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Short communication

Simultaneous determination of sulphamethoxazole and trimethoprim in powder mixtures by attenuated total reflection-Fourier transform infrared and multivariate calibration

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ABSTRACT

A partial least-squares calibration (PLS) procedure in combination with infrared spectroscopy has been developed for simultaneous determination of sulphamethoxazole (SMZ) and trimethoprim (TMP) in raw material powder mixtures used for manufacturing commercial pharmaceutical products. Multivariate calibration modeling procedures, interval partial least squares (*i*PLS) and synergy partial least squares (*si*PLS), were applied to select a spectral range that provided the lowest prediction error in comparison to the full-spectrum model. The experimental matrix was constructed using 49 synthetic samples and 15 commercial samples. The considered concentration ranges were 400–900 mg g^{-1} SMZ and 80–240 mg g^{-1} TMP. Spectral data were recorded between 650 and 4000 cm^{-1} with a 4 cm^{-1} resolution by Fourier transform infrared spectroscopy coupled with attenuated total reflectance (ATR-FTIR) accessory. The proposed procedure was compared with conventional procedure by high performance liquid chromatography (HPLC) using 15 commercial samples containing SMZ and TMP. The results showed that PLS regression model combined to ATR-FTIR is a relatively simple, rapid and accurate procedure that could be applied to the simultaneous determination of SMZ and TMP in routine quality control of powder mixtures. A root mean square error of prediction (RMSEP) of 13.18 mg g^{-1} for SMZ and 6.03 mg g^{-1} for TMP was obtained after selection of better intervals by *si*PLS. Using the proposed procedure it is possible to analyze each sample in less than 3 min considering two replicates (excluding the grinding step). Accuracy was checked by comparison to HPLC method and agreement better than 98.8% was achieved.

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1. Introduction

Sulphonamides are one of the oldest groups of antimicrobial compounds. Sulphamethoxazole [4-amino-N-(5-methyl-3-isoxazolyl)-benzenesulphonamide] and trimethoprim [5-(3,4,5-trimethoxybenzyl) pyrimidine-2,4-diyldiamine] have been currently used combined in a single pharmaceutical product to treat infections such as bronchitis, middle ear infection, urinary tract infection, and traveler's diarrhea [1]. Sulphamethoxazole (SMZ), a sulphonamide with a structure similar to p-aminobenzoic acid, inhibits p-aminobenzoic acid incorporation into folic acid. On the other hand, trimethoprim (TMP) prevents dihydrofolate to tetrahydrofolate reduction, which is essential bacterial DNA synthesis [2,3].

Several methods for SMZ and TMP determination in pharmaceutical preparations have been reported. Quantification of these substances has been described using simple spectrophotometric method based on red-colored product formation by diazotization of sulphonamides [4], flow injection systems [5–7], high-performance micellar liquid chromatography [8], high performance liquid chromatography (HPLC) [9], second derivative spectrophotometry [10], adsorptive stripping voltammetry [11], bivariate calibration [12] and chemometrics methods [13,14]. Also, a quantitative diffuse reflectance near infrared method was investigated to determine SMZ polymorphs in binary and multi-component powder mixtures [15]. Pharmacopoeial methods list HPLC as the official assay procedure for quality control in pharmaceutical preparations [16].

Analytical methodologies with high throughput should be considered in the analysis of drugs in pharmaceutical industries, particularly in monitoring processes area, allowing measurements in real time. Also, the search for free-solvent methods becomes essential because the environmental impact and large daily toxic waste produced by pharmaceutical industries. Infrared spectroscopy method has these characteristics, as mentioned above. Moreover, the use of infrared spectroscopy eliminates the stage of sample preparation [17].

