Degradation kinetics, *in-vitro* dissolution studies and quantification of praziquantel, anchored in emission intensity by spectrofluorimetry

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Short title: Quantification of praziquantel anchored in emission intensity

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Abstract:

A simple, rapid, specific and highly sensitive eco-friendly spectrofluorimetric method has been developed for the quantification of praziquantel. A linear relationship was found between fluorescence intensity and praziquantel concentration in the range of 1-20 µg/mL in water at emission wavelength of 286 nm after excitation wavelength at 263 nm with a good correlation coefficient (0.999). The proposed method was validated according to ICH guidelines and statistical analysis of the results revealed high accuracy and good precision with the percentage RSD values less than 2. The detection and quantification limits were found to be 0.27 and 0.81 µg/mL, respectively. The contemplated method was successfully applied for quantification of praziquantel in tablets, assay results were found to be within the limits and percentage recovery was in the range of 99.95 – 101.45. The proposed method was extended to investigate the stability of the drug and its degradation kinetics in the presence of acidic, alkaline and oxidative conditions. Moreover, the method was utilized to study the *in-vitro* dissolution studies of praziquantel tablet formulation. The suggested procedures could be used for the assessment of praziquantel in drug substance and drug products as well as in presence of its degradation products.

Key words: Praziquantel, excitation wavelength – 263nm, emission wavelength – 286 nm, dissolution, forced degradation.

Introduction

Chemically, praziquantel is 2-cyclohexanecarbonyl-1H,2H,3H,4H,6H,7H,11bH-piperazino [2,1-a] isoquinolin-4-one (Fig.1). It comes under the category of anthelmintic, used for the treatment of schistosome and many cestode infestations. Schistosomiasis is a parasitic disease caused by worms that penetrate the skin of people through water. Praziquantel is the only drug for treatment of schistosomiasis. Praziquantel is effective and safe. Work is ongoing to expand the treatment with praziquantel in young children as well[1, 2].

The extended literature survey revealed that few analytical methods (using HPLC and HPLC-MS) reported for estimation of praziquantel[3-7]. Till now, one spectrofluorimetric method was reported for praziquantel quantification in blood-plasma and urine. As the method was complicated because drug chemical derivatization with dansyl chloride, which involved various solvents with many extraction procedures. keeping above points into consideration we attempted this work with an objective to develop a simple method for praziquantel quantification in pharmaceutical dosage forms in presence of degradation products and *in-vitro* dissolution samples. Spectrofluorimetry has implicit a foremost role in drug analysis because of its greater sensitivity, furthermore specific than absorption spectrophotometry. Fluorimetry involve in the estimation of drug solution at two wavelengths, excitation and emission in fluorescence; an emission spectrum is acquired by scanning the emission monochromator at determined emission wavelengths (λ_{em}), at a particular excitation wavelength (λ_{ex})[8-13].

Nowadays, dissolution tests are routinely used in the pharmaceutical industry in a wide variety of applications, to identify which sort of formulation will produce the best results in the clinic, to release products to the market, to verify batch-to-batch reproducibility[14,15]. International conference on harmonization (ICH) Q1A guidelines suggested procedures to carry out stress degradation studies to ensure high quality, safety and efficacy of the pharmaceutical product during its storage. Forced degradation (FD) study/ Stress testing is a process in which the natural degradation rate of a pharmaceutical product is increased by the application of an additional stress. It is an important part of the drug development process as it provides knowledge about

the degradation chemistry of drug compounds to develop stability-indicating analytical methods and characterization of degradation products[16].

Keeping all these points into consideration, the present work was attempted with the objective to develop and validate the simple spectrofluorimetric method for quantification of praziquantel in pharmaceutical dosage forms. The application of proposed method to study the *in-vitro* dissolution profiles of praziquantel and further extended to stress degradation studies of praziquantel, degradation kinetics also calculated.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were of analytical grade. Praziquantel (99.50 %) pure was obtained as gift samples from Taj Pharmaceutical Ltd., Mumbai, India. Tablets (Zenticide) were obtained from local pharmacies. Glacial acetic acid (99.8 - 100.5 %), ethanol, methanol, hydrochloric acid (36.46 %)ortho phosphoric acid, sodium hydroxide, hydrogen peroxide, dimethyl sulphoxide (DMSO), dimethyl formamide(DMF),acetonitrile and potassium di-hydrogen phosphate were purchased from SD Fine Chemicals Ltd., Mumbai, India. Series of buffer solutions of acetate buffers (pH 3.7-4.7) and phosphate buffers (pH 5.8-7.8) were prepared as per Indian Pharmacopeia[17].

