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## ORIGINAL ARTICLE

# A rapid stability indicating LC-method for determination of praziquantel in presence of its pharmacopoeial impurities



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## KEYWORDS

Praziquantel;  
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**Abstract** This study reports for the first time about a stability indicating RP-HPLC method for quantitative determination of Praziquantel (PZQ) in bulk powder and dosage form and in presence of its pharmacopoeial impurities. The chromatographic separation was carried out on (Caltrex AI<sup>®</sup>) calixarene column, a relatively new packing material. Chromatography was done using an isocratic binary mobile phase consisting of ACN and 25 mM ammonium acetate (NH<sub>4</sub>Ac) in the ratio of 40:60 at flow rate of 1 mL min<sup>-1</sup>, 30 °C and 210 nm wavelength for detection. The elution time of PZQ was found to be 6.15 ± 0.03 min. The method was validated for system suitability, linearity, precision, limits of detection and quantitation, specificity, stability and robustness. The robustness study was done for small changes in temperature, flow rate, wavelength of detection and % of ACN in mobile phase. Stability tests were done through exposure of the analyte solution to five different stress conditions: Reflux with 1 N HCl, reflux with 1 N NaOH, reflux with 30% H<sub>2</sub>O<sub>2</sub>, thermal degradation of powder and exposure to UV radiation. Limits of detection and quantification were found to be 0.56 and 1.70 µg mL<sup>-1</sup>, respectively. The recovery value of this method was 100.30% ± 1.10 and the reproducibility was within 1.31.

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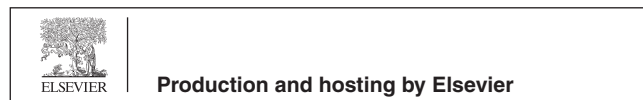
## 1. Introduction

PZQ is the drug of choice for the treatment of schistosomiasis and most of the trematode infections. It is an anthelmintic with a broad spectrum of activity against trematodes and against cestodes. PZQ has a good safety and therapeutic profile. It has been widely used in community-based schistosomiasis control programs. For example, in Egypt and China, more than 100 million doses have been administered over the past two decades (Chen, 2005; Fenwick et al., 2006).

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Many techniques have been described for the quantitative determination of praziquantel, including spectrofluorimetry (Putter, 1979; Putter and Held, 1979), gas chromatography (Saleh and Schnekenburger, 1992) and high-performance liquid chromatography (Xiao et al., 1983; Anurak and Kesara, 2004; Moutasim et al., 2004; Rubiana et al., 2006; Jia et al., 2007; Jiangeng et al., 2010; Kulik et al., 2011; British pharmacopoeia, 2011; Lucie Havlíková et al., 2012).

All drugs, according to current good manufacturing practice, must be tested with a stability-indicating assay before release. The intrinsic stability of the molecule and validation of the stability-indicating power of the analytical procedures used can be identified through stress testing. It was felt that a HPLC method of analysis that separates the drugs from the degradation products formed under ICH-suggested conditions (hydrolysis, oxidation, photolysis, and thermal stress) would be of general interest. These studies provide valuable information about the inherent stability of a drug and help in the validation of analytical methods to be used in stability studies (ICH, 2003). Chromatographic methods have taken priority over conventional analysis methods because of the requirement for separation of several components during stability analysis. In addition to separation of multiple components, chromatographic methods have other advantages, which are: they are more accurate and sensitive, even for small quantities of degradation products. In a previous study triethylamine was used for the separation of PZQ from other anthelmintics (Lucie Havlíková et al., 2012). Ion-pairing agents as triethylamine are known to deteriorate the stationary phases (Snyder and Kirkland, 1979; Snyder et al., 1997).