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Spectroscopy procedures involving NIR has received increasingly wider applications in analysis of different products [18–26]. However, MIR has been a technique relatively still little explored in pharmaceutical analysis [27–30]. Moreover, Fourier transformed infrared spectroscopy (FTIR) analysis provides faster (when compared with HPLC) and interesting quantitative information [31–33]. If this technique is coupled with an attenuated total reflectance (ATR) accessory some of the sample handing problems commonly associated with infrared analysis can be simplified. This accessory has been currently used to routine quality control applications and bulk polymorphs pharmaceutical materials quantitative analysis [28]. Multivariate calibration has been described as the most successful application in chemometrics and spectral data combination [34]. In addition, partial least square (PLS) regression is the most popular multivariate calibration technique to build prediction models using spectroscopic signals [34–36]. Recent applications had been published showing that spectral region selection using suitable algorithms can significantly improve the performance of these full-spectrum calibration techniques [37–39]. In this case, specific regions are selected generating models with lower prediction error. In practice, multivariate regression models optimization is based on the identification of a complete data subset that will produce the lowest prediction error. An optimized region can be found by reducing or increasing it by subtracting or adding new variables. One of the main advantages of this method is the possibility to represent a regression model in a graphical display, focusing on the better intervals choice and allowing a comparison among interval models and the full-spectrum model [40]. Interval partial least squares (*i*PLS) allows the building of models with spectral interval and root mean squared error of cross-validation (RMSECV) values can be used as the criterion to evaluate the prediction ability of this interval [47]. However, the exclusion of intervals with higher RMSECV values would loose useful information. This way, advanced models as synergy partial least squares (*si*PLS) could be applied to find favorable intervals combinations for calibration [45].

As an alternative to official methods, the main objective of this work was to investigate the feasibility of ATR-FTIR spectroscopy associated to *i*PLS and *si*PLS algorithms for the obtained regression models. These models were used as the multivariate linear calibration methods to predicting SMZ and TMP amount in tablets. Partial least square was employed to data modeling using full spectra information, while *i*PLS and *si*PLS were used to select variable intervals. The results by the proposed method were compared with those by using recommended procedures in official pharmacopoeias.

2. Materials and methods

2.1. Materials and sample preparation

Sulphamethoxazole and trimethoprim certified reference materials were acquired from Brazilian Pharmacopoeia (batches 1010 and 1011 for SMZ and TMP, respectively). Methanol, acetonitrile and triethylamine were HPLC grade. Forty-nine formulations (synthetic samples) containing SMZ (400–900 mg g^{-1} range), TMP (80–240 mg g^{-1} range) and diluents starch and magnesium stearate (99:1) were prepared in laboratory. Sulphamethoxazole (batch 22960805) and trimethoprim (batch 200504246) bulk drugs were purchased from Henrifarma (Sao Paulo, Brazil) and used for synthetic samples preparation. Fifteen commercial tablets formulations from nine manufactures (named commercial samples) were acquired in local drugstores. Calibration set was constructed with 32 synthetic samples and 9 commercial samples and the prediction set was constructed using 17 synthetic samples and 6 commercial samples. Synthetic and commercial samples were prepared by powder mixing in a cryogenic mill Spex Certiprep (model 6750 Freezer Mill, Metuchen, EUA). A time period of 2 min was enough to mixing each samples, that was ground up to particle size less than 80 μm.

2.2. Apparatus and software

All spectra were recorded from 4000 to 650 cm^{-1} using a PerkinElmer Model Spectrum One FTIR spectrometer with 16 scans and 4 cm^{-1}. This instrument is equipped with a universal ATR sampling accessory supplied with a top plate ZnSe crystal. For ATR data acquisition, 35.0 ± 0.3 mg of solid sample was placed onto the crystal and its spectrum was recorded. Data were handling using Matlab software 6.1 version (The Math Works, Natick, USA). For PLS multivariate calibration models, the "PLS Toolbox" 2.0 version was used (Eigenvector Technologies, Manson, USA). The *i*Toolbox for Matlab (http://www.models.kvl.dk, USA) was used to variables selection and develop multivariate models [41]. Software program was run on an IBM-compatible Intel Pentium 4 CPU 3 GHz and 1 Gbytes RAM microcomputer. For evaluation of the models generated from *i*PLS and *si*PLS algorithms, the spectral band was divided in 10, 25 and 50 intervals. The spectra of samples were preprocessed by multiplicative scatter correction (MSC), autoscalling (A) and mean centering (MC). A statistical *F*-test ($\alpha = 0.5\%$) was introduced in order to show if there was significant difference between prediction errors of constructed models.

