.2 Methodology

2.2.1 Identification of drug by UV spectroscopy

A) Preparation of Standard solution in Phosphate Buffer pH-7.4- Put one hundred milligrams of the drug into one hundred milliliters of phosphate buffer solution with a pH of 7.4, and then dilute the mixture until it has a concentration of ten milligrams per milliliter scanned utilizing a wavelength range of 200–400 nm with a blank solution serving as the reference point [16].

B) Preparation of Standard solution in Methanol solution (50:50) - Put one hundred milligram's of the drug into one hundred milliliters of methanol, and then dilute the mixture until it has a concentration of ten milligrams per milliliter. Scanned utilizing a wavelength range of 200–400 nm with a blank solution serving as the reference point [16].

2.2.2 Characterization of drug with excipients

These infrared tests were carried out utilizing an IR spectrometer manufactured by Thermo-Scientific, USA's Nicole 6700 Fourier Transform Infrared Spectrometer, which included a frequency range of 4000 to 6000 cm^{-1} and a resolution of 1 cm^{-1}. The generation of pure Pioglitazone, a physical mixture of drug and excipients, and a control sample were all accomplished via pelletization in KBr, which was done independently of one another. The IR peaks of the pharmaceutical, as well as its physical composition, were investigated by the researchers [17].

3. RESULTS AND DISCUSSION

3.1 Identification of Drug by UV Spectroscopy

A) Preparation of Standard solution in Phosphate Buffer pH-7.4: Put one hundred milligram's of the drug into one hundred milliliters of phosphate buffer solution with a pH of 7.4, and then dilute the mixture until it has a concentration of ten milligrams per milliliter. Scanned utilizing a wavelength range of 200–400 nm with a blank solution serving as the reference point. It was established that 268 nm is the wavelength at which absorption is at its peak. The calibration curve was produced by measuring the absorbance of the solutions at 268 nm against a blank (Table 1), which allowed for the calculation of the slope of the curve.

B) Preparation of Standard solution in Methanol solution (50:50) - Put one hundred milligrams of the drug into one hundred milliliters of methanol, and then dilutes the mixture until it has a concentration of ten milligrams per milliliter. Scanned utilizing a wavelength range of 200–400 nm with a blank solution serving as the reference point. It has been discovered that the optimal wavelength for absorption is 272 nm. In order to calibrate the solutions, it was necessary to measure their absorbance at 272 nm in comparison to a blank (Table 2).

Table 1. Calibration curve of Pioglitazone hydrochloride in phosphate buffer solution pH-7.4

S.No.	Concentration(µg/ml)	Absorbance(nm)
01.	0	0
02.	1	0.181
03.	2	0.338
04.	4	0.521
05.	6	0.688
06.	8	0.829
07.	10	0.999

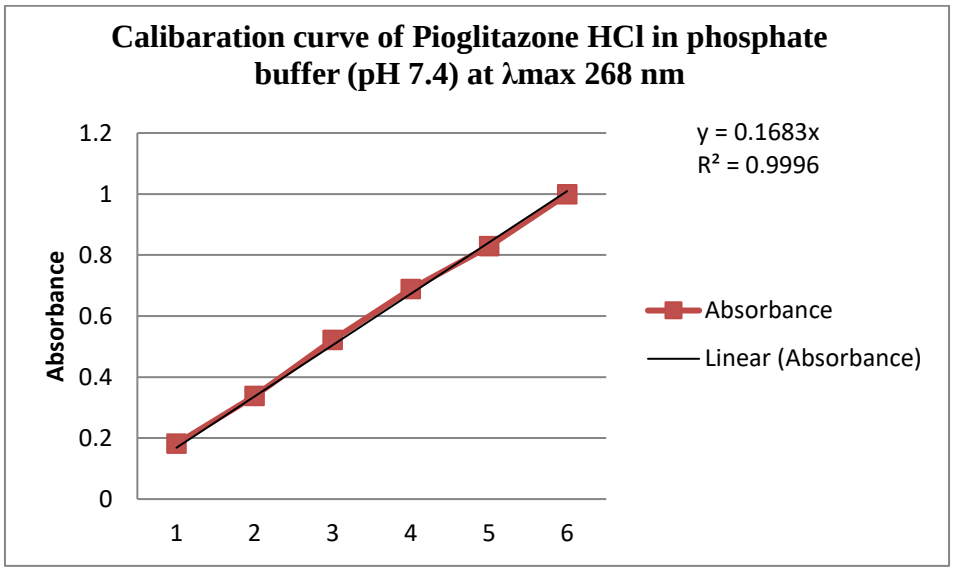

Fig. 1. Calibration curve of Pioglitazone hydrochloride in phosphate buffer solution pH-7.4