As far as we know, there is no previous stability indicating quantitation method in the literature for determination of PZQ. The alone study which reported about photo-thermal stability (Moutasim et al., 2004) aimed to find the best excipient to increase the stability of PZQ in the dosage form. The objective of this study was, therefore, to develop a new, simple, rapid, precise, accurate, and specific stability-indicating HPLC method for quantitative determination of PZQ in tablet formulation and in presence of its impurities without using ion-pairing agents.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All chemicals and reagents are at least of analytical grade. Bidistilled Water was used. Ammonium acetate ( $\text{NH}_4\text{Ac}$ ) was purchased from (Merck, Germany). ACN was of HPLC-grade (J.T. Baker, Holland).

100% purity pharmaceutical grade PZQ obtained from (EIPICo, Egypt) was used (EIPICo, Egypt). Pharmaceutical formulation, Distocid® tablets (containing 600 mg PZQ per tablet) were obtained from Egyptian market.

### 2.2. Instrumentation

Agilent HPLC series 1200 (Agilent technologies, Germany) consists of solvent pump (model G1311A), autosampler (model G1329A), column compartment (model G1316A) and UV detector (model G1314A).

SUNTEST CPS+® was used for UV-radiation for photo-degradation of PZQ.

### 2.3. Column

Calixarene stationary phase column (Caltrex AI®, Kromasil Si 100/5  $\mu\text{m}$ ,  $4.0 \times 125$  mm) was obtained from (Syntrex Roth and Menyes GbR., Germany).

### 2.4. Chromatography

The experiments were performed with isocratic elution. The binary mobile phase consisted of ACN in 0.02 M  $\text{NH}_4\text{Ac}$ , 40:60 V/V. The eluents were degassed before running, set at a flow rate of  $1 \text{ mL min}^{-1}$  and column temperature at  $30^\circ\text{C}$ . A volume of  $10 \mu\text{L}$  of samples was injected per run and eluates were detected using a UV-detector at  $\lambda = 210 \text{ nm}$ .

### 2.5. Solutions preparation

#### 2.5.1. Preparation of stock and standard working solutions

The stock solution of PZQ ( $1 \text{ mg mL}^{-1}$ ) was prepared by dissolving 100 mg of PZQ in (1:1) ACN:water to make 100 mL of solution. The standard working solutions were prepared by diluting aliquots of the stock solution to obtain concentrations ranging from 0.1 to  $100 \mu\text{g mL}^{-1}$ . The calibration graph was constructed by plotting the peak areas obtained at a wavelength of 210 nm versus the corresponding injected concentrations.

### 2.6. Sample preparation

The contents of 10 tablets of Distocid® 600 mg were accurately weighed and ground to a fine powder. To an accurately weighed portion of the powder equivalent to one tablet, 250 mL (1:1) ACN:H<sub>2</sub>O was added then the solution was left in the ultrasonic bath for 10 min. After that the solution was filtered and the first 10 mL was rejected then 2 mL of filtrate was diluted to 100 mL using same solvent.

### 2.7. Stability tests

Forced degradation studies were performed to ensure the stability-indicating properties and specificity of the method. Intentional degradation was attempted using acid, base, hydrogen peroxide, thermal and UV-radiation. A degradation sample was prepared by dissolving 25 mg of PZQ in 25 mL (ACN:H<sub>2</sub>O, 1:1) through shaking and sonication. Then 10 mL of this solution was taken in each of three 50 mL round bottomed flasks to perform the first three degradation tests. To the first flask, 10 mL of 1 N HCl was added for acidic degradation. To the second flask, 10 mL of 1 N NaOH was added for basic degradation. To the third flask, 10 mL of 30% H<sub>2</sub>O<sub>2</sub> was added for oxidative degradation. All the three flasks were refluxed for about 4 h. After completing degradation treatments, samples were allowed to cool to room temperature and treated as follows: The pH values of the first and second flasks were neutralized with 1 N NaOH and 1 N HCl, respectively. To the third flask 1 N sodium bisulfite solution was added to destroy H<sub>2</sub>O<sub>2</sub>. The volume of all the three flasks was adjusted to 50 mL with (ACN:H<sub>2</sub>O, 1:1).