2.3. HPLC reference method

Determination of SMZ and TMP content was carried out using high performance liquid chromatography (HPLC) procedure according to the method described in the United States Pharmacopoeia, USP 30 [16]. This procedure was chosen as reference and it was performed with a HPLC system consisting of Agilent 1100 Series system equipped with pump (model G1311A), detector (model G1315B DAD) and automatic sampling system (model G133A ALS). Detector was set at 254 nm and peak areas were integrated automatically using a Chemstation® software program (Agilent Technologies Inc., CA, USA). Separation was carried out at ambient temperature using a Zorbax® SBC-18 column (250 mm × 4.5 mm i.d., 5 μm particle size). A Zorbax® SBC-18 column (12.5 mm × 4.5 mm i.d., 5 μm particle size) guard cartridge system was used to safeguard the analytical column. The mobile phase was a mixture of water:acetonitrile:triethylamine (1400:400:2, v/v/v). The pH was adjusted for 5.9 and the volume was completed with distillated water. Commercial tablets were finely powdered and mixed. A mass corresponding to 160 mg of sulphamethoxazole and 32 mg trimethoprim for each formulation was accurately weighed and dissolved in 100 mL of methanol. Dissolution was carried out with aid of an ultrasonic bath (15 min) An aliquot of 5 mL of each sample was added to 50 mL volumetric flasks and mobile phase was used to complete the volume. All these determinations were performed in triplicate for synthetic and commercial samples.

2.4. Chemometrics models

Multivariate chemometrics methods were applied to obtain quantitative information from the measurements. Partial least square regression was applied to ATR-FTIR data to built calibration models enabling prediction of SMZ and TMP amount in pharmaceutical preparations. The root mean square error (RMSE) was calculated according to the following equation [42]:

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \quad (1)$$

where $\hat{y}_i$ is the predicted value for test set sample i, y_i the reference value for test set sample i and n is the number of observation in testing set.

Root mean squares error of cross-validation was used to evaluate the error of the proposed calibration models and to select the number of latent variables. Root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used to evaluate the prediction ability between different PLS models. Performance of the obtained calibration models was checked through relative standard error of prediction (RSEP) as calculated from the following equation [43]:

$$\text{RSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i)^2}} \times 100 \quad (2)$$

where $\hat{y}_i$ is the predicted value for test set sample i, y_i the reference value for test set sample i.

The systematic error ("bias") and the standard deviation of validation (SDV) were calculated from Eqs. (3) and (4), respectively:

$$\text{bias} = \frac{\sum_{i=1}^{n}(y_i - \widehat{y}_i)}{n} \quad (3)$$

$$\text{SDV} = \sqrt{\frac{\sum_{i=1}^{n}[(y_i - \widehat{y}_i) - \text{bias}]^2}{n-1}} \quad (4)$$

Thereafter, the t-test was applied, conformed the following equation [46]:

$$t_{\text{sist}} = \frac{|\text{bias}|\sqrt{n}}{\text{SDV}} \quad (5)$$

The systematic error was considered not significant for the t_{sist} values lower than critical value $(t_c)_\alpha = 0.5\%$ and $n-1$ d.f.

The *i*PLS models were built on spectra division into 10, 25 and 50 intervals. The *i*PLS routine generates graphical information indicating the optimum number of latent variables used in each interval model and RMSECV values. In this case, the subinterval than presented the minor RMSECV values was selected. Synergy PLS models were constructed with spectra set divided into 10, 25 and 50 intervals and combinations from 2 to 5 intervals. The combined subintervals than presented the minor RMSECV values were selected. The results obtained by the proposed method were compared with reference method (using HPLC) by t-test paired ($\alpha = 0.5\%$). The results obtained by ATR-FTIR were also in agreement to the interval allowed by Brazilian Pharmacopoeia (93–107% declared value).

3. Results and discussion

3.1. Preprocessing choice

MSC preprocessing showed the lower RMSEP and RMSECV values (F-test, $\alpha = 0.5\%$). For the A and MC preprocessing no significant difference was observed among RMSEP values. The A preprocessing tool was chosen in view of lower RMSEP value.

3.2. Full-spectrum PLS model

Initially, in order to have a measurement of the variable selection algorithms quality, models were built using ATR-FTIR full-spectrum information. Full-spectrum PLS models were obtained with six and four latent variables for SMZ and TMP, respectively, and results are shown in Tables 1 and 2. Accuracy was calculated for the proposed ATR-FTIR method and it reports the agreement between the reference value by HPLC and the value found by the calibration model (in this case evaluated by RMSEP value).

