ANALYTICAL SCIENCES AUGUST 2010, VOL. 26

2010 © The Japan Society for Analytical Chemistry

Simultaneous Determination of Albendazole and Praziquantel by Second Derivative Spectrophotometry and Multivariated Calibration Methods in Veterinary Pharmaceutical Formulation

César Soto,*† David Contreras,* Sandra Orellana, Jorge Yañez,* and M. Inés Toral****

**Department of Analytical and Inorganic Chemistry, Faculty of Chemical Sciences, University of Concepción, P. O. Box 160, Concepción, Chile*
***Department of Chemistry, Faculty of Sciences, University of Chile, P. O. Box 653, Santiago, Chile*

The simultaneous determination of albendazole (ABZ) and praziquantel (PZQ) was performed by different mathematical approaches: second derivative spectrophotometry (SDS), classical least squares, regression of partial least squares and principal components regression based on spectral data of drugs dissolved in methanol–hydrochloric acid solution. The detection limits for multivariate calibrations were determined by creating a surrogate variable signal. SDS presented the best analytical features. The recoveries of ABZ and PZQ from the synthetic samples were near to 100 ± 5%. The methods were applied in veterinary pharmaceutical formulation whose mass ratio ABZ:PZQ is 10:1; the results obtained were according to nominal content.

(Received April 3, 2010; Accepted May 29, 2010; Published August 10, 2010)

Introduction

The antiparasitic drugs are widely used in veterinary practice and human therapy. The antihelminthics are an important type of these drugs used in cattle and pets, bringing benefit and improved quality of life to such animals.[1] An important example of a disease treated with these drugs is helminthiases, a common parasitic disease of great economical and public health importance. Since the antihelminthic spectra of most drugs used for treatment is limited, combinations of more than one active ingredient are required to control helminthic infections effectively. In this context, albendazole, methyl 5-(propylthio)-2-benzimidazole carbamate (ABZ) (Fig. 1a), is an antihelminthic drug, it is active against most of the nematode worms and some of the cestode worms in humans and animals, and it acts by inhibiting fumarate reductase and the microtubular polymerization of the parasite.[2] Praziquantel, 2-cyclohexylcarbonyl-1,2,3,6,7,11b-hexahydro-4h-pyrazino(2,1-a) isoquinolin-4-one (PZQ) (Fig. 1b), acts on the parasite, increasing the permeability to calcium ions causing contractions and vacuolization. The mix of both drugs is efficiently used in veterinary treatment of parasitic diseases. Many publications describe the determination of ABZ and PZQ in pharmaceutical formulations. Individual and simultaneous determination with other drugs are possible by FIA using UV-detection and HPLC method in suspensions,[3] adsorptive stripping voltammetry (ASV) and linear sweep voltammetry (LSV), square-wave voltammetry (SWV), differential pulse voltammetry (DPV),[4,5] Vis-spectrophotometry,[6-10] HPLC,[11,12] derivative UV-spectrophotometry,[13,14] GLC[15] and PMR spectrometry.[16] In biological samples for individuals, simultaneous determinations with other drugs and metabolites have been reported by: HPLC,[17-24] LC-TMS,[25,26] LC-ES-SM,[27] nonaqueous capillary electrophoresis,[28] IR[29] and fluorometry.[30,31] The simultaneous determination of ABZ and PZQ has not been reported. In general, derivative spectrophotometry has been directly used to simultaneously determine organic and inorganic compounds.[32,33] Multivariate methods offer an alternative for spectroscopic data analyses. These methods have been widely applied to the simultaneous quantification of analytes in mixtures. Among multivariate calibration methods, principal component regression (PCR) and partial least-squares regression (PLS) have been successfully adopted in many quantitative

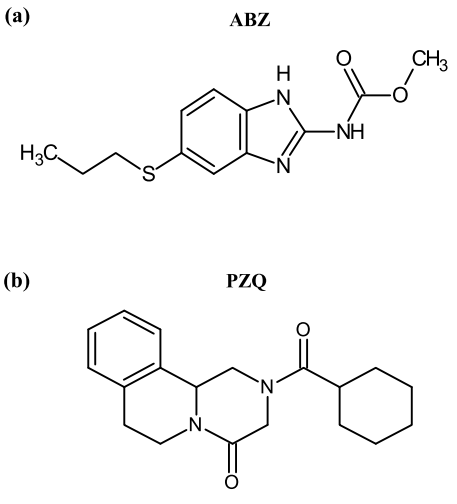

Fig. 1 Molecular structure for (a) ABZ and (b) PZQ.