Instrumentation

The Shimadzu (Japan) RF-5301 PC Spectrofluoro photometer used for the measurement of fluorescence intensity of selected drug, which is equipped with 150-watt Xenon arc lamp, 1 cm non-fluorescent quartz cell was used, connected to RFPC software. The instrument was operated both at low and high sensitivity with excitation and emission slit width set at 5 nm. Analytical balance (Shimadzu AUX 220, Japan), dissolution apparatus (Electro Lab, TDT-08L, India), pH meter (Elico), hot air oven and UV-cabinet (Bio-Technics, India) were used for the study.

Preparation of standard stock solutions

Praziquantel pure drug of 10.0mg was weighed and decamped into 10.0 mL volumetric flask and dissolved in methanol. The flask was shaken and volume was

contrived up to the mark with distilled water. 0.1 mL of prepared solution was diluted to 10.0 mL with distilled water to attain praziquantel end concentration of 10.0 µg/mL.

Construction of calibration graph

The standard stock solution of praziquantel (100 µg/mL) was used to prepare set of diluted standard solutions of various concentrations (1-20 µg/mL) prepared by pipetting appropriate volumes (0.1, 0.5, 1, 1.5 ,2 mL) of stock solution into 10.0 mL volumetric flasks and adjusting the volume to mark with distilled water. These solutions were scanned in Spectro fluorimeter at λ_{ex} of 263 nm.

Determination of praziquantel in tablet dosage form (assay)

Twenty tablets of marketed formulation (Zenticide®), each containing 600 mg of praziquantel were taken and accurately weighed. Average weight was determined and crushed into fine powder. An accurately weighed profusion of powder equivalent to 10.0 mg praziquantel was transferred to a 10.0 mL volumetric flask. Methanol was added to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with water. The above solution was filtered through whatmann filter paper (No.41). From the filtrate, a final concentration of solution was prepared for the estimation of drug content by the proposed method.

Dissolution studies

Dissolution testing of praziquantel tablet formulation (Zenticide®) was carried out in 0.1M HCl (900 mL) containing 2 mg of sodium lauryl sulphate according to the FDA dissolution data base, using paddle apparatus (Type 2) at 50rpm and 37±0.5°C for 60 min. Sampling aliquots of 5.0 mL were withdrawn at 10, 20, 30, 40,50 and 60 minutes, reconstituted with an equal volume of the fresh medium to maintain the sink conditions. At the end of each test time, sample aliquots were filtered, diluted with water and quantified. Samples withdrawn were evaluated with regression equation of contemplated analytical technique for quantification of dissolved drug followed by plot counter to time.

Forced Degradation studies

Forced degradation studies were carried out by exposing the sample solution to stress conditions like acidic, alkaline, thermal, oxidative and UV effects.

Sample solution (10 µg/ mL) was prepared by adding 1.0 mL of stock solution and 2.0 mL of 5M HCl to 10.0 mL volumetric flask. Then the volumetric flask was kept under

60°Creflux conditions for 5 h and neutralized with 5M NaOH then volume was made up to the mark with distilled water. Similarly, alkaline degradation (5 M NaOH), oxidative degradation (1% H_2O_2) and for thermal degradation (heated at 105° C) were performed. UV degradation was performed with 10 mg of praziquantel placed in UV cabinet at short wavelength (254 nm), then subjected to proposed sample procedure and fluorescence intensity was measured for every 1 hr upto 24hr.

