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Short communication

Chemometrics-assisted UV-spectroscopic strategies for the determination of theophylline in syrups

María J. Culzoni, María M. De Zan, Juan Carlos Robles, Víctor E. Mantovani, Héctor C. Goicoechea *

Laboratorio de Control de Calidad de Medicamentos, Cátedra de Química Analítica I, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Ciudad Universitaria, S3000ZAA Santa Fe, Argentina

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Abstract

Two spectrophotometric methods, assisted by chemometric tools, were developed for the determination of theophylline in syrups: derivative spectroscopy (DS) and partial least-squares regression (PLS), the latter using both artificial and natural calibration sets. HPLC technique was employed to apply a reference method in order to achieve a complete assessment of performance. Calibrations presented excellent analytical figures of merits (i.e. LOD ranged from 0.03 to 0.4 mg L^{-1} and analytical sensitivity ranged from 1.7 to 8.3 L mg^{-1}). The intermediate precision presented pooled coefficients of variation ranged between 0.54 and 1.08%. Accuracy was studied trough an elliptical joint confidence region test (EJCR) comparing the obtained values when analysing spiked real samples by the HPLC and the studied methodologies. Both studied methods can be considered acceptable for the pharmaceutical quality assurance of theophylline in syrup samples.

Keywords: Theophylline; Syrup samples; Chemometrics; Derivative spectroscopy; Partial least-squares regression

1. Introduction

Theophylline (1,3-dimethylxanthine) has been extensively used for asthma management during the last 70 years. It remains an important drug in the treatment of this disease, especially for patients with moderate to severe symptoms [1]. In addition, the methylxanthines aminophylline, theophylline and caffeine have been used widely to treat apnoea of prematurity. Today, they are among the most commonly prescribed drugs in neonatal medicine.

Theophylline has been determined in pharmaceutical preparations by several methods that include techniques like liquid cromatography [2,3], micellar electrokinetic cromatography [4], capillary electrophoresis [5] and ion chromatography [6]. Two chemometric–spectroscopic approaches were presented for its determination in blood serum [7] and coffee [8].

Derivative spectrophotometry (DS) offers greater selectivity than does classical spectrophotometry. It decreases spectral overlap and allows for better resolution because small differences between spectra are magnified. Baseline drifts are eliminated as well. Consequently, it has been widely applied in pharmaceutical analysis of drug mixtures [9,10]. Moreover, the conversion of zero-order into higher order UV derivative spectra may result in the elimination of the non-specific matrix interference [11]. This fact is possible because of the occurrence of zero-crossing wavelengths or minimization of the interference signal. Recently, a method based on this technique has been presented for the simultaneous determination of theophylline and ephedrine in tablets [12]. Interestingly, a review showing the latest applications and achievements of DS in chemical analysis has been recently presented by Karpinska [13].

A variety of linear regression methods have been proposed for multicomponent analysis, among which the most popular is partial least-squares (PLS). Currently, it has become the standard for multivariate calibration because of the quality of

* Corresponding author. Tel.: +54 342 4575205; fax: +54 342 4575205.
E-mail address: hgoico@fbcb.unl.edu.ar (H.C. Goicoechea).

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the calibration models, the easiness of its implementation and the availability of software [14–16]. PLS shows the advantage of using full spectral data points, which is critical for the spectroscopic resolution of complex mixtures of analytes. It allows for a rapid determination of components, usually with no need for prior separation. When applying PLS, calibration can be performed by ignoring the concentrations of all other components except the analyte of interest. Additionally, if the prediction sample contains unexpected interferences, the determination will be biased. Fortunately, this sample can be identified as an outlier because of the unusually large spectral residuals (this property is known as the first-order advantage) [17]. This fact may be especially significant when the analyte is immersed in a complex matrix like the one constituted by syrups. Very recently, a review showing multivariate calibration as a powerful tool in pharmaceutical analysis has been presented [18].

In the present report, as a part of a quality control program, we discuss different strategies to carry out the determination of theophylline in syrup samples, by applying electronic absorption measurements together with derivative spectroscopy, PLS-1 calibration and high-performance liquid chromatography (HPLC) as reference technique. As will be shown below, an important degree of spectral overlap exists among theophylline and syrup excipients (which may substantially vary among production laboratories) in the spectral region of interest. The problem thus consists of a single analyte embedded in a highly complex matrix, which is sometimes unknown or difficult to reproduce. We have thus explored the use of chemometric tools for accomplishing this goal.

