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Simultaneous determination of paracetamol, caffeine and acetylsalicylic acid by means of a FI ultraviolet pls multioptosensing device

A. Ruiz Medina, M.L. Fernandez de Córdova, A. Molina-Diaz *

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Paraje 'Las Lagunillas', E-230071 Jaén, Spain

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Abstract

A simple and rapid analytical procedure is proposed for the simultaneous determination of caffeine (CF), acetylsalicylic acid (ASA) and paracetamol (PCT) in pharmaceutical preparations by partial least-squares (PLS) treatment of a flow-through multisensor based on the integration of the retention and UV detection of the analytes on a solid support. Diode-array spectrophotometry has been used to obtain spectra (240–350 nm) of the analytes retained on C_{18} bonded phase beads packed in a flow cell. By using a 0.5% pH 1 $HClO_4$ solution as the carrier, the multisensor responds linearly in the measuring range without requiring additional reagents or derivatization processes and the active microzone is regenerated by using methanol as eluting agent. Spectra of the corresponding analytes were used to provide multivariate data for the multivariate procedure. The statistical parameters obtained by the application of PLS methods at different reaction times were analysed, from which the optimum reaction time for the simultaneous determination of the analytes was selected. In the analysis of real and synthetic samples, precise and accurate values were obtained.

Keywords: Partial least-squares; Flow-through multisensor; Caffeine; Acetylsalicylic acid; Paracetamol; Pharmaceutical preparations

1. Introduction

Computer-controlled instrumentation and multivariate calibration methods are playing a very important role in the multicomponent analysis of mixtures by UV/visible molecular absorption spectrophotometry. Both approaches are useful for the resolution of band overlapping in the quantitative analysis. In general, a multivariate calibration model is constructed from instrumental response data collected for a set of multicomponent samples of known concentrations with respect to the analytes of interest. Each method needs this calibration step, followed by a prediction step in which the results of the calibration are used to determine the component concentrations

* Corresponding author. Tel.: +34-9-53212147; fax: +34-9-53212526.
E-mail address: amolina@ujaen.es (A. Molina-Diaz)

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from the sample spectrum. In recent years, multivariate calibration techniques have been widely applied to UV/visible spectral data, and methods such as PCR [1] and partial least-squares (PLS) [2–4] are increasingly being used in conjunction with flow injection techniques. PCR and PLS are indirect calibration methods, i.e. they do not require individual spectra of each analyte and interferents to be known in advance. They, however, require the analysis of a comprehensive set of calibration samples that span all the expected physical and chemical phenomena that may influence the spectra of samples for prediction. Within this calibration set, the analyte concentration must be known, but the levels of any interference do not need to be known. This is in contrast to direct multicomponent analysis (DMA). In PCR, the spectra are decomposed on the basis of the maximum variance between spectral data without using information about the concentrations, whereas PLS uses both the spectral and concentration data in modelling. Hence, PLS sacrifices some fit of the spectral data relative to PCR in order to achieve better correlations to concentrations during prediction. The multivariate calibration techniques are discussed in more detail elsewhere [5].

Multivariate calibration methods have an increased importance in multicomponent analysis, specially those using PLS method with decomposition into latent variables [6]. The PLS method has already been used successfully in various instances: in fluorimetry [7], in spectrophotometry [8], in near-infrared spectrometry [9], in kinetic analysis [10], and for the evaluation of potentiometric acid–base tritrations [11].

Acetylsalicylic acid (ASA) and paracetamol (PCT) are used as analgesic and antipyretic agents. They appear associated with caffeine (CF) in many commercial formulations including tablets and capsules. In spite of its outstanding advantages, as sensitivity and low cost, the UV/visible spectroscopy presents limitations to analyze mixtures of organic compounds. However, the use of a solid support in combination with chemometric techniques, provides a high selectivity and a increase in sensitivity. This paper reports on the resolution of the ternary mixture of PCT, CF and ASA and its simultaneous determination by PLS flow-through multisensor.