† To whom correspondence should be addressed.
E-mail: cesarsoto@udec.cl

assays of pharmaceutical formulations.[34-37] The theoretical base of these methods has been fully described by several authors.[38] Classical least squares (CLS) analysis is one of the simplest multivariate methods and is easy to perform, although its results are not very accurate in the quantification of mixtures when the analyzed spectra have significant overlapping.[39] On the other hand, PCR and PLS regression have been used more successfully in quantification of those types of samples, although their use presents more complications than CLS and second derivative spectrophotometry (SDS). The aim of this work is to develop different mathematical approaches for the simultaneous determination of ABZ and PZQ in veterinary pharmaceutical formulation.

Experimental

Instruments

A Shimadzu UV-1603 spectrophotometer with 10 mm quartz cells was used for measurement of the absorbance and derivative absorption spectra. For all solutions, the second derivative spectra were recorded over the range between 190 – 350 nm against solvent, using sampling intervals of 0.2 nm with an scan speed of 480 nm min^{-1}. The spectral data were processed by Shimadzu software kit Ver. 3.7 (P/N 206-60570-04). The solid samples were weighted with a ±0.01 mg of uncertainty using a Sartorius R 200D balance.

Reagents

All reagents were of analytical reagent grade, ABZ and PZQ were purchased from Sigma-Aldrich®. Stock solutions of 1.0×10^{-3} mol L^{-1} of ABZ and PZQ were prepared of each drug by dissolving 13.3 and 15.6 mg and diluted to 50 mL in HCl 6.0×10^{-3} mol L^{-1} in methanol solution. Other ranges of concentrations were prepared by appropriate dilution using the same solvent. Furthermore, in order to carry out a study of the solvent effects on the spectral behavior, we prepared stock solutions of 1.0×10^{-3} mol L^{-1} of ABZ and PZQ by dissolving the same amount as described above of each drug in different solvents. Other ranges of concentration were prepared by appropriate dilution using the respective solvent.

Procedure for calibration graphs of ABZ and PZQ in mixtures

Aliquots of stock solutions of ABZ and PZQ were simultaneously diluted in HCl 6.0×10^{-3} mol L^{-1} in methanol solution in a concentration range between 2.0×10^{-6} to 3.5×10^{-5} mol L^{-1}. The calibration procedure was carried out for each compound in the presence of 3.0×10^{-6} mol L^{-1} of ABZ and PZQ, respectively. In all cases, the corresponding absolute values of the second derivative spectra at 327.5 nm for ABZ and 223.2 nm for PZQ were obtained and then these values were plotted against the corresponding concentrations.

Procedure for simultaneous determination of ABZ and PZQ in synthetic samples

The stock solutions of each compound were appropriately diluted to obtain solutions that contain molar ratios between 12:1 and 1:12 (ABZ:PZQ). For each solution, five repetitions were made. Then, the second derivative spectra were evaluated and the concentrations of each compound were calculated, allowing the determination of the corresponding values of recovery and relative standard deviation (RSD).

Procedure for photo stability studies of ABZ and PZQ

Individual solutions of ABZ and PZQ containing 6.0×10^{-6} mol L^{-1} of each drug in HCl 6.0×10^{-3} mol L^{-1} in methanol solution, were used in photo-stability studies. The solutions were exposed to direct light, indirect light and darkness, and the second derivative spectra were evaluated every 30 min over 5 h. Then all solutions were evaluated after 24 h.

