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PLS-UV spectrophotometric method for the simultaneous determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceutical formulations

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Abstract A simple and fast analytical procedure is proposed for the simultaneous determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceuticals by means the partial least square treatment of the spectrophotometric absorbance data between 216 and 300 nm, taken at 5 nm intervals. The method involves the use of 8 standard mixtures of the three compounds assayed, considered at two concentration levels, and the measurement of the absorbance of samples in a 20% (v/v) ethanol in water solution previously filtered. In the analysis of real and synthetic samples precise and accurate values were obtained by the aforementioned procedure, providing in all cases variation coefficients and accuracy errors lower than 5% which agree with the tolerance level established by the pharmacopoeia for this kind of samples which is $\pm$ 10%.

Introduction

Partial least-squares (PLS) is a powerful multivariate statistical tool that has been successfully applied to the quantitative pharmaceutical analysis by using ultraviolet [1–3], near infrared [4, 5], fluorometric [6, 7], Fourier-transform-infrared-attenuated total reflectance [8] and polarographic data [9, 10].

Paracetamol (PRC), acetylsalicylic acid (ASA) and caffeine (CAF) are active principles widely used and frequently combined in pharmaceutical preparations [11]. The simultaneous determination of these three active components and other additional ones can be performed by using thin layer chromatography (TLC) [12], high performance liquid chromatography HPLC [13, 14], and also by spectroscopy, having proposed various numerical methods such as Kalman filtering of ultraviolet data [15], multiple linear regression of the NIR reflectance data [16], multivariate programs using spectrophotometric data such as multicomponents analysis, Simplex and Multic [17], iterative-target transformation factor analysis [18] and stepwise regression method [19]. However, no attempt was made to use PLS for the simultaneous determination of the aforementioned compounds.

The main objective of this paper is to propose the use of a PLS procedure for the simultaneous spectrophotometric determination, using a reduced calibration matrix, of paracetamol, acetylsalicylic acid and caffeine in a series of pharmaceutical preparations with different proportions of these active principles.

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Experimental

Apparatus and software. A Perkin-Elmer Lambda 16 UV-Visible spectrophotometer (Norwalk, USA) equipped with a 10 mm path length quartz cell was employed to obtain the absorbance spectra of samples and standards which are transferred to a J-Camp format and manipulated using a PC 486 computer equipped with the ATI Mattson PLS software package (Madison, USA) which is employed for calibration, validation and prediction.

Reagents and samples. Paracetamol (PRC) and acetylsalicylic acid (ASA) were supplied by GUINAMA, (Valencia, SPAIN), caffeine (CAF) from FLUKA, (Buchs, SWITZERLAND). All this products are USP grade.

Analytical-reagent grade ethanol was obtained from PANREAC (Barcelona, SPAIN).

The following commercial samples were acquired from Spanish pharmacies: Fiorinal Sandóz, capsules with a nominal content of 300 mg PRC, 200 mg ASA, 40 mg CAF and excipient and Neocibalena tablets with 150 mg PRC, 200 mg ASA, 50 mg CAF and excipient (in both cases the value of excipient was not stated).

Additionally to the commercial samples available from the Spanish Pharmacopoeia, three simulated samples were prepared by

mixing exact weights of PRC, ASA and CAF and starch as excipient. The nominal content of each synthetic sample was: i) 250 mg PRC, 250 mg ASA, 30 mg CAF and 127 mg starch, ii) 150 mg PRC, 350 mg ASA, 40 mg CAF and 130 mg starch, and iii) 200 mg PRC, 250 mg ASA, 50 mg CAF and 100 mg starch following the composition of the old preparations produced in Spain by small pharmaceutical laboratories.

General procedure. An appropriate amount of sample, containing between 13.5 and 33 mg of PRC, from 18 to 38.5 mg of ASA and from 2.7 to 5.5 mg CAF, is accurately weighed and dissolved in 100 ml of 20% (v/v) ethanol in water by shaking in an ultrasonic water bath for 15 min. An aliquot (1.5–2.0 ml) of the filtered solution is diluted to 25 ml with the aqueous ethanolic solution and UV spectra recorded between 216 and 300 nm taking absorbance data at 5 nm intervals and using 20% (v/v) ethanol in water as a blank.

For calibration 8 synthetic mixtures of PRC, ASA and CAF at two concentrations levels dissolved in 20% (v/v) ethanol in water were employed (see Table 1). The upper and lower limits of the studied concentrations used for calibration were selected in order to include the maximum levels of the studied active principles in all the pharmaceuticals commercialized in Spain.