RESULTS AND DISCUSSION

Selection of wavelength

To attain sensitive and specific spectrofluorimetric method for quantification of praziquantel, several solvent systems were investigated such as methanol, di methyl sulfoxide (DMSO), ethanol, di methyl formamide (DMF) and glacial acetic acid. Fluorescence intensity was higher in methanol- water (1:9) than the other solvent systems (Fig.2) and exhibits native fluorescence at emission wave length 286 nm after excitation at 263 nm in the presence of polycyclic aromatic system like pyrazino and isoquinolin rings with more π electrons (Fig.3). Hence methanol- water (1:9) was selected as solvent for quantification, forced degradation studies and *in-vitro* dissolution studies of praziquantel.

Analytical method validation

Linearity and Range

The linearity was verified qualitatively by running the plot; at several concentrations anon, plot was observed that amplitudes gradually changed (increased) at 286 nm (Fig.4), this was prime face evidence on the suitability of the method for analysis. The linearity was assessed by the regression equation of calibration curve further the data and regression equation of calibration curves were proclaimed in Table 1 and Fig.5, denotes responses for praziquantel at 286 nm were found to be linear in the concentration range of 1-20 µg/mL, with a correlation co-efficient (R^2) value of 0.996.

Selectivity

Fluorescence spectrum attained from the commercial formulation solution was compared with the spectrum of standard solution (praziquantel). Both commercial formulation spectrum and the standard solutions spectrum were superimposed;

denoted no interference from excipients with response of praziquantel at analytical wavelength286 nm. Hence the method was found to be specific and selective.

Accuracy (Recovery studies)

The accuracy was assessed by standard addition method at three distinct levels (80%, 100% and 120%) of standards coalesced to commercial tablets in triplicate anon the mean of percentage recoveries, % RSD values was computed and proclaimed in Table 2. The % RSD values at relevant concentration level was 2 and % recoveries of praziquantel were assessed to be in the range of 99 – 101 and thus suggesting the accuracy of the method[18].

Precision

Intra-day and inter-day precision harmony was appraised as per ICH guidelines, in which samples containing praziquantel (5/10 and 15µg/mL) were analyzed six times on the same day (intra-day precision) and for three consecutive days (inter- day precision), anon % RSD was calculated. Results of intra-day and inter-day precision data were proclaimed in Table 3, it revealed that there was no significant distinction between the % RSD values of intra-day and inter-day analysis, which indicates the contemplated method, is highly precise.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection (LOD) and Limit of quantification (LOQ) parameters were discretely appraised based on standard calibration curve, results proclaimed in Table 1.

Applications

Assay

The contemplated method was employed to the assay of commercial tablets (Zenticide®), containing praziquantel (600 mg). The results were correlated with consequent labelled amounts and proclaimed in Table 4, best peaks of the assay in tablets found to be 100.1(% RSD < 2), which indicates the accuracy of the proposed method.

In-vitro dissolution testing

Dissolution studies on praziquantel tablets were enforced under the FDA dissolution test medium as USP dissolution apparatus type 2 containing 900 mL of 0.1 M HCl with 2.0 mg of sodium lauryl sulphate as dissolution medium at a paddle speed of 50 rpm at temperature 37 ± 0.5 ˚C. These results and profiles were proclaimed in Fig6, revealed that amplified than 95% of praziquantel was dissolved from the tablet dosage form.

Forced degradation studies

The stability of praziquantel was studied to elucidate the inherent stability characteristics of it in terms of fluorescence intensity in the incidence of acid/alkali hydrolysis, oxidative, thermal and UV light, anon counter the plot between % drug degraded Vs time interval. The degradation was observed in 5M HCl (81 % up to 60 min) and 5MNaoH (1.0 % up to 60 min) and 1% H_2O_2 (84% up to 60 min) but there was no evidence of degradation under UV light and thermal conditions (data not shown). The acidic and oxidative degradation was observed that relative fluorescence intensity decreased gradually with increased heating time (Fig 7). Countered the plot between logarithmic concentration against time shown in Figures 8 & 9 and calculated the half-life and first order rate constants for acid and oxidative degradation (Table 5).

CONCLUSION

The contemplated spectrofluorimetric method has the advantages of being economical, simple, sensitive, specific and extraction free for quantification of praziquantel in tablets and *in-vitro* dissolution samples. Furthermore, the work was also utilized to study degradation kinetics of praziquantel under stress conditions as per ICH guidelines. Fortunate, considerable degradation was observed and calculated the rate constant, half-life under acidic and oxidative stress conditions were calculated. The results of contemplated spectrofluorimetric method was prime face evidence to employ fruitfully for quantification of praziquantel in tablet dosage form, *in-vitro* dissolution samples without any interference and in the presence of degradation studies.