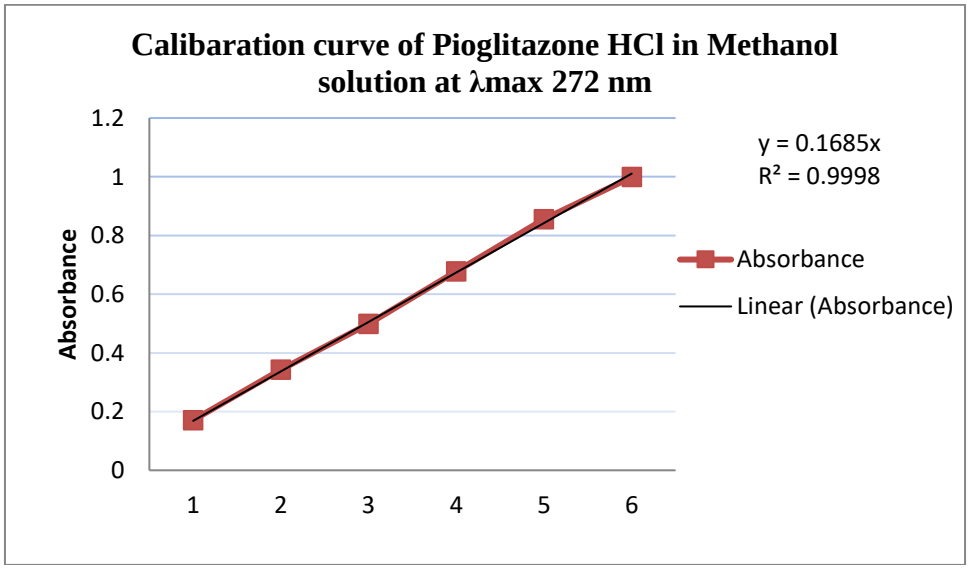

Fig. 2. Calibration Curve of Pioglitazone hydrochloride in Methanol solution

Table 2. Calibration Curve of Pioglitazone hydrochloride in Methanol solution

S.No.	Concentration(µg/ml)	Absorbance(nm)
01.	0	0
02.	1	0.171
03.	2	0.343
04.	4	0.499
05.	6	0.677
06.	8	0.855
07.	10	0.999

Maximum absorbance, Beer's law limitations, and molar absorptivity, along with other optical properties, are tabulated below. Tables 3 and 4 summaries the results of a least-squares regression analysis performed on the slope (b), intercept (a), and correlation (r) obtained from the various concentrations.

Table 3. Validation parameters in phosphate buffer solution pH-7.4

S.No.	Parameter	Result
01.	Absorption maxima	268 nm
02.	Linearity Range	1-20 µg/ml
03.	Standard Regression Equation	y = 0.168x
04.	Correlation coefficient	0.998
05.	Molar absorptivity	9.98× 10^4/mol.cm
06.	Specificity	A 2 µg/ml of drug in Phosphate buffer solution at UV detection wavelength of 268 nm shows an absorbance value of 0.343

Table 4. Validation parameters in Methanol solution

S.No.	Parameter	Result
01.	Absorption maxima	272
02.	Linearity Range	1-20 µg/ml
03.	Standard Regression Equation	y=0.168x
04.	Correlation coefficient	0.999
05.	Molar absorptivity	9.99× 10^4/mol.cm
06.	Specificity	A 10 µg/ml of drug in methanol solution at UV detection wavelength of 272 nm shows an absorbance value of 0.999

3.2 Linearity

In the concentration range of 1–20 µg/ml, Pioglitazone hydrochloride followed Beer's Law and showed maximal absorption at 268nm. Absorbance vs. concentration was linearly regressed, and the resulting equation (in Phosphate Buffer, pH-7.4) was y = 0.168x, with a correlation coefficient of 0.998. Maximum absorption was observed at 272nm, and the concentration range of Pioglitazone hydrochloride obeyed Beer's Law (1-20 µg/ml). In a Methanol solution, the linear regression equation for absorbance versus concentration is y=0.168x, with a correlation coefficient of 0.999.

3.3 Pioglitazone HCl FTIR Analyses

The test known as Fourier transform infrared spectroscopy was utilized in order to examine the Pioglitazone's functional groups. The peaks in the infrared spectrum of Pioglitazone that are attributable to N- stretching are located at 3173.95 cm^{-1} and 3296.42 cm^{-1}. The peaks that are attributable to aliphatic C-H stretching are located at 2918.44 cm^{-1} and 2850.25 cm^{-1}. A powerful absorption peak may be traced back to the medication's carbonyl stretching vibration, denoted by the symbol C=O and located at 1627.49 cm^{-1}. The peak at 1568.85 cm^{-1} is representative of the aromatic ring, and the peak at 1167.63 cm^{-1} is related to the C-O Ar group (1) (Fig. 3) (5).

