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Solid-phase spectrofluorimetric determination of acetylsalicylic acid and caffeine in pharmaceutical preparations using partial least-squares multivariate calibration

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Abstract

PLS-1, a variant of the partial least-squares algorithm was used for the solid-phase spectrofluorimetric determination of acetylsalicylic acid (ASA) and caffeine (CF) in pharmaceutical formulations. The method allows the simultaneous quantification of the analytes, as the closely overlapping spectral bands are efficiently solved. Sample preparation prior to analysis is not required. The calibration set consisted of 83 samples with 50–170 mg g^{-1} ASA plus 5–20 mg g^{-1} CF; another set of 25 samples was used for external validation. Agreement between predicted and experimental concentrations was fair (r = 0.987 and 0.974 for ASA and CF models). For both models, the prediction performance was evaluated in terms of the coefficient of variability (CV), relative predictive determination (RPD), and ratio error range (RER). The final PLS-1 models were used for the determination of ASA and CF in pharmaceutical formulations.

Keywords: Solid-phase analysis; Fluorescence spectroscopy; Acetylsalicylic acid; Caffeine; PLS

1. Introduction

Exploitation of multivariate calibration as a means to improve multicomponent analysis has been increasing, especially in relation to samples of biological [1], environmental [2] and/or pharmaceutical [3] interest. In this context, chemometrics is nowadays considered an useful approach to improve the determination of pharmaceutical compounds by different techniques [4–6].

Acetylsalicylic acid (ASA) and caffeine (CF) are active principles widely used and frequently combined in pharmaceutical preparations [7]. The simultaneous determination of these chemical species can be performed by high performance liquid chromatography [8], and other techniques relying on spectrophotometry [9–11] and Fourier transform infrared spectrometry [12]. However, requirements of cost, time, labor and reagent consumption are inherent to most of the above mentioned procedures. Moreover, the need for sample preparation prior to the determination makes them cumbersome and, thus, less attractive for large-scale analyses.

Other techniques such as Near-infrared (NIR) [13,14] and Raman [15] spectroscopy have been also widely used for single or multi-analyte determination. Regarding NIR spectroscopy, some parameters, such as sample humidity, may influence the analytical results, mainly in relation to powdered samples [16]; moreover, a low spectral resolution is inherent to this technique. Raman spectroscopy may present limitations in sensitivity, often requiring powerful and costly laser sources for excitation [17]. Another possibility for analysis of powdered pharmaceutical tablets is the recently proposed solid-phase fluorescence, already applied to ASA

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determination [18]. This new approach has not been applied to simultaneous determinations.

Multiple linear (MLR), principal component (PCR) and partial least squares (PLS) regressions are receiving increasing attention as calibration techniques, as they generally lead to a simplification in sample handling and sometimes to the unneed for separation steps [19]. They have been often applied to analysis of pharmaceutical formulations containing two or more components yielding overlapping spectra. In this context, exploitation of multivariate calibration algorithms, especially PLS, which makes use of decomposition into latent variables, has increased in recent years [20,21]. Thus, PLS has been used in combination with different spectroscopic techniques such as, e.g. UV–vis spectrophotometry [22,23], NIR [24] or fluorescence spectroscopy, FS [25,26].

FS procedures have been widely used due to their favorable characteristics of sensitivity, selectivity and instrumental cost. As FS procedures may present limitations in the analysis of components yielding strongly overlapping spectra, a prior separation step is usually required. When a linear relationship between analyte concentration and fluorescence intensity is observed, PLS can be successfully applied for quantitative analysis of multicomponent mixtures that cannot be easily solved by univariate spectrofluorimetry [27,28].

This paper reports the development of two models (for ASA and CF) using PLS-1 for the simultaneous spectrofluorimetric analysis of solid matrices. The native ASA and CF fluorescences are exploited; therefore the intended procedure should not require any prior separation or derivatization step.