2. Experimental

2.1. Apparatus

Electronic absorption measurements were carried out on a Perkin-Elmer Lambda 20 spectrophotometer, using 1.00 cm quartz cells, 2 nm of slit width, a scan speed of 120 nm/min and a wavelength interval of 1.0 nm. UV spectra of working solutions were recorded in the range 210–350 nm. All spectra were saved in ASCII format, and transferred to a PC for subsequent manipulation. First derivative spectra were calculated with a Svitzky-Golay filter using a second-order polynomial and a five-point window. PLS-1 was implemented using the MVC1 MATLAB toolbox [19]. Chromatographic measurements were carried out in a high-performance liquid chromatograph Perkin-Elmer Series 200 pump, 785A UV detector, NCI900 interface and manual injector with a 20 μl loop.

2.2. Reagents

The experiments were performed with analytical-reagent grade chemicals for spectroscopy and HPLC grade chemicals for chromatography. A theophylline standard was provided by the Instituto Nacional del Medicamento, Buenos Aires, Argentina (INAME). Stock solution of theophylline ($1.015\,g\,L^{-1}$) was prepared by dissolving the standard of theophylline in doubly distilled water. Solutions of the excipients usually present in commercial theophylline syrups: NaOH, sodium methylparaben, sodium propylparaben, saccharin, sorbitol and saccharose were also prepared in doubly distilled water. Two mixture solutions of the excipients present in the commercial theophylline syrup were also prepared in doubly distilled water, one containing NaOH, saccharin, sorbitol and saccharose, and the other one containing sodium methylparaben and sodium propylparaben.

2.3. Calibration and validation sets

2.3.1. Calibration set for applying derivative spectroscopy

Univariate calibration solutions of theophylline were prepared by diluting appropriate amounts of stock solution with NaOH $0.1\,mol\,L^{-1}$ (pH 13.0) in 50.00 ml volumetric flasks. Seven standards were performed (three replicates) at the following concentrations: 6.5, 9.6, 12.8, 16.0, 19.2, 22.4 and $25.6\,mg\,L^{-1}$. The absorption spectra were recorded in random order with respect to analyte concentrations against a blank consisting of NaOH $0.1\,mol\,L^{-1}$. First derivative spectra were obtained, selecting the working wavelength of 284 nm.

2.3.2. Multivariate calibration PLS sets

In the presently studied syrup samples, the analyte of interest is embedded in a complex mixture of a large number of components. Moreover, the analyst occasionally has access to the information corresponding to the composition of the syrup, but usually this information is not accessible. Taking into account these considerations, two sample sets were built to be used as calibration sets for applying PLS-1, in the following way:

2.3.2.1. Calibration set number 1. Artificial, a training set of 19 samples corresponding to a central composite design with five centre samples (in these case mixtures of the studied component with five different levels of blank syrup sample, i.e. syrup without theophylline) was prepared for calibration, with the concentrations of theophylline lying in the linear absorbance-concentration range of 0–$20\,mg\,L^{-1}$. The excipients were added from two separated solutions in order to better model the sources of variability: those excipients which present absorbance in the UV region and those that do not. The corresponding concentration of theophylline and excipients can be seen in Table 1.

2.3.2.2. Calibration set number 2. Natural/artificial, a commercial sample whose theophylline concentration was obtained by HPLC was used. Concentration values of the syrup matrix excipients were taken from the formulation