Procedure for simultaneous determination of ABZ and PZQ in pharmaceutical formulation

The content of ten tablets of Albendazol Plus® (Laboratorio Vetanco, Argentina) were weighed and powdered. A quantity of 58.6 mg of powder was weighed and diluted in HCl 6.0×10^{-3} mol L^{-1} in methanol solution. The solution was shaken for 20 min, centrifuged and 50 μL from supernatant was diluted with same solvent to 5 mL and then this mixture was evaluated by SDS.

Multivariate calibration methods

Software. CLS, PCR and PLS analyses were performed by the software Pirouette Ver. 3.11 (Infometrix).

Data pretreatment. Seventy four independent standards were used for the calibration data set, ranging over the same values as those described in section *Procedure for calibration graphs of ABZ and PZQ in mixtures*. The recorded spectra data were preprocessing by mean-centering and transformed by second derivative (15 data) and smoothing by mobile media (25 data). The wavelength region between 218 and 450 nm was selected for multivariate calculation in order to minimize the influence of variables that do not contribute to the model but could contribute to the error component.[38] The spectra resolution was 0.2 nm.

Outlier diagnosis. The outlier diagnose for CLS was performed considering the Leverage, Sample Residual and Mahalanobis distance. The outlier diagnosis for PCR and PLS was carried out through the cross validation performed, considering the Studentized residual together with Leverage with thresholds based on a 95%. The unusual samples were eliminated, and then the models were reconstructed, being optimized, validated and utilized for all the calculations.[40]

Validation and optimization. All the multivariate models utilized cross validation, removing in turn each standard from the training set and then predicting the excluded sample with the calibration, similar to procedures utilized in the literature.[41] The optimal baseline order for CLS was determined by minimizing the model error sum of squares (ESS).

$$\mathrm{ESS} = \sum_{i}^{n} \mathbf{e}_i \mathbf{e}_i^{T}$$

Where **e** is the error matrix.

The prediction residual error sum of squares (PRESS), standard error of cross-validation (SECV) as well as the correlation coefficient (rVal) for the predicted *Y* values *versus* known *Y* values were calculated, considering the cross validation.[40] In the PCR and PLS calibration methods, these parameters were calculated according to Ragno *et al*.[41] and Afkhami *et al*.[42]

$$\mathrm{PRESS} = \mathbf{f}^{T}\mathbf{f}$$

Where **f** is the prediction residual matrix.

Detection limit for multivariated methods. The detection limits of (DL) for multivariate calibrations were determined according to Soto *et al*.[43] creating a surrogate signal variable (SSV)[38,44] in order to create a pseudo-univariated model. The selected SSVs were the model predicted values. Thus, the LOD was calculated in a manner similar to that used for SDS.

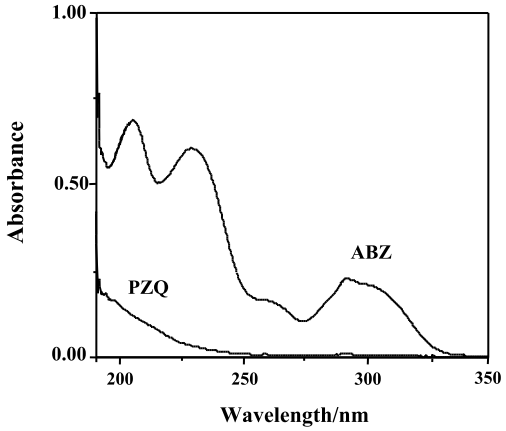

Fig. 2 Zero-order spectra in HCl 6.0×10^{-3} mol L^{-1} in methanol solution of ABZ 2.0×10^{-5} mol L^{-1} and PZQ 2.0×10^{-6} mol L^{-1}.

Results and Discussion

Solvent selection and spectral behavior

Because the objective of this work is the simultaneous determination of ABZ and PZQ, it is necessary to satisfy the solubility of both drugs in the same medium to prepare the solution standards and dilutions. The spectral behaviors should also be adapted to carry out the simultaneous determination for derivative spectrophotometry, so that the method can be precise and the obtained results can be exact. The selection of solvent was based on solubility values reported,[45] a mixture of HCl of 6.0×10^{-3} mol L^{-1} in methanol solution is suitable to dissolve ABZ. This mixture also dissolves PZQ. The spectral behavior of both drugs in this solvent shows the possibility of the simultaneous determination of the two drugs (Fig. 2).

