ASSAY OF SOME ORAL HYPOGLYCEMIC AGENTS BY UV-VISIBLE SPECTROSCOPIC MEASUREMENTS

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ABSTRACT

Pharmaceutical chemistry is a science that makes use of the general laws of chemistry to study drugs, their preparation, chemical nature, composition, structure, methods of quality control, etc. UV-Visible spectrometry is one of the important analytical methods employed in the qualitative and quantitative analysis of pharmaceutical drugs. Qualitative analysis of the compound is done through UV-Visible spectroscopy by identifying the λ_{max}. UV-Visible spectrophotometers are more significantly employed for the quantitative estimation of assay of drugs. The actual drug content of a tablet is calculated by comparing the absorbance at λ_{max} of pure substance and the drug. In this work, the quantitative analysis of three hypoglycemic agents used for the treatment of type 2 diabetes namely, gliclazide, pioglitazone hydrochloride and metformin hydrochloride has been carried out (to estimate assay) using UV-Vis spectrophotometry. Tablets of different strengths from same manufacturer were used for the analysis. The estimation was found to be satisfactory.

Key words: UV-Visible spectrometry, Estimation of assay, Oral hypoglycemic agents, Gliclazide, Pioglitazone hydrochloride, Metformin hydrochloride.

INTRODUCTION

A drug is defined as a chemical substance used in the treatment, cure, prevention or diagnosis of disease or used to otherwise enhance physical or mental well being in man or animals. Pharmacology is the science, which deals with the detailed study of drugs and their action on living animals, organs and tissues. The two major classification of pharmacology are pharmacodynamics and pharmacokinetics. The former deals with the biological effects of drugs and the latter explain absorption, distribution, metabolism and excretion of drugs consumed. Spectacular development in physiology, biochemistry, organic chemistry and molecular biology has accelerated the advances in drug design and development. Drug

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development in turn has helped to elucidate many basic physiological and pathological mechanisms in health and disease.

Pharmaceutical chemistry makes use of the general chemistry to study drugs, their preparation, chemical nature, composition, structure and conditions of their usage. The term pharmaceutical means any chemical that finds use in medical treatment for internal or external administration in human body. All pharmaceutical samples including the intermediates and final products have to be carefully controlled for their quality for which specifications of quality have been listed. A collection of such descriptions is the pharmacopoeia. It is an official code containing a selected list of established drugs with description of their physical properties and tests for their identity, purity and potency.

The descriptive material pertaining to any of the drugs in the pharmacopoeia is known as the monograph. A pharmacopoeial monograph constitutes the drug name, formula weight, molecular weight, category, dose, description, solubility, standards, limits for impurities, assay and storage of the drugs[1-3]. Each monograph is very important in the identification of the drug. Among these, assay is the estimation of potency of an active substance. It gives a step by step description of a chemical analytical method for the estimation of potency of an active substance. When the assay method given in the pharmacopoeia is applied to a chemical, the standards prescribed in the monograph earlier should be attained. Different methods of assay are titrimetric, gravimetric, spectrophotometric, chromatographic etc. Each of the methods is prescribed to a particular compound. The present work deals with the application of spectrophotometric measurements to the quantitative estimation of the active substance in a drug.

A dosage is the amount of the drug designed for administration to the patients for the diagnosis and treatment of diseases. Many drugs are more conveniently marketed and administered in solid dosage forms like powders, capsules, tablets, pellets and pills. Tablets are the solid dosage forms containing granulated or powdered drug substance with suitable binders and diluents that are compressed into round or disc shapes. Additives are included to enhance the physical appearance, improve stability and aid in disintegration after administration. These additives are inert ingredients and care is taken in their selection to ensure that the physiological availability and therapeutic efficiency of the active drug is not decreased.[4-6].