3.4 Physical mixture of Drug and Guar Gum

In the IR spectra of Pioglitazone HCl-Guar gum, the amide-NH stretching vibration at 3173.95 cm^{-1} was not recognized in a broad band. This may have been because the drug's N-H band co-appeared with Guar gum's OH enhanced band at 3370.96 cm^{-1}. Based on these findings, it seemed likely that Pioglitazone HCl formed a compound with guar gum due to their robust interaction. The C-H stretching of Pioglitazone HCl caused the absorption band that occurred at 2918.13 cm^{-1} to migrate to a higher wave number, which caused Pioglitazone HCl-guar gum to both broaden the band and move it. The formation of persistent hydrogen bonds between Pioglitazone HCl and guar gum, which was observed in the IR spectra, is evidence of a drug-excipient interaction (2) (Fig. 4) (6).

3.5 Physical Mixture of Drug and Chitosan FTIR Analysis

The N-H and O-H stretching, as well as the intramolecular hydrogen bonds, are all represented by a prominent band in the area 3291-3361 cm^{-1} in the IR spectrum of Pioglitazone HCl-Chitosan. The drug's N-H band appeared together with the Chitosan band at 3372.08 cm^{-1}, which may explain why the amide-NH stretching vibration at 3174.05 $cm^{-1}$was not found on a broad band. C-H symmetric

and asymmetric stretching are responsible for the absorption bands about 2918.86 cm^{-1}. Bands near 1628.31 cm^{-1} (C=O stretching of amide I) confirmed the presence of lingering N-acetyl groups. Asymmetric stretching of the C-O-C bridge is responsible for the 1166.27 cm^{-1} absorption band. The IR spectra showed that Pioglitazone HCl and Chitosan interacted with one another, forming stable hydrogen bonds (3) (Fig. 5) (7).