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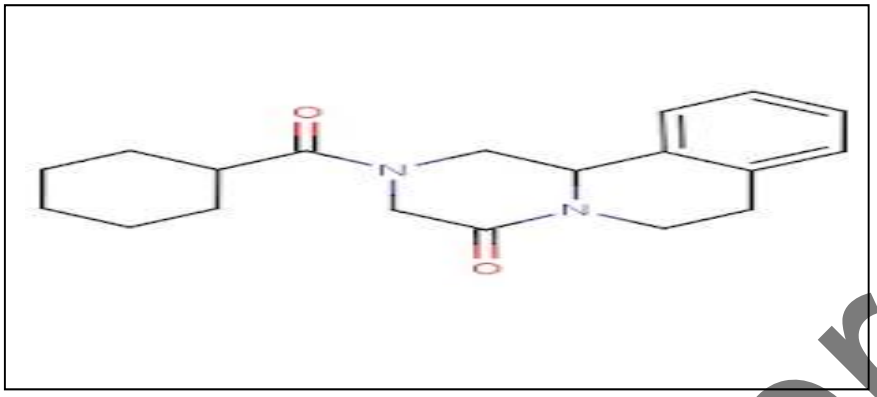

Fig.1. Chemical structure of praziquantel

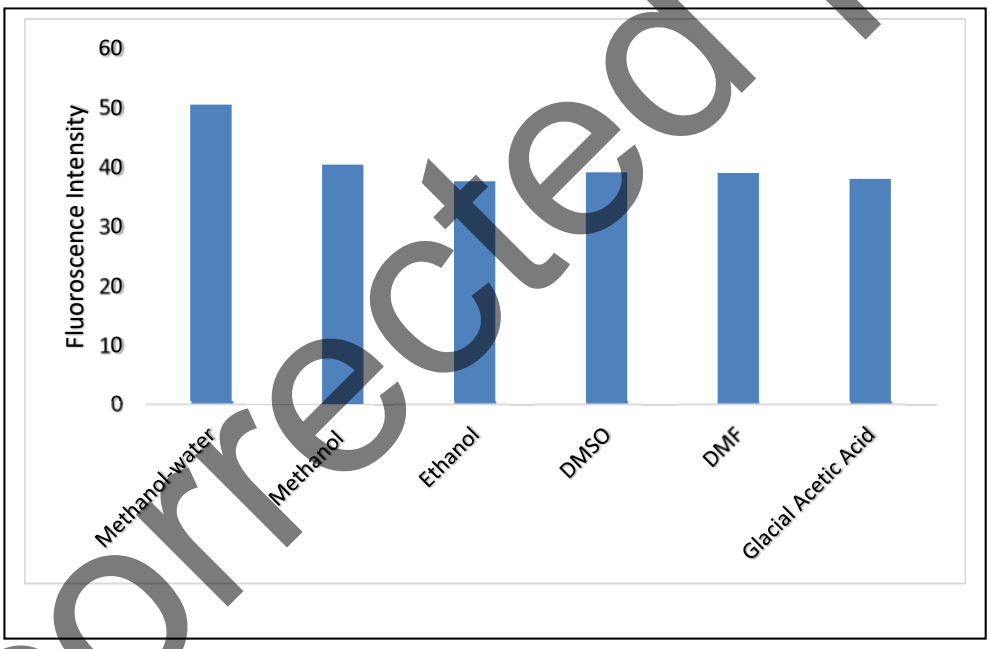

Fig.2. Effect of various solvents on fluorescence intensity of praziquantel

