DETERMINATION OF TERNARY MIXTURES OF DEXTROMETHORPHAN HYDROBROMIDE, PSEUDOEPHEDRINE HYDROCHLORIDE AND TRIPROLIDINE HYDROCHLORIDE IN SYRUPS BY PARTIAL LEAST-SQUARES MULTIVARIATE CALIBRATION

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ABSTRACT

PLS-2, a partial least-squares multivariate calibration, was used for the determination of dextromethorphan hydrobromide (DEX), pseudoephedrine hydrochloride (PSE) and triprolidine hydrochloride (TRP) in syrup samples. The method allows the simultaneous quantification of the analytes that the closely overlapping spectral bands are not efficiently solved. The calibration set consisting of 27 samples with 60, 80, 100 $\mu\text{g mL}^{-1}$ of DEX, 180, 240, 300 $\mu\text{g mL}^{-1}$ of PSE and 8, 10, 12 $\mu\text{g mL}^{-1}$ of TRP was used for calibration matrix. The absorbances were recorded between 230 and 320 nm every 5 nm. The accuracy and precision of the method have been determined and the method has been validated by synthetic mixtures.

Keywords: Dextromethorphan hydrobromide, pseudoephedrine hydrochloride, triprolidine hydrochloride, multivariate calibration.

INTRODUCTION

The combination of TRP with PSE and DEX is used to relieve the symptoms of colds and flu, such as nasal congestion, cough and runny nose,

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where these are associated with a dry cough.

The simultaneous analysis of these components has been performed by a few methods, including UV spectrophotometry /1,2/ and HPLC /3/. In spite of widespread use of HPLC and other conventional spectrophotometric methods, these methods have some disadvantages such as expensive instruments and extra pure solvents (i.e. HPLC). On the other hand, the simultaneous determination of three compounds with overlapped spectra is not possible using conventional spectrophotometric methods. So, developing alternative analytical techniques is very important for pharmaceutical samples because of the demand for simple, cheap and fast analytical methods.

Multivariate calibration methods are receiving considerable attention as quantitative analysis techniques. Recently, the PLS method has been applied for analysis of pharmaceutical and biomedical samples containing two or more components yielding overlapping spectra /4-14/.

In this study, we present a simple spectrophotometric method for simultaneous determination of DEX, PSE and TRP in Actidem[®] syrup pharmaceutical formulations using PLS regression of PLS-2 formalism. In order to appraise the results obtained by PLS-2, a derivative spectrophotometric method was used and the results of two methods were compared.

EXPERIMENTAL

Materials

The DEX was supplied from Ilsan İlaç Drug Industry (Istanbul, Turkey), PSE was supplied from Plantafarma Drug Industry (Istanbul, Turkey), TRP and Actidem[®] syrup were obtained from Glaxo Smith Kline Drug Industry (Istanbul, Turkey) . Other chemicals were of analytical reagent grade, and redistilled water was used throughout the work. The absorbance measurements were performed with an Agilent 8453 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 /15/.

Stock solutions of DEX (1000 $\mu\text{g mL}^{-1}$), PSE (3000 $\mu\text{g mL}^{-1}$) and TRP (100 $\mu\text{g mL}^{-1}$) were prepared by dissolving in water. To obtain standard solutions for preparation of calibration matrix, stock solutions were diluted

with water to 10 ml containing 1mL of 1M H₂SO₄.

Calibration set for PLS-2

For preparation of calibration set, 27 proposed sample mixtures from a full-factorial three-level design for the three analytes were used /16/. The selected levels were 60, 80, 100 µg mL⁻¹ for DEX, 180, 240, 300 µg mL⁻¹ for PSE and 8, 10, 12 µg mL⁻¹ for TRP in this design (Table 1). The absorbances were recorded between 230 and 320 nm every 5 nm.

Table 1
Composition of the calibration matrix for DEX, PSE and TRP (µg mL⁻¹)

Standards	DEX	PSE	TRP
1	60	180	8
2	60	240	8
3	60	300	8
4	80	180	8
5	80	240	8
6	80	300	8
7	100	180	8
8	100	240	8
9	100	300	8
10	60	180	10
11	60	240	10
12	60	300	10
13	80	180	10
14	80	240	10
15	80	300	10
16	100	180	10
17	100	240	10
18	100	300	10
19	60	180	12
20	60	240	12
21	60	300	12
22	80	180	12
23	80	240	12

24	80	300	12
25	100	180	12
26	100	240	12
27	100	300	12

Validation set

The validation set was prepared with three different levels of DEX, PSE and TRP, also a single level of excipients (100%). Three groups of all samples were prepared and analysed three times a day for four consecutive weeks. This procedure allowed us to assess intra- and inter-assay accuracy and precision.