Table 1
Composition of both calibration sets for applying PLS-1

Calibration sample	Theophylline added (mg L^{-1})	Syrup blank (A) added[a] (%)[c]	Syrup blank (B) added[b] (%)[c]	Theophylline actual (mg L^{-1})	Syrup blank actual (%)[d]
Set 1–1	11.7	100.0	100.0	–	–
Set 1–2	20.2	100.0	100.0	–	–
Set 1–3	16.0	74.8	100.0	–	–
Set 1–4	16.0	125.2	100.0	–	–
Set 1–5	16.0	100.0	74.8	–	–
Set 1–6	16.0	100.0	125.2	–	–
Set 1–7	13.5	85.0	85.0	–	–
Set 1–8	18.6	85.0	85.0	–	–
Set 1–9	13.5	115.0	85.0	–	–
Set 1–10	18.6	115.0	85.0	–	–
Set 1–11	13.5	85.0	115.0	–	–
Set 1–12	18.6	85.0	115.0	–	–
Set 1–13	13.5	115.0	115.0	–	–
Set 1–14	18.6	115.0	115.0	–	–
Set 1–15	16.0	100.0	100.0	–	–
Set 1–16	16.0	100.0	100.0	–	–
Set 1–17	16.0	100.0	100.0	–	–
Set 1–18	16.0	100.0	100.0	–	–
Set 1–19	16.0	100.0	100.0	–	–
Set 2–1	–	–	–	12.0	80.0
Set 2–2	–	–	–	12.0	100.0
Set 2–3	–	–	–	12.0	120.0
Set 2–4	–	–	–	15.0	80.0
Set 2–5	–	–	–	15.0	100.0
Set 2–6	–	–	–	15.0	120.0
Set 2–7	–	–	–	18.0	80.0
Set 2–8	–	–	–	18.0	100.0
Set 2–9	–	–	–	18.0	120.0

[a] Syrup blank (A) containing the excipients that have absorbance in the UV region.
[b] Syrup blank (B) containing the excipients that not have absorbance in the UV region.
[c] The % is relative to the commercial syrup blank taken as 100%.
[d] The % is relative to the commercial syrup blank taken as 100%.

declared by the manufacturing laboratory. A training set of 9 samples was prepared for calibration, consisting in mixtures of three levels of theophylline and excipients whit concentrations ranging from 80.0 to 120.0% of the target one (see Table 1). In order to prepare the different standard solutions, the following cases were considered:

a) Samples Set 2–1, Set 2–5 and Set 2–9 of Table 1, dilutions were performed on the commercial sample with NaOH 0.1 mol L^{-1}.
b) Samples Set 2–4, Set 2–7 and Set 2–8 of Table 1, spiking with theophylline and suitable dilution were performed.
c) Samples Set 2-2, Set 2–3 and Set 2–6 of Table 1, spiking excipients and suitable dilutions were performed.

The spectra for all sets were recorded in random order with respect to analyte concentrations.

2.3.3. Validation samples

Two validation sets were built with samples containing different levels of theophylline and excipients. Each sample was prepared in different days in order to take into account the maximum possible data variability. Both the composition and purpose of each set are the following:

2.3.3.1. Set number 1. Precision set, a commercial sample was proportionally partitioned into three sub-samples, and analysed applying the studied methods ten times in three different weeks in order to carry out statistical tests for intra-day precision and to estimate the intermediate precision.

2.3.3.2. Set number 2. Recovery assay set, obtained by spiking the commercial sample with five levels of theophylline from 105 to 125% of the target value declared for the manufacturing laboratory. This set was employed to further test the performance of the methods through the slope and intercept of the linear regression of calculated versus added concentration values.

2.4. Sample

The analysed samples were different batches of Syrup Crisasma nf 800 (Sintesina Laboratories, Argentine). This pharmaceutical contains 8.0 g L^{-1} of theophylline and was undergone to dilution 1/500 with NaOH 0.1 mol L^{-1} using volumetric material previous to analysis.

2.5. Chromatographic method

Chromatographic separation was performed on a LiChrospher® 100, RP-18, 5 μm, 250 × 4 mm i.d., Merck column at ambient temperature. The mobile phase consisted of a filtered and degassedified mixture of acetonitrile in 0.01 mol L^{-1} acetate buffer pH 3.2 (7/100). The analysis was done under isocratic conditions at a flow rate of 1 ml min^{-1} and the effluent was monitored by UV measurements at 280 nm. The standard was prepared by dissolving an accurated amount of theophylline in mobile phase and diluting in the same solvent to have a work solution of 80 μg mL^{-1}. Samples were diluted 1/100 in mobile phase. All solutions were filtered through 0.22 μm Millipore filter before inyected. Triplicate injections were made for each solution and the peaks areas were recorded to calculate concentration in samples.

3. Results and discussion

3.1. Optimization of the working conditions

One of the first steps in applying UV spectrophotometry is the selection of the most convenient working pH. Three conditions were assayed: NaOH 0.1 mol L^{-1} (pH 13), HCl 0.1 mol L^{-1} (pH 1) and buffer solutions of sodium acetate (pH 4.5) and phosphate (pH 8.0). NaOH 0.1 mol L^{-1} was selected owing to the high solubility, excellent spectral response and good stability of theophylline.