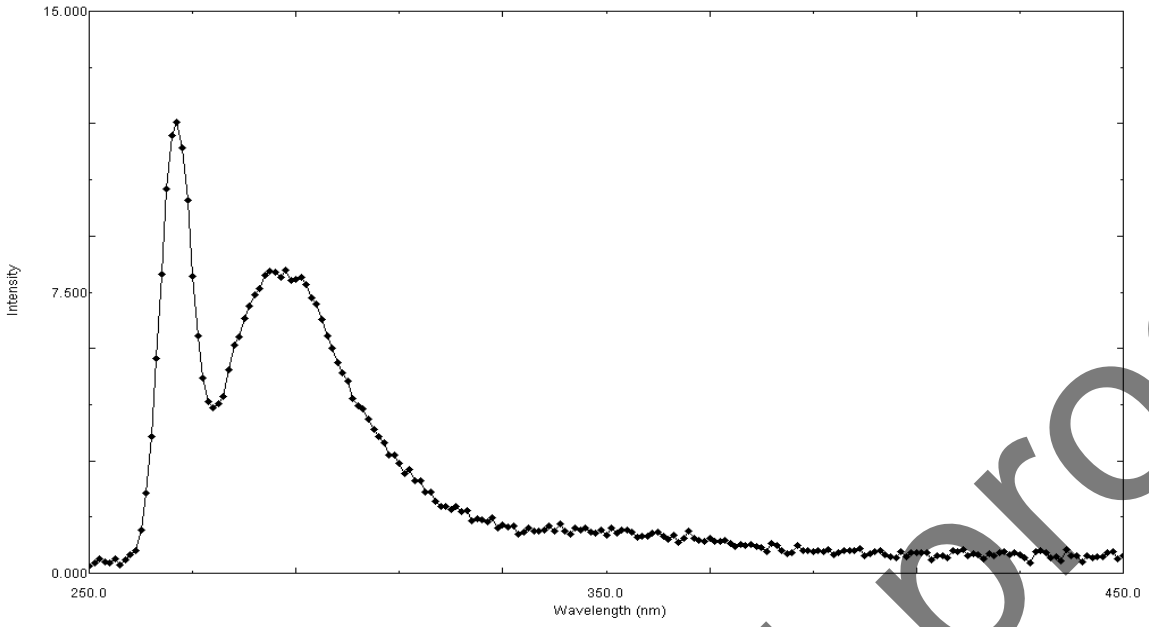

Fig.3. Emission spectrum (286 nm) of praziquantel in methanol water as solvent

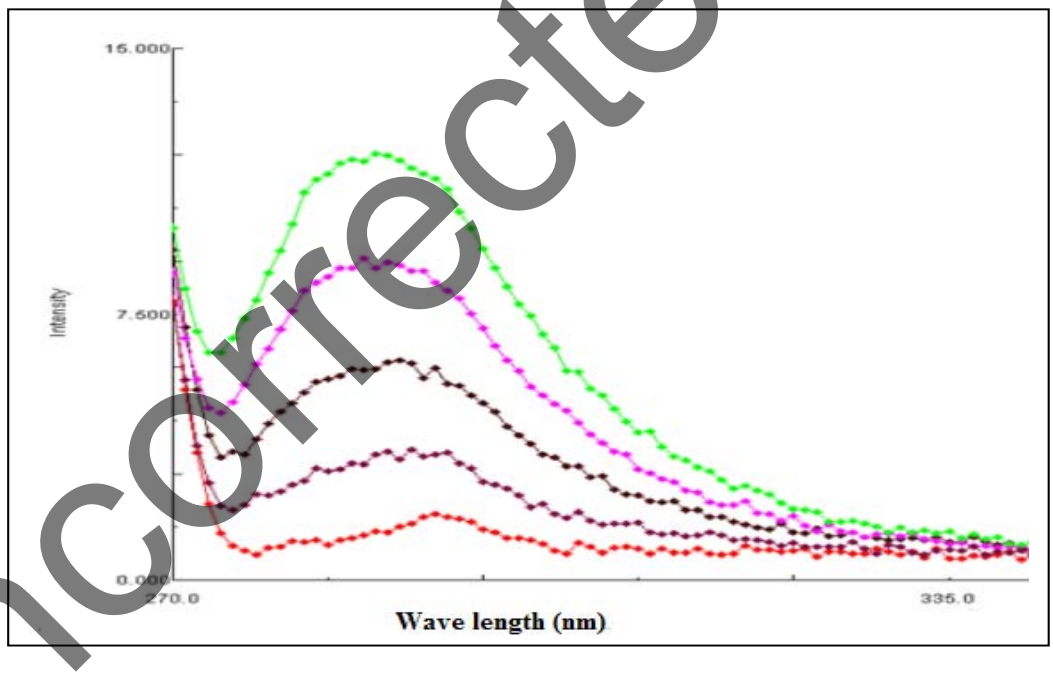

Fig.4. Linearity spectra of praziquantel at 286 nm (1-20 µg mL^{-1})