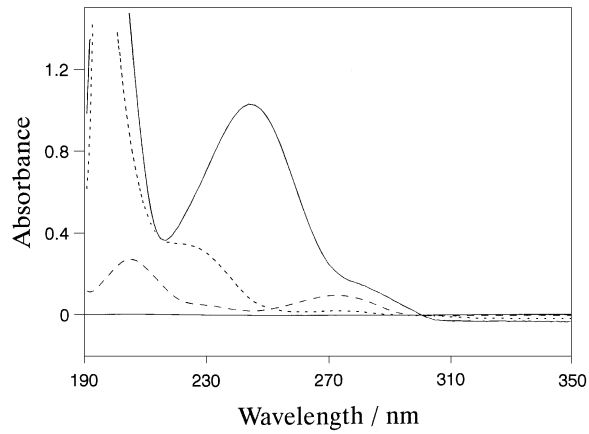

Fig. 1 Zero order absorbance spectra of PRC (15 μg/ml) (——), ASA (10 μg/ml) (— —) and CAF (2 μg/ml) (. . . . .) at the nominal concentration of Fiorinal Sandóz sample. All spectra were obtained in 20% (v/v) ethanol in water

Results and discussion

Ultraviolet spectra of the compounds studied

Figure 1 shows the absorbance spectra of PRC, ASA and CAF at the nominal analytical concentration in the Fiorinal Sandóz sample. PRC presents a high absorption band around 200 nm and a well established band at 245 nm, ASA absorbs around 200 nm and has a shoulder at 224 nm and CAF has two well defined bands at 205 nm and 272 nm. For practical reasons, the bands between 224 nm and 272 nm have been selected for the spectrophotometric determination of these compounds in aqueous ethanolic solutions.

It can be seen that the overlapping between the spectra of the three compounds under study is very high and cannot well resolved by the traditional procedures using univariate calibrations.

PLS matrix calibration

The PLS method requires a carefully experimental design of the standard composition of the calibration set in order to provide good predictions. In this study, the calibration set was generated by using two level factorial design, thus 8 standards were employed to construct the model. As it has indicated before, the upper and lower concentration levels considered were established from the reported concentration of the different pharmaceutical preparations available in the Spanish pharmacopoeia and taking also in consideration the $\pm$ 10% tolerance level accepted in the preparation of this type of pharmaceuticals (see Table 1).

Table 1 Composition of the calibration matrix

Sample No	Content (μg/ml)		
	PRC	ASA	CAF
1	33.20	18.00	2.70
2	13.60	38.16	2.70
3	13.60	18.00	5.50
4	33.20	38.16	2.70
5	33.20	18.00	5.50
6	13.60	38.16	5.50
7	13.60	18.00	2.70
8	33.20	38.16	5.50

PLS treatment of the UV data

From the UV absorbance data between 190 and 350 nm, obtained for the calibration set, data are mean centred, the spectral region defined, and the number of latent variables selected using the minimum value of (PRESS), predicted residual error sum of squares, criterion.

To establish the appropriate conditions for calibration, the effect of the number of working wavelengths and the spectral range on the relative standard error of prediction were studied, selecting in all cases the most adequate number of latent variables of the model for the determination of each one of the compounds studied.

Using a spectral range from 216 to 300 nm, the use of experimental absorbance data obtained at singular intervals from 1 to 10 nm provides a total number of wavelengths employed which varies from 84 to 9 values. As it can be seen in Table 2 the use of a big amount of information reduces the relative standard error of prediction, found by cross validation, for PRC and ASA. However, in the case of caffeine prediction errors are higher than those obtained for the other compounds assayed, and in this latter case, it seems that the use of 17 wavelength values, taken at intervals

Table 2 Effect of the number of working wavelengths on the relative standard error of prediction obtained by cross validation using a spectral range between 216 and 300 nm

Wavelength interval of experimental data	Number of working wavelength	Errors of prediction %			Number of latent variables[a]
		PRC	ASA	CAF	
1	84	0.9	1.6	3.6	(4, 3, 3)
2	42	1.0	1.6	3.2	(4, 3, 3)
3	28	1.0	1.8	3.8	(4, 3, 3)
4	21	1.0	1.5	3.1	(4, 3, 3)
5	17	1.0	1.6	2.4	(4, 3, 3)
6	14	1.6	1.7	2.9	(4, 3, 3)
10	9	1.9	1.9	7.5	(4, 3, 3)

[a] The number of latent variables was chosen for the minimum PRESS value

of 5 nm, provides a minimum error of prediction of the order of 2.4%. The number of latent variables corresponding to each one of the analytes are chosen in order to obtain the minimum value of the sum of squares of the standard error of prediction. It can be seen in Table 2 that in all cases the number of latent variables corresponds to 4 for PRC and 3 for ASA and CAF.