3.2. Application of zero-crossing methodology

Electronic absorption spectra for diluted and alkalinised samples were recorded in the range 210–350 nm at 120 nm min^{-1} scanning speed and subjected to both derivative and multivariate analysis. The zero-crossing methodology is recommended in the literature when the interfering spectrum overlaps the analyte spectrum and its derivative signal passes through zero at a defined wavelength, in which the vertical distance from the zero line is measured and is proportional to the analyte concentration [20]. The present method is based on the zero-crossing first derivative spectroscopy. As can be seen in Fig. 1A, the absorption spectra of theophylline and sample excipients are severely overlapped. This fact hinders the resolution of the task by using conventional spectrophotometry. Alternatively, first derivative spectra and the zero-crossing wavelength are presented in Fig. 1B. In this case, the zero-crossing point at 284 nm was selected as the optimun wavelength for determination of teophylline in the presence of syrup excipients.

The statistical parameters obtained by least-squares fit of the calibration line of theophylline by zero-crossing spectrophotometry can be seen in Table 2. A statistical test was also performed to check linearity of the calibration line according to the test proposed in reference [21]. It was concluded that the graph is linear in the studied range ($p < 0.05$). Table 2 also presents three analytical figures of merit which are very useful in comparing methodologies (see bellow).

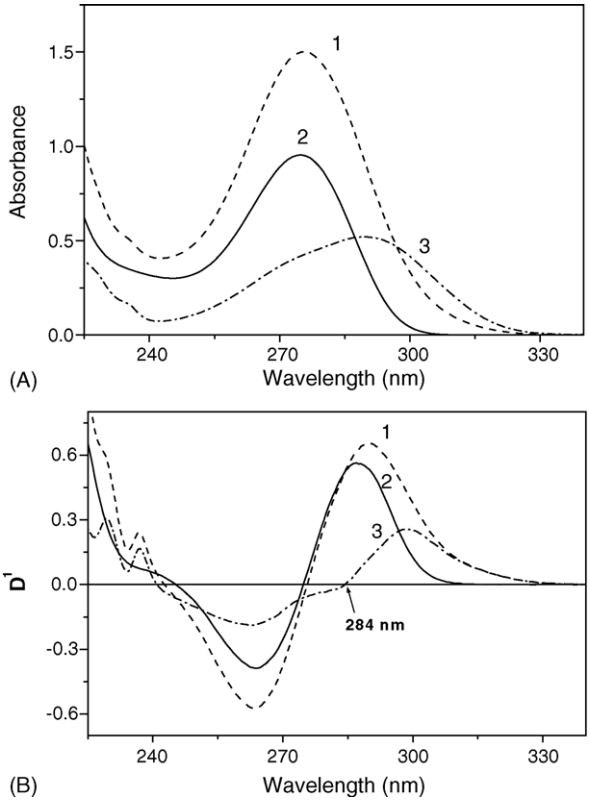

Fig. 1. (A) Absorption spectra of syrup sample containing theophylline (15.0 mg L^{-1}) and all the excipients (see text), diluted (1/500) (1, dash line), teophylline standard solution of (15.0 mg L^{-1}) (2, solid line) and excipients solution (dilution 1/500 of the sirup blank) (3, dash dotted line). (B) First-derivative spectra corresponding to zero-order spectra presented in (A). All analytes were dissolved in NaOH 0.1 mol L^{-1}.

3.3. Application of multivariate methodology

In the last years, PLS has become a routine multivariate method for solving tasks such as the presently studied problem. This multivariate method involves a calibration step in which the relation between spectra and component concentrations is estimated from a set of reference samples, and a prediction step in which the results of the calibration are used to estimate the component concentrations in an unknown sample spectrum [15]. Here we implemented the PLS-1 version that is optimised for the determination of a single analyte of interest (theophylline) [14].