Few studies have been reported about the simultaneous determination of analytes by flow injection spectrophotometry in solid phase. Therefore, we extended this analytical procedure to the simultaneous continuous solid phase flow determination of PCT, CF and ASA by using PLS multivariate calibration and UV detection, that is, by means of a PLS UV optosensing device. One of the most promising aspects of this type of sensors is the possibility of performing multicomponent analysis using photometric diode-array detector to monitor simultaneously the absorbance at various wavelengths or record the entire spectra in a fraction of a second. This paper deals with the use of the sorbing retention for the optosensing of the three analytes and their subsequent determination by exploiting not only their spectral features on the solid support with the aid of a multicalibration chemometric approach but also their different kinetic behaviour in the retention–elution process onto the solid phase by selecting the spectra at two adequate time values which provides the most different behaviour by themselves.

2. Experimental

2.1. Apparatus and software

A Milton Roy Spectronic 3000 diode-array spectrophotometer was used for spectra recording, using the Milton Roy software Rapid Scan 2.01 for all data acquisition. The GRAMS/32 (from Galactic) software package, with the PLSplus/IQ application software [12] running in a 486 PC was used for the statistical treatment of the data and the application of the PLS method. The equipment was also used to import to its own format the absorbance spectra of samples and standards.

Spectra were recorded in the range 240–350 nm using a quartz flow-through cell Hellma model 138-QS of 1 mm optical path length (50 μl inner volume). The cell was packed with the solid support (C_{18} gel) by introducing it in a methanol suspension with the aid of a syringe. The outlet of

the cell was blocked with glass wool to prevent the moving of the solid particles from the flow stream. A four channel peristaltic pump (Gilson Minipuls 3) was employed as impelling system. Two six-way rotary injection valves Rheodyne 5041 (one of which acted as a selecting valve), PTFE tubing of 0.5 mm i.d., an ultrasonic bath (Selecta), and a digital Crison model 2002 pH-meter fitted with a glass/saturated calomel electrode assembly and a temperature probe, were used also.

2.2. Reagents

All chemicals were of analytical-reagent grade.

Stock solutions of paracetamol (Fluka), caffeine (Merck) and acetylsalicylic acid (Fluka), containing 1000 μg ml^{-1} were prepared in 100 ml volumetric flasks, by dissolving 100 mg of each compound in doubly distilled water. Only freshly prepared solutions of PCT and ASA were used due to their low stability. Working standard solutions were prepared daily by diluting the stock solutions. HPLC grade methanol was obtained from Panreac and it was used as the eluting agent. A 0.5% v/v of $HClO_4$ (Panreac) was used as carrier. All chemicals such as nitric, hydrochloric and ortophosphoric acids, acetonitrile, ethanol and acetone, were obtained from Panreac.

C_{18} bonded silica (Waters) with average particle sizes of 55–105 μm was used as solid support; ion-exchange resins Sephadex SP C-25 and QAE A-25 (Fluka) were also tested. All pharmaceuticals containing PCT, CF, ASA and excipients were from Spanish Pharmacopoeia, and all solutions were prepared in doubly distilled water.

2.3. Treatment of samples (tablet formulations)

Tablets containing the three or someone of the analytes, were directly dissolved in doubly distilled water with shaking for 10 min in an ultrasonic bath. The solution was filtered through a 0.45-μm pore size Millipore membrane filter, and the filtrate plus washings were diluted to the mark in a 100 ml calibrated flask. Appropriate dilutions were made from this solution.

2.4. Procedure

The continuous-flow manifold used is shown in Fig. 1. The sample (250 μl) containing between 1 and 15 μg ml^{-1} of CF, 5 and 100 μg ml^{-1} of ASA and, 5 and 70 μg ml^{-1} of PCT, was inserted into the carrier stream (0.5% $HClO_4$ v/v) at a flow-rate of 1.15 ml min^{-1}. The analytes were retained on the solid support (C_{18}) when passing through the flow cell, while spectra were being recorded between 240–350 nm (using data resolution of 0.35 nm) at 4.2 s intervals during all the retention process (2.5 min). After that, selecting valve was then switched to the eluent stream (methanol) during 30 s, which removed the analytes from the support. Optimized calibration matrix, calculated by application of the PLS-1 method (Table 1), were applied to analyse the spectra of the samples and calculate the concentrations of PCT, CF and ASA in the mixture.

