http://dx.doi.org/10.5935/0103-5053.20150107

J. Braz. Chem. Soc., Vol. 26, No. 7, 1387-1395, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Comparison between Ultraviolet and Infrared Spectroscopies for the Simultaneous Multivariate Determination of Pyrantel and Praziquantel

Mário S. Piantavini,[a] Flávia L. D. Pontes,[a] Leandro X. Weiss,[a] Marcelo M. Sena[b,c] and Roberto Pontarolo[a,]*

[a]*Laboratório de Controle de Qualidade, Departamento de Farmácia, Universidade Federal do Paraná, 80210-170 Curitiba-PR, Brazil*

[b]*Departamento de Química, ICEx-Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte-MG, Brazil*

[c]*Instituto Nacional de Ciência e Tecnologia em Bioanalítica (INCT Bio), 13083-970 Campinas-SP, Brazil*

Methods based on multivariate calibration and diffuse reflectance infrared Fourier transform (DRIFT) and ultraviolet (UV) spectroscopies were developed for the simultaneous determination of two veterinary pharmaceutical drugs, pyrantel pamoate and praziquantel, in commercial tablets. The best UV model was obtained with the full spectra, 200-400 nm, and partial least squares (PLS). The best DRIFT model was optimized by selecting the most predictive spectral regions with synergy interval PLS, 3998-3636 cm^{-1}, 3274-1824 cm^{-1} and 1100-735 cm^{-1}. Both methods were validated according to Brazilian and international guidelines through the estimate of figures of merit, such as trueness, precision, linearity, analytical sensitivity, bias and residual prediction deviation (RPD). These methods were applied to the determination of the drugs in three different veterinary formulations commercialized in the Brazilian market and the results were compared with high performance liquid chromatography (HPLC). DRIFT was considered more suitable for the quality control of these formulations, because it is faster, does not use solvents and does not generate chemical waste.

Keywords: DRIFT, multivariate calibration, quality control, PLS, ultraviolet spectroscopy

Introduction

Praziquantel (PZ) is an anthelmintic highly active against a wide range of cestodes and all species of *schistosoma* pathogenic to man.[1] The most obvious and immediate modification that can be observed in schistosomes exposed to this drug, either *in vitro* or *in vivo*, causes a spatic paralysis of the worm musculature.[2] Pyrantel pamoate (PP) is a cholinergic agonist that acts by inhibiting the neuromuscular transmissions of the parasite,[3] and it is effective against infestations with *Enterobius vermicularis*, *Ascaris lumbricoides*, *Ancylostoma duodenale*, and *Necator americanus*, among others, in children and adults.[4] Considering the limited effect of these drugs individually, combined formulations of anthelmintics with different mechanisms of action have been effectively used in veterinary practice to extend the spectrum of antiparasitic activity.[5] These compounds are widely applied in veterinary treatments and formulations containing PZ or PP and are the most used in Brazil for preventing parasitic diseases in pets. However, PZ and PP also have applications in human therapy, since these diseases are transmissible to humans.[3] The structures of these two compounds are shown in Figure 1.

The literature has described several analytical methods for the determination of PP or PZ in pharmaceutical formulations based on techniques, such as voltammetry,[6] high performance liquid chromatography (HPLC),[7-9] nuclear magnetic resonance (NMR) spectroscopy,[7] derivative ultraviolet (UV) spectrophotometry,[10] titrimetry[11] and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).[12] Nevertheless, the simultaneous determination of PZ and PP has been carried out only in a few papers, primarily based on chromatographic techniques.[8,9,12] Though well established, chromatographic methods are slow, of relative high cost and demand a large amount of pure solvents, also generating a

*e-mail: pontarolo@ufpr.br

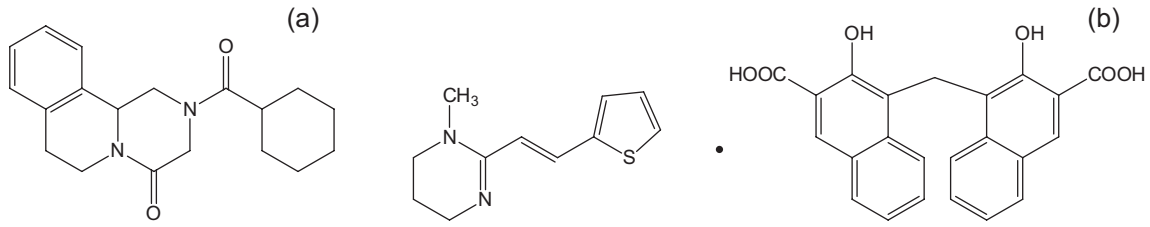

Figure 1. Chemical structures of praziquantel (a) and pyrantel pamoate (b).

lot of chemical waste. An alternative for direct and rapid determinations is the use of spectrophotometric methods. Spectral interferences are a substantial problem, often making the direct determination of some analytes almost impossible. This simultaneous quantification is a challenge for analysts when significant spectral overlapping of the analytes is presented. In recent years, chemometric tools have been employed jointly with UV-Visible,[13-16] near (NIR)[17-19] and mid infrared (MIR)[20-23] spectroscopies in the development of methods for the direct and simultaneous determinations of active pharmaceutical ingredients (API) in several formulations. The most widely applied multivariate calibration method is the partial least squares (PLS).[24] In this paper, synergy interval PLS (siPLS), a variant of PLS, was also employed. It divides the data set into a number of continuous wavelength intervals and combines two or more of them for providing the best predictive model.[22]