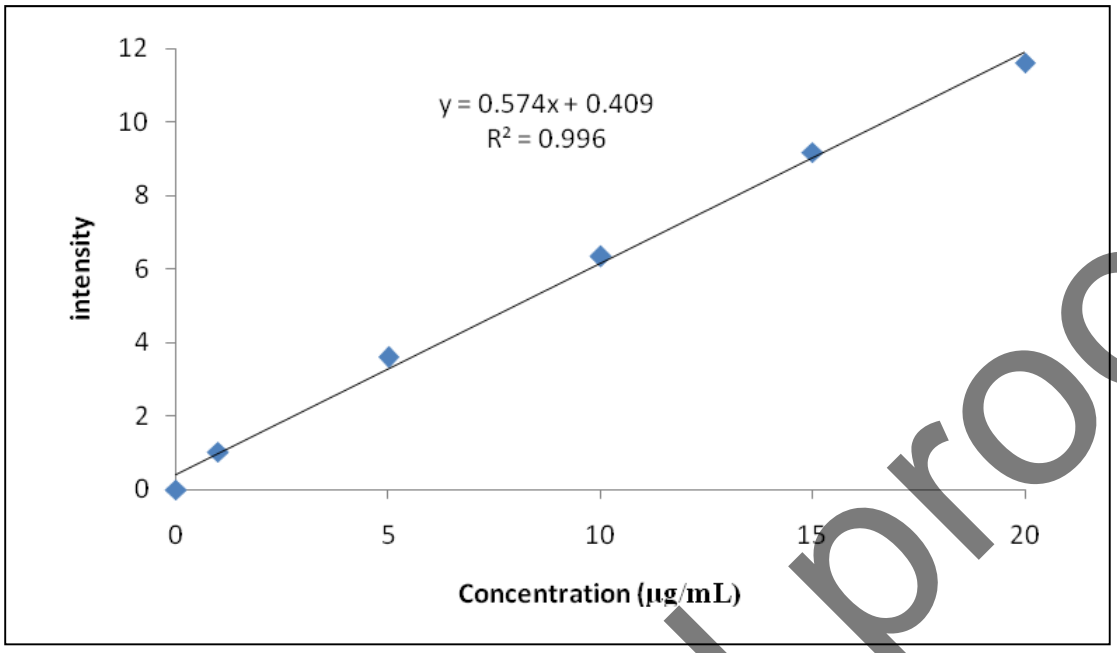

Fig.5. Linearity plot of praziquantel

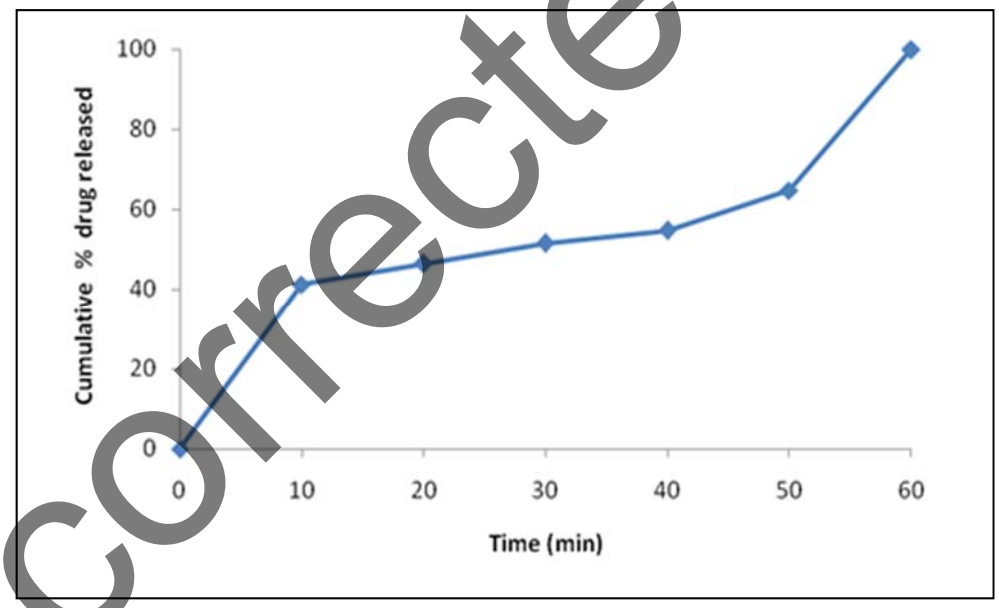

Fig.6. Cumulative % drug release of praziquantel from zenticide tablets

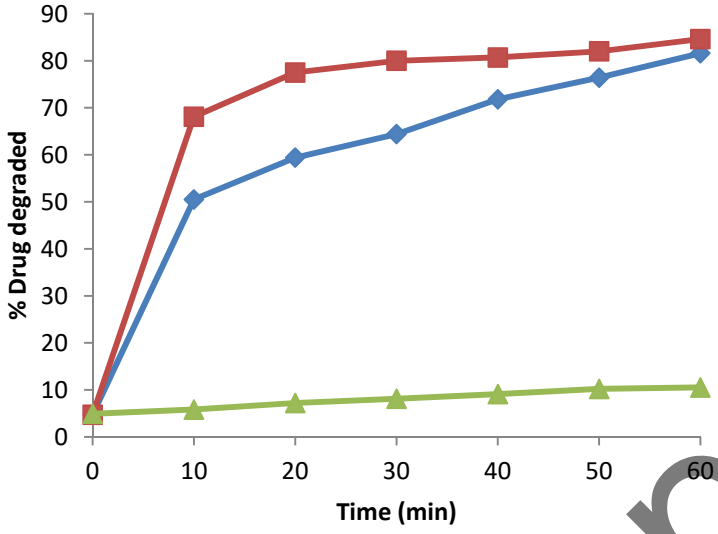

Fig.7. Stability profile of praziquantel in the presence of peroxide (—■—) basic (—◆—) and acidic (—▲—) degradation conditions