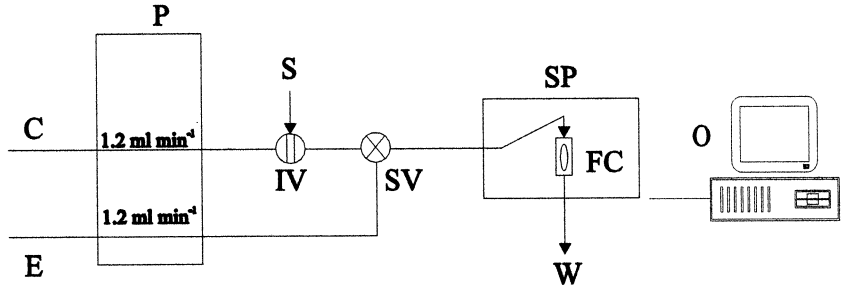

Fig. 1. Flow system diagram. C, carrier solution; S, sample; E, eluent; W, waste; IV, injection valve; P, peristaltic pump; SV, selection valve; SP, spectrophotometer; FC, flow-cell; O, computer.

Table 1
Calibration matrix (concentration in μg ml^{-1})

Sample	Caffeine	Acetylsalicylic acid	Paracetamol
1	5	–	–
2	15	–	–
3	–	30	–
4	–	100	–
5	–	–	25
6	–	–	40
7	10	20	–
8	5	100	–
9	–	75	20
10	–	50	30
11	3	–	50
12	8	–	20
13	2	80	40
14	10	50	10
15	4	40	60
16	6	60	15
17	3	20	60
18	6	70	5
19	9	15	25
20	12	15	30
21	15	10	10
22	2	90	10
23	1	8	70
24	4	5	60
25	5	25	15
26	7	20	25
27	2	25	35
28	10	15	15
29	15	10	10
30	5	80	5

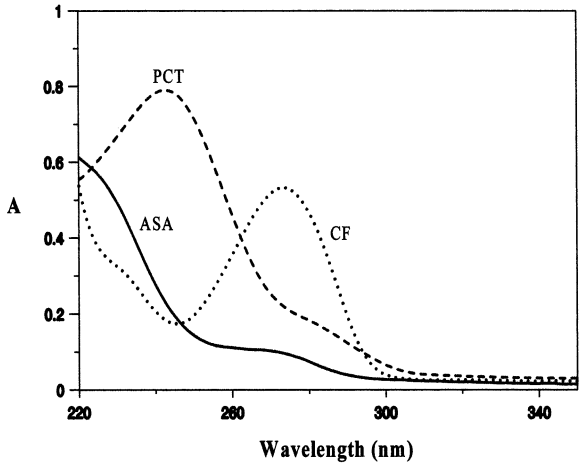

Fig. 2. Spectra of PCT, ASA and CF in solution: 10 μg ml^{-1} of each analyte; 1-cm optical path length.

3. Results and discussion

Fig. 2 shows the spectra of PCT, ASA and CF for 10 μg ml^{-1} solutions, respectively. All of them are absorbing substances in the UV region. These spectra were obtained using conventional spectrophotometry (cell of 1-cm optical path length) in homogeneous solution. It can be seen that the degree of overlapping is high and there is no selective wavelength that allows their determination by classical univariate calibration. Furthermore, since CF is a minor constituent in most commercial pharmaceutical preparations, this makes the resolution of the mixture more difficult. In addition, the overlap of their first derivative spectra does not permit the mixture to be resolved by the zero-crossing technique. No changes in the positions of absorption maxima were observed when the species were retained on the sorbent. However, there are some spectral differences that can be used to resolve this mixture by multivariate calibration methods (PLS).

As the analytes to be determined are retained on the resin, it is possible to obtain high sensitivity in on-line detection. Besides, the tolerance to possible interfering species in the proposed method is increased drastically by the selective retention of the analytes on the solid support in the cell. However, in order to develop the on-line resolution of the mixture the analytes to be determined should be desorbed easily and completely by an appropriate desorbing agent solution, simultaneously.