For thermal degradation, PZQ powder was dispersed onto a Petri-dish and left in oven at  $60^\circ\text{C}$  for 4 h then the solution was prepared from it in a concentration of 0.2 mg/mL using (ACN:H<sub>2</sub>O, 1:1) as solvent. For degradation through UV-radi-

ation 2 mL of the sample solution was left in UV radiation for 4 h then the radiated solution was diluted with (ACN:H<sub>2</sub>O, 1:1) to 10 mL, then finally injected into chromatograph and compared with control sample.

The specified pharmacopoeial PZQ impurities A and B (US pharmacopoeia, 2011) prepared in a concentration of 0.1 mg/mL (ACN:H<sub>2</sub>O, 1:1) as solvent were also separated in the presence of PZQ.

### 3. Results and discussion

When separation was done using binary mobile phase consisting of ACN and H<sub>2</sub>O, there was no complete separation between the intact drug and the degradants or impurities; so inorganic salts were added to water. The best baseline separations were obtained when NH<sub>4</sub>Ac was used. When the content of ACN in mobile phase was greater than 40%, the retention time shortened but the peak of the intact drug was co-eluted with the peaks of degradants. On contrast, when the content of ACN in the mobile phase was less than 40%, the retention time became longer without any improvement in separation; so 40% ACN was used as organic modifier. 210 nm was used as the wavelength of detection because it has the advantage over the greater wavelength in that at it all peaks of degradants and intact drug can be detected.

#### 3.1. System suitability

The results of three runs indicate high system suitability (Table 1). The  $t_R$ -value of PZQ is  $6.15 \pm 0.03$  min. The RSD of peak area is 0.51%.

**Table 1** System suitability and linearity and regression data.

Parameters	
<i>System suitability</i>	
$t_R \pm SD$ (min)	$6.15 \pm 0.03$
<i>N</i>	2000
<i>k</i>	5.5
Linearity range ( $\mu\text{g mL}^{-1}$ )	5–100
Detection limit ( $\mu\text{g mL}^{-1}$ )	0.56
Quantitation limit ( $\mu\text{g mL}^{-1}$ )	1.70
<i>Regression data</i>	
Slope (b)	44.6
Intercept (a)	-11.797
Coefficient of determination ( $R^2$ )	0.9996

#### 3.2. Linearity and range

Seven concentrations of PZQ solution ranging from 0.1 to 100  $\mu\text{g mL}^{-1}$  were analyzed. The graph of the peak area against concentration proved linearity in the range of 5–100  $\mu\text{g mL}^{-1}$  and the linearity equation is:  $Y = 44.601X - 11.797$ . Regression coefficient was found to be 0.9996. The limit of detection (LOD) defined as the injected quantity giving  $S/N$  of 3 (in terms of peak height), was found to be 0.56  $\mu\text{g mL}^{-1}$ . The limit of quantification (LOQ), defined as the injected quantity giving  $S/N$  of 10 (in terms of peak height), was found to be 1.70  $\mu\text{g mL}^{-1}$  (Table 1).

#### 3.3. Accuracy and specificity of the method

The accuracy of the method was determined by recovery experiments applying standard addition technique ( $n = 5$ ) and showed high accuracy with recovery of  $100.30 \pm 1.10\%$ .