The various components in a tablet apart from the active therapeutic ingredient are anti-adherents, binders, diluents, fillers, disintegrators, coating agent, lubricants, polishing agents etc.[7] Diluents are added to increase the weight of the tablet in order to make it of a

practical size, as the active ingredient is small in quantity. Commonly used diluents are dicalcium phosphate, calcium sulphate, lactose, sodium chloride, dry starch and powdered sugar. Binders are agents used to impart cohesive qualities to the powdered material, which are starch, gelatin, sucrose, glucose or lactose. Lubricants improve the rate of flow of the tablet granulation, prevent adhesion of the tablet and facilitate its ejection. Hydrogenated vegetable oils, stearic acid, calcium and magnesium stearates are the commonly used lubricants. Disintegrators are added to a tablet to facilitate its breakup after administration so that the active ingredient is released from the tablet as efficiently as possible to allow for its rapid dissolution. Active disintegrators are corn and potato starch, cellulose, algins and gums[8-10].

Tablets remain a popular dosage form among drug manufacturers because of their simplicity, economy of preparation, stability and convenience in packaging, transporting and dispensing. In addition, these tablets provide many more advantages to patients like compactness, portability, accuracy of dosage and ease of administration. In the present work, three antidiabetic tablets, gliclazide, pioglitazone hydrochloride and metformin hydrochloride are employed for the quantitative estimation of the drug content in the tablet, using UV-Visible spectroscopic technique.

EXPERIMENTAL

Methods and materials

High grade samples of gliclazide, pioglitazone hydrochloride and metformin hydrochloride and their tablet forms were procured from Sun Pharmaceutical Industries Limited, Mumbai, India. For gliclazide, 40 mg tablet is used for assay estimation. Similarly, the tablet containing pioglitazone alone as the active substance was pioglit 30 mg. The tablets containing metfomin as the active ingredient used in assay is of 500 mg strength. The UV-Visible spectra have been recorded for all the drugs in the pure form and for the tablets using SL 159 UV-Visible spectrophotometer at Spectrophysics Research Laboratory, Pachaiyappa's College, Chennai, India. The sources that produce ultra-violet rays in the spectrometer were deuterium and tungsten halogen lamps with the monochromator as Czerny-Turner type with 1200 lines/mm holographic grating. The detector has a wide range of photodiode with greater efficiency and stray light loss is about < 0.1% at 220 nm with NaI 10 g/L. The scanning range capability of the device was 200-1000 nm with an accuracy of ± 0.5 nm. Quartz cuvettes of 10 mm path length were used. The amount of absorption of UV-Visible radiation was measured for each concentration at the characteristic wavelength maximum λ_{max}. By comparing the absorbance in the pure and the tablet form of the sample, the quantitative estimation of the drug content was carried out.

RESULTS AND DISCUSSION

UV-Visible spectral analysis

UV-Visible spectrophotometry plays a significant role in analytical chemistry, especially in the pharmaceutical field. The introduction of UV-Visible spectrophotometer paved a new opportunity for the qualitative and quantitative analysis of a great variety of drugs. The identification of λ_{max} ensures the quality of the drug, which is an inevitable step.[11] In addition to the qualitative analysis; it is the quantitative analytical application, which is of greater significance in drug analysis. Many of the pharmaceutical samples are assayed spectrophotometrically. A solution of the test substance is made of a known concentration in a suitable solvent. The absorbance is noted at the wavelength maxima of the UV-Visible spectrum and recorded. By making a parallel determination of a solution prepared from a pure reference sample for the same concentration, the amount of the active ingredient in the test substance is calculated. All the measurements are based on the fundamental law of spectrophotometry i.e. Beer-Lambert's law.[12] The drug content of the tablet is calculated using Beer law as

Assay = (Test absorption / Standard absorption) x

(Standard weight / Test Weight) x (Average weight of one tablet)

Gliclazide

Gliclazide is a second generation of hypoglycemic sulfonylurea,[13] a useful drug for a long-term treatment of non-insulin dependent diabetes mellitus (NIDDM) because of its good general tolerance, low incidence of hypoglycemia [14,15] and a low rate of secondary failure[16].