The optimum number of factors to be used within the PLS-1 algorithm is an important parameter to achieve better performance in prediction. This allows one to model the system with the optimum amount of information, avoiding overfitting. The well-known cross-validation leave-one-sample-out procedure was applied in the present work. The optimum numbers of factors are shown in Table 2. As can be seen, the obtained latent variables (four and five) are indicating the

Table 2
Working wavelength, optimum number of factors, optimal data region and statistical parameters corresponding to the calibration graph of the theophylline by using zero-crossing derivative spectrophotometry and calibration sets numbers 1 and 2 when applying PLS-1

Statistical parameters and region	Zero-crossing derivative	PLS-1	
		Set 1	Set 2
λ (nm)	284	–	–
Intercept	−0.003	–	–
Standard deviation on intercept	0.002	–	–
Slope	0.0344	–	–
Standard deviation on slope	0.0001	–	–
R	0.9999	–	–
s_{fit}	0.0028	–	–
Factors[a]	–	4	5
Data region (nm)	–	281–320	210–350
RMSECV (mg L^{-1})[b]	–	0.13	0.24
REP(%)[c]	–	0.81	1.58
SEN	–	0.034	0.136
SEL	–	0.220	0.053
γ^{-1} (mg L^{-1})	0.58	0.59	0.12
LOD (mg L^{-1})	0.4	0.13	0.033
LOQ (mg L^{-1})	1.1	0.38	0.10

[a] Factors were selected following the Haaland criterion [17].

[b] RMSECV $= \sqrt{\frac{\sum (x_{act}-x_{pred})^2}{I}}$, where I is the number of calibration simples, x_{act}, the actual concentration in calibration samples, x_{pred}, the predicted concentration with the PLS models and $\bar{x}_{act}$ is the average concentration in the calibration set.

[c] REP(%) = RMSECV × 100 /$\bar{x}_{act}$.

large number of variability sources in the presently studied system. This latter table also gives other important statistical parameters and figures of merit such as the root mean square error of cross-validation (RMSECV), the relative error of prediction (REP%), the sensitivity (SEN), the selectivity (SEL), the inverse of the analytical sensitivity (γ^{-1}) and the limits of detection (LOD) and quantification (LOQ). These latter figures of merit can be calculated and used for method comparison or to study the quality of a given analytical technique. Convenient definitions for the latter figures of merit can be found in the literature [22,23].

Finally, Table 2 presents the values of the optimal data regions used for the multivariate method in the corresponding calibrations (calibration sets numbers 1 and 2). The region of sensors to be selected is a critical step for increasing the predictive ability of multivariate analysis, and should ideally eliminate both uninformative and/or highly correlated data. In the present report we have applied a moving window strategy to the calibration set itself, in order to find the most informative range in the time profile by localisation of the minimum prediction error sum of squares (PRESS). The method consists of building both a three- and a two-dimensional contour plot of the calculated values of PRESS for the calibration set as a function of first sensor and window width. The values of PRESS are calculated using the optimum number of calibration factors in each spectral region. A visual inspection of both plots (not shown) allowed us to detect the smaller PRESS and the region in which it is located. This latter method has shown excellent results in several applications [19,24]. As regard the selected regions, an important observation can be made: when using calibration set number 1, the selected region is reduced (281–320 nm), and eliminates highly correlated data (see Fig. 1), but for calibration set number 2 the selected region is almost identical to the complete spectrum. This latter fact produces the increase in both selectivity and sensitivity which is observed in Table 2 when calibrating with set number 2. On the other hand, the quality of the calibration 1 fit (REP% 50% smaller) is better than that for calibration 2. This is probably due to the fact that the former was built using a more suitable experimental design that comprises a larger number of calibration samples (19 versus 9). When comparing γ^{-1}, LOD and LOQ, better figures are obtained for calibration set number 2. This can be explained by the use of more extensive analytical information. Even though differences on the parameters shown in Table 2 are found, these are not significant, and the performance of both calibrations (DS and PLS) can be considered acceptable.

3.4. Comparative study of the precision obtained when applying derivative spectroscopy and both PLS-calibrations

Table 3 shows the results obtained when validation set number 1 was analysed. As can be seen, the results obtained analysing the same sample during three consecutive weeks by using the three methodologies are excellent. Both the repeatability (intra assay variability) and the intermediate precision presented coefficients of variation near to 1%. The ANOVA showed that the inter assay and the intra assay variances were comparable for the three methods ($p > 0.05$).

