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Rapid and Simple Method for Simultaneous Determination of Escin and Diethylamine Salicylate in Pharmaceutical Preparations by Partial Least-Squares Multivariate Calibration

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Summary. A partial least-squares calibration (PLS) method has been developed for simultaneous quantitative determination of escin (*ES*) and diethylamine salicylate (*DAS*) in pharmaceutical preparations. The resolution of these mixtures has been accomplished without prior separation or derivatisation, by using partial least-squares (PLS-2) regression analysis of electronic absorption spectral data. The experimental calibration matrix was constructed with 9 samples. The concentration ranges considered were 10, 20, 30 (*ES*) and 40, 50, 60 (*DAS*) μg cm^{-3}. The absorbances were recorded between 200 and 325 nm every 5 nm. Proposed method was compared with conventional spectrophotometric method. The results show that PLS-2 is a simple, rapid, and accurate method applied to the determination of these compounds in pharmaceuticals.

Keywords. Spectrophotometry; Multivariate calibration; Escin; Diethylamine salicylate.

Introduction

Escin (*ES*) is a mixture of triterpene glycosides naturally occuring mostly in horsechestnut (*Aesculus hippocastanum L.*) seeds. The pharmacological properties of this saponin are well known and it is widely used in the prevention and treatment of various peripheral vascular disorders. Diethylamine salicylate (*DAS*) is a topical analgesic with high penetrative value commonly prescribed for the pains of fibrosis, muscular and arthritic rheumatism. Several methods have been reported for the determination of *ES* [1–8] and *DAS* [9–14] alone or in other combinations. But, no standard analytical procedures exist for analysis of these components simultaneously. Ultraviolet visible spectrophotometry is widely used in different fields of chemical analysis on account of its rapidity, simplicity, applicability, and relativity low cost. However, as is usual in other spectroscopic techniques, when analyzing mixtures of

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components that show overlapping spectra, they often cannot be successfully resolved and it requires resolution by using sample clean-up and separation procedures.

Recently, to solve these problems, multivariate calibration methods are being widely used for biomedical and pharmaceutical analyses [15–21]. We have recently reported the simultaneous determination of mixtures of some components in pharmaceutical preparations and food products by PLS regression using the PLS-2 formalism [22–25]. However, the potential of this method for the assay of *ES* and *DAS* in gel preparations has not been studied in the past. Therefore, the present paper describes the use of PLS-2 method in the simultaneous analysis of these components in gel preparations.

Results and Discussion

The UV spectra of *ES* ($10\,\mu g\,cm^{-3}$) and *DAS* ($50\,\mu g\,cm^{-3}$) in ethyl alcohol are shown in Fig. 1. As can be seen in this figure, *ES* and *DAS* show high overlapping and the extinction coefficients of *DAS* are larger than that of the minor component *ES* in the useful spectral range. This fact makes a difficult task for the resolution of these binary mixtures for absorption spectroscopic techniques. Therefore, PLS-2 method was applied to resolution of mixtures of two components in this study.

According to PLS-2 algoritm, PLS-2 calibration equations for two drugs were found as follows:

$$\begin{aligned} C_{ES} = {} & 35.3889 - 45.20A_1 + 12.49A_2 - 39.71A_3 + 132.09A_4 - 71.52A_5 + 93.09A_6 \\ & + 33.58A_7 + 29.33A_8 + 17.46A_9 + 3.41A_{10} - 3.90A_{11} - 0.29A_{12} - 7.10A_{13} \\ & - 10.78A_{14} - 21.27A_{15} - 27.12A_{16} - 41.42A_{17} - 51.54A_{18} - 47.51A_{19} \\ & - 44.60A_{20} - 45.01A_{21} - 23.73A_{22} - 8.94A_{23} - 6.77A_{24} - 21.48A_{25} \end{aligned}$$