Photo stability studies

In order to establish the photo stability of ABZ and PZQ in HCl 6.0×10^{-3} mol L^{-1} in methanol solution, we used 6.0×10^{-6} mol L^{-1} solution of each individual drug. All solutions were exposed to direct light, indirect light and darkness. For both drugs no photochemical degradation was evidenced because the analytical signals remained constant.

Spectral features

Evaluated directly against solvent selected, ABZ showed 3 maximum absorption peaks centered at 210, 229 and 299 nm; under identical conditions PZQ spectrum presented an absorption band between 190 to 240 nm (Fig. 2). Because the spectral bands of the two drugs are strongly overlapped, the derivative spectrophotometry technique was used; it is a good approach to resolve this type of spectra, making possible the simultaneous determination.

Selection of spectral variables

Derivative order. In this work the digital derivative spectrophotometry technique used is based on a mathematic computational model proposed by Savitzky and Golay,[46] where the spectral resolution is sacrificed in order to increase strongly the signal/noise ratio, favoring the accuracy of the results and the precision of the method. To optimize the derivative order, the first to the fourth derivative spectra of the solution containing separately the respective drugs were recorded. In Figs. 3a and 3b are shown the first and second derivative; higher derivative orders were discarded because the sensibilities decrease and the noise is also increased. Comparing these spectra derivative, one finds that the first derivative presents the higher sensitivities than the second; however, this derivative order does not present zero crossing characteristics for PZQ determination, so only ABZ determination by graphic method between 251.0 and 325.0 nm is possible. The simultaneous determination is possible by second derivative because there are characteristic zones for each compound; for this reason, this order of derivative was selected.