Table 1
Statistical results to SMZ better calibration models and SMZ full-spectrum PLS model.

Model	VN[a]	Intervals	LV[b]	RMSECV, SMZ (mg g^{-1})	R^2_{cal}	RMSEP, SMZ (mg g^{-1})
PLS	3351	All	6	34.75	0.978	22.29
*i*PLS10	335	10	3	40.15	0.947	35.49
iPLS25	135	22	4	49.05	0.932	37.12
*i*PLS50	68	49	6	33.20	0.976	31.24
*si*PLS10	670	1 and 10	6	30.05	0.981	22.06
*si*PLS10	1005	1, 2 and 3	7	26.32	0.988	26.42
*si*PLS10	1340	1, 2, 3 and 4	8	23.34	0.991	23.46
*si*PLS10	1675	1, 2, 3, 4 and 5	9	24.46	0.992	22.86
*si*PLS25	270	9 and 23	9	21.52	0.996	13.18
*si*PLS25	405	6, 9 and 23	8	23.35	0.995	16.25
*si*PLS50	136	39 and 49	6	19.52	0.993	18.09
*si*PLS50	204	11, 23 and 49	7	15.75	0.995	19.28

[a] VN: total variables numbers.
[b] LV: latent variables.

3.3. Sulphamethoxazole iPLS models

Interval PLS algorithm principle is to split the spectra into smaller equidistant regions and develop models for each subinterval. Thereafter, the subintervals RMSECV are compared with full-spectrum RMSECV values. This algorithm allows the comparison among intervals model and the full-spectrum model. Interval PLS plots (and latent variables used for each constructed model, represented by numbers above interval number), RMSECV values for each interval selected and the RMSECV values for the full-spectrum model (dotted line) using six latent variables are demonstrated in Fig. 1. It is possible to observe that the developed model using interval number 22 to *i*PLS with 25 intervals produces the minor RMSECV values (when compared with the others 24 intervals) but does not produce better results than the value of full-spectrum PLS model. Therefore, the *i*PLS models using the spectrum subdivided in 50 intervals were developed. Table 1 shows the statistical indicators to better SMZ *i*PLS calibrations models developed with 10, 25 and 50 intervals. The developed model using the interval 49 for *i*PLS with 50 intervals produced better results according to lower results for RMSECV values (using 6 latent variables and only 68 total variables numbers). To evaluate the prediction ability of developed models in relation to full model the RMSEP values were compared. The RMSEP value for the full-spectrum model was lower than those obtained for developed models after iPLS algorithm application (F-test, $\alpha = 0.5$). It is possible that information was spread on the whole spectral range and a variable selection per interval could automatically reduce the information and induce an increase of RMSEP compared with full-spectrum PLS [40].

Table 2
Statistical results to TMP better calibration models and TMP full-spectrum PLS model.

Model	VN[a]	Intervals	LV[b]	RMSECV, TMP (mg g^{-1})	R^2_{cal}	RMSEP, TMP (mg g^{-1})
PLS	3351	All	4	13.51	0.938	7.86
*i*PLS10	335	3	3	14.60	0.912	10.82
*i*PLS25	135	19	9	13.08	0.953	15.84
*i*PLS50	68	49	5	17.40	0.936	10.82
*si*PLS10	670	2 and 10	7	12.51	0.961	6.80
*si*PLS10	1005	3,7 and 9	4	12.68	0.938	8.2
*si*PLS10	1340	1, 2, 3 and 10	4	12.40	0.943	9.07
*si*PLS10	1675	1, 2, 3, 9 and 10	4	12.24	0.943	7.83
*si*PLS25	270	14 and 25	11	10.01	0.994	9.47
*si*PLS25	405	7, 19 and 23	9	9.25	0.985	8.28
*si*PLS50	136	34 and 49	4	8.75	0.975	7.08
*si*PLS50	204	14, 39 and 49	6	8.17	0.983	6.03

[a] VN: total variables numbers.
[b] VL: latent variables.