Considering the increasing number of multivariate spectrophotometric methods developed for the quality control of API in formulations and the stringent requirements of the pharmaceutical industry, there is a need to change the official regulation, because the vast majority of the guidelines are based on univariate methods.[25-27] Thus, an important issue raised in this work is the multivariate analytical validation, aiming at the harmonization between official guidelines and multivariate methods. Some important peculiarities of these methods are the absence of calibration curves (signal as a function of the API content) and the lack of requirement for total signal selectivity. Since multivariate methods are only useful in practice when there is signal overlapping, their selectivity has a different meaning from univariate methods, just indicating how much of the spectroscopic signal is used for building the model. Another important multivariate concept is the net analyte signal (NAS), which allows separating the specific information of the analyte from the interferences. Multivariate figures of merit (FOM), such as selectivity (SEL), sensitivity (SEN), limits of detection and quantification are estimated based on the NAS. A more thorough discussion on these topics can be found elsewhere.[18,19,28,29]

The aim of this work is to develop and validate different approaches for the simultaneous quantification of PP and PZ in veterinary pharmaceutical formulations, based on ultraviolet and DRIFT diffuse reflectance spectroscopies. These methods will be compared based on the estimation of the multivariate FOM, such as linearity, trueness, precision, range, SEL, SEN, analytical sensitivity (γ), bias and residual prediction deviation (RPD).

Experimental

Reagents and samples

Standards of PP and PZ were purchased from Sigma-Aldrich (St. Louis, USA) and stored protected from light. The analyzed excipients, microcrystalline cellulose was from DEG (≥ 99.0%, São Paulo, Brazil), corn starch was from Embrafarma (≥ 95.0%, São Paulo, Brazil), colloidal silicon dioxide was from Sigma-Aldrich (≥ 99.5%, St. Louis, USA), sodium lauryl sulphate was from Via Farma (≥ 90.0%, São Paulo, Brazil), butylated hydroxytoluene and talc were from Sigma-Aldrich (both ≥ 99.0%, St. Louis, USA), were obtained from certified suppliers and used without further purification. Tablets were purchased from local pharmacies (Curitiba, Brazil). All the determined commercial veterinary formulations have the same composition of API, 145.0 mg of PP and 50.5 mg of PZ, and their contents of excipients are not publicly available. Acetonitrile and methanol were from J. T. Baker, (≥ 99.9%, Deventer, Netherlands) with HPLC grade. Deionized water was obtained with a Milli-Q system from Millipore (Bedford, USA).

Apparatus and software

DRIFT spectra were recorded in a Bruker Alpha Fourier transform infrared (FTIR) spectrophotometer (Bruker Bioscience, Germany), equipped with a diffuse reflectance accessory. The spectra were obtained in a room under control of temperature (20.0 ± 0.2 ºC) and humidity (45-55%) and the equipment was controlled by OPUS software for windows (version 6.0) from Brucker Optik (Bremen, Germany). UV spectra were recorded in

an Agilent 8453 UV-Visible spectrophotometer (Agilent Technologies, Santa Clara, USA), with a 1.00 cm quartz cell. Data were treated using MATLAB software, version 7.13 (The Math-Works, Natick, USA), and PLS Toolbox, version 6.5 (Eigenvector Technologies, Manson, USA).

Experimental designs

For the DRIFT model, built with solid samples, twenty seven powder mixtures were prepared according to a central composite design with two factors, PZ and PP, and seven concentration levels (Figure 2a). The total mass of each mixture was fixed at 100.00 mg. The range of PZ and PP were varied from 5.64 to 17.00 mg *per* 100.00 mg and from 14.60 to 45.00 mg *per* 100.00 mg, respectively. The central point of this design corresponds to the nominal composition of the analyzed formulations (Reagents and samples section). Each mixture sample was completed to 100.00 mg with the addition of excipients according to other experimental design. This design consisted of two factors varying at certain ranges (not specified here for reasons of commercial interest). These two factors were a mixture of the two major excipient components, microcrystalline cellulose and corn starch, and a mixture of the others (colloidal silicon dioxide, sodium lauryl sulphate, butylated hydroxytoluene and talc). These designed mixtures of excipients were randomly mixed with the samples from the first design aiming at achieving a robust model.