3.1. Optimization of procedure

Optimization studies were carried out for each individual constituent and optimum values of the experimental variables were selected. Typical variables of the flow-through sensor such as the type of cell and the type of support in the flow cell were optimized. In previous works [13,14] we found a Hellma 138 QS cell of 1-mm optical path to be the most appropriate cell in question. Several exchange resins (Sephadex QAE A-25 and Sephadex SP C-25) and C_{18} gel were tested. Of all of them, C_{18} (a non-polar sorbent) gave the best results and fixation became quicker, due its large

interaction capability with all the three analytes. Decreasing particle size of the support increased the compaction of the solid in the cell caused a decrease on permeability of the support, subsequently, an increase of both the background and pressure in the system was observed. Therefore, the greatest C_{18} particle size commercially available (55–105 μm) was selected because an acceptable absorbance background was obtained without causing pressure problems. The level of the sorbent in the flow cell was a very important and a key variable: for too high support level a large amount of the analytes is retained on the C_{18} just on the top of the cell, where the incoming flow just contacts the solid support, falling outside of the detection area (above the light beam dark zone A in Fig. 3). On the other hand, with too low levels (dark zone B in Fig. 3), the light beam pass through the solution. Therefore, the optimum height of packing level was found to be 15 mm.

After above indicated, the variables influencing the system can be divided into two groups: chemical variables and flow system variables.

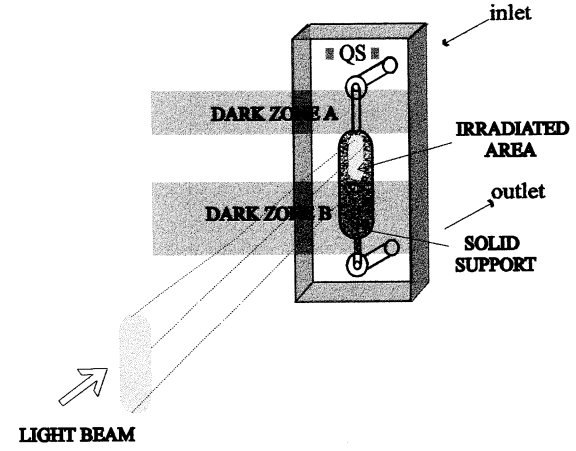

Fig. 3. Flow cell (Hellma 138-QS) containing the active solid support.

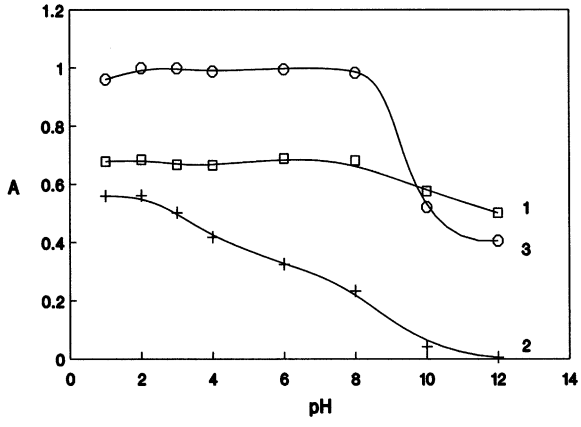

Fig. 4. Influence of the pH of carrier solution: (1) 15 μg ml^{-1} of caffeine ($\lambda = 275$ nm); (2) 75 μg ml^{-1} of acetylsalicylic acid ($\lambda = 275$ nm); (3) 30 μg ml^{-1} of paracetamol ($\lambda = 250$ nm).

3.1.1. Chemical variables

The influence of the pH of the carrier solution was studied in the range 1–12 by adjusting it with HCl or NaOH (Fig. 4). The retention of CF was found to be independent of pH from 1 to 8 whereas signal of PCT decreased drastically at $pH > 8$ due to its ionization. As can be seen, the strongest dependence on pH was that of the ASA retention, where a strong acid medium might favour the retention and detection steps (above pH 3 the progressive dissociation of the carboxylic groups makes the retention of ASA on the hydrophobic support impossible). Therefore, pH 1 was selected to further experiences. Several acids (hydrochloric, ortophosphoric, perchloric and nitric acids) were tested, and the best results were obtained by using perchloric acid (at a 0.5% v/v concentration).

In order to regenerate the solid phase and make the sensor reusable, a study of the effect of different solvents as eluting agents was carried out. Several water–organic solvent mixtures ranged between 10 and 100% from acetonitrile, ethanol methanol and acetone were tested. The use of methanol resulted in the fastest elution, the speed of which increased by increasing its content in the eluting solvent, therefore, pure methanol was selected in order to achieve a complete and fast (30 s) regeneration of the sensor.