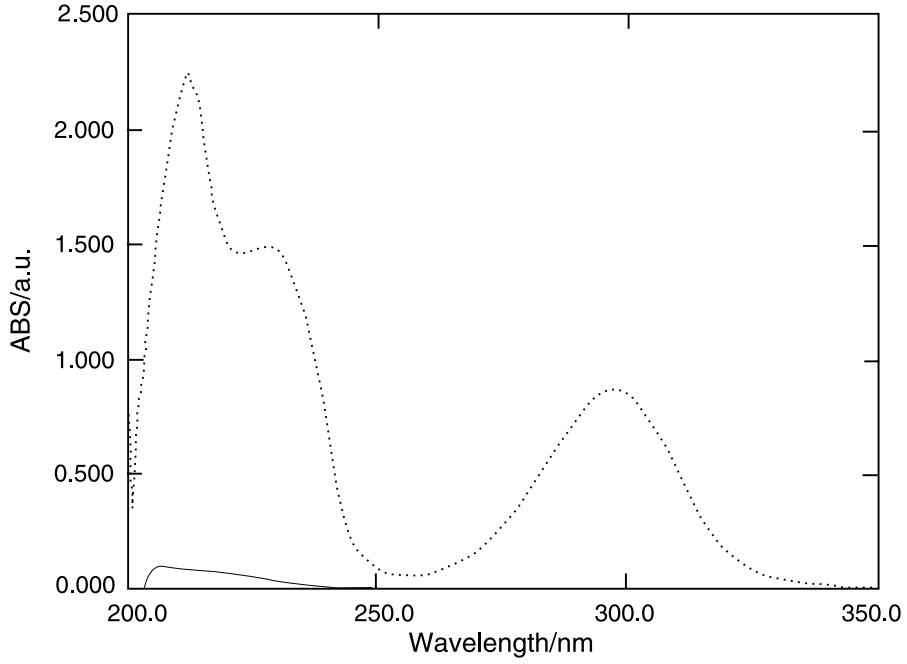

Fig. 1. Spectra of *ES* (——) $10\,\mu g\,cm^{-3}$ and *DAS* (·····) $50\,\mu g\,cm^{-3}$ in ethyl alcohol

$$C_{DAS} = -14.6675 + 7.29A_1 - 0.36A_2 + 10.94A_3 - 12.62A_4 + 16.26A_5 - 7.79A_6 - 0.24A_7 - 1.42A_8 - 1.72A_9 - 0.26A_{10} + 0.63A_{11} + 0.13A_{12} + 0.19A_{13} + 1.93A_{14} + 3.75A_{15} + 5.10A_{16} + 7.68A_{17} + 9.67A_{18} + 9.46A_{19} + 9.03A_{20} + 8.75A_{21} + 5.05A_{22} + 2.26A_{23} + 1.45A_{24} + 3.23A_{25}$$

where C_{ES} and C_{DAS} are the concentrations of *ES* and *DAS*, and $A_{1,}\ A_{2,} \ldots A_{25}$ are the absorbance values measured at 25 points between 200 and 325 nm for samples. These absorbance values were replaced in the above equation and the amount of each drug in the samples was computed.

The results obtained by three-factor PLS-2 solution in both placebo and real (Reparil-gel) samples are given in Tables 1 and 2. Table 1 shows the results for all

Table 1. Validation results[a]