For the UV model, fifty one solutions were prepared according to a full factorial design (Figure 2b) with two factors and seven levels (with triplicate of the central point). The range of PZ and PP were varied from 3.00 to 5.00 μg mL^{-1} and from 9.10 to 15.20 μg mL^{-1}, respectively. The central point of this design corresponds to the appropriate dilution of the nominal composition of the analyzed formulations. A larger number of samples were used for building the UV model, because there was no addition of excipients, since all of them are filtrated during the sample preparation or do not absorb in the spectral

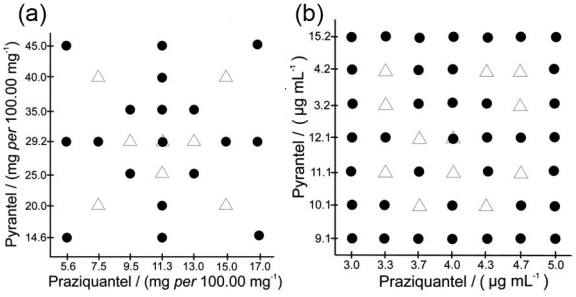

Figure 2. Experimental designs used in this work: (a) central composite design for DRIFT model and (b) full factorial design for UV model. Calibration (full circles) and validation (empty triangles) samples.

range of interest. For both models the calibration set was built aiming at ensuring a representative and homogeneous distribution of the samples in the composition ranges, including the extreme points.

Procedure

DRIFT spectra

The powder mixture samples were prepared by weighing the appropriate masses in an analytical balance (± 0.00001 g). In the sequence, they were manually homogenized and directly measured. The spectra were registered between 4000 and 400 cm^{-1}, with 64 scans and a resolution of 4 cm^{-1}. Replicates of samples from the central point were obtained for evaluating repeatability and intermediate precision. Twenty reflectance spectra of spectroscopic-grade KBr previously ground in agate mortar were also recorded for estimating the instrumental noise.

Ultraviolet spectra

Stock solutions of PZ and PP at concentrations of 1 mg mL^{-1} were prepared separately in a diluent solution of methanol and acetonitrile (50:50, v/v) and stored protected from light at 4 °C. From these solutions, an intermediate solution of each standard was prepared at a concentration of 150 μg mL^{-1} in the same diluent solution. These intermediate standards were used to prepare mixtures of the standard solutions for the calibration and validation sets. The spectra were registered between 200 and 400 nm, with a spectral resolution of 0.5 nm. Twenty spectra of a blank solution were also recorded for estimating the instrumental noise.

Analysis of commercial samples

For each formulation, twenty tablets were pulverized using a mortar and pestle, and mixed into a homogeneous powder. In the DRIFT analysis, the diffuse reflectance spectra were acquired directly on the solid mixtures. For UV and HPLC analyses, a mass equivalent to one tablet was dissolved, sonicated in a Branson 2510 ultrasonic bath (Danbury, USA) at 130 W for 15 minutes and filtered through a Millipore Millex PVDF (Darmstadt, Germany). Then, the filtered solution was transferred to a volumetric flask containing a diluent solution of methanol and acetonitrile (50:50, v/v), according to the appropriate concentration of each technique.

HPLC analysis

Commercial samples were also analyzed by HPLC, based on a procedure adapted from the literature.[8] This analysis was performed using an Agilent 1100 HPLC System (Wilmington, USA) composed of a G1311A

quaternary pump, a G1379A degasser, a G1329A autosampler, a G1316A column oven and a G1315B diode array detector. An XBridge C18 150 × 4.6 mm (5 µm particle size) column coupled with an XBridge C18 20 × 4.6 mm (5 µm particle size) both from Waters Corporation (Milford, USA) guard column were employed. The mobile phase was composed of water (pH 3.5, adjusted with H_3PO_4) and acetonitrile in a gradient mode. The injection volume was 10 µL, the flow rate was 1.2 mL min^{-1}, the detection was at 215 nm and the column temperature was 40 °C. Each sample was analysed in triplicate and all the injections were repeated three times. Data acquisition was performed using ChemStation A.10.02 software.

Results and Discussion

PP and PZ spectra

DRIFT spectra of PP and PZ standards are shown offset from the baseline in Figure 3. In order to interpret them, spectral assignments were carried out based on the relevant literature.[30] For PP, the most distinctive assignments were a broad band with three peaks at 3067, 2969 and 2892 cm^{-1} (related to =C–H and –C–H stretching); a strong peak at 1659 cm^{-1} (aromatic carboxylic, C=O stretching); a peak at 1613 cm^{-1} (cyclic imine, C=N stretching); peaks at 1265, 1150 and 1047 cm^{-1} (all related to C–N stretchings); and a peak at 713 cm^{-1} (out-of-plane C–H bending of a tiophene ring).[31] For PZ, the most relevant assignments were two small peaks at 3291 and 3234 cm^{-1} (first overtones of C=O stretching); two strong peaks at 2898 and 2839 cm^{-1} (stretching of C–H bound to tertiary amines in the lactam ring); a strong band between 1600 and 1700 cm^{-1}, centered at 1666 cm^{-1} (C=O stretching of two amide carbonyl groups); a strong band centered around 1450 cm^{-1} (C–H bending of di-substituted amides); and a peak at 1301 cm^{-1} (C–N stretching).

UV spectra of pure solutions of PP (12.1 µg mL^{-1}) and PZ (4.0 µg mL^{-1}) in MeOH/ACN (50:50, v/v) are shown in Figure 4. PP presents a large and intense absorption band between 200 and 260 nm, with a maximum at 237 nm, while PZ exhibits absorption in the range between 200 and 233 nm. It is not possible determine simultaneously PP and PZ in their mixtures by univariate methods due to the observed spectral overlap. Thus, the use of multivariate calibration is required.