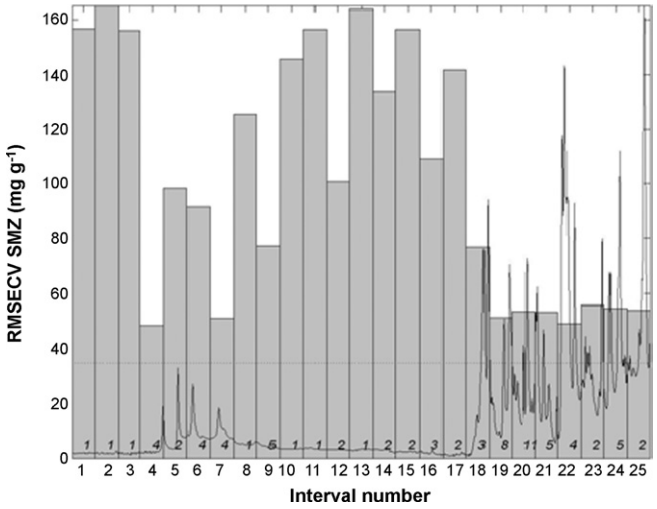

Fig. 1. Cross-validated prediction errors (RMSECV) values to full-spectrum model and interval models (bars) to SMZ determination using PLS and *i*PLS algorithms (dotted line and numbers above interval numbers refer to full-spectrum RMSECV and latent variables using in each model, respectively).

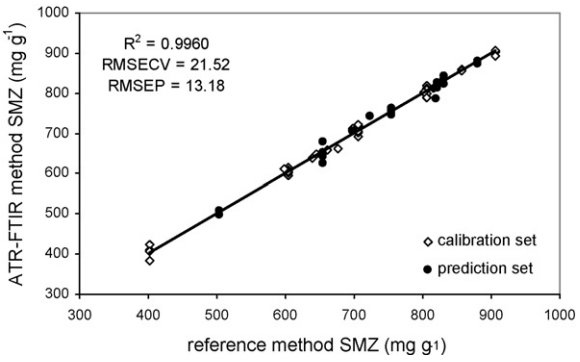

Fig. 2. Reference HPLC values versus predicted SMZ values for siPLS model, using intervals 9 and 23 and 9 latent variables.

3.4. Sulphamethoxazole *si*PLS model

The *si*PLS algorithm principle is to split the data set into a number of intervals (variable-wise) and to calculate all possible PLS model combinations of two, three or more intervals. Thereafter, the combined subinterval RMSECV is compared with full-spectrum RMSECV values. For the developed PLS models using spectra full information, the including uninformative wavenumbers could negatively affect the calibration by producing both large relative bias. In this case, a judicious selection of spectral regions would improve the predictive ability of the PLS model [37]. Therefore, variables selection by *si*PLS was implemented to verify if the combination of more than one interval would result in models with better predictive capacity. The spectrum was divided in 10, 25 or 50 intervals combined in up to 5 subintervals. The spectrum divided in 10 intervals was combined in up to 5 subintervals and the spectrum divided in 25 and 50 intervals was combined in up to 3 intervals. Table 1 shows the statistical indicator to SMZ *si*PLS better calibration models.

Models obtained by spectra division in 10 intervals showed predictive capacity similar to the full-spectrum model (model siPLS 10 with intervals numbers 1, 2, 3, 4 and 5). All models using the spectrum subdivided in 25 and 50 intervals, after the variable selection by *si*PLS, showed lower RMSEP values (*F*-test, $\alpha = 0.5$). The minor RMSEP for *si*PLS was obtained when the spectra set was split in 25 intervals and the intervals number 9 and 23 had been combined. For this *si*PLS model, results showed good correlation between reference and predicted values indicated by a correlation coefficient of 0.996, as shown in Fig. 2. The selected intervals included the regions of 2794–2928 cm^{-1} (interval 9) and 918–1052 cm^{-1} (interval 23) that corresponds to CH_3 and S–N stretching vibrations [44], respectively. These groups are constituents of SMZ chemical structure. In a general way, the combination of intervals 9 and

Table 3
Average prediction results to selected models by *si*PLS algorithm.