Week	*ES* (μg cm^{-3})		*DAS* (μg cm^{-3})		Within-day[b]	*ES* (μg cm^{-3})		*DAS* (μg cm^{-3})	
	Added	Found	Added	Found		Added	Found	Added	Found
1	10.00	12.40	40.00	40.11	1	10.00	12.05	40.00	39.11
1	20.00	20.00	50.00	50.01	1	20.00	20.03	50.00	50.29
1	30.00	30.57	60.00	59.12	1	30.00	31.94	60.00	58.75
2	10.00	10.33	40.00	40.14	2	10.00	11.00	40.00	39.54
2	20.00	20.02	50.00	50.04	2	20.00	19.84	50.00	48.82
2	30.00	27.48	60.00	53.48	2	30.00	26.27	60.00	59.51
3	10.00	9.13	40.00	40.09	3	10.00	11.25	40.00	44.30
3	20.00	19.98	50.00	49.92	3	20.00	19.91	50.00	49.57
3	30.00	27.48	60.00	53.48	3	30.00	28.73	60.00	58.77
4	10.00	9.29	40.00	37.03					
4	20.00	19.96	50.00	49.74					
4	30.00	29.83	60.00	58.30					
Recovery%	99.14		98.06			103.5		99.91	
RMSD	1.62		2.15			1.70		1.64	
REP%	8.10		4.30			8.50		3.28	

[a] $RMSD = \left[\frac{1}{m}\sum_1^m \left(c_{\text{act}} - c_{\text{pred}}\right)^2\right]^{\frac{1}{2}}$; $\text{REP}\% = \frac{100}{\bar{c}}\left[\frac{1}{m}\sum_1^m \left(c_{\text{act}} - c_{\text{pred}}\right)^2\right]^{\frac{1}{2}}$, $\bar{c}$ is the avarage component concentration in the m mixtures; [b] three different levels of each compound analysed three times (1, 2, 3) within-day

Table 2. Results of the determination of active components in Reparil-gel preparations[a]

Sample	ES		*DAS*	
	Found (mg $\cdot$ g^{-1})	Recovery %	Found (mg $\cdot$ g^{-1})	Recovery %
1	0.90	90.00	5.03	100.60
2	1.07	107.00	4.93	98.60
3	1.08	108.00	5.07	101.40
4	0.94	94.00	5.15	103.00
5	1.13	113.00	4.95	99.00
Mean value $\pm$ *SD*	1.02 ± 0.10		5.03 ± 0.09	

[a] Amount labeled (g/100) *ES* (1) and *DAS* (5)

Table 3. Comparison of results in two method analysis of commercial formulation Reparil-gel

$n_1 = n_2 = 5$	*ES* (g/100 g)		*DAS* (g/100 g)	
	PLS-2	Comparison Method	PLS-2	Comparison Method
Mean ± *SD*	1.02 ± 0.10	1.10 ± 0.12	5.03 ± 0.09	4.92 ± 0.07
t test of significance		1.15		2.18
		$t_8^{0.05} = 2.31$		

the components studied in the validation set. The analyses were performed in four consecutive weeks and three times a day, in order to evaluate inter- and intra-assay accuracy and precision. As regards the results provided by PLS-2 on the validation set, good recoveries were obtained for *ES* and *DAS*.

Table 2 shows the results obtained by applying PLS-2 analyses to Reparil-gel samples. As can be assumed from this table, the recoveries can be considered as reasonably good in view of the usual limits of 90–110% established by regulatory agencies [26, 27].

The PLS-2 method proposed in this study was compared with known spectrophotometric methods. It is seen from Table 3, *t*-test shows that there is no significant difference between the mean values obtained from PLS-2 and the methods given in literature [1].

Conclusions

PLS-2 method for the simultaneous assay of *ES* and *DAS* has been developed. Prior sample preparation and extraction procedures are not required as compared with conventional spectrophotometric methods. Therefore PLS-2 is a simple, inexpensive and very fast procedure for the analysis *ES* and *DAS* in gel preparations and can be proposed for a routine analysis and quality control of pharmaceutical formulations.

Methods

Partial Least-Squares

In this study, the absorbances ($A_1, A_2, \ldots A_{25}$) measured at the selected wavelengths were used as *X*-variables. The corresponding *ES* and *DAS* concentrations (C_{ES}, C_{DAS}) were used as *Y*-variables. Thus, for *n* samples, two matrices are formed and their inter-relationship must be calculated. The model can then be used to analyse samples of unknown composition.

