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Pharmacokinetic Studies on Diazepam Using UV-Visible Spectroscopy

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The aim of the present investigation is to study the bound blood-drug interaction of diazepam using UV-Vis spectroscopic method. Diazepam is an anti-epileptic drug which can be useful in the treatment of epilepsy. Bound blood-drug interaction is a method of analysis to understand the pharmacokinetic parameters with regard to the plasma drug concentration in the blood. The pharmacokinetic properties of a particular drug are basically related to its absorption, distribution, metabolism and excretion. When a drug is given, it reaches its peak level in the blood pretty quickly and the drug level then decreases as the drug is broken down and removed from the blood. UV-Vis spectral measurements have been used to study the pharmacokinetic interaction of the drug with the living system (dog as a model); the results of clinical significance have been discussed.

Key Words: Pharmacokinetic interaction, Diazepam, UV-Vis Spectroscopy.

INTRODUCTION

UV-Vis spectroscopic methods have been employed to investigate the samples of biological interest[1–4]. Gunasekaran *et al.*[5] studied the ultraviolet absorption spectra of diseased subjects and normal healthy subjects belonging to the same blood group, age and sex. Arulmozhichelvan[6] analyzed the bound blood-drug interaction of dopamine hydrochloride on rat and rabbit models with different dosages at different time intervals using UV-Vis spectrometric method. Recently UV-Vis spectroscopic method has been employed successfully to study the bound blood-drug interaction of pentobarbitone sodium with dog as a model[7]. A single dose pharmacokinetic study in human volunteers using carbamazepine (anti-epileptic drug) has been analyzed by fluorescence polarisation immunoassay[8]. Pharmacokinetic interaction of phenytoin sodium has been analyzed in mice and rats by several researchers using conventional bio-assay methods. In the present work, a bound blood-drug interaction study is made on emergency drug diazepam using UV-Vis spectrophotometry. Diazepam is a member of the benzodiazepine family. Benzodiazepines are sedatives that cause dose-related

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depression of the central nervous system. It is an anti-epileptic drug. One of the brand names of diazepam is Valium. The molecular formula of diazepam is $C_{16}H_{13}ClN_2O$. Diazepam is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzo-diazepin-2-one.

Blood Components

Normally, 7–8% of human body weight is due to blood. This essential fluid carries out the critical functions of transporting oxygen and nutrients to our cells and getting rid of carbon dioxide and other waste products. In addition, it plays a vital role in our immune system and in maintaining a relatively constant body temperature. Blood is a highly specialized tissue composed of many different kinds of components produced in bone marrow. Four of the most important ones are red cells, white cells, platelets and plasma. All humans produce these blood components and there are no populational or regional differences. Red cells, or erythrocytes, are relatively large microscopic cells without nuclei. These cells normally make up 40–50% of the total blood volume. They transport oxygen from the lungs to all the living tissues of the body and carry away carbon dioxide. The red cells are produced continuously in our bone marrow from stem cells. People who are anemic generally have a deficiency in red cells. The colour of blood is primarily due to the red cells. White cells, or leukocytes, exist in variable numbers and types but make up a very small part of blood's volume—normally only about 1%. Most are produced in our bone marrow from the same kind of stem cells that produce red cells. Some white cells (called lymphocytes) are a major part of the immune system. Other white cells (called granulocytes and macrophages) protect our bodies from infection by surrounding and destroying bacteria, viruses, fungi or other parasites. Platelets, or thrombocytes, are cells that clot blood at the site of wounds. They do this by adhering to the walls of blood vessels, thereby plugging the rupture in the vascular wall. Plasma is the relatively clear liquid protein and salt solution which carries the red cells, white cells and platelets. Normally, 55% of our blood's volume is made up of plasma. About 95% of it consists of water. As the heart pumps blood to cells throughout the body, the plasma brings them nourishment and removes the waste products of metabolism. Plasma also contains blood clotting factors, sugars, lipids, vitamins, minerals, hormones, enzymes, antibodies and other proteins.