2. Experimental

2.1. Apparatus

Measurements were performed by a LS-55 model Perkin-Elmer luminescence spectrometer, equipped with a xenon discharge lamp (20 kW, 8 μs), two Monk–Gillieson monochromators, a Hamamatso photomultiplier, a reference photodiode, an optical-fiber accessory and a 96-well plate (compartment for the powdered samples). Slits for excitation and emission were set as 10 nm, the photomultiplier voltage was adjusted to 775 mV, and the monochromator scan rate was kept as 500 nm min^{-1}. Some parameters influencing the fluorescence intensity, such as sample amount and distance between optical fiber and sample had already been established [18]. For data acquisition and treatment, a PC microcomputer running commercial Pirouette software (version 3.02, Infometrix Inc.) was used.

2.2. Reagents and samples

Lactose, maize starch, talc and magnesium stearate were of pharmaceutical grade (GALENA), whereas ASA and CF (ACROS) were of analytical-reagent grade. The ingredients were used for diluting the investigated mixtures in accordance with the intended concentrations.

The samples were prepared according to the British Pharmacopoeia recommendations [7]. The sample dilutions were carried out after powdering the samples until a homogeneous particle size was attained; this powder was further mixed and homogenized with the solid ingredients.

2.3. Procedure

Sample amounts with 50–170 mg g^{-1} ASA plus 5–20 mg g^{-1} CF were accurately weighed and mixed with lactose, maize starch, talc, and magnesium stearate in the 70:15:10:5 or 80:10:7:3 w/w proportions. As the ASA to CF ratios in commercial tablets (usually around 10:1 w/w) is not always kept, different proportions (9:1, 10:1 and 11:1 w/w) were tested. A 25 mg sample amount was placed into the 96-well plate as previously described [18]. The fluorescence spectra of the mixture were recorded between 310 and 375 nm, maintaining the excitation wavelength at 275 nm and the ambient temperature at $25 \pm 1\,^{\circ}C$. For both models, the multivariate calibration was performed by PLS with one dependent variable (PLS-1).

Data from the recorded spectra were mean centered in order to remove any offset. The numbers of latent variables were determined by the leaving one out approach.

The ASA and CF models were developed from 83 samples prepared in the laboratory; 25 additional samples were used to evaluate model performance (validation step). The validation sample set was prepared with ASA and CF concentrations different than those employed for calibration, following a random design, but keeping all values within the corresponding calibration ranges for each analyte. After dimensioning, the procedure was applied to the analysis of pharmaceutical formulations and the main figures of merit were evaluated.

3. Results and discussion

An analytical produce should not be susceptible to slight variations in the nominal value of experimental variables, such as concentration, environmental and sample conditions [29,30]. In this regard, several definitions related to robustness and ruggedness have been proposed, and the expression “robustness” is differently used, according to the specific problem [31]. In the present work, different ASA/CF mass proportions as well as different ingredient amounts were tested. Corroborating the previous study [18] it was also noted that each component used as ingredient presents different effects on the fluorescence signal of ASA. However, it has shown a good robustness for small variations of the ingredients with a relative standard deviation of 2.3% for $n = 6$. Larger variations in the ingredient proportions were prepared for the models construction in order to decrease the effect of the ingredients, thus minimizing the prediction errors due to variations in the ingredient or active substance proportions.

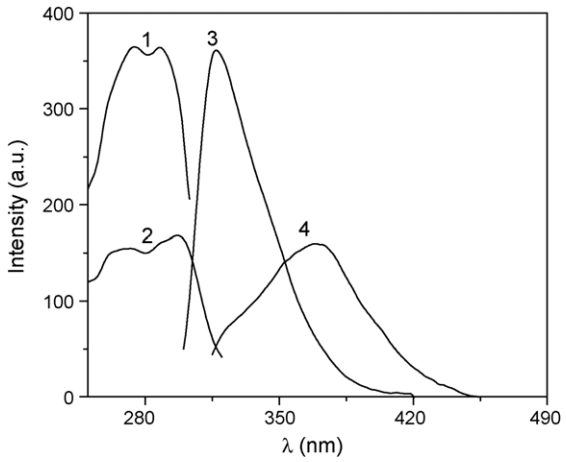

Fig. 1. Excitation (1 and 2) and emission (3 and 4) spectra for individual ASA and CF, respectively. Spectra recorded with 80 mg g^{-1} for each compounds.