Multivariate calibration models

DRIFT model

Since DRIFT spectra were obtained from solid mixture samples, it is necessary to incorporate the excipient composition in these mixtures for building multivariate calibration models. Based on a specific knowledge of the most common excipients used in veterinary formulations and on biopharmaceutics classification system (BCS),[32] two major (microcrystalline cellulose and corn starch) and four minor excipients (colloidal silicon dioxide, sodium lauryl sulphate, butylated hydroxytoluene and talc) were chosen. As described in Experimental designs section, an experimental design was employed for incorporating a range of the excipient composition to the samples, in order to obtain a more robust and representative model.

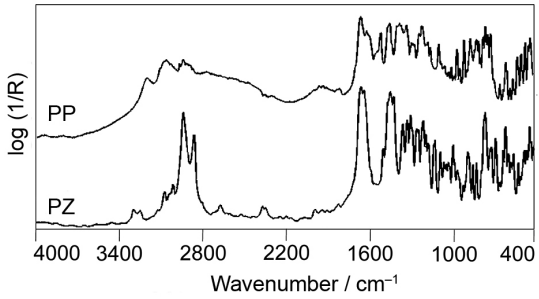

Figure 3. DRIFT spectra of pure PZ and PP.

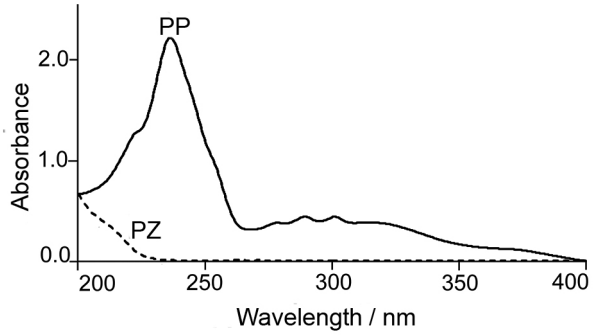

Figure 4. UV absorption spectra of pure PZ (4.0 µg mL^{-1}) and PP (12.1 µg mL^{-1}) in methanol:acetonitrile (50:50, v/v).

DRIFT calibration models were developed in the PLS2 mode (both analytes are predicted simultaneously from the same set of loadings) and optimized for PZ and PP. The spectra of 27 samples were split in twenty for the calibration set and seven for the validation set (Figure 5a), according to an experimental design (Figure 2a). The validation samples were chosen in order to represent homogeneously the analytical ranges. Different preprocessing methods were tested for correcting baseline deviations typically observed in diffuse reflectance measurements of solids, due to the multiplicative light scattering. First derivative with smoothing, multiplicative scatter correction (MSC), standard normal variate (SNV) and vector normalization were tested,[33] and the best model was obtained with first derivative followed by Savitsky-Golay smoothing (15 points and first order fit), MSC and mean centering. Leave-one-out cross-validation was used to

select the number of latent variables (LV) in the best PLS model.

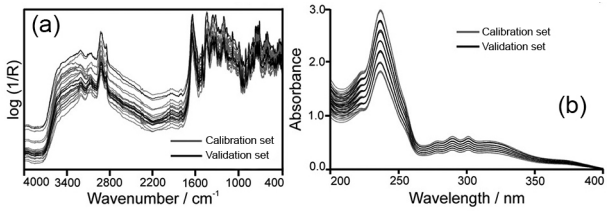

Figure 5. (a) DRIFT spectra of 27 samples of mixtures of PP and PZ; (b) UV absorption spectra of 49 samples of mixtures of PP and PZ in methanol:acetonitrile (50:50, v/v).

Considering that the use of the full spectra may include non predictive wavenumber regions, the model was optimized by variable selection with siPLS. This is a method of continuous variable selection that searches for selecting the most informative spectral regions.[22] siPLS is appropriate for variable selection in multivariate infrared methods, considering the continuous nature of these spectra and it is also more robust than the better known interval PLS (iPLS). In fact, the optimization of siPLS encompasses iPLS when models with only one subinterval are evaluated. In this article, it were tested models with the spectra divided in 8, 10, 20, 30, 40 and 50 intervals combined up to 6 subintervals. The best siPLS model was selected with a combination of six subintervals (from the spectra divided in 10 intervals), using 5 LV and accounting for 99.7% in the **X** block and 97.9% in the **Y** block. The root mean square errors of cross-validation (RMSECV) were decreased from PLS to siPLS model, going from 1.63 to 1.31 mg *per* 100.00 mg and from 1.13 to 0.81 mg *per* 100.00 mg for PP and PZ, respectively. Although F tests at 95% confidence level have indicated no significant differences between the RMSECV values (for PZ there is significant difference at 75% confidence level), siPLS models were considered better and adopted. Outlier detection based on high values of leverage, X and Y residuals at 95% confidence level[19] was applied to the final model, but no outlier was detected. The wavenumber selected by siPLS were the intervals 3998-3636 cm^{-1}, 3274-1824 cm^{-1}, and 1100-735 cm^{-1}. These spectral regions contain specific vibrations of PP and PZ, such as the peaks at 2898 and 2839 cm^{-1}, assigned to stretching of C–H bound to a tertiary amine of PZ, and three characteristic peaks of PP at 3067, 2969 and 2892 cm^{-1}, assigned to C–H stretching.