Hemoglobin is one of the best characterized of all proteins, in terms of both its structure and function. UV-Vis spectroscopy has been widely used in these studies. The oxyhemoglobin exhibits absorbance maxima[9] at 417 nm, 542 nm and 577 nm. The two most important coenzymes are the pyridine nucleotide coenzyme NAD^+ (nicotinamide adenine dinucleotide) and $NADP^+$ (nicotinamide adenine dinucleotide phosphate)[10]. The reduced forms of NAD^+ and $NADP^+$ are NADH and NADPH[11, 12]. The pyridine nucleotide coenzymes upon reduction to NADH and NADPH absorb at 349 nm due to their quinoid structure[13]. The changes in blood under pathophysiological conditions can be detected by the characteristic absorptions in blood.

Pharmacokinetics

Pharmacokinetics is the study of the time course of drug and metabolite levels in different body fluids during the absorption, distribution, metabolism and elimination phases of the drug. After administration by any route, a drug will reach the blood stream. This process is known as "absorption". The drug in the blood distributes rapidly between the plasma and blood cells and also between plasma proteins. This process of transferring a drug from blood to various tissues is called "distribution". A drug is eliminated either directly through an excretory route such as urine, bile and etc. which is known as "elimination", or indirectly through enzymatic or biochemical transformation by the liver. The latter path of elimination is called "metabolism".The study of this whole process of absorption, distribution, metabolism and elimination of a drug is presented schematically as shown in Fig. 1.

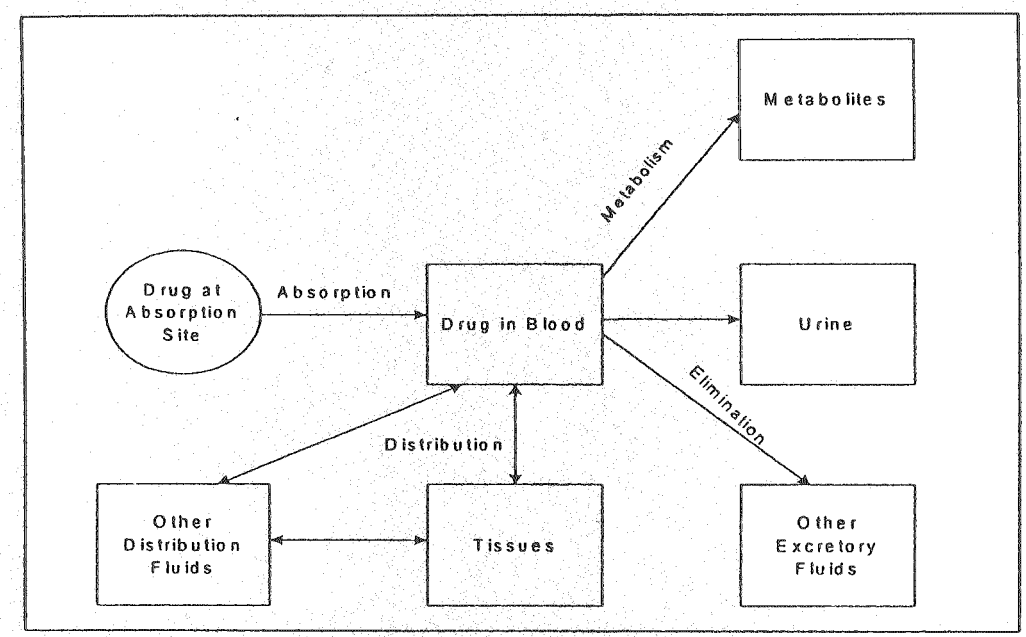

Fig. 1. Schematic representation of drug's path from blood

Pharmacokinetic Parameters

Volume of distribution (V_d): This is the volume into which a drug appears to have been dissolved after administration to an organism, symbolized by V_d. Suppose a drug has been completely absorbed from its site of application, has reached an equilibrium in its distribution among the several tissues of the body and no biotransformation or excretion of the drug has occurred. If one knew the mass (dose) of drug administered and the average concentration of the drug in the body, the apparent volume into which the drug had been dissolved could be determined from the relationship: Concentration = Mass/Volume.

Clearance (C_l): The clearance of a drug is the volume of plasma from which the drug is completely removed per unit time. The amount eliminated is proportional to the concentration of the drug in the blood.