It is chemically known as 3-(7-azabicyclo [3.3.0] oct-7-yl)-1-(4-methylphenyl) sulphonyl-urea.[17] It has the molecular formula $C_{15}H_{21}N_3O_3S$ and molecular weight of 323.4. The structure of gliclazide is presented in Fig. 1.
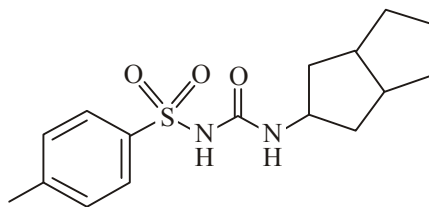

Fig. 1: Chemical structure of gliclazide

It is a white powder, practically insoluble in water, freely soluble in dichloromethane, methanol and sparingly soluble in acetone. Gliclazide stimulates insulin secretion from functional pancreatic β cells and increases the sensitivity of β cells to glucose stimulus. The pharmacokinetics and pharmacodynamics of gliclazide have been studied in Caucasians and Australian aborigines with type 2 diabetes by Davis *et al*.[18] Assay of gliclazide by chromatographic techniques has been already reported[19, 20].

For the assay of gliclazide, the tablet of strength 40 mg was used. As a first step, the pure form of the drug was standardized by UV-Visible spectral measurements. Methanol was used as solvent. The 1 mg/mL solution of gliclazide was prepared initially by dissolving 5 mg of pure sample in 5 mL of methanol, and by successive dilution, the other concentrations were obtained. The characteristic wavelength maximum for gliclazide was observed at around 232 nm (Fig. 2).
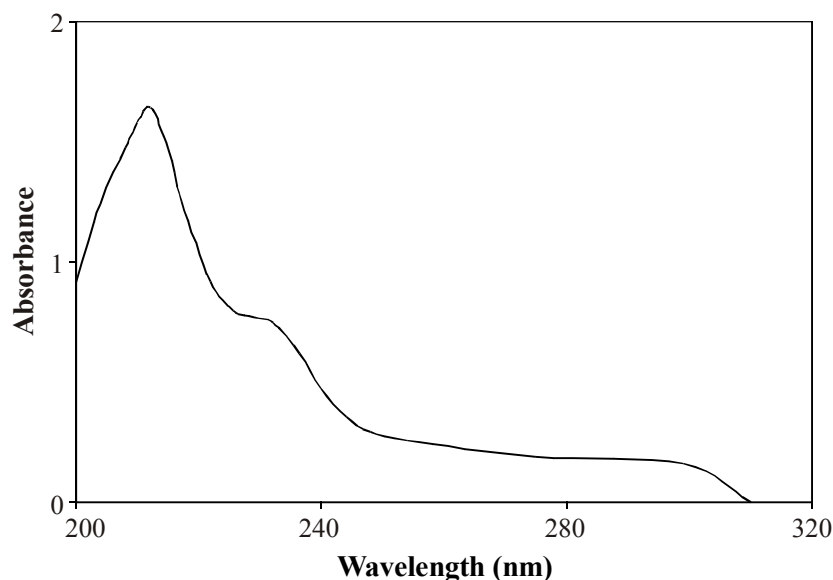

Fig. 2: UV-Visible spectrum of gliclazide

The presence of CONH group in the compound results in the n $\rightarrow$ π^* transition producing a λ_{max} at 232 nm.[21] The absorbance values at λ_{max} were noted. For doing the test absorption, 10 tablets of gliclazide 40 mg were weighed and the average weight of one tablet was calculated as 147 mg. To obtain the same concentration as that of the standard, 5 mg of standard gliclazide was equivalent to 18.375 mg of tablet. Hence, 18.375 mg of powdered tablet was dissolved in 5 mL of double distilled water and the solution was filtered using a fine grade filter paper. The stock solution of tablet also had the concentration of 1 mg/mL.