Calibration with use of the PLS-2 method is done by decomposition of both the concentration and absorbance matrices into latent variables (Eqs. (1) and (2)) where F_y is the latent concentration matrix with *n* rows (mixtures) and *d* columns (number of dimensions), L_y represents the concentration loading matrix with *d* rows and *m* columns (number of components), F_x is the *nxd* latent absorbance matrix, L_x is the *dxp* absorbance loading matrix (where *p* is the wavelength), and E_y and E_x are error matrices that have the same dimensions as the original

concentration matrix (*nxm*) and absorbance matrix (*nxp*), respectively.

$$Y = F_y L_y + E_y \tag{1}$$

$$X = F_x L_x + E_x \tag{2}$$

Relating the latent variable matrix from Eq. (1) to that in Eq. (2), one obtains a diagonal regression matrix V according to Eq. (3) where E_e is an error matrix.

$$F_y = F_x \cdot V + E_e \tag{3}$$

The matrix V is used in the prediction step for estimation of the unknown concentrations from the absorbance spectrum X_0 of the sample as follows from Eq. (4).

$$Y_0 = X_0(F_y{}'X)'VL_y \tag{4}$$

There the matrices F_y and L_y are known from calibration.

Experimental

Apparatus

The absorbance measurements were performed with a Unicam UV2-100 UV-Vis Spectrophotometer, using 1.00 cm quartz cells. PLS-2 was applied with an in-house program written according to the algorithm given by *Martens* and *Naes* [28].

Reagents and Samples

The *ES*, *DAS*, and Reparil-gel were supplied from Abdi İbrahim İlaç San. A. Ş. Stock solutions of *ES* and *DAS* with 1000 μg cm^{-3} in ethyl alcohol were prepared.

Procedure

a) Calibration set for PLS-2: The calibration set was generated by a 3-level full factorial design [29] and thus 9 samples were used to construct the model. The levels selected were for *ES* 10, 20, 30 μg cm^{-3} and for *DAS* 40, 50, 60 μg cm^{-3} in this design. By this choice of design, possible interactions can be accounted for. Table 4 shows the composition of the binary mixtures used in the calibration set.

Calibration solutions were prepared by dissolving *ES* and *DAS* in ethyl alcohol. The absorbances were recorded in 1.00 cm cuvettes between 200 and 325 nm every 5 nm. In this way a signal concentration matrix which could be subjected to data analysis was obtained. The calibration samples were measured in random order, so that experimental errors due to drift were not introduced.

Table 4. Composition of the calibration matrix for *ES* and *DAS* (μg cm^{-3})

Standards	*ES*	*DAS*
1	10	40
2	10	50
3	10	60
4	20	40
5	20	50
6	20	60
7	30	40
8	30	50
9	30	60

b) Validation set: A validation set was built with three different levels of each compound, also containing a single level of excipients (100%). All the components were similar to those in the calibration sets (Table 1). Three groups of nine samples were prepared and analysed in three times a day and four consecutive weeks. This procedure allowed us to assess intra-and inter-assay accuracy and precision.

c) Commercial sample preparation: In analyzing the active components of Reparil-gel, an accurately weighed amount of 0.1 g gel was transferred to a 100 cm^3 volumetric flask, dissolved by shaking for 10 min with 50 cm^3 ethyl alcohol and diluted to the mark with ethyl alcohol. The solution was filtered and the absorbances of this solution were recorded between 200 and 325 nm.

Comparison Method

ES in gel preparations was analysed according to the DAB-8 procedure [1]. In this procedure, a sample was extracted with ether and a mixture of chloroform:propanol (100:40). After adding a reagent of $FeCl_3$–*Ac*OH–H_2SO_4, absorbance values of complexes were measured at 540 nm.

For the analysis of *DAS* in gel preparations, the absorbance value of *DAS* in ethyl alcohol at 297 nm was used. As can be seen from Fig. 1 escin in ethyl alcohol has no absorption between 250–350 nm.

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