Samples	SMZ		TMP	
	Reference HPLC method (mg g^{-1})	ATR-FTIR method (mg g^{-1})	Reference HPLC method (mg g^{-1})	ATR-FTIR method (mg g^{-1})
1[a]	503.59	507.20	175.73	169.99
2[a]	503.59	498.19	220.91	217.80
3[a]	654.67	644.16	100.41	93.37
4[a]	654.67	641.54	150.62	159.36
5[a]	654.67	652.47	220.91	222.82
6[a]	755.38	752.44	100.41	101.08
7[a]	755.38	757.02	175.72	181.46
8[a]	755.38	762.81	220.91	233.02
9[a]	830.92	836.43	100.41	95.66
10[a]	830.92	841.74	125.52	123.70
11[a]	830.92	821.58	175.72	181.70
12[a]	881.28	871.36	125.52	119.89
13[b]	698.12	704.88	145.10	135.51
14[b]	655.30	677.53	129.00	133.64
15[b]	722.95	743.29	141.83	138.71
16[b]	819.79	785.98	155.44	144.41
17[b]	820.66	826.37	156.23	160.23
18[b]	820.88	811.28	155.60	152.63
19[a]	503.59	497.70	125.52	123.16
20[a]	654.67	624.57	175.72	183.65
21[a]	755.38	746.55	125.52	126.30
22[a]	755.38	750.12	150.62	157.76
23[a]	881.28	880.74	100.41	101.02
RMSEP (mg g^{-1})	–	13.18	–	6.03
RSEP (%)	–	1.79	–	3.90

[a] Synthetic samples.
[b] Commercial samples.

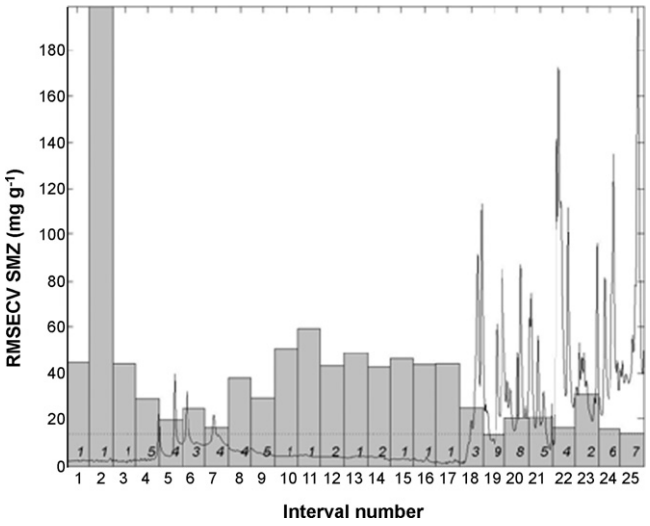

Fig. 3. Cross-validated prediction errors (RMSECV) values to full-spectrum model and interval models (bars) to TMP determination using PLS and *i*PLS algorithms (dotted line and numbers above interval numbers refer to full-spectrum RMSECV and latent variables using in each model, respectively).

23 by *si*PLS algorithm reduced RMSECV and RMSEP values. Therefore, it was possible to find a narrow region to SMZ determination with small prediction errors and reduced variable numbers (270 variables compared with 3351 used in the full-spectrum model). Interval 6 inclusion (model siPLS25 with interval numbers 6, 9 and 23) does not modify significantly the quality of model. This interval can refer on a spectral region that does not contain relevant information. Average prediction results and RMSEP to the selected *si*PLS calibration models are shown in Table 3. Synergy PLS model developed using intervals 9 and 23 resulted in low relative standard error of prediction (RSEP = 1.79%), suggesting that the method used is accurate as also described in Table 3. The systematic error provided by model was not significant (bias = 2.75 and $t_{sist} < t_{crit}$) due to the non-tendency for the prediction errors.

3.5. Trimethoprim iPLS models

Fig. 3 shows the central *i*PLS plots, the RMSECV values for each interval selected (bars) and the RMSECV values to full-spectrum model (dotted line) using four latent variables. Table 2 shows the statistical indicator to TMP *i*PLS calibrations models using the spectrum subdivided in 10, 25 and 50 intervals. The developed model using interval 19 for *i*PLS with 25 intervals produced the minor RMSECV values (when compared with the others 24 intervals) but not the significant minor RMSEP values than the full-spectrum PLS model (*F*-test, $\alpha = 0.5$). As in the previous case, an increase of RMSEP can be attributed to the limited information generated by the selection of spectrum specific regions [40].