Aiming at complementing the spectral interpretation of the developed model, the variable importance in projection (VIP) scores are shown in Figure 6. VIP scores measure the importance of each variable in the projection used by a particular PLS model.[34] As can be observed, there is a reasonable agreement between the highest VIP scores values and the spectra of PP and PZ. This is a qualitative interpretation of the developed models that consistently indicates that the most important variables are related to the most characteristic vibration bands of the analytes. For PP (Figure 6a), one of the most important VIP scores is at 2969 cm^{-1}, related to –C–H stretching, while for PZ (Figure 6b) the second highest peak is at 2839 cm^{-1}, related to the C–H bound to a tertiary amine.

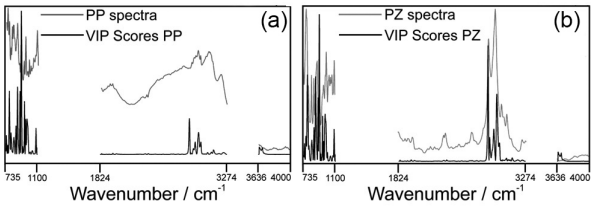

Figure 6. Comparison between the VIP scores for siPLS model and the DRIFT spectra of PP (a) and PZ (b).

UV model

As mentioned in Experimental designs section, there is no need to include excipients in the UV model, since all of them do not interfere, being retained during the filtration process or not absorbing in the spectral range of interest. This is an advantage of the UV model, since it is not necessary to know the quantitative excipient composition. However, UV model has the disadvantage of being destructive, requiring sample dissolution and extraction of the analytes.

UV model was developed using PLS2 and the 51 sample spectra were split in 38 for the calibration set and 13 for the validation set (Figure 5b), according to a full factorial design (Figure 2b). The best model was obtained with only mean centering as preprocessing and variable selection by siPLS did not provide better models than using the full spectra. By using leave-one-out cross-validation, a model with 2 LV accounted for 99.9% in the **X** block and 99.8% in the **Y** block, and provided RMSECV of 0.06 and 0.07 μg mL^{-1} for PP and PZ, respectively. No outlier was detected based on leverage and X and Y residuals. The VIP

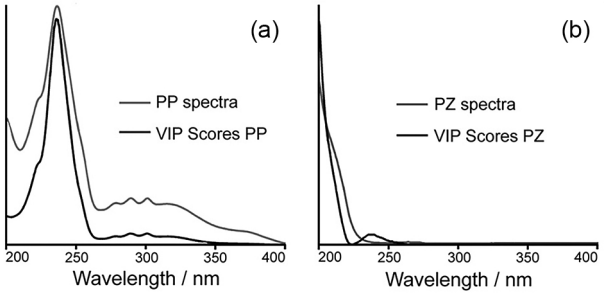

Figure 7. Comparison between the VIP scores for PLS model and the UV spectra of PP (a) and PZ (b).

scores for this model are shown in Figure 7, in which it is possible to note the close agreement between them and the spectral profiles of PP and PZ.

Multivariate analytical validation

DRIFT model

The estimated FOM used to validate the developed DRIFT model are summarized in Table 1. The parameters commonly used to evaluate trueness of multivariate models are the root mean square errors of calibration (RMSEC) and prediction (RMSEP), which were estimated at 0.35 and 0.65 mg *per* 100.00 mg for PP, and 0.17 and 0.43 mg *per* 100.00 mg for PZ, respectively. The trueness is also evaluated through the individual relative errors of prediction, which were all in the range of ± 5%, with mean values of 1.01% for PP and 1.42% for PZ. Precision was evaluated at the levels of repeatability (the same operating conditions) and intermediate precision (two different analysts in two days) by estimating the relative standard deviation (RSD) for six replicates of one sample (central point). All RSD were below 5%, as prescribed by the Brazilian guidelines.[25] These results of trueness and precision corroborate the accuracy of this method.

The model fit was evaluated by plotting the reference *versus* predicted values (Figures 8a and 8b). The linearity was represented by the residuals of these fits, which presented random behaviors and whose regression parameters are shown in Table 1. The correlation coefficients (r) were 0.996 for PP and 0.999 for PZ, all above the minimum acceptable value of 0.99, prescribed by the Brazilian guidelines.[25] Considering the linearity and the accuracy of the method, its analytical ranges were found to be 14.60-45.00 mg *per* 100.00 mg for PP, and 5.60-17.00 mg *per* 100.00 mg for PZ.