This was diluted to the same concentrations of the pure sample. The UV-Visible spectrum has been recorded in each case. From the formula given, the quantitative estimation of the active substance gliclazide was estimated in the tablet and found to be around 38 mg approximately. The experiment was repeated at least five times and the average values of the test and standard absorption are tabulated. Table 1 presents the estimation of assay of tablet gliclazide. In the present work, a single component system was chosen for the assay. From the spectroscopic point of view, a single component system is the one for which, at the wavelength selected for the measurement, the determination of the analyte is not influenced either by another substance or by background absorption.

Table 1: Estimation of assay in gliclazide

Wavelength maximum (nm)	Concentration (mg / mL)	Average absorbance at wavelength maximum for		Estimation of assay (mg)
		Pure sample	Tablet	
232	0.33	1.43	1.366	38.2
232	0.25	0.923	0.905	39.22
232	0.20	0.54	0.521	38.59
232	0.166	0.405	0.389	38.41

Pioglitazone

Pioglitazone is an oral antidiabetic agent that belongs to the class of thiozolidinedione. It is used in the management of type 2 diabetes mellitus and acts primarily by decreasing insulin resistance.[22] It is chemically designated as [5-[[4 - [2 - (5 - ethyl - 2 pyridinyl) ethoxy] phenyl] methyl]-2, 4-] thiazolidine-dione monohydrochloride having a molecular formula $C_{19}H_{20}N_2O_3S$. HCl. It has a molecular weight of 392.9. It is a white crystalline powder, soluble in N, N′-dimethylformamide, methanol, very slightly soluble in acetone and acetonitrile, practically insoluble in water and ether. The structure of pioglitazone is presented in Fig. 3.
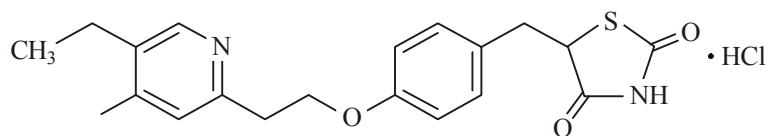

Fig. 3: Chemical structure of pioglitazone hydrochloride

Pioglitazone enhances insulin sensitivity in the peripheral organs and the liver, resulting in improved glycemic control in patients with type 2 diabetes.[23, 24] In these patients, pioglitazone also lowers elevated plasma free fatty acids and improves diabetic dyslipidemia (low HDL - cholesterol and high triglycerides).[25] The effect of pioglitazone on glycemic control and indication of insulin sensitivity in patients with type 2 diabetes was studied by Pavo *et al.*[26] Assay of pioglitazone hydrochloride in tablets has been already reported by HPLC methods[27, 28].

For the assay of pioglitazone, the tablet of strength 30 mg was used. The UV-Visible spectral analysis of pure pioglitazone was carried out first to find the absorbance values at the characteristic λ_{max} at various concentrations. The λ_{max} value was found to be 269 nm. The presence of benzene and pyridine rings in the structure of pioglitazone produces the λ_{max} at 269 nm.[21] The UV-Visible spectrum of pioglitazone is presented in Fig. 4.
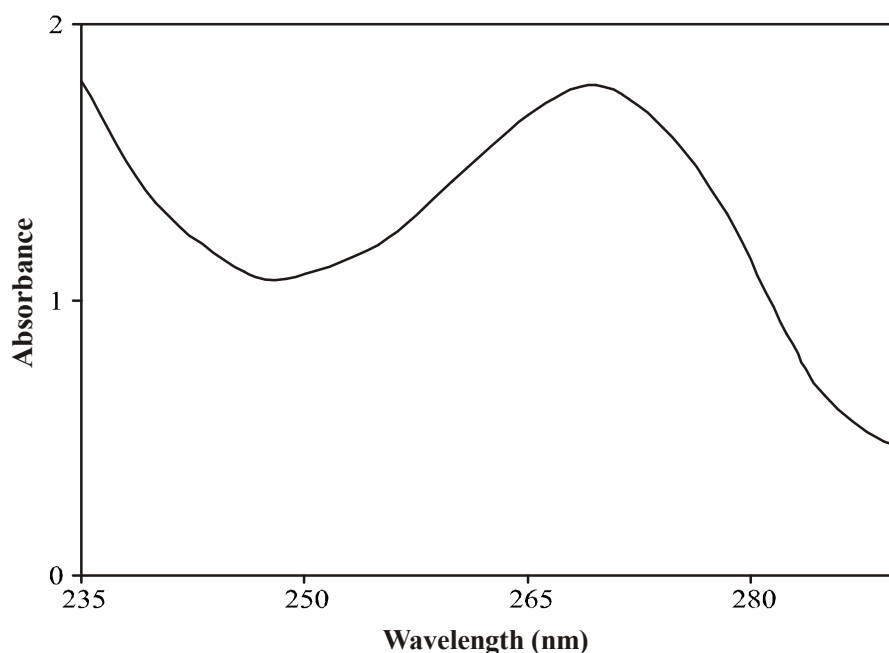