Half life ($t_{1/2}$): This is the time required by the body, tissue or organ to metabolize or inactivate half the amount of a substance taken in. This is an important consideration in determining the proper amount and frequency of dose of drug to be administered. Mathematically, it can be described by the following equation: $t_{1/2} = 0.693/K$, where K is the elimination rate constant.

Blood (or serum or plasma) concentration time curve: Following the oral administration of a medication, if blood samples are drawn from the patient at specific time intervals and analyzed for drug content, the resulting data may be plotted on ordinary graph paper to yield the type of drug blood level curve presented in Fig. 2. The vertical axis of this type of plot characteristically presents the concentration of drug present in the blood (or serum or plasma) and the horizontal axis presents the time the samples were obtained following the administration of the drug. When the drug is first administered to the body (time zero), the blood concentration of the drug should also be zero. As the drug passes into the stomach and/or intestine, it is released from the dosage form, eventually dissolves and is absorbed. As the sampling and analysis continue, the blood samples reveal increasing concentrations of drug until the maximum (peak) concentration (C_{max}) is reached. Then, the blood level of the drug progressively

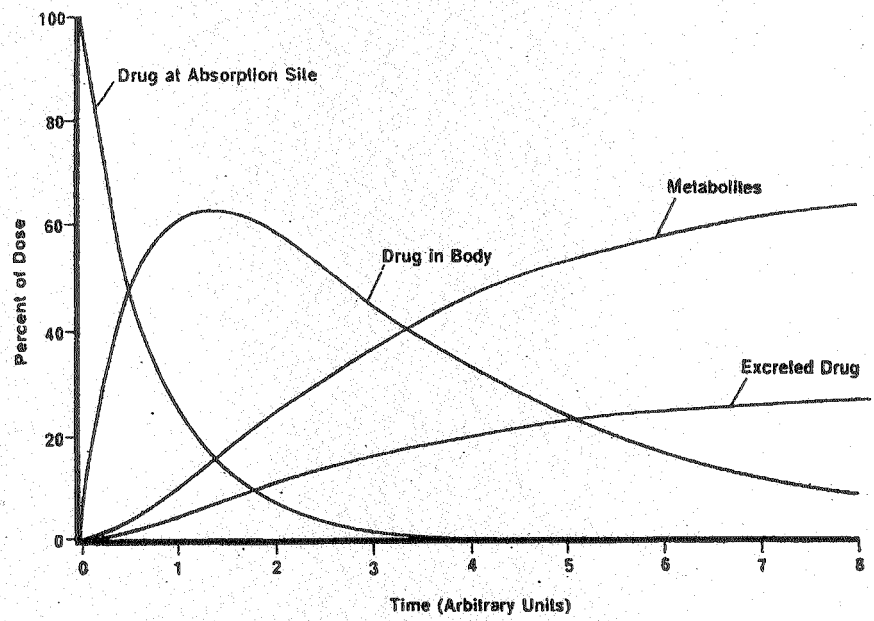

Fig. 2. Serum concentration-time curve

decreases and if no additional dose is given, eventually falls to zero. The diminished blood level of drug after the peak height is reached indicates that the rate of drug elimination from the blood stream is greater than the rate of drug absorption into the circulatory system. It should be understood that drug absorption does not terminate after the peak blood level is reached, but may continue for some time. Similarly the process of drug elimination is a continuous one. It begins as soon as the drug first appears in the blood stream and continues until the entire drug has been eliminated. When the drug leaves the blood it may

be found in various body tissues and cells for which it has an affinity until ultimately it is excreted as such or as drug metabolites in the urine or *via* some other route (Fig. 3). A urinalysis for the drug or its metabolites may be used to indicate the extent of drug absorption and/or the rate of drug elimination from the body.

