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Spectrophotometric determination of pharmaceutical dosages by partial least-squares calibration

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Abstract: Partial least-squares calibration was used for the spectrophotometric determination of the active compound and preservative in a syrup also containing several light absorbing excipients. The calibration matrix was constructed from laboratory-made mixtures of the analytes and excipients and was used to quantify three samples from different batches. The most suitable conditions for quantitation were determined and the results obtained are compared with those provided by HPLC.

Keywords: *Partial least-squares calibration; simultaneous determination; UV spectrophotometry; methylparaben; ebastin.*

Introduction

The robustness, ease of operation, rapid response and low purchase and maintenance costs of UV–vis spectrophotometry make it the technique of choice for control analyses of pharmaceutical preparations since most potential analytes absorb in the ultraviolet region. However, the fact that such preparations are usually in the form of complex mixtures of one or more active compounds, preservatives and excipients, some of which absorb in the same region and result in severe spectral overlap, has so far limited application of the UV spectrophotometric technique to control analyses on pure compounds (raw materials) or very simple preparations, the remainder being addressed by HPLC despite its relative modest throughput and higher analytical costs.

The above picture has been markedly altered by the advent of chemometric procedures for treatment of complex analytical signals and the consolidation of personal computers in the laboratory. The impact of chemometrics on pharmaceutical analysis was recently reviewed [1], showing that spectrophotometric techniques are among those which have benefited most from the inception of chemometric procedures, now making possible the quantitation of mixtures of active compounds and the removal of the spectral interference of any excipients. Derivative spectra are widely used in this context [2] as they alleviate spectral overlap by providing specific wavelengths for measurement of each analyte or implementing the zero-crossing method — both alternatives, though, are basically applicable to binary mixtures only. More severely overlapped spectra of highly complex mixtures call for more powerful computational procedures such as those of least-squares methods [3–7], Kalman filtering [8, 9] and the simplex method [5, 10], all of which rely on statistical fitting of standard spectra to the sample spectrum.

Notwithstanding the wide use of the above procedures, they occasionally result in small, though significant differences between calculated values and those provided by reference analytical procedures. On the other hand, quality of the results is influenced both by the spectral mode used (absorbance or derivative spectra) and by the wavelength range employed to resolve the mixture concerned [11, 12]. This last problem has been studied in depth [13, 14], yet is rather difficult to manage, so the best wavelength range for a given application is usually determined empirically.

In a recent paper [15], the small differences between reference and least-squares regression values were assigned to the seemingly negligible absorbance of the excipients or interactions that give rise to small spectral changes which are not easily detected by the analyst but

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result in deviations in the computational estimation of the concentrations. These problems can be, at least partially, solved by using soft multivariate calibration (SMC) procedures [16]. Thus, a method based on principal component regression (PCR) [15] was used to simultaneously quantify the active compound and preservative in a pharmaceutical preparation containing a fairly high concentration of non-absorbing excipients. Recently, partial least-squares (PLS) methodology was applied to the determination of acetaminophen and caffeine in tablet preparations [17] and of acetaminophen and phenobarbital in suppositories following extraction in ethanol [18]. In both cases, the final solutions contained the two active compounds only.

This paper reports on the use of PLS methodology for the simultaneous determination of the active compound and preservative in a complex preparation also containing high concentrations of several excipients that exhibit considerable absorption in the same spectral region as the analytes.

Theoretical Background

The foundation of PLS methodology is described in detail in the literature [16], so the ensuing discussion is limited to its usage in this work.