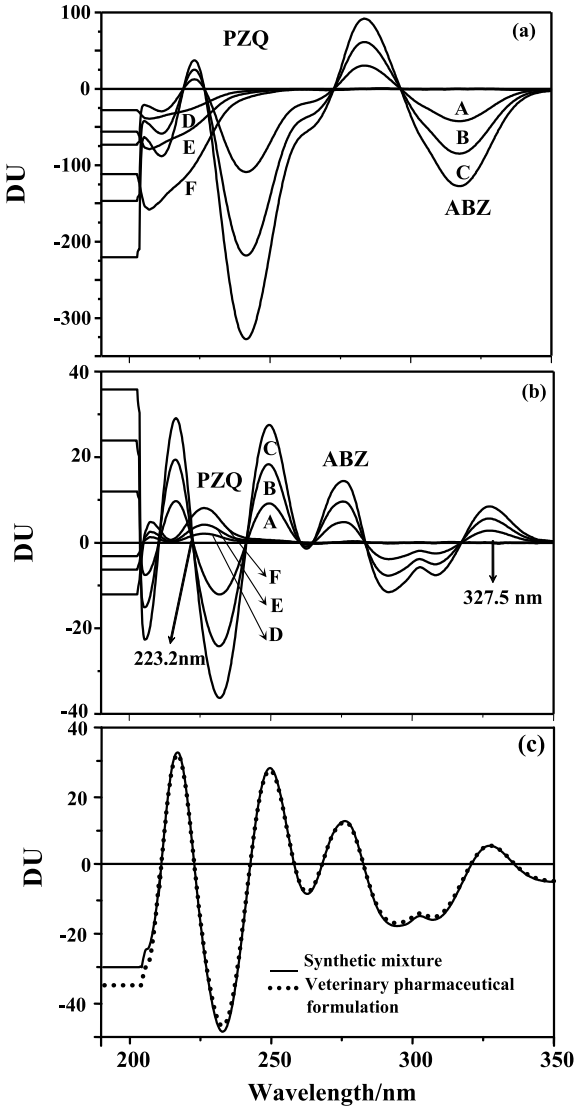

Fig. 3 Derivative spectra of ABZ and PZQ in HCl 6.0×10^{-3} mol L^{-1} in methanol solution. (a) First order. (b) Second order. For both derivatives, ABZ: (A) 1.0×10^{-5}, (B) 2.0×10^{-5}, (C) 3.0×10^{-5} mol L^{-1}, PZQ: (D) 2.5×10^{-6}, (E) 5.0×10^{-6}, (F) 1.0×10^{-5} mol L^{-1}. (c) Comparison between the spectra of synthetic mixture of both drugs with the veterinary pharmaceutical formulation. (——) Synthetic mixture and (····) veterinary pharmaceutical formulation. Concentrations for both samples: ABZ, 3.5×10^{-5} mol L^{-1}; PZQ, 3.0×10^{-6} mol L^{-1}.

Selection of the smoothing and scale factor. By using the second derivative, the smoothing factor could be optimized when the values defined by defect are varied, according to $\Delta\lambda$ value used to obtain the spectra. For a $\Delta\lambda$ values of 160 nm the smoothing factor can be studied by taking the following values:

2000, 4000, 8000 and 16000. When the smoothing factor increases, the heights of derivative signal decreased, but the noise decreased more significantly, yielding a superior signal/noise ratio. A value of 16000 was selected, because in these conditions the signal/noise ratio was favored and the sensitivities were sacrificed in order to decrease the error of the results. Further, when this smoothing spectral factor was used, distortion effects in derivative spectra are not present. A value of 10000 was selected as the scale factor because it favored the reading of the analytical signal, but did not affect the sensibility, since the analytical signal and the standard deviation of the blank increase in the same proportion.

Selection of wavelength. The second derivative spectra ABZ presents two zero crossing points at 223.2 and 241.4 nm (Fig. 3b), which could be used for PZQ determination; we selected 223.2 nm for PZQ determination, however, because the signal are higher. The second derivative spectrum of PZQ presents two zero crossing points at 255.4 and 272.2 nm, between 326.0 to 329.0 nm PZQ does not absorb and the ABZ analytical signals are higher and well defined. Consequently, 327.5 nm was selected for the determination of ABZ (Fig. 3b). The pharmaceutical formulation (Albendazol Plus®) has a molar ratio ABZ:PZQ corresponding to 12:1; therefore it is important to study the effect of the concentration of each drug on the other, in order to select suitable analytical wavelengths. We used different mixtures of both drugs, with varying concentrations of ABZ between 2.0×10^{-6} to 3.5×10^{-5} mol L^{-1} and constant PZQ 3.0×10^{-6} mol L^{-1}, including the relationship contained in pharmaceutical formulations; these solutions were evaluated by second-order derivative, with values of 16000 and 10000 as smoothing and scale factor, respectively. Figure 3c shows a spectrum obtained for a mixture of both drugs and also includes the spectrum of a solution prepared from the tablet dissolved under same conditions selected, containing the following concentrations: PZQ 3.0×10^{-6} mol L^{-1} and ABZ 3.5×10^{-5} mol L^{-1}. The spectrum of the molar ratio of ABZ:PZQ 12:1 is analogous with that of the tablet. The values of zero crossing points at 223.2 and 327.5 nm selected for the determination of PZQ and ABZ, are independent of the concentrations. The simultaneous determination of these drugs is not affected by the other compounds present in the sample prepared from the tablet.

Analytical features

Calibration graphs were created by plotting the second derivative values for ABZ, DU_1 (λ = 327.5 nm) and for PZQ, DU_2 (λ = 223.2 nm), using a smoothing factor of 16000, a scale factor of 10000 and a $\Delta\lambda$ of 160 nm, *versus* the respective drug concentrations. The analytical features were obtained used the criteria given by ICH Harmonized Tripartite Guideline,[47] to calculate the detection and quantification limits (DL, QL). The following equations were used: $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation corresponding to the response of 11 blanks. The repeatability, expressed as relative standard deviation (RSD), was obtained by using 9 standard samples containing 3.0×10^{-6}, 6.0×10^{-6} and 9.0×10^{-6} mol L^{-1} of each drug and 3 replicates of each concentration. The determination range was defined between the QL and the loss of linearity. In this context, it is possible to obtain an acceptable linearity degree with accuracy and precision in the results when the proposed method is applied. The results are shown in Tables 1 and 2.