Figure 2 shows, as an example, the evolution of the predicted versus actual concentration values found by cross validation for ASA, taking into consideration 1, 2 and 3 latent variables and, as it can be seen the correlation coefficient found varies from 0.411 to 0.999.

For a selected wavelength interval of 5 nm a series of wavelength ranges, from complete UV spectra to selected ranges covering the main absorbance bands of all the compounds under study, were employed to evaluate the relative standard error of prediction regarding the concentration of PRC, ASA and CAF. As it can be seen from data in Table 3 the consideration of the wavelength range between 190 and 210 nm extremely affects the cross validation, providing errors higher than 10% in the prediction of CAF concentration. On the other hand the use of selected bands a little bit improves the prediction for PRC, ASA, but the best prediction for CAF is obtained using 17 absorbance data from 216 to 300 nm.

For all the wavelength ranges assayed the minimum number of latent variables required for each compound corresponds to 4 for PRC. However, for ASA and CAF the introduction of low wavelengths increases the number of latent variables from 3 to 4.

Table 3 Effect of the spectral range on the relative standard error of prediction of each compound studied, for a wavelength interval of experimental data of 5 nm

Spectral range (nm)	Standard error of prediction %			Number of latent variables[a]
	PRC	ASA	CAF	
(190–350)	1.6	1.5	10.7	(4, 4, 4)
(195–210) (223–232) (235–254) (266–281)	1.5	1.7	12.6	(4, 4, 4)
(223–232) (235–254) (266–281)	0.9	1.5	3.2	(4, 3, 3)
(216–300)	1.0	1.6	2.4	(4, 3, 3)

[a] The number of latent variables was chosen for the minimum PRESS value

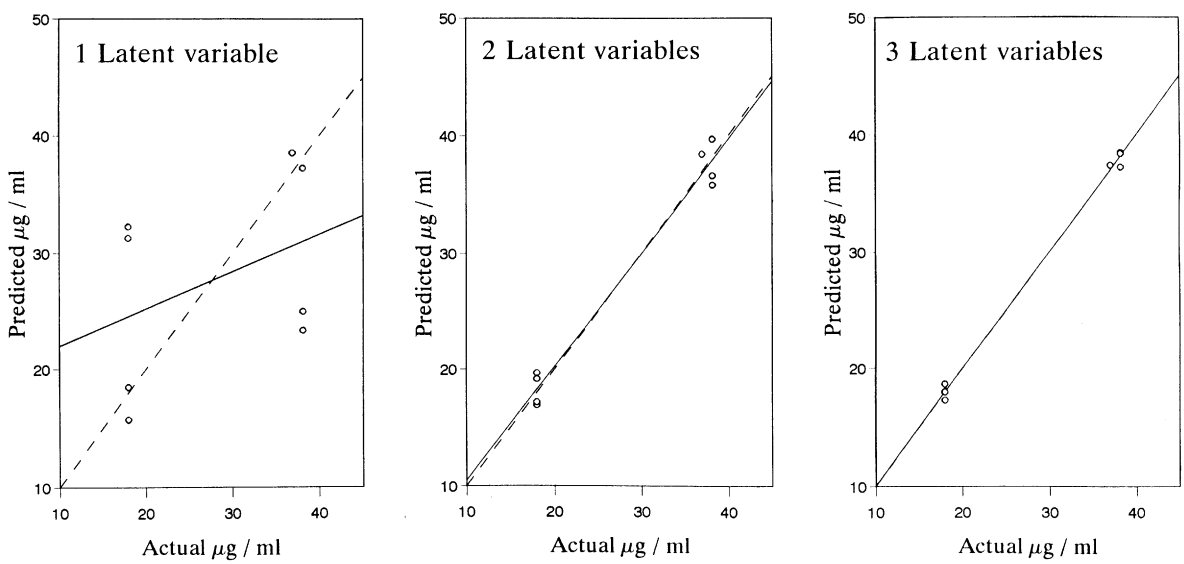

Fig. 2 Evolution of correlation between predicted and actual concentration values of ASA for 1, 2 and 3 latent variables. The solid line represents the experimental correlation line and the dotted line is the ideal fitting

Table 4 Simultaneous PLS-spectrophotometric determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceuticals

Commercial and synthetic samples	Label claim mg/unit			Found[a] mg/unit					
	PRC	ASA	CAF	PRC	E%	ASA	E%[b]	CAF	E%
Fiorinal Sandóz	300	200	40	308 ± 4	+ 2.6	202 ± 1	+ 1	38 ± 1	− 5
Neocibalena	150	200	50	154 ± 1	+ 2.6	202 ± 4	+ 1	49 ± 1	− 2
Sample (1)	250	250	30	258 ± 1	+ 3.2	250 ± 1	0	29 ± 1	− 3.3
Sample (2)	150	350	40	154 ± 1	+ 2.7	354 ± 4	+ 1.1	41 ± 2	+ 2.5
Sample (3)	200	250	50	203 ± 5	+ 1.5	253 ± 4	+ 1.2	49 ± 2	− 2