3.6. Trimethoprim siPLS models

The algorithm *si*PLS was implemented using the spectrum subdivided in 10, 25 or 50 intervals combined in up to 5 subintervals. Table 2 shows the statistical indicators to TMP *si*PLS calibration models. With exception of siPLS10 model (with 4 intervals) and siPLS25 model (with 2 intervals), the other models not provided significant difference in RMSEP values (*F*-test, $\alpha = 0.5$). The siPLS10 model (with intervals numbers 1, 2, 3 and 10) showed higher RMSEP value when compared with full-spectrum model. However, with the addition of interval number 9 (siPLS10 model with intervals numbers 1, 2, 3, 9 and 10) the RMSEP value was reduced. The siPLS10 model (with 5 intervals) showed RMSEP equivalent to the full-spectrum model. The additional interval (interval 9) could be due to spectral region that does contain relevant information.

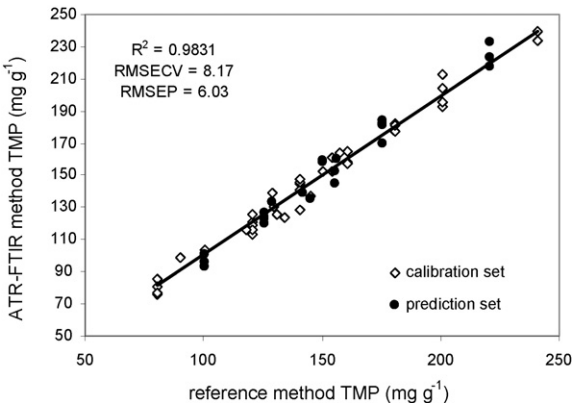

Fig. 4. Reference HPLC values versus predicted TMP values for siPLS model using 14, 39 and 49 intervals and 6 latent variables.

The minor RMSEP value was obtained when the spectrum was split in 50 intervals and the intervals 14, 39 and 49 were combined. For this *si*PLS model, the results showed a good correlation between reference and predicted values, indicated by a correlation coefficient of 0.983, as shown in Fig. 4. The selected intervals included the regions of 3064–3130 cm^{-1} (interval 14) and 1389–1455 cm^{-1} (interval 39). Both intervals include =C–H, =C–C and =C–N stretching vibrations of the pyrimidine ring presented in structure of TMP and the interval 49 (717–784 cm^{-1}) corresponds to out-of-plane N–H bending vibration [44]. The *si*PLS model combined the intervals 14, 39 and 49 allowing better predictive ability when compared with *i*PLS models and full-spectrum PLS model. Therefore, it was possible to find a narrow region for TMP determination with small prediction errors and reduced variable numbers. Average prediction results, RMSEP and RSEP for the selected *si*PLS calibration model are shown in Table 3. This synergy PLS model combining three intervals resulted in low prediction errors (RSEP = 3.90%). The systematic error provided by model was not significant (bias = −0.13 and $t_{sist} < t_{crit}$), showing that the prediction errors have not tendency.

3.7. Comparison of PLS, iPLS and siPLS models

Comparing the results from PLS, iPLS and siPLS models in determination of pharmaceuticals by FTIR-ATR, siPLS models showed better predictive capacity (lower RMSEP). This result could be explained by three reasons: (1) PLS models included noisy spectral information; (2) iPLS models can reduce noise by selecting specific spectral regions, however useful spectral information can be lost; (3) with intervals combination performed by siPLS is possible to obtain models with reduced total variables numbers (removing noisy spectral) and better predictive capacity (without information loosing).

4. Conclusions

Using the PLS regression algorithm combined with ATR-FTIR data it was possible to develop multivariate models for simultaneous determination of SMZ and TMP in commercial pharmaceutical products. Assay results, expressed as the percentage of the label claim were found to be 95–103% for SMZ and 93–105% for TMP. These results were in agreement to the content of SMZ and TMP in powder mixtures according to the USP 30 requirements (93–107%) for the solid preparations. The variable selection techniques used in this work produced models with better predictive ability compared to full-spectrum PLS models. The *si*PLS algorithm demonstrated to

be more suitable in the combined regions selection having relevant information. The proposed method is simple, free solvent and allows potential applications to simultaneous, fast and reliable determination of SMZ and TMP in solid pharmaceutical dosage forms.

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