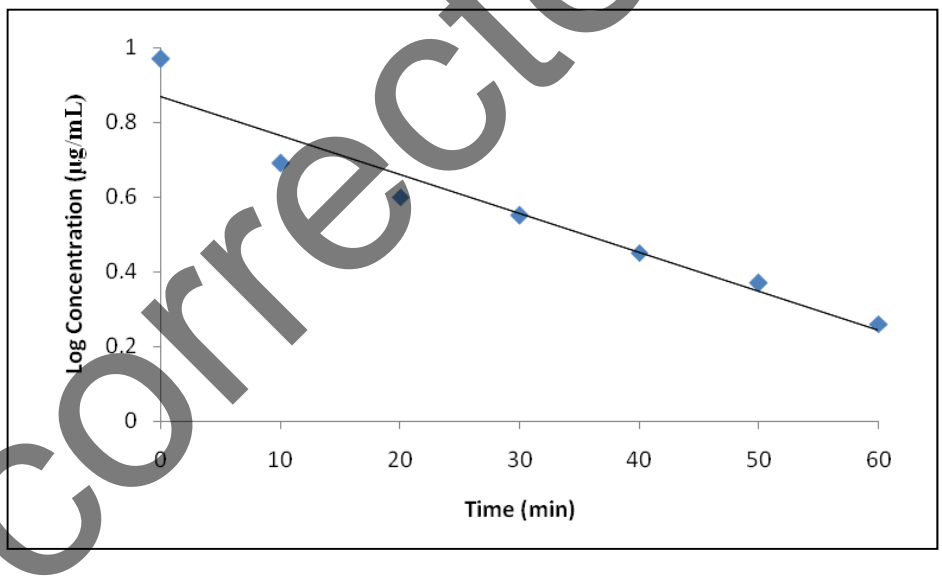

Fig.8. Plot of praziquantel degradation in the presence of 5 N HCl solution

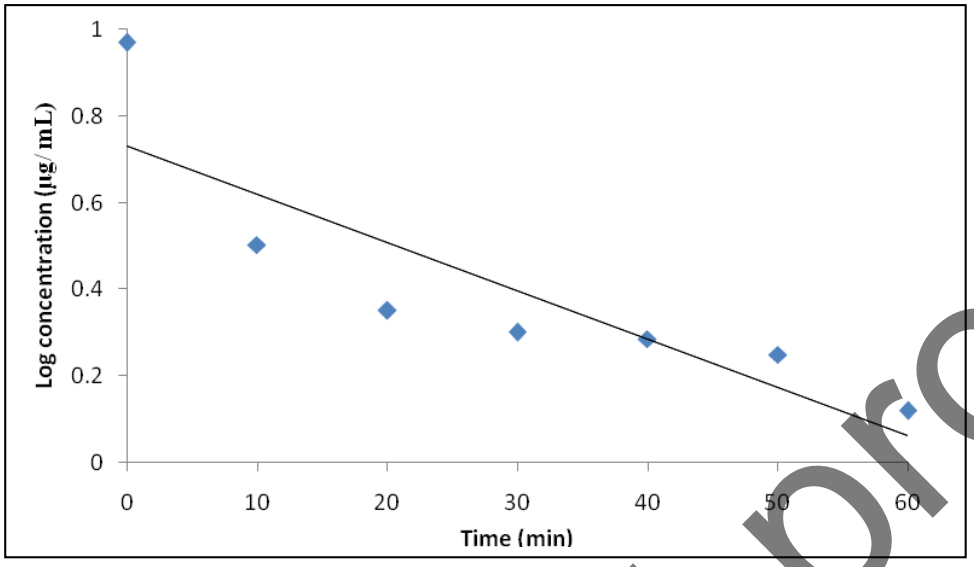

Fig.9. Plot of praziquantel degradation in the presence of 1% H_2O_2 solution

Table 1. Optimized parameters for proposed method

Parameter	Value
Excitation wavelength (nm)	263
Emission wavelength (nm)	286
Linearity range (µg ml^{-1})	1-20
Limit of detection (µg ml^{-1})	0.27
Limit of quantification (µg ml^{-1})	0.81
Correlation coefficient (r^2)	0.996
Slope (m)	0.574
Intercept (c)	0.409
Regression equation	Y=0.574x+0.409

Table 2. Accuracy (% recovery) of proposed method

Formulation	Recovery Level (%)	Theoretical Content (µg ml^{-1})	Concentration found (µg ml^{-1}) (Mean± SD)	% Amount Recovered (mean ± SD)	% RSD
zenticide®	80	18	17.93 ±0.072	99.6 ±0.677	0.679
	100	20	20.12 ±0.164	100.6 ± 1.361	1.352
	120	22	22.45 ±0.390	102.0 ± 0.264	0.258

Table 3. Precision data of proposed analytical method

[a.] Mean values of six different standards for each concentration.

[b.] Inter-day reproducibility was quantified from six different standards of each concentration for three consecutive days.

Theoretical concentration (µg ml^{-1})	Intra- day		Inter-day	
	Concentration (Mean[a] ± S.D)	% RSD	Concentration (Mean[b] ± S.D)	% RSD
5	4.9 ±0.018	0.36	5.0 ±0.016	0.32
10	9.8 ±0.0169	0.17	9.9 ±0.024	0.24
15	15.1 ±0.023	0.15	14.9 ±0.035	0.23

Table 4. Assay results of marketed tablets of praziquantel

Formulation	Praziquantel			
	Label claim (mg)	Amount found (mg) (Mean ± SD) (n=3)	%Assay	% RSD
Zenticide	600	600.8 ±0.08	100.1%	0.013

Table 5. Results of the degradation study of praziquantel under acidic and oxidative conditions on the kinetic parameters of praziquantel

Degradation condition	Reaction Rate constant (K min^{-1})	Half Life Time ($t_{1/2}$, min)
Acidic Degradation (5N HCl, 60°C)	0.027	25.6
Oxidative Degradation (1% H_2O_2)	0.030	23