The sample pH value (injected volume 250 μl) did not influence the analytical signal of any of the analytes when its value was maintained in the 2–9 range, and hence, there was no need to adjust the sample pH.

3.1.2. Flow system variables

The flow injection variables studied were the sample volume, which influenced the sensitivity of the method, and the flow-rate, which determines both the residence time and the rate at which the analyte can be eluted, and hence, the sampling frequency. Very low flow-rate, increased retention of the analytes and subsequently the signals, but it was incompatible with short residence times and rapid baseline restoration; thus, in all subsequent experiments, the total flow-rate was maintained at 1.15 ml min^{-1}. On the other hand, large sample volumes resulted in decreasing sampling frequency but increased sensitivity as a result of the retention of a larger amount of analytes in the detection area. This made possible to select the most appropriate volume of sample taking into consideration the concentration of the samples that are going to be analyzed. A volume of 250 μl was chosen. We can increase the sensitivity, by increasing the sample volume, because the absorbance increases linearly with the increase in the sample volume (as usually in this type of sensors [13–15]) from 100 to 1500 μl for the three analytes. The increasing is not linear for a greater sample volume than 1500 μl.

3.2. Application of the PLS method

Contradictory results about the convenience of applying differentiation techniques prior to the use of multicomponent calibration methods can be found in the literature. MacLaurin et al. [16] and Durán-Merás et al. [17] applied several multivariate calibration methods to UV/visible spectra to resolve ternary mixtures. They made a comparative study of applying methods based on the use of the absorbance and first and- second-derivative spectral data. Both groups found no significant differences in the predictions from the absorbance and first-derivative data with PLS and PCR, but second-derivative data had less precise predictions because of its much poorer signal-to-noise ratio, compared with that of the direct absorbance signal. We used the measurement of absorbance to develop this paper.

Multivariate calibration methods require a suitable experimental design of the standards belonging to the calibration set in order to provide good predictions. A synthetic set of 30 solutions of mixtures of PCT, ASA and CF were prepared (Table 1). The upper and lower limits of the concentrations used for calibration were selected in order to include all common levels of the studied active principles in all pharmaceuticals commercially available. In order to select the optimum retention time of the analytes for PLS resolution, six matrices at different detection times after injection were optimized (at 25, 34, 42, 50, 55 and 59 s). These matrices were designated as matrices 1, 2, 3, 4, 5 and 6, respectively. The model was mean-centred in every case and PLS calibration was carried out using the PLS-1 mode.

In order to select the number of factors, a cross-validation method [18], leaving out one sample at a time, was used. For each selected retention time, PLS calibration was performed on the 30 calibration spectra set. From this set of calibration spectra, the calibration on 29 calibration spectra was carried out and, using this calibration, the concentration of the analytes in the sample left out during calibration was predicted. This process was repeated 30 times until each calibration sample had been left out once. The concentration of the analytes in each sample was then predicted and compared with the known concentration of the reference sample; therefore, the prediction error sum-of-squares (PRESS) was calculated each time a new factor was added to the PLS-1 model. This parameter is a measure of how well a particular model fits the concentration data.

One reasonable choice for the optimum number of factors would be that number which yielded the minimum PRESS. However, PRESS calculation is based on a finite number of samples, and, therefore, it is subject to error. Haaland and Thomas [19] have determined empirically than an F-ratio probability of 0.75 is a good choice. The number of factors for the first PRESS value that had an F-ratio probability falling below 0.75 was selected as the optimum. The statistical results obtained for all the matrices and analytes are summarized in Table 2. The values of the root mean squares difference (RMSD), the square of the correlation coefficient (R^2) and the relative error of prediction

Table 2
Statistical parameters

Matrix	Caffeine				Acetylsalicylic Acid				Paracetamol			
	No. factors	R^2	RMSD	REP	No. factors	R^2	RMSD	REP	No. Factors	R^2	RMSD	REP
1	1	0.1497	4.402	64.4	4	0.5711	21.837	51.7	10	0.9648	3.168	11.1
2	3	0.7208	2.526	36.9	7	0.8215	13.974	33.1	4	0.9229	5.662	19.8
3	12	0.8857	1.856	27.1	9	0.9550	6.411	15.1	4	0.9312	5.437	19.0
4	12	0.9200	1.484	21.7	2	0.7985	14.722	34.8	1	0.1266	19.644	68.8
5	5	0.8568	1.966	28.7	5	0.8791	11.985	28.4	1	0.1547	19.255	67.4
6	7	0.9711	0.503	7.3	5	0.9901	2.578	6.1	1	0.1816	18.962	66.4