Fig. 4: UV-Visible spectrum of pioglitazone

For doing the test absorption, 10 tablets of pioglitazone (30 mg) were weighed and the average weight of one tablet was calculated as 69 mg. To prepare 1 mg/mL solution of the tablet, 11.5 mg of the powdered tablet was dissolved in 5 mL of double distilled water and the solution was filtered using a fine grade filter paper. This solution was used to get the

other concentrations by successive dilution procedure. The UV-Visible spectrum has been recorded in each case. Using the formula for assay, the active substance pioglitazone in the tablet was estimated and found to be 29.4 mg. Table 2 presents the estimation of assay of tablet pioglitazone.

Table 2: Estimation of assay in pioglitazone

Wavelength maximum(nm)	Concentration (mg/mL)	Average absorbance at wavelength maximum for		Estimation of assay (mg)
		Pure sample	Tablet	
269	0.166	1.216	1.210	29.85
269	0.142	0.803	0.805	30.07
269	0.125	0.574	0.569	29.73
269	0.111	0.385	0.412	28.03

Metformin

Metformin is an exciting drug that has a major impact on the treatment of type 2 diabetes for the past three decades.[29] It belongs to the class of biguanides and it is chemically known as N,N'-dimethyl biguanide hydrochloride. It is a white hygroscopic crystalline powder with a bitter taste with a molecular formula $C_4H_{11}N_5HCl$. Fig. 5 presents the molecular structure of metformin. The assay of metformin has been reported by high-performance liquid chromatography in human plasma and urine.[30, 31] Also, the estimation of pioglitazone hydrochloride and metformin hydrochloride in tablets by derivative spectrophotometry and liquid chromatographic methods has been reported by Shankar *et al.*[32].
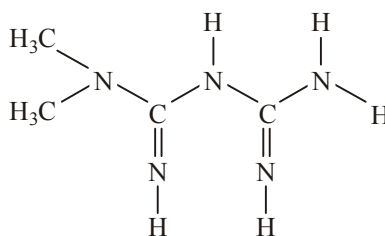
Fig. 5: Chemical structure of metformin hydrochloride