The detection limits for the multivariate methods are presented in Table 2. The lowest DL was achieved by CLS method for both drugs. The PLS and PCR reach similar values for DL. However, a lower value for ABZ was achieved with the PCR method and a lower value for PZQ was achieved with the PLS method.

Table 1 Analytical features for SDS

Parameter	ABZ	PZQ
Determination range (mol L^{-1})	2.98×10^{-7} – 8.00×10^{-5}	3.78×10^{-7} – 8.00×10^{-5}
Repeatability (RSD, %)	1.3	1.2
Linear regression (C, mol L^{-1})	$DU_1 = 2.82 \times 10^5 C$ – 0.0939	$DU_2 = 7.36 \times 10^5 C$ – 0.206
Correlation coefficient	r = 0.998	r = 0.999

Table 2 Detection and quantification limits (DL and QL) of the different methods developed

	Drug	CLS	PCR	PLS	SDS
DL/mol L^{-1}	ABZ	1.02×10^{-7}	2.05×10^{-7}	2.91×10^{-7}	8.94×10^{-8}
	PZQ	1.77×10^{-7}	3.56×10^{-7}	2.11×10^{-7}	1.13×10^{-7}
QL/mol L^{-1}	ABZ	3.39×10^{-7}	6.81×10^{-7}	9.70×10^{-7}	2.98×10^{-7}
	PZQ	5.88×10^{-7}	1.18×10^{-6}	7.02×10^{-7}	3.78×10^{-7}

Validation and optimization of multivariate calibrations

CLS. The criteria used to adjust the optimal baseline for CLS was to reach the best ESS. Consequently, the best values were obtained without a baseline.

PCR and PLS. For PCR and PLS, the optimal numbers of PCs were estimated considering validation based criteria. We chose six and two PCs for ABZ and PZQ, respectively, for both calibrations: PCR and PLS. These numbers include lower values of PRESS and SECV with a rVal over 0.9. The lowest number of PC was selected in order to not over fit the models.

Recovery and comparison methods

Owing to the ABZ:PZQ ratio contained in the Albendazol Plus® tablet, it was necessary for each method to establish the ratios at which one analyte can be accurately measured in the presence of the other; the recoveries of samples containing standard solutions of mixtures of ABZ and PZQ in different concentration ratios were carried out. The results shown in Table 3 correspond to the averages of all ratios used. According to the results, the content of each compound can be determined for developed methods in different relationships. It is important to highlight that the 12:1 molar ratio for ABZ:PZQ, corresponds to a 10:1 mass ratio. Thus we conclude that this method has a wide range of use and is applicable when the contents of these drugs are present in this proportion of the veterinary pharmaceutical formulation. The recoveries obtained from the multivariated methods, in general, were poorer than those from SDS considering both compounds together. The poorest recovery was reached for PZQ. The principal components (PCR and PLS) based method reach the poorest recovery for PZQ; this can be due to the higher relationship between the ABZ:PZQ molar absortivity constant at different wavelengths. In this way, when the first PC's are generated for the calibrations, important information for PZQ concentration could not been associated with these PC's.

Application of the proposed methods

Finally, the content of ABZ and PZQ in Albendazol Plus®

Table 3 Average values of all ratios used for recoveries of ABZ and PZQ, by SDS, CLS, PCR and PLS (including the ratio present in commercial tablets)

Drug	Recovery[a]			
	CLS, %	PCR, %	PLS, %	SDS, %
ABZ	97.5 ± 2.9	100.0 ± 2.8	101.0 ± 2.4	100.2 ± 3.1
PZQ	96.6 ± 7.9	127.0 ± 6.6	119.0 ± 7.2	99.5 ± 3.1

a. The values correspond to the mean of 5 different determinations made from mixtures of ABZ and PZQ at different proportions.