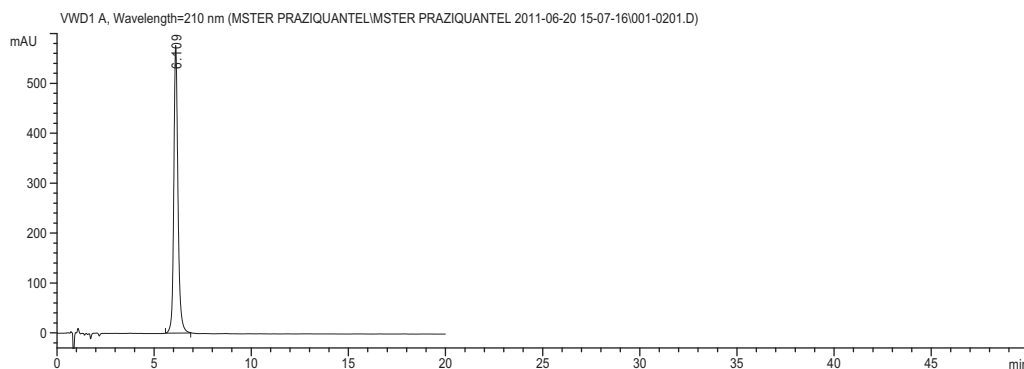
The comparison between the chromatogram of the raw PZQ (Fig. 1a) and that of extracted PZQ from tablets (Fig. 1b) indicates that the excipients in the formulation did not interfere with the determination of PZQ. Also USP impurities A and B, which come through chemical synthesis of the drug, did not interfere (Fig. 1c).

When diloxanide furoate, paracetamol, trimethoprim and albendazole were injected simultaneously with PZQ, only albendazole interfered.

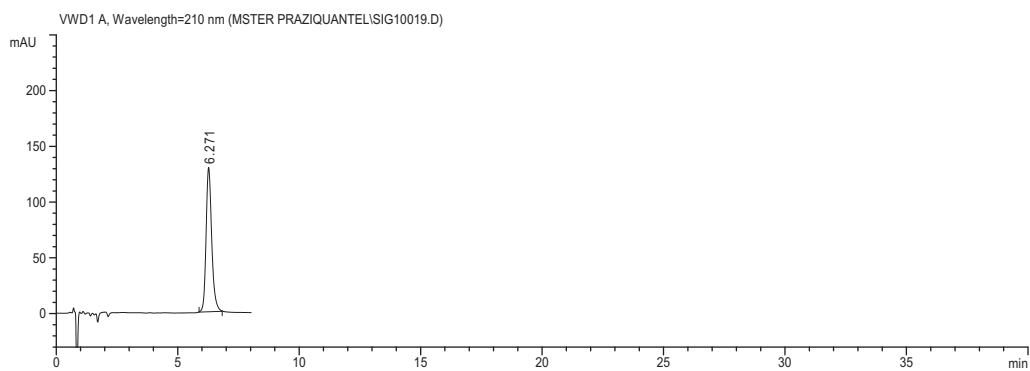
#### 3.4. Stability of the analytical solution and stability tests

Stability of the standard solution was studied by injection of the prepared solution at periodic intervals into the chromatograph up to about five days. The results indicate that the RSD of the peak area was within 1.50%.

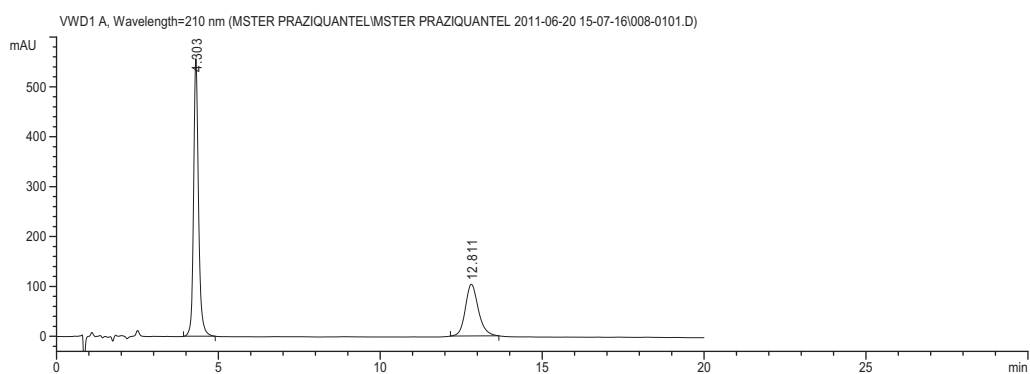
The results of stress degradation indicate that PZQ is strongly affected with reflux with NaOH (Fig. 2a) and H<sub>2</sub>O<sub>2</sub> (Fig. 2b). Moutasim et al. (2004) found that the increase of pH led to increase of degradation of PZQ. This ensures the stronger effect of NaOH than that of HCl in this study. The strong effect of oxidative degradation can be due to the presence of amino-groups in PZQ structure. Reflux with HCl (Fig. 2c) led to degradation of PZQ but the effect here is weaker than in cases of H<sub>2</sub>O<sub>2</sub> and NaOH. Thermal (Fig. 2d) and UV-light exposure (Fig. 2d) gave the minimum effect on PZQ. Although there were several degradants, there was not any interference with the peak of the intact drug indicating that the method is stability indicating. (see Fig. 2e).