Table 3
Validation set

Sample	Caffeine			Acetylsalicylic Acid			Paracetamol		
	C_{added} [a]	C_{found} [b]	Recov $\pm \sigma$ (%)	C_{added} [a]	C_{found} [b]	Recov $\pm \sigma$ (%)	C_{added} [a]	C_{found} [b]	Recov $\pm \sigma$ (%)
1	10.00	9.72	97 ± 5	25.00	25.51	102 ± 4	10.00	10.10	101 ± 3
2	5.00	4.64	93 ± 1	40.00	41.18	103 ± 3	20.0	19.69	98 ± 3
3	3.00	3.23	107 ± 2	75.00	77.58	103 ± 2	20.0	19.68	98 ± 2
4	8.00	8.41	105 ± 1	50.00	52.03	104 ± 2	8.00	8.14	102 ± 2
5	15.00	14.02	94 ± 3	15.00	16.32	109 ± 2	10.0	10.03	100 ± 4
6	15.00	14.42	96 ± 4	20.00	21.40	107 ± 6	30.00	32.56	108 ± 1
7	10.00	10.18	102 ± 1	40.00	42.19	105 ± 3	20.00	21.14	106 ± 2
8	2.00	1.90	95 ± 2	50.00	54.19	108 ± 1	50.00	44.90	90 ± 3
9	5.00	5.32	106 ± 6	60.00	60.35	100 ± 2	40.00	37.77	94 ± 3
10	6.00	5.82	97 ± 3	60.00	59.19	99 ± 2	15.00	15.06	100 ± 2
11	2.00	1.90	95 ± 3	25.00	23.82	95 ± 3	35.00	32.60	93 ± 4
12	10.00	9.96	100 ± 2	12.00	11.90	99 ± 5	50.00	53.43	107 ± 2
13	15.00	15.00	100 ± 5	15.00	14.59	97 ± 2	50.00	48.82	98 ± 2
14	7.00	7.39	105 ± 2	50.00	46.86	94 ± 1	30.00	32.63	109 ± 3
15	10.00	10.01	100 ± 1	20.00	20.01	100 ± 6	30.00	27.79	93 ± 1
16	4.00	4.27	107 ± 5	40.00	39.22	98 ± 2	45.00	45.18	100 ± 6
20	15.00	15.02	100 ± 4	–	2.12	–	–	0.29	–
18	5.00	4.59	92 ± 6	100.00	91.68	92 ± 5	–	0.17	–
19	–	0.56	–	100.00	86.52	87 ± 3	20.00	17.97	90 ± 1
20	–	−0.20	–		−2.89	–	30.00	30.59	102 ± 2

[a] C_{added}, real concentration (μg ml^{-1}).
[b] C_{found}, found concentration (μg ml^{-1}).

(REP) are included in order to give an indication both of the average error in the analysis and the quality of fit of all data to a straight line for each component.

By application of PLS-1 model, the simultaneously best R^2 and REP values are obtained from matrix 1 (25 s after injection) for PCT, and from matrix 6 (59 s after injection) for ASA and CF. So the best results for PCT are obtained when it begins to be eluted from the solid support, meanwhile the best results for the other analytes are found when PCT is rather completely desorbed. Therefore, it was not possible to quantify satisfactorily the three analytes by using only one of the matrices with acceptable REP values for all of them, therefore, it is necessary the use of two different calibration matrices. It must be emphasized that two different experiences had not to be done, but spectra at two different time values recorded with only one injection of sample had to be selected in order to build the two matrices.

The PLS-1 cross-validation performed with respect to the optimum number of factors affecting the prediction of each of the compounds individually, (Table 2) showed seven factors for CF, five for ASA, and ten factors for PCT. The use of the solid support probably causes non-linear effects on the system and it could be the reason of additional factors being three (the ideal number of them in a linear system of three components) to be necessary. Once the optical number of PLS factors is determined, it is necessary to perform the final calibration, using all calibration samples with the optical number of factors.