Calibration is done by using a set of mixtures of known composition, the spectra of which are recorded over a preset wavelength range. In this way, the absorbance matrix $\mathbf{X}(m,n)$ and the concentration matrix $\mathbf{Y}(m,k)$, where m denotes the number of mixtures used, n that of wavelengths and k that of analytes to be determined, are obtained. The mixtures should be similar to that of the unknown samples so that all possible interactions can be considered in the calibration; also, the analyte concentrations should span the potential concentration range one may encounter in the samples. In the operational mode used in this work, PLS1, the regression is performed on a single analyte, so matrix $\mathbf{Y}$ simplifies to the corresponding concentration vector. PLS methodology breaks down matrices $\mathbf{X}$ and $\mathbf{Y}$ into their latent variables.

$$\mathbf{Y} = \mathbf{F}_y\mathbf{L}_y + \mathbf{E}_y,$$

$$\mathbf{X} = \mathbf{F}_x\mathbf{L}_x + \mathbf{E}_x,$$

where $\mathbf{F}_y(m,a)$ and $\mathbf{F}_x(m,a)$ are the score matrices, $\mathbf{L}_y(a,l)$ and $\mathbf{L}_x(a,n)$ the loading matrices, and $\mathbf{E}_y(m,l)$ and $\mathbf{E}_x(m,n)$ the residual matrices, a being the number of principal components or factors. Unlike principal component regression, where only the absorbance matrix is broken down, PLS methodology breaks down both matrices and takes into account the latent variables of matrix $\mathbf{Y}$ in calculating those of matrix $\mathbf{X}$.

By relating $\mathbf{F}_x$ and $\mathbf{F}_y$ one obtains a diagonal relation matrix $\mathbf{V}$ such that

$$\mathbf{F}_y = \mathbf{F}_x\mathbf{V} + \mathbf{E}.$$

Matrix $\mathbf{V}$ is used in the prediction step to estimate the unknown concentration from the absorbance spectrum $\mathbf{x}_0$ of the sample:

$$\mathbf{y}_0 = \mathbf{x}_0\,(\mathbf{F}'_y\mathbf{X})'\mathbf{V}\mathbf{L}_y.$$

In addition to the concentration value, the programme used provides a measure of the uncertainty associated with the predicted value which is a function of the ratio of the residual variance of the measured spectrum to the average X-residual variance in the calibration objects. The significantly wider confidence ranges than those obtained for the predicted samples used for calibration indicate that the sample is 'different' from the mixture used, which suggests the presence of an overlooked interference, solution turbidity, etc.

Experimental

Reagents and sample

The sample assayed was the pharmaceutical Ebastel® (Laboratorios Almirall, Barcelona), which consists of ebastin {4-diphenylmethoxy-1-[3-(4-*t*-butylbenzoyl)propyl]piperidine} as the active compound, methyl and propyl *p*-hydroxybenzoate as preservatives and various excipients including sweeteners and flavourings. The pharmaceutical is commercially available as syrup.

All samples and reagents were supplied by Laboratorios Almirall and were assayed with no further purification.

Apparatus and software

UV–visible spectra were recorded on a Hewlett–Packard HP 8452A diode array spectrophotometer using HP 89530 MS-DOS UV–vis software. All measurements were

made at 2 nm intervals in the 230–360 nm region, using an integration time of 1 s. Spectra data were processed by using the PLS1 algorithm included in the software package Unscrambler 3.54.

Procedure

The UV spectra of methyl and propyl *p*-hydroxybenzoate are virtually indistinguishable, so they were quantified jointly and the combined result was expressed as methyl *p*-hydroxybenzoate (methylparaben), which was used to prepare the calibration mixtures.

The calibration set was composed of 20 mixtures, prepared in 0.01 M NaOH, 1:1 v/v ethanol–water, with the composition given in Table 1. Prior to PLS processing, absorbance values were mean centred and scaled at unit variance. The calibration model was obtained by cross-validation (20 segments) and the number of factors was chosen as the lowest yielding a prediction error statistically not different to the minimum prediction error [19].

The sample spectrum was recorded from a solution obtained by diluting *ca* 1 g of syrup in 250 ml of 0.01 M NaOH, 1:1 v/v ethanol–water.

The high viscosity of the preparation compelled us to measure the amount of syrup added by weight rather than volume. Weights were then converted into volumes after the specific gravity of the syrup was determined.