3.1. Spectra of the studied compounds

Fig. 1 shows the individual ASA and CF excitation and emission spectra. It can be observed that ASA presents a strong emission band around 320 nm and CF a less intense band at 367 nm. A great overlapping of the spectra is verified, which cannot be well solved by traditional procedures. Besides, CF is a minor constituent in most of the commercial pharmaceutical preparations; this makes resolution of the mixture even more complex. However, there are some spectral differences that can be used to solve this mixture by multivariate calibration methods. The spectral region between 310 and 375 nm was set from the range of 300 up to 420 nm, because it provides the maximum spectral information of the components in the mixture.

3.2. Calibration and number of factors

The data set was mean centered to build the calibration models and their performances were evaluated by leaving one out cross validation, in which each sample was left out once, and its concentration was estimated by a model built with the remaining samples [32]. In order to find the optimum number of factors for the PLS-1 model the prediction residual error sum of square (PRESS) [32,33] was calculated according to Eq. (1):

$$\text{PRESS} = \sum_{i=1}^{m} (\hat{C}_i - C_i)^2 \quad (1)$$

where m is the total number of samples, $\hat{C}_i$ the estimated concentration, and C_i the reference concentration. It was verified that the optimum number of latent variables for the PLS-1 algorithm were 4 and 3 for ASA and CF, respectively, as yielded the minimum PRESS values.

3.3. Validation set

In order to test the prediction performance of the proposed method, the constructed models were used for ASA and CF determinations in a situation where all the constituents of the samples are known, including the different concentrations of ASA and CF. For this task, 25 samples containing the same ingredients as the mixtures for the calibration set were used. From the experimental and predicted values in Table 1 , it can be seen that only two samples for each model (ASA and CF) were predicted with residuals close to 10%.

A way to evaluate the prediction ability of a model is the coefficient of variability (CV) [34], expressed as a percentage of the mean of the true concentrations, given by

$$\text{CV} = \text{S.D.}_{\text{RES}} \frac{100}{\text{mean}_{\text{EXP}}} \quad (2)$$

where S.D._{RES} is the standard deviation of the residuals and mean_{EXP} is the mean of the experimental concentration.

Table 1
Results of evaluation of the prediction ability

Sample	Experimental (mg g^{-1})		Predicted (mg g^{-1})		Relative residuals (%)	
	ASA	CF	ASA	CF	ASA	CF
1	94.8	10.5	96.1	9.9	−1.4	5.7
2	83.8	8.4	92.6	8.8	−10.5	−4.8
3	83.8	8.4	89.5	9.0	−6.8	−7.1
4	75.7	7.6	74.6	7.4	1.5	2.6
5	104.5	10.5	111.3	10.4	−6.5	1.0
6	104.5	10.5	96.5	9.6	7.7	8.6
7	120.7	12.1	116.1	11.6	3.8	4.1
8	144.3	14.5	146.2	13.6	−1.3	6.2
9	141.5	12.9	143.9	14.2	−1.7	−10.1
10	82.5	7.5	81.9	8.1	0.7	−8.0
11	171.6	15.6	169.6	16.8	1.2	−7.7
12	72.8	8.1	72.0	6.9	1.1	14.8
13	101.9	11.3	107.0	10.9	−5.0	3.5
14	118.6	13.2	129.0	12.9	−8.8	2.3
15	82.2	7.5	77.6	7.2	5.6	4.0
16	142.5	15.9	139.8	14.8	1.9	6.9
17	93.7	9.4	96.8	10.0	−3.3	−6.4
18	93.7	9.4	98.4	9.3	−5.0	1.1
19	122.2	12.2	132.3	12.9	−8.3	−5.7
20	144.7	14.5	146.6	14.2	−1.3	2.1
21	144.7	14.5	146.6	14.2	−1.3	2.1
22	82.5	7.5	72.5	7.1	12.1	5.3
23	96.1	8.7	88.9	8.7	7.5	0.0
24	117.4	10.7	113.0	11.1	3.7	−3.7
25	144.9	13.2	137.9	13.0	4.8	1.5
Mean	110.6	11.0	111.1	10.9	−0.4[a]	0.1[a]
S.D.	27.6	2.7	28.2	2.8	5.7[a]	0.7[a]
Range	71.9	8.4				
RPD					4.8	3.9
RER					12.6	12.0
CV					5.2	6.4
R					0.987	0.974

S.D.: standard deviation (n = 25); RPD: relative predictive deviation; RER: ratio error range; CV: coefficient of variability; R: correlation coefficient.
[a] The values are expressed in relation to the absolute residuals.