[a] Values indicated are the average of three independent analysis ± the corresponding standard deviation. The synthetic sample composition corresponds to the concentration of the active principles in old formulations produced by small Spanish pharmaceutical laboratories having employed starch as excipient

[b] E: accuracy error in percentage

Analysis of real and synthetic samples

Commercially available and synthetic samples, described in the experimental section, were analyzed by the recommended procedure and results obtained are summarized in Table 4. It can be seen that accurate values, with errors generally lower than ± 3% were found in all cases, except for the caffeine determination at concentrations of the order of 30 and 40 mg/unit, for which errors of − 3.3% and − 5% were found. On the other hand the precision of these data correspond to variation coefficients from 0.4 to 2.5% for PRC and ASA determinations at concentrations varying from 150 to 350 mg/unit, being the variation coefficients of CAF determination between 2 and 4.9% for concentrations ranges from 30 to 50 mg/unit. Thus it can be concluded that data found by the proposed method are appropriate for practical analysis taking into account the tolerance level established in the USP Pharmacopoeia for this type of drugs which is ± 10% [11].

On the other hand data obtained by PLS are more accurate and precise than those reported for the simultaneous wavelength spectrophotometric quantitation of these three active principles by using multicomponents analysis, Multic and Simplex strategies [17]. Additionally, the use of ethanol, instead of methanol, for sample preparation avoids the toxic effects of the solvent, thus providing a more environmentally friendly methodology.

Conclusions

The PLS spectrophotometric procedure developed for the simultaneous determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceuticals is very fast, requiring only the previous dissolution of the active principles in ethanol 20% (v/v) aqueous solution and sample filtration, using only 17 absorbance data for samples and a reduced number of 8 standard solutions. Additionally, the method developed provides accurate and precise results in a wide range of concentrations which varies from 30 mg/unit of caffeine to 350 mg/unit of acetylsalicylic acid.

For all the aforementioned reasons the procedure developed can be very useful for quality control analysis of pharmaceuticals.

References

1. Bozdogan A, Acar M, Kunt G, Caglar H (1994) Pharmazie 49 H6:457–458
2. Bautista RD, Jimenez F, Jimenez AI, Arias JJ (1993) Talanta 40 (11):1687–1694
3. Bozdogan A, Acar M, Kunt G (1992) Talanta 39(8):977–979
4. Blanco M, Coello J, Iturriaga H, Maspoch S, De la Pezuela C (1993) Talanta 40(11):1671–1676
5. Jouan-Rimbaud D, Walczak B, Massart DL, Last IR, Prebble KA (1995) Anal Chim Acta 304(3):285–295
6. Sanchez-Peña M, Muñoz-de la Peña A, Salinas F, Mahedero MC, Aaron J J (1994) Analyst 119(6):1177–1181
7. Muñoz-de la Peña A, Durna-Meras J Moreno MD, Salinas F, Martinez-Galera M (1995) Fresenius J Anal Chem 351(6): 571–576
8. Hartauer KJ, Guillory JK (1989) Pharm Res 6 (7):608–611
9. Guiberteau-Cabanillas A, Galeano-Diaz T, Espinosa-Mansilla A, Salinas-Lopez F (1994) Talanta 41(11):1821–1832
10. Guiberteau-Cabanillas A, Galeano-Diaz T, Espinosa-Mansilla A, Lopez-de Alba PL, Salinas-Lopez F (1995) Anal Chim Acta 302(1):9–19
11. United States Pharmacopeia XXIII revision (1990) USP Convention Inc Rockville, MD
12. Tomankova H, Vasatova M (1989) Pharmazie 44(3):197–198
13. Das Gupta V (1980) Pharm Sci J 69(1):110–113
14. Shah S, Taylor LT (1989) LC GC 7(4):340 342–344 346
15. Van Loosbroek A, Debets HJG, Coenegracht PMJ (1984) Anal Lett 17(B9):779–792
16. Molt K, Egelkraut M (1988) GIT Fachz Lab 32(12):1311–1313
17. Sala G, Maspoch S, Iturriaga H, Blanco M, Cerda V (1988) Pharm Biomed Anal J 6(6–8):765–772
18. Shen H, Cai S (1993) Gaodeng Xuexiao Huaxue Xuebao 14 (7): 918–921
19. Shen H, Cai S (1994) Fenxi Huaxue 22(7):716–719