The SEL of the method was estimated as 23.6% for PP and 16.8% for PZ, indicating how much of the analytical signal from each analyte was used for building the models. Considering that the SEN is dependent on the analytical technique and thus not appropriate for comparison between methods, the analytical sensitivity (γ) was estimated as the ratio between SEN and instrumental noise (ε = 0.0023). This last value was calculated as the pooled standard deviation of twenty replicated spectra of the blank (spectroscopic-grade KBr powder). The inverse of γ, 0.007 mg *per* 100.00 mg for PP and 0.01 mg *per* 100.00 mg for PZ, are the minimum concentration differences that the method were able to discriminate, considering the random instrumental noise as the only source of errors. These values also define the number

Table 1. Parameters estimated for validating the developed siPLS-DRIFT and PLS-UV methods

Figure of merit	Parameter	Value			
		DRIFT		UV	
		PP	PZ	PP	PZ
Trueness	RMSEC	0.35[a]	0.17[a]	0.05[b]	0.07[b]
	RMSEP	0.65[a]	0.43[a]	0.07[b]	0.08[b]
	Mean relative error	1.01%	1.42%	0.52%	1.41%
Precision	$RSD_{repeatability}$	0.32%	0.69%	0.65%	0.51%
	$RSD_{intermediary\ precision}$	0.32%	1.19%	0.63%	0.56%
Linearity	Slope[c]	1.00	0.94	0.99	0.98
	Intercept[c]	0.01	0.39	0.08	0.04
	r	0.996	0.999	0.999	0.992
Range		14.60-45.00[a]	5.60-17.00[a]	9.10-15.20[b]	3.00-5.00[b]
Selectivity		23.6%	16.8%	82.3%	24.3%
Sensitivity[d]		0.33	0.24	16.11	2.34
Anal. sens. (γ)		143.5[e]	104.3[e]	2876[f]	417[f]
γ^{-1}		0.007[a]	0.01[a]	4×10^{-4}[b]	2×10^{-3}[b]
Bias		–0.192[a]	0.082[a]	0.0031[b]	0.0015[b]
SDV		0.5134[a]	0.1392[a]	0.0728[b]	0.0806[b]
RPD	Calibration	7.4	4.5	39.6	9.6
	Validation	8.5	4.7	20.9	6.9

[a]mg *per* 100.00 mg; [b]μg mL^{-1}; [c]values for the lines fitted to the calibration samples; [d]values expressed as the ratio between units of absorbance and (mg *per* 100.00 mg); [e](mg *per* 100 mg)$^{-1}$; [f](μg mL)$^{-1}$.

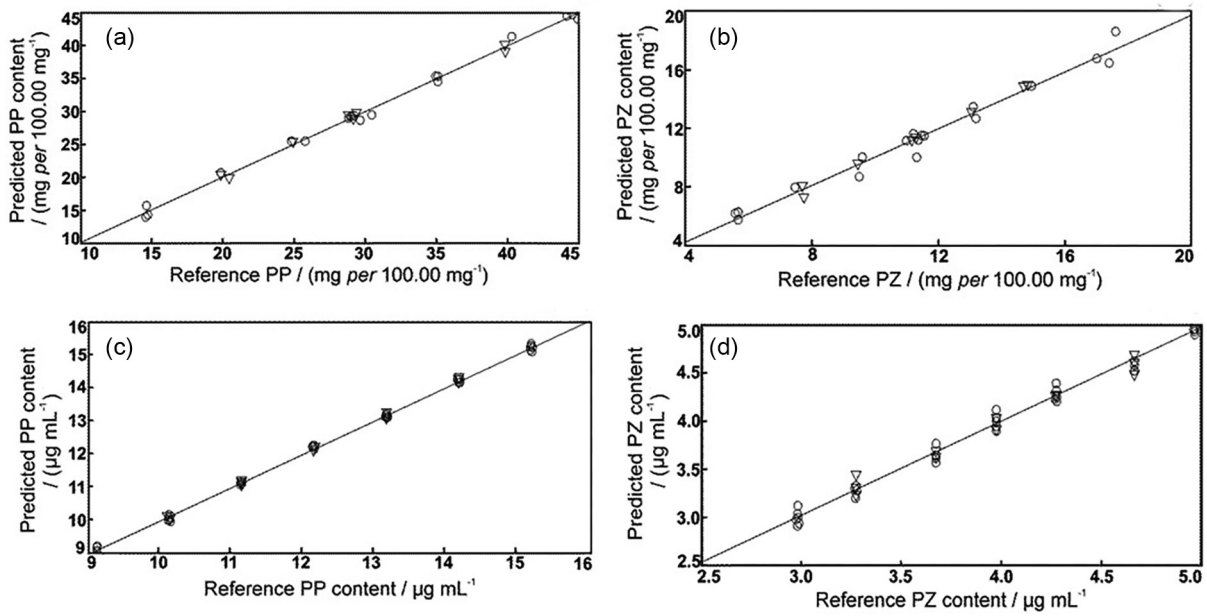

Figure 8. Plots of reference *versus* predicted values for the calibration (circles) and validation (down triangles) samples. siPLS- DRIFT model for PP (a), PZ (b), PLS-UV model for PP (c) and PZ (d).

of significant digits used to express the results. The bias was estimated only for the validation samples, according to ASTM.[35] Jointly with the standard deviation of the validation (SDV) samples, these values were used in two *t*-tests with seven degrees of freedom and at 95% of confidence level, allowing concluding for the absence of bias in this model. RPD[36] is a FOM originally proposed for multivariate NIRS models for comparing trueness in absolute terms, independent on the analytical range. It is estimated dividing the standard deviations of the calibration and validation sets by the RMSECV and RMSEP, respectively. For the DRIFT model, RPD between 4.5 and 8.5 were obtained, indicating its good quality, since values above 2.4 were considered satisfactory and the higher the better.