For the assay of metformin hydrochloride, the tablet of strength 500 mg was used. The pure sample of metformin was standardized by UV-Visible spectral measurements. 25 mg of pure metformin was dissolved in 25 mL of double distilled water to get the concentration of 1 mg/mL. The UV-Visible spectra for the solutions were recorded for various concentrations by successive dilution method. Fig. 6 gives the UV-Visible spectrum of metformin hydrochloride with a peak at 234 nm. The characteristic wavelength maximum of UV-Visible spectrum provides a wealth of structural information of the drug metformin. The peak at 234 nm is the R band (Radical - like) due to n $\rightarrow$ π^* electronic transition, indicating the presence of unconjugated acetoxime (C=N) chromophore. After noting the standard absorbance values at different concentrations, the test absorbance was found. By weighing ten tablets and finding the average, the weight of one tablet metformin was found as 514 mg, 25.7 mg of powdered tablet was dissolved in 25 mL of double distilled water to get the stock solution of the tablet metformin. Similar procedure has been adopted for the assay estimation. Table 3 presents the average values of test and standard absorptions measured at least five times and hence, the assay of the tablet metformin hydrochloride was estimated to be around 491 mg.
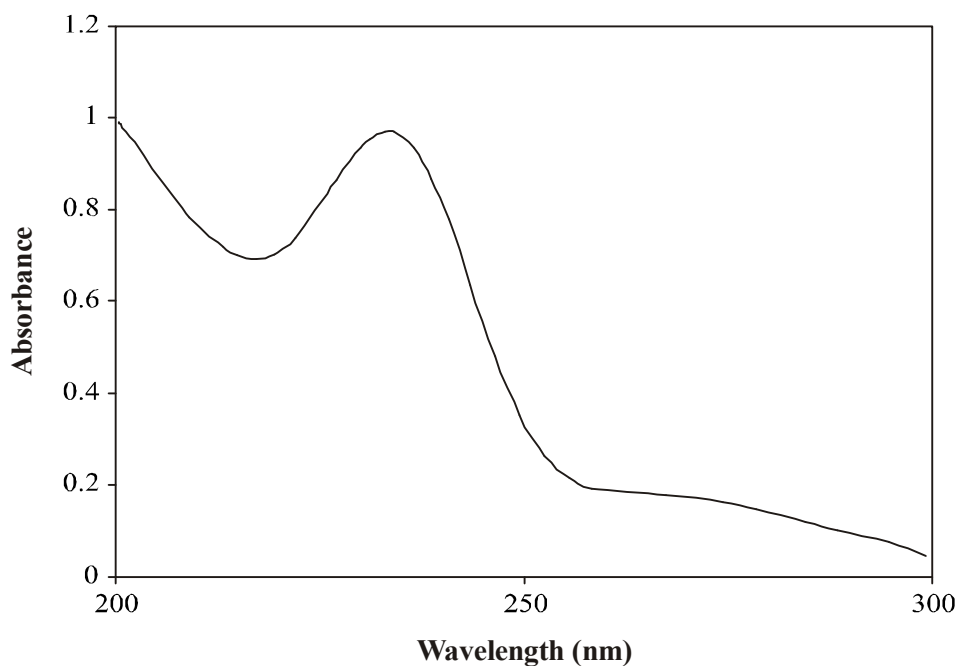

Fig. 6: UV-Visible spectrum of metformin hydrochloride

Table 3: Estimation of assay in metformin hydrochloride

Wavelength maximum (nm)	Concentration (mg / mL)	Average absorbance at wavelength maximum for		Estimation of assay (mg)
		Pure sample	Tablet	
234	0.125	1.972	1.934	490.30
234	0.111	1.193	1.170	490.36
234	0.100	0.700	0.690	492.85
234	0.909	0.391	0.385	492.32

CONCLUSION

UV-Visible spectroscopy has been successfully employed in the quantitative analysis of some pharmaceutical samples. The estimation of the active substance of a drug is one of the monographs specified by pharmacopoeia, which has to be checked periodically. The UV-Visible spectrophotometric method of assay has been employed in case of three antidiabetic tablets, gliclazide, pioglitazone hydrochloride and metformin hydrochloride. Using the absorbance values at wavelength maximum of tablet and the pure substance, the amount of active substance in each tablet was found out. In the case of gliclazide tablet of strength 40 mg, the experimental determination was found to be 38.6 mg. In 30 mg pioglitazone hydrochloride tablet, the active content of the drug was found to be 29.4 mg. For metformin hydrochloride 500 mg tablet, the assay was estimated to be around 491.5 mg. The estimation was found to be satisfactory.

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