3.2.1. Validation set

Examining the prediction capability of the model on samples not included in the calibration sample set, it is possible to test the validity of a calibration model. The above mentioned model was employed to predict the concentration of the analytes in 20 synthetic samples, and data are summarized in Table 3, in which the confidence

intervals found have been included also. As can be seen, the amounts added and found were consistent and the relative standard deviations low, with an error of less than 6% in every instance.

3.2.2. Applications

The proposed sensing device allows the determination of CF, ASA and PCT in ternary mixtures in spite of the much lower concentration from CF in the real samples as compared with the other two components. Accuracy and utility were checked by analysing commercial tablet formulations containing one, two or the three analytes. The results obtained and the percentage of recovery are summarized in Table 4, where the values declared by the producer are also reported. All the active principles were predicted with acceptable errors. The highest errors were about the tolerance level established in the USP Pharmacopoeia [20] for these type of drugs (10%).

Although the relative prediction errors are higher than those obtained with the validation set, it can be said that the sensing device shows a good prediction if we take into account the absence, in the calibration standards, of a compensation involving the presence of excipients and active principles which are present in some of the formulations.

4. Conclusions

The PLS flow-through multioptosensing device here proposed, which operates in the UV region, has demonstrated the simultaneous determination of PCT, ASA and CF in pharmaceutical preparations as well as in synthetic mixtures. The proposed method has very important advantages over methods which require a separation technique, such as HPLC or gas chromatography: (1) low cost and more rapidity (less than 3 min/analysis); (2) the solid support placed in the flow cell acts both on increasing the sensitivity and selectivity of the detection; (3) the proposed method can be applied for the rapid, routine simultaneous determination of the analytes by simply recording the spectra after dissolution of the samples in water and filtration (without any previous reac-

Table 4
Analytical applications

Pharmaceutical [a]	Caffeine			Acetylsalicylic Acid			Paracetamol		
	$C_{pharmac}$ [b]	C_{found} [c]	Recov $\pm \sigma$ (%)	$C_{pharmac}$ [b]	C_{found} [c]	Recov $\pm \sigma$ (%)	$C_{pharmac}$ [b]	C_{found} [c]	Recov $\pm \sigma$ (%)
1. Aspirina	–	–	–	50	48.9	98 ± 4	–	–	–
2. Tromalyt	–	–	–	300	272.6	91 ± 3	–	–	–
3. Apiretal	–	–	–	–	–	–	100	94.6	95 ± 2
4. Cafiaspirina	50	52.4	105 ± 3	500	543.0	108 ± 5	–	–	–
5. Analgilasa	30	35.0	116 ± 4	–	–	–	500	477.0	95 ± 2
6. Veganin	–	–	–	250	282.0	112 ± 4	250	220.5	88 ± 3
7. Actrón Compuesto	40	37.3	93 ± 6	267	274.4	103 ± 2	133	112.8	85 ± 7
8. Neocibalena	50	55.4	110 ± 3	200	177.4	89 ± 6	150	132.4	88 ± 5
9. Cerebrino Mandri	20	18.2	91 ± 5	250	237.7	95 ± 2	200	204.5	102 ± 2

[a] Nominal contents of pharmaceuticals (per comp.): 1. (Ltd. Bayer), ASA 500 mg; 2. (Ltd. Madaus Cerafarin), ASA 300 mg, saccharose 23 mg; 3. (Ltd. Ern), PCT 100 mg, saccharin 5 mg; 4. (Ltd. Bayer), ASA 500 mg, CF 50 mg; 5. (Ltd. Lasa), PCT 500 mg, CF 30 mg, codeine phosphate 10 mg; 6. (Ltd. Parke Davis), PCT 250 mg, ASA 250 mg, codeine phosphate 10 mg; 7. (Ltd. Bayer), PCT 133 mg, ASA 267 mg, CF 40 mg; 8. (Ltd. Zyma Farmacéutica), PCT 150 mg, ASA 200 mg, CF 50 mg; 9. (Ltd. Mandri), PCT 200 mg, ASA 250 mg, CF 20 mg.

[b] $C_{pharmac}$, content claimed (mg) of the active principle per comprimide.

[c] C_{found}, content (mg) of the active principle found per comprimide.

tion or derivatization process being necessary) before injecting them into the flow system.

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