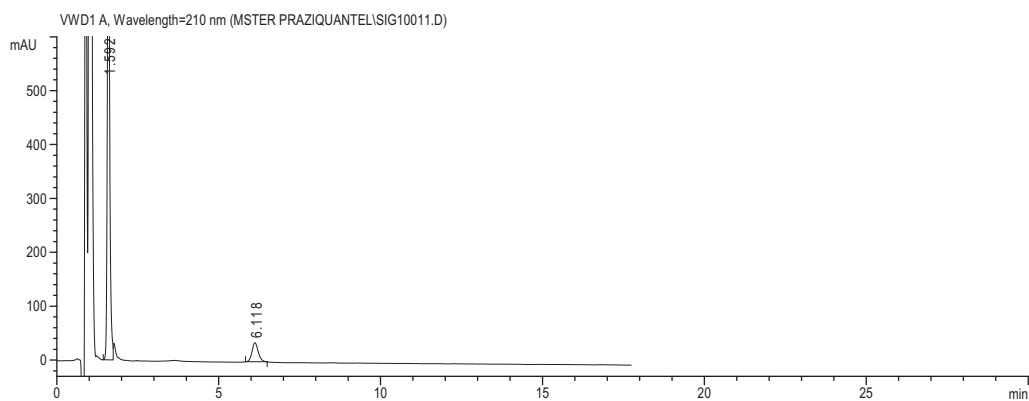
**Figure 1a** Praziquantel from bulk powder.



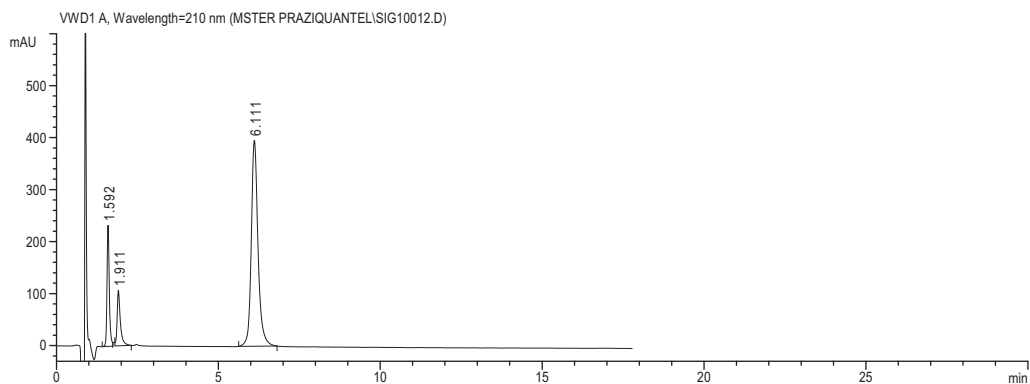
**Figure 1b** Praziquantel from Distocid® tablets.