Table 2
ASA and CF concentrations (added, found), expressed in mg g^{-1} are mean of three determinations; proportions 1 and 2 = 70:15:10:5 and 80:10:7:3, w/w

Sample	Proportion	Acetylsalicylic acid			Caffeine		
		Added	Found	Recovery (%)	Added	Found	Recovery (%)
1	1	83.1	84.0	101.1 ± 2.2	8.3	8.0	96.4 ± 3.9
2		83.0	82.3	99.2 ± 1.4	8.3	8.6	103.6 ± 4.6
3		92.0	90.9	98.8 ± 1.3	9.2	9.3	101.1 ± 2.3
4		92.1	88.4	96.0 ± 3.8	9.2	8.9	96.7 ± 1.3
5	2	93.2	90.8	97.4 ± 3.1	9.4	9.9	105.3 ± 4.7
6		93.1	92.1	99.0 ± 2.4	9.4	9.7	103.2 ± 3.8
7		93.2	91.2	97.9 ± 2.2	9.4	9.5	101.1 ± 1.4
8		93.0	91.8	98.7 ± 1.4	9.4	9.7	103.2 ± 3.1

Furthermore, for the same purpose of evaluation of the prediction ability relative prediction deviation (RPD) and ratio error range (RER) were also calculated, according to Eqs. (3) and (4) [34]:

$$\mathrm{RPD} = \frac{\mathrm{S.D.}_{C_{\mathrm{EXP}}}}{\mathrm{S.D.}_{\mathrm{RES}}} \quad (3)$$

$$\mathrm{RER} = \frac{C_{\mathrm{EXP_{MAX}}} C_{\mathrm{EXP_{MIN}}}}{\mathrm{S.D.}_{\mathrm{RES}}} \quad (4)$$

$\mathrm{S.D.}_{C_{\mathrm{EXP}}}$ is the standard deviation of experimental concentrations, $\mathrm{S.D.}_{\mathrm{RES}}$ the standard deviation of the residuals, $C_{\mathrm{EXP_{MAX}}}$ and $C_{\mathrm{EXP_{MIN}}}$ represents the maximum and minimum experimental concentrations, respectively. Having in mind the strong overlap between the spectra of two compounds under investigation, the results presented in Table 1 can be considered satisfactory.

3.4. Precision

The precisions of the models were checked by means of one-way ANOVA by performing ten successive prediction for a typical sample containing 80 mg g^{-1} ASA and 10 mg g^{-1} CF, and the relative standard deviations were calculated as 3.1 and 4.0%, respectively, confirming the good repeatability associated with the proposed models.

3.5. Applications

In order to check the performance of the proposed models, the method was applied to the simultaneous determination of ASA and CF in eight pharmaceutical formulations. Known amounts of the both analytes were added at mg g^{-1} level to the mixture of ingredients with two proportions tested as described in Section 2. The compounds were determined by applying the proposed models and the results are presented in Table 2. Good recoveries were obtained with acceptable prediction errors for all assayed samples.

4. Conclusions

The solid-phase spectrofluorimetric procedure for simultaneous determination of ASA and CF in pharmaceutical formulations was rapid and precise, yielding satisfactory results. Prior sample preparation and ASA hydrolysis [1,9,22] are not required, therefore some limitations inherent in traditional analytical procedures, such as intensive laboratory steps, increased analysis time and high reagent consumption are lessened. The approach permits the simultaneous quantification of ASA and CF, even though these compounds present closely overlapping spectral bands, therefore cumbersome separation steps are not required. A simple reading of the fluorescence spectrum in the solid sample enables an easy and fast determination, once the calibration parameters have been established.

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