Table 3
Results obtained when analysing precision on zero-crossing spectrophotometry and PLS-1 by using calibration sets numbers 1 and 2

	Derivative spectroscopy	PLS-1 (Set 1) (g L^{-1})	PLS-1 (Set 2)
Week 1[a]	7.44 (4)	7.76 (8)	7.48 (8)
Week 2[a]	7.48 (4)	7.76 (8)	7.46 (7)
Week 3[a]	7.45 (4)	7.72 (9)	7.44 (8)
(ANOVA)[b]	$F = 2.708$ ($p = 0.085$)	$F = 0.766$ ($p = 0.475$)	$F = 0.678$ ($p = 0.516$)
Intermediate precision (CV%)	0.54	1.03	1.07

[a] Values between parenthesis correspond to standard deviation (10 replicates).

[b] Critical $F_{(3-1);(30-3);0.05}$ value equal to 2.960.

Table 4
Comparative study of the results obtained when applying HPLC, derivative univariate calibration and PLS-1 on the validation set number 3

Samples	HPLC	Theophylline found ($g\,L^{-1}$)[a]		
		Derivative spectroscopy	PLS-1 (Set 1)	PLS-1 (Set 2)
1	7.54 (2)	7.42 (4)	7.61 (8)	7.50 (8)
2	7.94 (2)	7.92 (4)	8.06 (8)	8.01 (8)
3	8.39 (2)	8.21 (4)	8.38 (8)	8.19 (8)
4	8.68 (2)	8.62 (4)	8.76 (8)	8.63 (8)
5	9.12 (2)	9.06 (4)	9.18 (8)	9.00 (8)
6	9.50 (2)	9.47 (4)	9.59 (8)	9.46 (8)
Recovery (pooled) (%)[b]	–	99.1	100.8	99.3

[a] Values between parenthesis correspond to standard deviation (five replicates).
[b] The recovery was calculated taking the HPLC value as 100%.

3.5. Comparative study of the results obtained when applying HPLC, derivative univariate calibration and PLS

In order to evaluate the accuracy and precision of the different methods herein analysed, linear regression analysis of HPLC versus concentration values found by other methods was applied utilising the results presented in Table 4. The estimated intercept and slope ($\hat{a}$ and $\hat{b}$, respectively) were compared with their ideal values of 0 and 1 using the elliptical joint confidence region (EJCR) test, in this case by using a bivariate least-squares fitting (BLS) [25]. The equation describing the joint region is $N(a-\hat{a})^2 + 2\left(\sum x\right)(a-\hat{a})(b-\hat{b}) + \left(\sum x\right)^2(b-\hat{b})^2 = 2s^2F_{\alpha,2,N-2}$, where N is the number of data points, s^2, the regression variance and $F_{\alpha,2,N-2}$ is the statistical F value with 2 and $(N-2)$ degrees of freedom at a given $100\times(1-\alpha)$ confidence level, usually 95%. If the point (1,0) is inside the EJCR, it can be concluded that constant and proportional biases are absent. Fig. 2 shows the EJCR plots for all the studied methods. As can be seen, the ellipses contain the theoretical ($a=0$, $b=1$) point for the three alternatives.

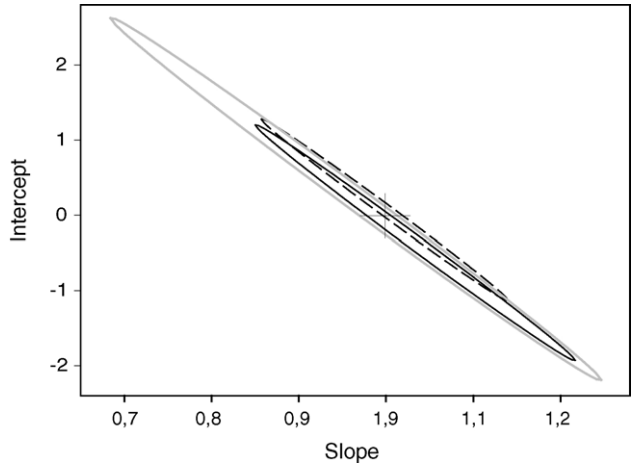

Fig. 2. Elliptical joint confidence regions for the slope (b) and intercept (a) corresponding to regressions of HPLC method predicted vs. chemometric assisted predicted concentrations of theophylline. Derivative spectroscopy (black solid line), PLS-1/calibration set 1 (black dashed line) and PLS-1/calibration set 1 (gray solid line). The black cross marks the theoretical ($a=0$, $b=1$) point.

Interestingly, the recoveries obtained when comparing the studied methods against the HPLC reference were $100\pm1\%$, indicating the high-performance of the methods herein analysed.