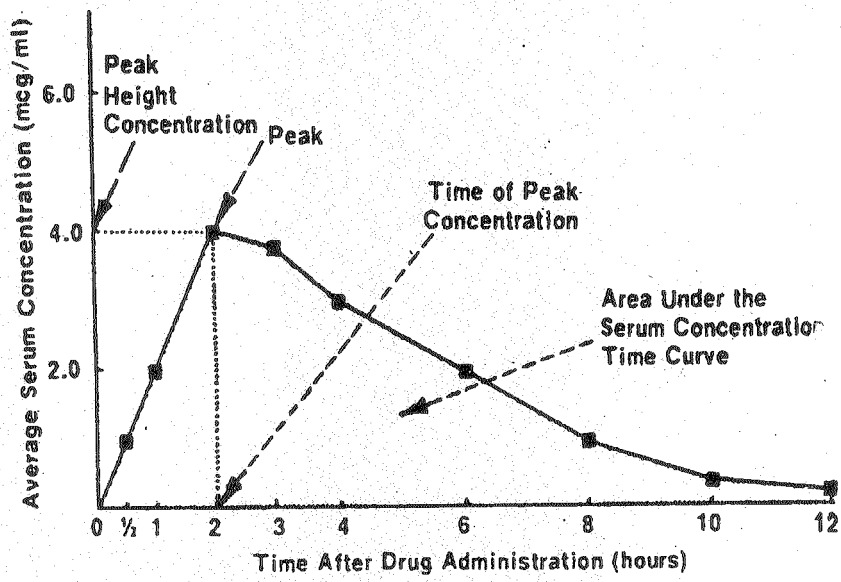

Fig. 3. Time course of drug in the body

EXPERIMENTAL

The present investigation deals with the study of the interaction of the drug diazepam with dog blood by UV-Vis spectral measurements. The drug is administered intravenously so that it enters the blood stream of the dog directly. The literature of the dog deals with weight, sex, colour, breed, age, etc. Out of these various parameters, the weight of the dog is taken into consideration in deciding the dosage of the drug to be injected. In the present work, a healthy dog of weight 5.7 kg has been taken for the bound blood-drug interaction study. Before administering the drug, the normal blood was collected intravenously from the dog and stored with anticoagulant at suitable constant temperature. Then the drug of dosage 10 mg/2 mL was administered intramuscularly. The blood samples were collected every 10 min for 50 min duration and stored at a suitable constant temperature. One drop of blood was diluted in 15 mL of normal saline making it suitable for UV-Vis absorption.

RESULTS AND DISCUSSION

The UV-Vis spectrum of bound-blood drug (diazepam) for normal blood is presented in Fig. 4. The variation of absorbance with time for different wavelengths for the drug diazepam is presented in Table-1. The spectra recorded at different time intervals for diazepam are presented in Fig. 5 and the variation of absorbance with time for different wavelength maxima is presented in Fig. 6.

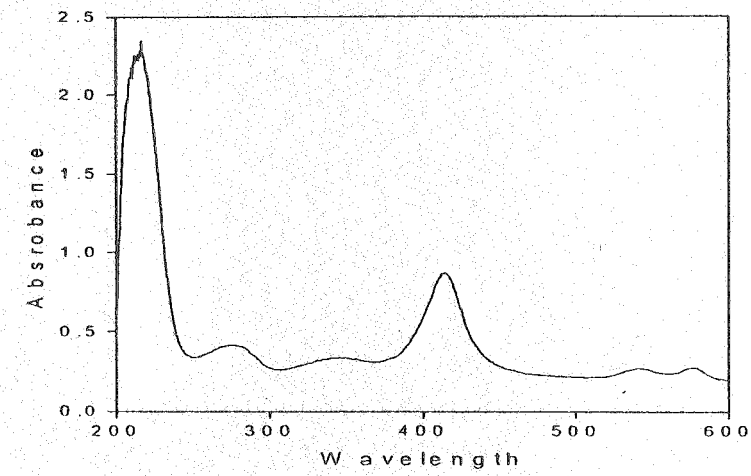

Fig. 4. UV-Vis spectrum of normal blood

TABLE-1
ABSORBANCE AT DIFFERENT WAVELENGTH
MAXIMA OF DIAZEPAM

Time (min)	Wavelength maxima (Absorbance)					
	210 nm	276 nm	341 nm	417 nm	540 nm	576 nm
Normal	2.173	0.413	0.330	0.845	0.267	0.272
10	2.379	0.665	0.562	0.951	0.490	0.494
20	2.389	0.777	0.663	1.061	0.603	0.601
30	2.541	0.833	0.781	1.085	0.718	0.728
40	2.859	1.691	1.455	2.096	1.298	1.309
50	2.458	0.806	0.703	0.929	0.669	0.674