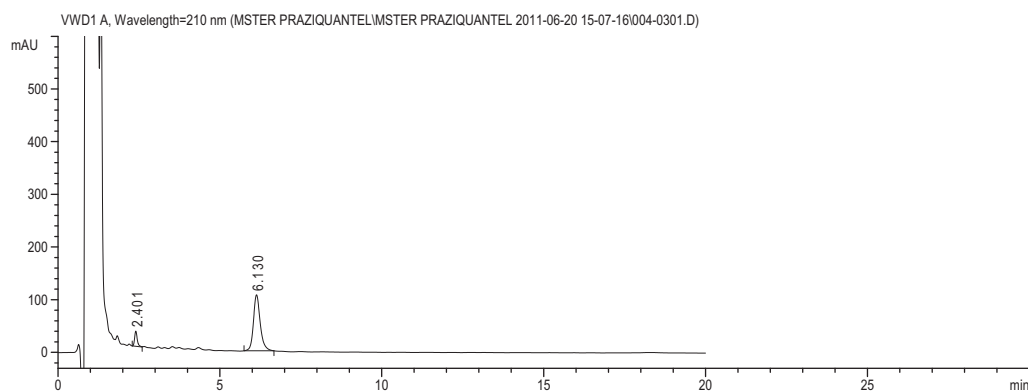
**Figure 1c** PZQ USP related impurity A (4.3 min) and PZQ USP related impurity B (12.8 min).



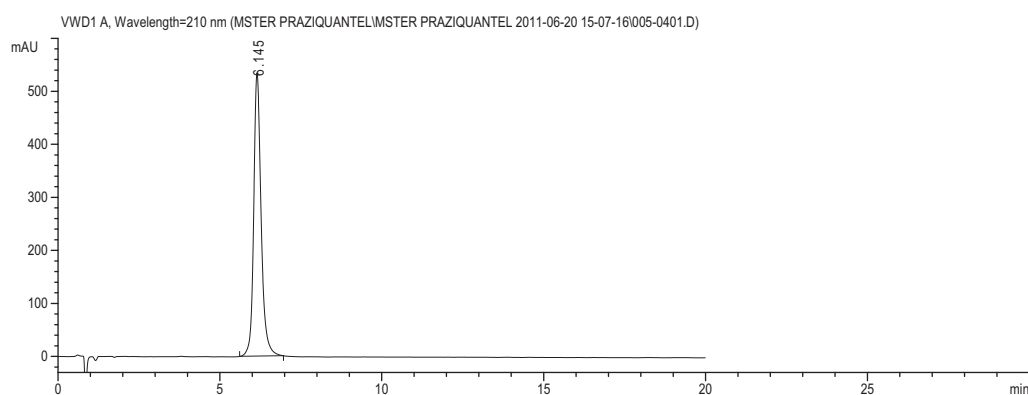
**Figure 2a** Praziquantel after treatment with 1 N NaOH.



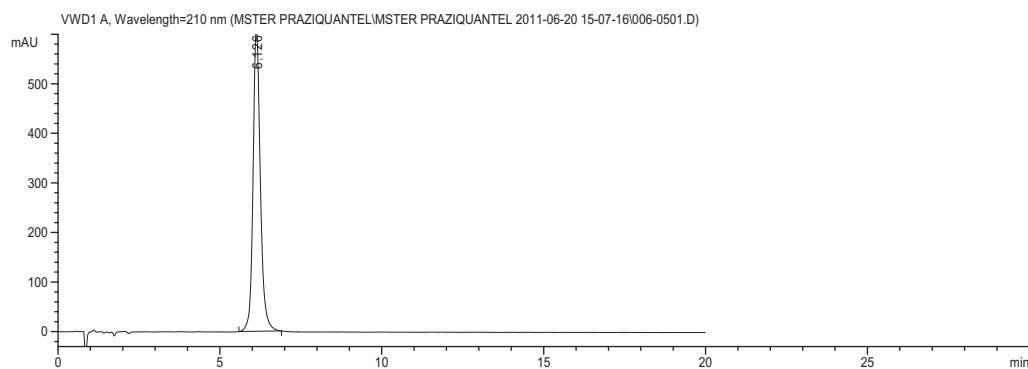
**Figure 2b** Praziquantel after treatment with 1 N HCl.