UV model

The estimated FOM for the UV model were also shown in Table 1. RMSEC and RMSEP were 0.05 µg mL^{-1} and 0.07 µg mL^{-1} for PP, and 0.07 µg mL^{-1} and 0.08 µg mL^{-1} for PZ, respectively. The relative errors of prediction were all in the range of ± 5%, with mean values of 0.52% for PP and 1.41% for PZ. RSD for repeatability and intermediate precision were of a maximum of 0.63%. The plots of reference versus predicted values for PP and PZ are shown in Figures 8c and 8d, without no systematic behavior in the residuals and with r of 0.999 for PP and 0.992 for PZ. All the results of trueness, precision and linearity were satisfactory and in accordance with the Brazilian guidelines.[25] Thus, the analytical ranges of the method were defined as 9.10-15.20 µg mL^{-1} and 3.00-5.00 µg mL^{-1} for PP and PZ, respectively. The SEL of was 82.3% for PP and 24.3% for PZ. The higher SEL for PP is related to the higher absorption of this analyte (Figure 4). The instrumental noise was estimated as 0.0056, from the pooled standard deviation of spectra of a blank solution. The inverse of γ was 0.0004 µg mL^{-1} for PP and 0.002 µg mL^{-1} for PP. However, the use of only two decimal places for expressing the results was considered more realistic and adopted. The bias for both the analytes were also considered not significant at 95% confidence level. Finally, RPD between 6.9 and 39.6 indicated the high quality of the models. These RPD values were better than the ones estimated for the DRIFT method, but this better prediction ability were obtained at the cost of a more laborious and destructive procedure.

Analysis of commercial samples

The best multivariate models for DRIFT and UV were applied to the simultaneous determination of PP and PZ in commercial samples, three different brands of tablets. These results (Table 2) were compared with HPLC and indicated a good agreement between multivariate and chromatographic methods. According to non-paired *t*-test, for twelve comparisons (HPLC *versus* UV or HPLC *versus* DRIFT for both analytes in three different samples), eleven results presented no significant differences at 95% confidence level. The only exception was between the result of HPLC *versus* DRIFT for PP in commercial brand #1.

Table 2. Mean values and standard deviations (n = 3) for the simultaneous quantification of PP and PZ in three different brand formulations with HPLC, DRIFT and UV methods. All the results are in mg *per* tablet

Label claim / mg *per* tablet		HPLC / mg *per* tablet		Multivariate method / mg *per* tablet		
PP	PZ	PP	PZ	Model / Brand	PP	PZ
145.0	50.5	140.98 ± 0.80	47.78 ± 0.04	DRIFT / #1	144.30 ± 0.32	47.90 ± 0.36
				UV / #1	141.98 ± 0.07	47.94 ± 0.45
		138.98 ± 1.35	46.13 ± 3.64	DRIFT / #2	139.87 ± 2.08	47.18 ± 3.24
				UV / #2	137.80 ± 0.27	47.71 ± 0.84
		158.71 ± 0.54	44.97 ± 2.99	DRIFT / #3	160.45 ± 1.56	45.92 ± 4.13
				UV / #3	157.47 ± 1.35	43.96 ± 4.66

Conclusions

Two spectrophotometric methods using multivariate calibration were developed for the simultaneous determination of praziquantel and pyrantel pamoate in veterinary pharmaceutical formulations. The first method was based on diffuse reflectance DRIFT spectroscopy and its predictive performance was improved by selecting the most selective spectral regions with siPLS. The second method, based on UV spectroscopy, was not improved by variable selection and the best model was obtained with PLS using the full spectra. Both methods were validated through the estimate of FOM, such as trueness, precision, linearity, analytical sensitivity, bias and RPD. Their validation performances were similar in relation to almost all the FOM, with the exception of RPD, for which the UV method was superior. These methods were also used for the determination of both the analytes in three different commercial formulations and these results were verified by HPLC. In comparison with HPLC, both the spectroscopic methods are less expensive, simpler, more rapid and suitable for routine quality-control in industries and handling pharmacies. Considering that the most of the veterinary antiparasitic formulations are commercialized in tablets, we concluded that the DRIFT method is the most advantageous for this quality control. Since there is no need to dissolve the samples, this method does not use reagents or solvents and does not generate chemical waste.

Acknowledgements

The authors are grateful to CNPq and to CAPES-REUNI for granting research fellowships.