Results and Discussion

Figure 1 shows the UV–vis spectrum for the syrup and ebastin, methylparaben and the most highly absorbing excipients at their nominal concentrations. As can be seen, ebastin contributes very little to the overall absorption of the sample; also, the absorption bands of the preservative and the excipients are extensively overlapped.

In order to assess the precision and accuracy one could expect from the syrup analysis we used five initial mixtures that were quantified by using the model constructed from the other 15 samples.

Selection of the number of factors

Appropriate selection of the number of

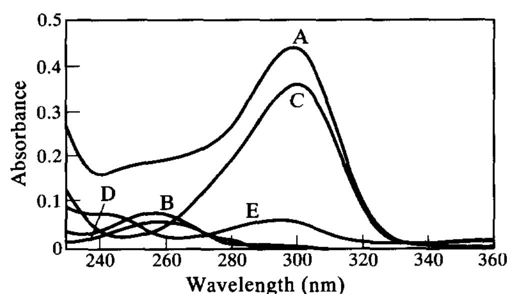

Figure 1
Absorption spectra of dilute syrup (A) and the most strongly absorbing components. B, Ebastin; C, methylparaben; D and E, excipients 3 and 4, respectively.

Table 1
Composition of the different solutions used. [B]: Ebastin; [M]: methylparaben; [E]: excipients. All concentrations are expressed in μg ml^{-1} except those of excipients E5 and E6, which are given in mg ml^{-1}

Sample	[B]	[M]	[E1]	[E2]	[E3]	[E4]	[E5]	[E6]
1	2.11	7.77	11.8	19.2	1.25	6.50	1.06	0.96
2	3.16	8.63	35.4	28.8	1.87	3.90	1.38	0.53
3	4.22	5.17	59.0	9.6	1.25	6.50	0.95	0.48
4	5.27	6.47	35.4	19.2	1.87	5.20	0.92	0.39
5	6.33	3.45	59.0	9.6	1.25	5.20	1.42	0.88
6	7.38	4.31	11.8	28.8	1.87	3.90	1.08	0.53
7	8.44	4.31	35.4	19.2	1.25	3.90	1.03	0.91
8	3.16	7.77	59.0	9.6	1.87	6.50	0.68	0.45
9	5.27	3.45	11.8	28.8	1.25	5.20	1.86	0.73
10	7.38	5.17	65.4	19.2	1.87	3.90	1.10	0.45
11	2.63	7.34	19.4	9.0	0.00	5.20	0.98	0.64
12	3.69	8.25	32.4	18.0	0.00	3.90	1.32	0.54
13	4.74	4.75	19.4	9.0	1.87	0.00	0.95	0.65
14	5.80	6.04	32.4	27.0	1.25	0.00	0.98	0.67
15	6.85	3.89	6.5	18.0	1.87	3.90	0.82	0.89
16	7.91	3.88	19.4	9.0	1.25	6.50	1.68	0.35
17	8.96	4.75	32.4	27.0	1.87	5.20	0.74	0.65
18	2.63	8.25	6.5	18.0	1.87	6.50	1.39	0.41
19	4.74	3.88	32.4	18.0	1.87	6.50	1.39	0.41
20	6.85	5.60	6.5	27.0	1.25	3.90	1.03	0.45

factors to be used to construct the model is a key to achieving correct quantitation in PLS calibrations. The most usual procedure for this purpose involves choosing the number of factors that result in the minimum mean prediction error (MSEP) expressed as

$$\text{MSEP} = \frac{1}{m} \sum (C_{\text{added}} - C_{\text{calc}})^2,$$

where m is the number of mixtures used.

However, this criterion is subject to some constraints since, occasionally, the MSEP does not reach a sharp minimum, but decreases very gradually above a given number of factors. On the other hand, it is calculated from a finite number of samples, so it is error-prone. For these reasons, we chose to use the procedure developed by Haaland and Thomas [19], which involves selecting that model including the smallest number of factors that results in an insignificant difference between the corresponding MSEP and the minimum MSEP. The significance of such a difference is determined by means of the F statistics, $\alpha = 0.25$.