**Figure 2c** Praziquantel after treatment with H<sub>2</sub>O<sub>2</sub>.



**Figure 2d** Praziquantel after exposure to UV light at suntest® for 4 h.



**Figure 2e** Praziquantel powder after exposure to heat at 60 °C for 4 h.

**Table 2** Reproducibility and precision ( $n = 5$ ).

Injected amount ( $\mu\text{g mL}^{-1}$ )	Intra-day ( $n = 5$ )			Inter-day ( $n = 5$ )		
	Observed amount ( $\mu\text{g mL}^{-1}$ )	CV <sup>a</sup>	Accuracy (%) <sup>b</sup>	Observed amount ( $\mu\text{g mL}^{-1}$ )	CV <sup>a</sup>	Accuracy (%) <sup>b</sup>
20.0	20.07	0.51	100.3	19.42	1.86	97.08
40.0	40.24	0.38	100.6	38.94	1.22	97.35
50.0	49.83	0.23	99.7	48.16	0.98	96.32

<sup>a</sup> Coefficient of variation (CV) = S.D.  $\times$  100/mean.

<sup>b</sup> Accuracy (%) = observed concentration  $\times$  100/used concentration.

**Table 3** Statistical analysis of results obtained by the proposed method applied on Distocid® tablets compared with a reported method.

Parameters	Distocid® tablets	Reference method (Rubiana et al., 2006)
<i>n</i>	5	8
Mean recovery	98.70	99.33
Variance	3.90	2.41
± S.D.	1.97	1.55
± R.S.D.	2.00	1.56
Student's- <i>t</i> test	0.64 (2.20) <sup>a</sup>	
<i>F</i> -test	1.62(4.12) <sup>b</sup>	

a and b are the theoretical Student's-*t* test values and *F*-ratios at  $p_{0.05}$ .

**Table 4** Robustness of the proposed method.

Slight changes in	Temp. (C°)	Flow rate (mLmin <sup>-1</sup> )	ACN (%)	Detection wavelength (nm)	Buffer conc. (mM)	
Affected factor	28, 30 and 32	0.9, 1.0 and 1.1	38, 40 and 42	208, 210 and 212	20, 25 and 30	
C.V. (%)	Peak area	K'	Peak area	<i>t</i> <sub>R</sub>	Peak area	K'
	0	0.94	1.0	0.42	2.0	0.18

### 3.5. Reproducibility and precision of the method

Results (Table 2) show that there were high intra- and inter-day precisions (both within 2.0%). Intra-day precision was assessed through the injection of three different concentrations of the standard solution five times during a day. The same procedure was done for inter-day precision test except that the injection of the samples was every day for five days.

### 3.6. Application

The analysis of PZQ in the formulation Distocid® tablets by the proposed method (Table 3) showed a mean recovery of  $98.70 \pm 2.0\%$  which was statistically comparable to reported method (Rubiana et al., 2006).

### 3.7. Robustness of the method

The robustness of the present method was evaluated in terms of small changes in temperature, flow rate, content of ACN in mobile phase, wavelength of detection, salt concentration and injection volume (Table 4). The results show that the slight variations in the examined factors had no significant effect on the shape of the peak.

## 4. Conclusions

A valid and fast stability indicating HPLC-method for quantification of PZQ is established. Compared with the reported methods, this method represents the first report about a stability indicating method for the determination of PZQ in short time. With the proposed method a satisfactory separation of PZQ from the degradation products and impurities, extended linear range and rapid analysis time were carried out. A high recovery of PZQ in formulation was achieved. The proposed method ensured a precise and accurate determination of PZQ in oral tablet formulation. No interference from the excipients was noticed.

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