The UV-Vis spectra of samples of the dog's blood have been recorded at different time intervals and each spectrum shows six bands observed at wavelengths 210, 276, 341, 417, 540 and 576 nm. The absorption band at 210 nm is due to the amide backbone of the proteins. The absorption occurring at 276 nm is due to the amino acids tryptophan and tyrosine. Unlike many other proteins, hemoglobin exhibits intense absorption radiation at wavelengths above 320 nm. These absorptions arise from π-π^* electronic transitions within the porphyrin molecule. Especially strong absorptions occurring near 400 nm are named the soret band. Both the wavelength maxima (λ_{max}) and the molar absorptivity coefficients (ε) for these electronic transitions are influenced by the redox state and ligands of the central ion. The absorbance at 540 and 576 nm are due to Fe(II)-hemoglobin moiety. Periodic recording of the UV-Vis spectra of the blood samples every ten minutes after administering the drug reveal that the maximum absorbance is observed at 40th minute for all the wavelengths. After the 40th minute the absorbance at wavelengths 210, 276, 341, 417, 540 and 576 nm is

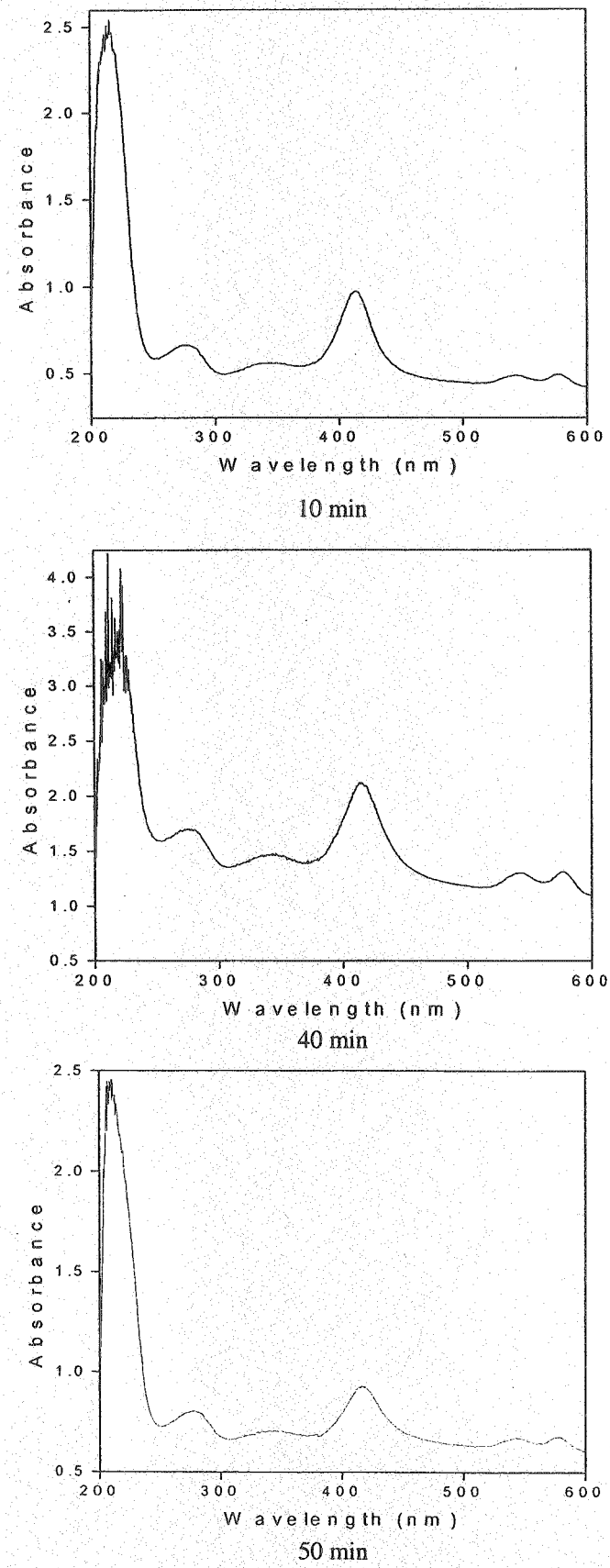

Fig. 5. UV-Vis spectra of bound-blood drug at different time intervals

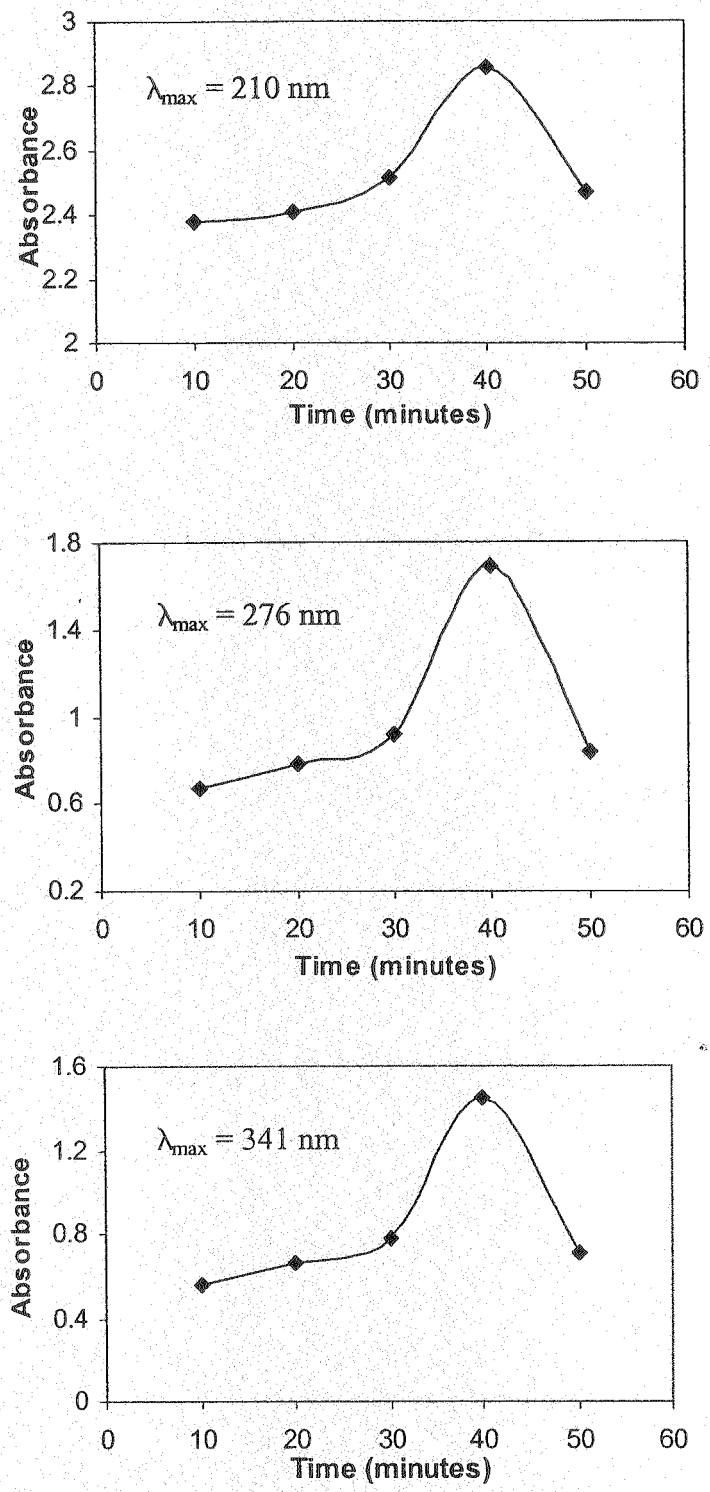

Fig. 6. Variation of absorbance with time for different wavelength maxima

decreased. So the pharmacokinetic interaction of the drug has been analyzed through UV-Vis spectroscopic method. In future, the study may be further extended to investigate the plasma half-life of the drug.

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