Figures 2 and 3 show the variation of the MSEP as a function of the number of factors for the determination, respectively, of ebastin and methylparaben over various wavelength ranges. A clear minimum is only obtained for ebastin determination when using large wavelength ranges, while flat valleys are always found in modelling methylparaben determination. In the former case, the difference between minimum MSEP and the other MSEP values was found to be significant, and, thus, the number of factors used to build up the model coincided with that of the minimum. In the other cases, the significance criteria proposed by Haaland and Thomas proved to be very useful in order to choose a stable model.

The number of factors required for correct quantification of ebastin exceeds that for methylparaben, which is consistent with its smaller contribution to the mixed spectrum. Using a narrower wavelength range for both compounds reduced the number of factors required to construct the model.

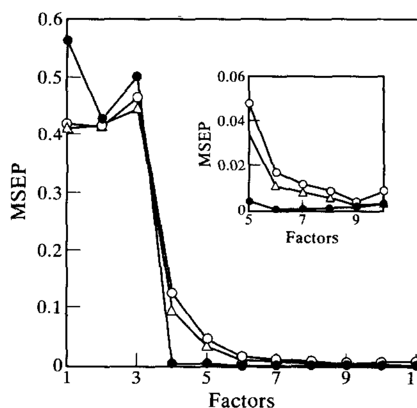

Figure 2
Variation of the MSEP as a function of the number of factors used for calibration of ebastin. (○) 230–330 nm; (△) 230–320 nm; (●) 234–276 nm.

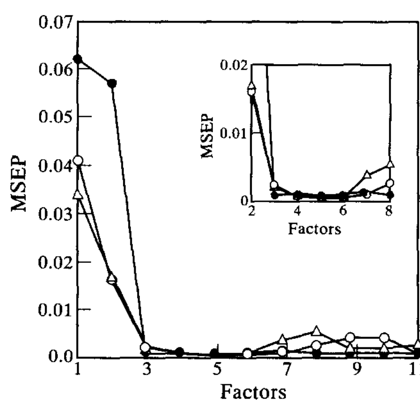

Figure 3
Variation of the MSEP as a function of the number of factors used for calibration of methylparaben. (○) 230–330 nm; (△) 230–320 nm; (●) 260–330 nm.

Wavelength range

PLS procedures are designated to be full-spectrum computational procedures; however, using highly noisy, scarcely informative wavelengths detracts from precision. This can be lessened by using a large number of mixtures to build the calibration matrix or, more inexpensively, by discarding particularly noisy wavelengths. Using this last choice in UV–vis spectrophotometry is quite sensible as the pure spectra of the analytes are very often available and the positions of their bands are not usually greatly affected by the presence of the excipients, so one can predict which spectral region in the sample spectrum will contain the information relevant to the analyte.

Table 2 lists the results obtained in the quantitation of the five mixtures that made up the test set using the selected model for the three different wavelength range studies. As can be seen, both analytes are accurately determined in any case, however the precision of the calculation is considerably affected by the wavelength range used.

Table 2
Results and confidence intervals (μg ml^{-1}) obtained using different wavelength ranges in the PSL1 quantitation of five mixtures using the other 15 to construct the calibration matrix

		Found		
			Ebastin	
Mixture	Added	230–330 nm[a]	230–320 nm[a]	234–276 nm[b]
3	4.22	4.23 ± 0.1	4.23 ± 0.07	4.22 ± 0.03
5	6.33	6.35 ± 0.09	6.34 ± 0.06	6.35 ± 0.03
6	7.38	7.44 ± 0.09	7.45 ± 0.07	7.43 ± 0.02
8	3.16	3.05 ± 0.16	3.05 ± 0.11	3.08 ± 0.04
20	6.85	7.02 ± 0.14	7.03 ± 0.09	6.96 ± 0.02
			Methylparaben	
Mixture	Added	230–330 nm[c]	230–320 nm[c]	260–330 nm[d]
3	5.17	5.14 ± 0.03	5.16 ± 0.03	5.15 ± 0.05
5	3.45	3.41 ± 0.03	3.41 ± 0.04	3.44 ± 0.03
6	4.31	4.30 ± 0.04	4.32 ± 0.04	4.28 ± 0.04
8	7.77	7.75 ± 0.05	7.74 ± 0.04	7.85 ± 0.04
20	5.60	5.65 ± 0.03	5.66 ± 0.02	5.58 ± 0.03

Factors used to construct each model: a, 9; b, 7; c, 4; d, 3.