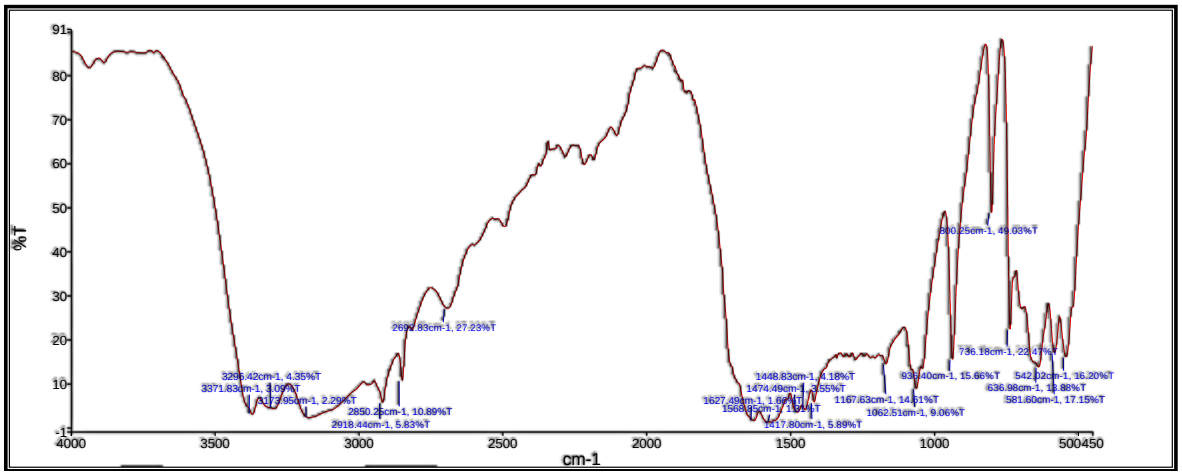

Fig. 3. FTIR spectrum of Pioglitazone HCl

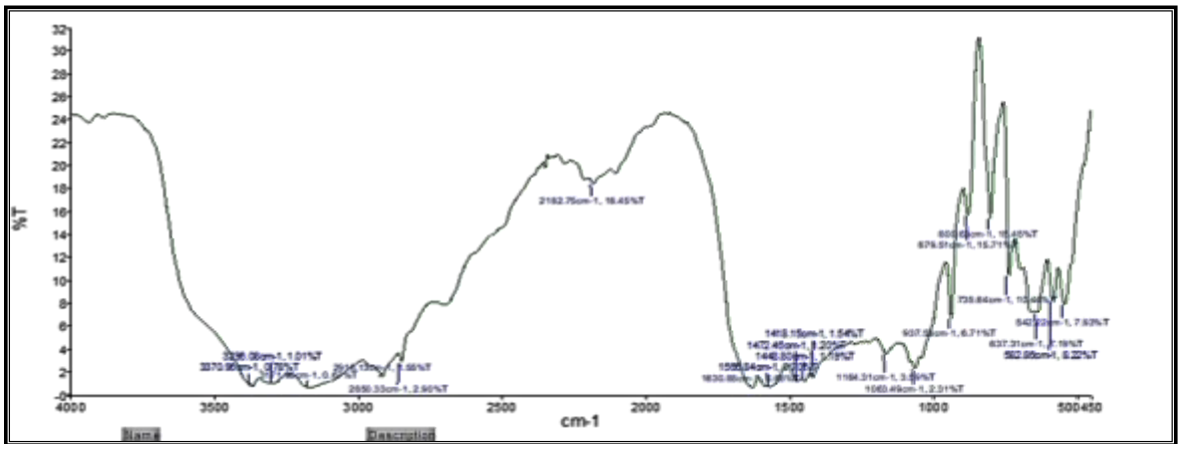

Fig. 4. FTIR Spectrum of Drug and Guar Gum

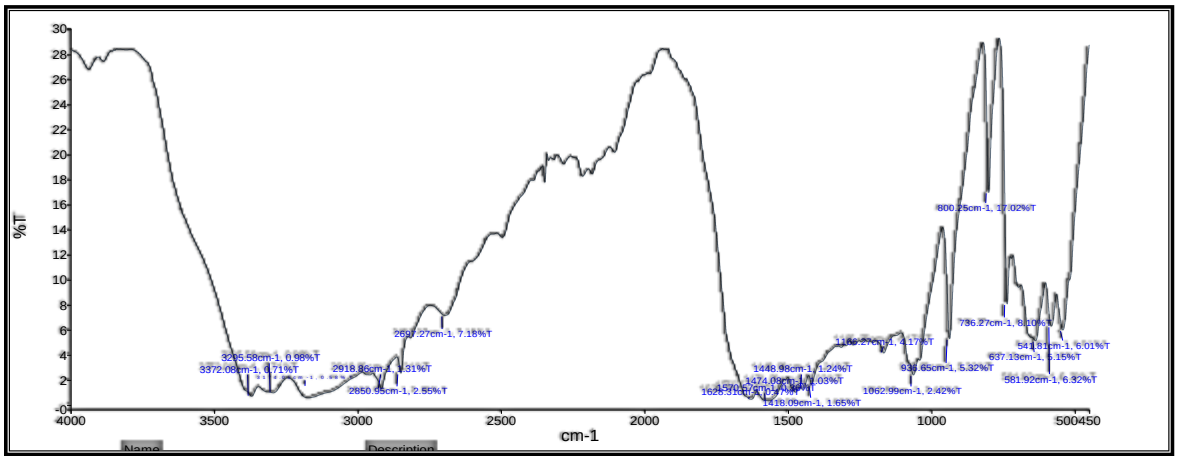

Fig. 5. FTIR spectrum of Drug and Chitosan

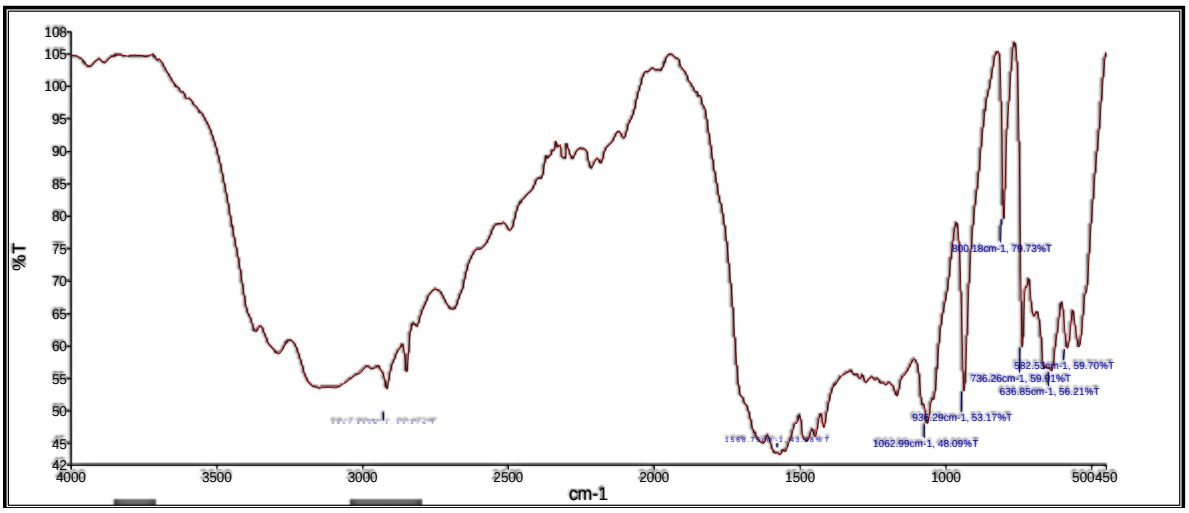

Fig. 6. FTIR spectrum of Drug and Sodium Alginate

Table 5. Various principle absorption peaks for different functional groups in Pioglitazone HCl, Guar Gum, Chitosan and Sodium Alginate

S.no.	Principle absorption peak (/cm)	Functional group and vibration
A. Pioglitazone HCl(Drug)		
01.	3173.95 cm^{-1} and 3296.42 cm^{-1}	N-H stretching
02.	2918.44 $cm^{-1}$and 2850.25cm^{-1}	C-H stretching
03.	1627.49 cm^{-1}	C=O stretching
04.	1568.85cm^{-1}	aromatic ring
05.	1167.63 cm^{-1}	C-O-Ar group
B. Drug and Guar Gum		
01.	3370.96 cm^{-1}	O-H stretching
02.	at2918.13 cm^{-1}	C-H stretching
03.	1630. 85cm^{-1}	C=O stretching
04.	1566.84 cm^{-1}	Aromatic ring
05.	1448.8 cm^{-1}	C-H bending
C. Drug and Chitosan		
01.	3291–3361 cm^{-1}	N-H and O-H stretching
02.	2918.86 cm^{-1}	C-H symmetric and asymmetric stretching
03.	1628.31 cm^{-1}	C=O stretching of amide I
04.	1166.27 cm^{-1}	C-O-C bridge
D. Drug and Sodium Alginate		
01.	2917.85 cm^{-1}	CH_2 stretching
02.	1568.75cm^{-1}	Aromatic ring
03.	1062.99 cm^{-1}	C-O stretching

3.6 Physical Mixture of Drug and Sodium Alginate

The NH functional group, at 3200–3400 cm^{-1}, and the CH_2 stretching at 2917.85 cm^{-1}, was not detected. The aromatic ring is represented by a peak at 1568.75 cm^{-1}. Interaction between medication and excipient was detected by IR spectroscopy (Fig. 6).

4. CONCLUSION

This study has successfully developed a UV spectrophotometric method for the analysis of Pioglitazone hydrochloride in phosphate buffer (pH 7.4) and methanolic solutions. The investigation revealed that Pioglitazone exhibited maximum absorption at 268 nm in phosphate buffer and 272 nm in a methanolic solution,