Table 4 Simultaneous determination of ABZ and PZQ in veterinary pharmaceutical formulation

Drug	Albendazol Plus®/mg	Amount found per tablet/mg[a]			
		SDS	PLS	PCR	CLS
ABZ	500	495.0 ± 3.4	503.0 ± 12.2	503.0 ± 10.6	487.5 ± 15.0
PZQ	50	49.8 ± 0.5	48.4 ± 6.5	56.4 ± 4.0	48.9 ± 6.3

a. Each value is the average of 6 determinations.

(Laboratorio Vetanco, Argentina) was analyzed by developed methods; the results were satisfactory (Table 4).

Conclusions

According to the results obtained with these proposed methods, simultaneous determination of the binary mixtures of ABZ and PZQ by different mathematical approaches based on spectral data is effective and accurate. Derivative spectrophotometry and recently multivariate calibration methods have been used. The proposed methods are simpler, less time-consuming and more sensitive. The difference in the values of DL for the two drugs, including multivariate calibration (PLS, CLS, PCR) and SDS was minimal (less than one order of magnitude). The recoveries of ABZ and PZQ from the synthetic samples, including the proportion contained according to veterinary pharmaceutical formulation, were near 100%. The amounts found for both drugs are very similar to the nominal content in commercial tablets. The pharmaceutical matrix had no significant effect on the determination of the two drugs. Moreover the mass ratio problem present in tablets (ABZ:PZQ (10:1)) was overcome successfully.

Acknowledgements

The authors are grateful to the projects: DIUC No. 210.021. 029-10 and FONDECYT Nos. 1070905 and 1100103, for the financial support.