Table 3
Determination of ebastin and methylparaben in three different batches of Ebastel. The PLS1 confidence interval was 0.01 g l^{-1} in every case. HPLC values are average values for each batch as obtained by the manufacturer's quality control laboratory

	Ebastin[a] (g l^{-1})		Methylparaben[b] (g l^{-1})	
Batch	234–276 nm	HPLC	230–320 nm	HPLC
1	0.97, 0.99, 0.98	1.00	1.45, 1.47, 1.46	1.39
2	0.97, 0.98, 0.98	0.97	1.47, 1.47, 1.47	1.42
3	1.03, 1.03, 1.03	1.03	1.48, 1.48, 1.48	1.47

Factors used to construct the model: a, 7; b, 4.

Under the experimental conditions used in this work, ebastin shows an absorption band centred at 256 nm and virtually no absorption above 280 nm. By using the whole spectrum (230–330 nm) one obtains concentration values that are quite consistent with the actual concentrations, but the computations are not too precise, which reflects in a relatively wide confidence interval. In reviewing the residual variance of each wavelength it was seen that those corresponding to wavelengths above 324 nm were significantly higher than the rest, which is not surprising taking into account that the absorbance was virtually nil at such wavelengths (i.e. they consisted almost solely of noise). The results obtained by using the truncated range (230–320 nm) were essentially similar, but their precision was substantially higher. The best precision was obtained by applying the PLS procedure over the wavelength of its absorption band (234–276 nm).

The precision of methylparaben quantitation was not affected by the wavelength range, since, even though its absorption intensity peaks at 294 nm, it exhibits significant absorbance throughout the wavelength range.

Analysis of Ebastel

Once we had checked that quantifying both components in Ebastel was feasible, the 20 mixtures were used to construct the calibration matrix and their content in the pharmaceutical was determined. The number of factors needed to build up the model coincided with those found before, just proving that a stable model has been reached. To test the reliability of the procedure, we chose samples from three different manufactured batches and analysed them in triplicate. The results obtained are shown in Table 3 alongside those provided by the HPLC technique. The stated values for methylparaben are the sums of the two esters,

which were determined separately by HPLC and are expressed as the methyl ester.

As can be seen, the ebastin concentrations obtained by using the two methods are quite consistent, whereas the methylparaben contents determined for the first and second batches were somewhat higher than the HPLC values.

The small differences encountered in the methylparaben content can be ascribed to intrinsic differences between the two methods (namely separate determinations in HPLC and a single, joint determination on the assumption that both components have the same absorptivity coefficient in UV–vis spectrophotometry). The small divergence between the two sets of values is thus acceptable, particularly if the proportions of the two esters varies from batch to batch. On the other hand, the spectrophotometric value can be considered a good estimate for the overall content.

Conclusions

Control analyses on pharmaceutical preparations by use of UV–vis spectrophotometry and PLS calibration has proved to be a valid alternative to HPLC, even with such highly complex samples as that assayed in this work.

Compared to multicomponent analysis methods based on multiple linear regression, PLS calibration is more robust and reliable, and enables quantitation even in those cases where extensive spectral overlap is involved. In addition, it is much less sensitive to the particular wavelengths used for the analysis and furnishes criteria to discard those that mainly contain noise and thus contribute little or no relevant analytical information.

Best precision is found when all the wavelengths used mainly contain information about the analyte, however, it does not seem sensible or convenient to use very narrow wavelength ranges since they can result in loss of overall information on the sample.

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