References

1. Davis, A.; Wegner, D. H.; *Bull. W. H. O.* **1979**, *57*, 767.
2. Cioli, D.; Pica-Mattoccia, L.; *Parasitol. Res.* **2003**, *90*, S3-9.
3. Ballweber, L. R.; *Veterinary Parasitology: The Practical Veterinarian,* 1st ed.; Butterworth–Heinemann: Boston, 2001.
4. Rahmatoola, R. J.; Halim, M. A.; Lari, F. A.; Ahmed, T.; Shamin, R.; Suleman, M.; *Arch. Dis. Child.* **1972**, *47*, 465.
5. McKellar, Q. A.; Jackson, F.; *Trends Parasitol.* **2004**, *20*, 456.
6. Jain, R.; Jadon, N.; Radhapyari, K.; *Talanta* **2006**, *70*, 383.
7. Li, J.; Wang, Y.; Fenwick, A.; Clayton, T. A.; Lau, Y. Y.; Legido-Quigley, C.; Lindon, J. C.; Utzinger, J.; Holmes, E.; *J. Pharm. Biomed. Anal.* **2007**, *45*, 263.
8. Bialecka, W.; Kulik, A.; *Acta Pol. Pharm.* **2010**, *67*, 463.
9. Havlíková, L.; Brabcová, I.; Satínsky, D.; Matysová, L.; Luskacivá, A.; Osicka, Z.; Solich, P.; *Anal. Methods* **2012**, *4*, 1592.
10. Soto, C.; Contreras, D.; Orellana, S.; Yañez, J.; Toral, I.; *Anal. Sci.* **2010**, *26*, 891.
11. European Pharmacopoeia, 6th ed.; Council of Europe: Strasbourg, 2007.
12. Pontes, F. L. D.; Pontarolo, R.; Campos, F. R.; Gasparetto, J. C.; Cardoso, M. A.; Piantavini, M. S.; Trindade, A. C. L. B.; *Asian J. Pharm. Clin. Res.* **2013**, *6*, 191.
13. Sena, M. M.; Chaudhry, Z. F.; Collins, C. H.; Poppi, R. J.; *J. Pharm. Biomed. Anal.* **2004**, *36*, 743.
14. Khoshayand, M. R.; Abdollahi, H.; Shariatpanahi, M.; Saadatfard, A.; Mohammadi, A.; *Spectrochim. Acta A* **2008**, *70*, 491.
15. Abdelwahab, N. S.; *J. AOAC Int.* **2012**, *95*, 1629.
16. Palabiyik, I. M.; Göker, E.; Çaglayan, M. G.; Onur, F.; *Curr. Pharm. Anal.* **2013**, *9*, 404.
17. Sarraguça, M. C.; Soares, S. O.; Lopes, J. A.; *Vib. Spectrosc.* **2011**, *56*, 184.
18. Silva, M. A. M.; Ferreira, M. H.; Braga, J. W. B.; Sena, M. M.; *Talanta* **2012**, *89*, 342.
19. Ferreira, M. H.; Braga, J. W. B.; Sena, M. M.; *Microchem. J.* **2013**, *109*, 158.
20. Bunaciu, A. A.; Aboul-Enein, H. Y.; Fleschin, S.; *Appl. Spectrosc. Rev.* **2010**, *45*, 206.

21. Kandhro, A. A.; Laghari, A. H.; Mahesar, S. A.; Saleem, R.; Nelofar, A.; Khan, S. T.; Sherazi, S. T. H.; *Spectrochim. Acta A* **2009**, *495*, 800.
22. Silva, F. E. B.; Ferrão, M. F.; Parisotto, G.; Muller, E. I.; Flores, E. M. M.; *J. Pharm. Biomed. Anal.* **2009**, *49*, 800.
23. Muller, A. L. H.; Flores, E. M. M.; Muller, E. I.; Silva, F. E. B.; Ferrão, M. F.; *J. Braz. Chem. Soc.* **2011**, *22*, 1903.
24. Brereton, R. G.; *Analyst* **2000**, *125*, 2125.
25. Agência Nacional de Vigilância Sanitária (ANVISA); *Guia para Validação de Métodos Analíticos e Bioanalíticos*, Resolution-RE No 899, 2003.
26. International Conference on Harmonization (ICH); *Tripartite Guideline-Q2A Text on Validation of Analytical Procedures*; ICH: London, 1995.
27. International Conference on Harmonization (ICH); *Tripartite Guideline-Q2B Validation of Analytical Procedures: Methodology*; ICH: London, 1995.
28. Valderrama, P.; Braga, J. W. B.; Poppi, R. J.; *Quim. Nova* **2009**, *32*, 1278.
29. Olivieri, A. C.; Faber, N. M.; Ferré, J.; Boqué, R.; Kalivas, J. H.; Mark, H.; *Pure Appl. Chem.* **2006**, *78*, 633.
30. Silverstein, R. M.; Webster, F. X.; Kiemle, D. J.; *Spectrometric Identification of Organic Compounds,* 7th ed.; John Wiley & Sons: New York, 2005.
31. Hegelund, F.; Larsen, R. W.; Palmer, M. H.; *J. Mol. Spectrosc.* **2008**, *247*, 100.
32. TRSL, Therapeutic Systems Research Laboratories. BCS (Biopharmaceutics Classification System). Available in http://www.tsrlinc.com/resources/services/ accessed in March 2015.
33. Rinnan, A.; van den Berg, F.; Engelsen, S. B.; *Trends Anal. Chem.* **2009**, *28*, 1201.
34. Chong, I. G.; Jun, C. H.; *Chemom. Intell. Lab. Syst.* **2005**, *78*, 103.
35. ASTM E1655-05: *Standard Practices for Infrared Multivariate Quantitative Analysis*, West Conshohocken, 2012.
36. Williams, P. In *Near-infrared Technology in the Agricultural and Food Industries*, 2nd ed.; Williams, P.; Norris, K., eds.; American Association of Cereal Chemists Inc: St. Paul, 2001.

Submitted: January 19, 2015
Publishe online: May 5, 2015

FAPESP has sponsored the publication of this article.