References

1. W. Campbell, *Parasitol. Today*, **1990**, *6*, 130.
2. T. Barragry, *N. Z. Vet. J.*, **1984**, *32*, 191.
3. Z. Atkosar and G. Altioka, *J. Liq. Chromatogr. Relat. Technol.*, **2006**, *29*, 849.
4. A. Abu Zuhri, A. Hussein, and M. Musmar, *Anal. Lett.*, **1999**, *32*, 2965.
5. M. F.de Oliveira and N. R. Stradiotto, *Anal. Lett.*, **2001**, *34*, 377.
6. G. Z. Zhao, H. K. Li, Y. Liu, and Y. H. Wanh, *Chin. J. Anal. Chem.*, **2001**, *29*, 400.
7. N. M. Fregonezi, M. M. Bacarat, E. R. Kedor, and R. M. Pinheiro, *Anal. Lett.*, **2001**, *34*, 1255.
8. K. Basavaiah and H. C. Prameela, *Oxid. Commun.*, **2004**, *27*, 177.
9. S. Hanaa and J. Schnekenburger, *Analyst*, **1992**, *117*, 87.
10. E. Hackmann, M. Santoro, and S. Benetton, *Rev. Cienc. Farm.*, **1991**, 131.
11. E. van Tonder, M. de Villiers, J. Handford, C. Malan, and J. du Preez, *J. Chromatogr., A*, **1996**, *729*, 267.
12. P. Bonato, A. Oliveira, F. de Santana, B. Fernandes, V. Lanchote, A. Gonzalez, H. Garcia, and O. Takayanagui, *J. Pharm. Biomed. Anal.*, **2007**, *44*, 558.
13. Y. Wu, F. Liu, and C. Li, *Zhongguo-Yiyao-Gongye-Zazhi*, **1991**, *22*, 75.
14. X. Yuan, *Yaowu-FenxiZazhi*, **1985**, *5*, 120.
15. H. Saleh and J. Schnekenburger, *Analyst*, **1992**, *117*, 1457.
16. S. El Khateeb, N. El Ragehy, F. Khattab, and A. Ahmad, *Spectrosc. Lett.*, **1991**, *23*, 505.
17. J. Garcýa, F. Bolás Fernández, and J. Torrado, *J. Chromatogr., B*, **1999**, *723*, 265.
18. G. Morovján, P. Csokán, L. Makranszki, E. Abdellah-Nagy, and K. Tóth, *J. Chromatogr., A*, **1998**, *797*, 237.
19. P. Chiap, B. Evrard, M. Binazubute, P. de Tullio, P. Hubert, L. Delattre, and J. Crommen, *J. Chromatogr., A*, **2000**, *870*, 121.
20. E. Unvermis, H. Colak, I. Tumer, and O. Ergun, *Medy. Wetery.*, **2005**, *61*, 398.
21. V. Hormazabal and M. Yndestad, *J. Liq. Chromatogr.*, **1995**, *18*, 589.
22. D. Gonzalez-Esquivel, C. Morano-Okuno, M. Sanchez-Rodriguez, J. Sotelo-Morales, and H. Jung-Cook, *J. Chromatogr., Biomed. Appl.*, **1993**, *124*, 174.
23. A. Rogstad, V. Hormazabal, and M. Yndestad, *J. Chromatogr.*, **1987**, *391*, 328.
24. V. Hormazabal and M. Yndestad, *J. Liq. Chromatogr.*, **1995**, *18*, 1231.
25. P. Bonato, V. Lanchote, and O. Takayanagui, *J. Chromatogr., B*, **2003**, *783*, 237.
26. H. De Ruyck, E. Daeseleire, H. De ridder, and R. Van Rentergehem, *J. Chromatogr., A*, **2002**, *976*, 181.
27. T. Msagati and M. Nindi, *Talanta*, **2006**, *69*, 243.
28. A. Prochazkova, M. Chouki, and R. Theurillat, *Electrophoresis*, **2000**, *21*, 729.
29. Y. Yang, C. Ye, and G. Yao, *Zhongguo-Yiyao-Gongye-Zazhi*, **1991**, *22*, 544.
30. H. Gao, C. Wang, and G. Yang, *Yaowu-Fenxi-Zazhi*, **1984**, *4*, 144.
31. J. Puetter, *Eur. J. Drug Metab. Pharmacokinet.*, **1979**, *4*, 143.
32. M. Toral, C. Soto, J. Rivas, M. Saldias, and S. Orellana, *J. Chil. Chem. Soc.*, **2008**, *53*, 1543.
33. M. Toral, C. Soto, A. Tassara, and P. Richter, *J. AOAC Int.*, **2003**, *86*, 241.
34. D. Contreras, J. Freer, and J. Rodriguez, *Int. Biodeterior. Biodegrad.*, **2006**, *57*, 63.
35. J. Kalivas, *Anal. Lett.*, **2005**, *38*, 2259.
36. H. Goicoechea and A. Olivieri, *Talanta*, **1998**, *47*, 103.
37. J. Lemus and J. Perez, *Anal. Chim. Acta*, **2002**, *460*, 85.
38. A. Olivieri, N. Faber, and J. Ferre, *Pure Appl. Chem.*, **2006**, *78*, 633.

39. S. Haswell and A. Walmsley, *Anal. Chim. Acta*, **1999**, *400*, 399.
40. I. T. I. A. C. Inc., 3.11 ed., Bothell, WA, **2003**.
41. G. Ragno, G. Ioele, and A. Risoli, *Anal. Chim. Acta*, **2004**, *512*, 173.
42. A. Afkhami, N. Sarlak, and A. Zarei, *Talanta*, **2007**, *71*, 893.
43. C. Soto, D. Contreras, M. Toral, L. Basáez, and J. Freer, *J. Chil. Chem. Soc*., **2009**, *54*, 113.
44. J. Saurina, C. Leal, R. Compano, and M. Granados, *Anal. Chim. Acta*, **2001**, *432*, 241.
45. W. Hou, *Yaoxue-Tongbao*, **1986**, *21*, 455.
46. A. Savitzky and M. Golay, *Anal. Chem*., **1964**, *36*, 1627.
47. ICH-Q2B Validation of Analytical Procedures, Methodology International Conference on Harmonization, **1996**.