3.6. Final remarks

Taking into account the good results obtained by applying the DS and PLS methods, either of them can be recommended. Which method is adopted will depend on the availability of the software and the analyst's knowledge about the theoretical considerations on the chemometric methods. If the analyst wants to be independent of the excipients used for the manufacturing laboratory, the best choice is to build a natural calibration set, with the subsequent application of PLS. However, this option needs previous HPLC measurements. Once the HPLC concentration is obtained, the calibration can be used for a routine quality control activity of this kind of pharmaceuticals. On the other hand, the simplest choice is the implementation of DS.

4. Conclusions

A simple strategy including derivative univariate or PLS multivariate spectrophotometric calibration for the determination of theophlylline in syrup has been developed. It involves the use of zero-crossing derivative or PLS multivariate calibration using artificial or natural calibration sets. The results were acceptable considering the complexity of the analysed sample and the speed in getting the results when comparing with the HPLC method. In conclusion, the combination of DS-univariate and -multivariate calibrations with spectrophotometric measurements constitutes a valuable tool towards the development of simple methods for monitoring theophylline in pharmaceutical formulations presenting complex matrices.

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References

[1] A. Goodman-Hilman, T. Rall, A. Nier, P. Taylor, The Pharmacologycal Basis of Therapeutics, McGraw-Hill, New York, 1996.
[2] P. Sun, G.J. Mariano, G. Barrer, R.A. Hartwick, Anal. Lett. 27 (1994) 927–937.
[3] L. Oi-Wah, M. Chuen Shing, J. Chromatogr. A 693 (1995) 45–54.
[4] W. Shan Huang, S. Jin Lin, H. Lung Wu, S. Hwei Chen, J. Chromatogr. B 795 (2003) 329–335.
[5] G. Chen, Q. Chu, L. Zhang, J. Ye, Anal. Chim. Acta 457 (2000) 225–233.
[6] Q. Chuan Chen, J. Wang, J. Chromatogr. A 937 (2001) 57–64.
[7] H.C. Goicoechea, A.C. Olivieri, A. Muñoz de la Peña, Anal. Chim. Acta 384 (1999) 95–103.
[8] L. López Martínez, P.L. López de Alba, R. García Campos, L.M. de León Rodríguez, Anal. Chim. Acta 493 (2003) 83–94.
[9] B. Morelli, J. Pharm. Biomed. Anal. 33 (2003) 423–433.
[10] N. Erk, J. Pharm. Biomed Anal. 23 (2000) 255–261.
[11] P.L. López de Alba, L. López Martínez, M. González Leal, Y. Estrada Hernández, Quim. Anal. 18 (1999) 291–297.
[12] Z. Sentűrk, N. Erk, S. Ozakan, J. Pharm. Biomed. Anal. 29 (2002) 291–298.
[13] J. Karpinska, Talanta 64 (2004) 801–822.
[14] E.V. Thomas, D.M. Haaland, Anal. Chem. 60 (1988) 1193–1202.
[15] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, 1989.
[16] T. Galeano Diaz, I. Durán Merás, A. Guiberteau Cabanillas, M.F. Alexandre Franco, Anal. Chim. Acta 511 (2004) 231–238.
[17] K.S. Booksh, B.R. Kowalsky, Anal. Chem. 66 (1994) 782A–791A.
[18] P.C. Damiani, G.M. Escandar, A.C. Olivieri, H.C. Goicoechea, Curr. Pharm. Anal. 1 (2005) 145–154.
[19] A.C. Olivieri, H.C. Goicoechea, F.A. Iñón, Chemom. Intell. Lab. Syst. 72 (2004) 189–197.
[20] G. Talsky, Derivative Spectrophotometry, VCH, Weinheim, 1994.
[21] K. Danzar, L.A. Currie, Pure Appl. Chem. 70 (1998) 993–1014.
[22] H.C. Goicoechea, A.C. Olivierri, Chemom. Intell. Lab. Syst. 56 (2001) 73–81.
[23] K. Danzar, L.A. Currie, Pure Appl. Chem. 70 (1998) 993–1014.
[24] M.S. Collado, V.E. Mantovani, H.C. Goicoechea, A.C. Olivieri, Talanta 52 (2000) 909–920.
[25] J. Riu, F.X. Rius, Anal. Chem 68 (1996) 1851–1857.