establishing optimal wavelengths for its quantitative analysis. The concentration range of Pioglitazone (1-20 µg/mL) followed Beer's Law in both solvents, demonstrating its linearity. The validation parameters, including standard regression equations, correlation coefficients, and molar absorptivity, were determined, further affirming the reliability and accuracy of the developed method. Specificity tests confirmed the method's ability to distinguish Pioglitazone even at low concentrations in the respective solvents. The FTIR analyses of Pioglitazone in physical mixtures with commonly used excipients, namely Guar Gum, Chitosan, and Sodium Alginate, indicated the presence of drug-excipient interactions. These interactions were revealed through shifts and modifications in the characteristic IR peaks associated with functional groups of Pioglitazone. Notably, the formation of stable hydrogen bonds between Pioglitazone and these excipients was observed, suggesting potential impact on drug stability and performance in pharmaceutical formulations. In conclusion, this study provides a valuable UV spectrophotometric method for the accurate and reproducible analysis of Pioglitazone in pharmaceutical preparations. Additionally, the characterization of Pioglitazone interactions with excipients sheds light on the potential effects of these interactions on the drug's behavior in formulation development. Understanding drug-excipient interactions is vital for ensuring the quality, stability, and efficacy of Pioglitazone-containing pharmaceutical products. The insights gained from this study can be instrumental for pharmaceutical scientists and formulators in the development of Pioglitazone-based medications, ultimately benefiting patients who rely on these drugs to manage diabetes and related conditions. Further research in the field of drug-excipient interactions and their impact on pharmaceutical formulations holds promise for improving drug development processes and optimizing therapeutic outcomes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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