Commercial sample preparation

In analyzing the active components of Actidem[®] syrup /1/, an accurately weighed amount of 10 mL of syrup to a 100 ml separating funnel containing 30 mL of water. The solution was made alkaline by the addition of 2 mL of 5 M NaOH and then extracted with three 20 mL volumes of chloroform. The extracts were combined in a second separating funnel and then extracted with 20, 15 and 10 mL of 0.1 M H₂SO₄. The acidic extracts were combined and diluted to 50 mL in a volumetric flask. A 2 volume of the acidic extract was transferred to a 10 mL volumetric flask containing 0.6 mL of 1M H₂SO₄ and the contents of flask were diluted to 10 mL with water. The absorbances of this solution were recorded between 230 and 320 nm.

RESULTS AND DISCUSSION

The absorption spectra of the DEX, PSE and TRP are presented in Figure 1. As can be seen, the spectra were obviously overlapped and these compounds cannot be determined in the presence of each other by conventional spectrophotometric techniques.

In the present work, DEX, PSE and TRP were analysed in syrup samples by using PLS calibration. The PLS calibration is done by the composition of both concentration and absorbance matrix into latent variables, $A = TP' + E$ and $C = UQ' + F$. The PLS calculations also give an auxiliary matrix W (PLS

weights) which expresses the correlation between U and A and use to calculate T . The vector b is given as $b = W(P'W)^{-1}Q$.

Fig. 1: Spectra of DEX (—) $80 \mu\text{g mL}^{-1}$, PSE (···) $240 \mu\text{g mL}^{-1}$ and TRP(– · –) $10 \mu\text{g mL}^{-1}$ in $0.1 \text{ M H}_2\text{SO}_4$

According to the PLS-2 algorithm, equations for three drugs were found as follows:

$$C_{\text{DEX}} = 14.0060 + 8.23A_1 + 10.10A_2 + 14.34A_3 + 20.46A_4 + 23.50A_5 + 20.93A_6 + 15.59A_7 + 5.48A_8 + 3.24A_9 + 3.16A_{10} + 2.84A_{11} + 1.72A_{12} + 1.14A_{13} + 0.96A_{14} + 0.79A_{15} + 1.09A_{16} + 0.67A_{17}$$

$$C_{\text{PSE}} = 62.1346 + 22.19A_1 + 27.22A_2 + 30.66A_3 + 55.15A_4 + 63.34A_5 + 56.41A_6 + 42.02A_7 + 14.78A_8 + 8.73A_9 + 8.51A_{10} + 7.65A_{11} + 4.63A_{12} + 3.08A_{13} + 2.59A_{14} + 2.13A_{15} + 2.94A_{16} + 1.79A_{17}$$

$$C_{\text{TRP}} = -0.3093 + 1.29A_1 + 1.58A_2 + 2.24A_3 + 3.20A_4 + 3.67A_5 + 3.27A_6 + 2.44A_7 + 0.86A_8 + 0.51A_9 + 0.49A_{10} + 0.44A_{11} + 0.27A_{12} + 0.18A_{13} + 0.15A_{14} + 0.12A_{15} + 0.17A_{16} + 0.10A_{17}$$

where C_{DEX} , C_{PSE} and C_{TRP} are the concentrations of DEX, PSE and TRP, $A_1, A_2, \dots, A_{25}$ are the absorbance values measured at 17 points between 230 and 320 nm for samples. These absorbance values were replaced in the above equation and the amount of each drugs in samples was computed.

Table 2
Validation results

Week	DEX ($\mu\text{g mL}^{-1}$)			PSE ($\mu\text{g mL}^{-1}$)			TRP ($\mu\text{g mL}^{-1}$)			Within-day ^a			DEX ($\mu\text{g mL}^{-1}$)			PSE ($\mu\text{g mL}^{-1}$)			TRP ($\mu\text{g mL}^{-1}$)		
	Added	Found		Added	Found		Added	Found		Added	Found		Added	Found		Added	Found		Added	Found	
1	60.0	65.3		180.0	193.88		8.0	7.47		1			60.0	64.71		180.0	192.71		8.0	7.39	
1	80.0	78.25		240.0	234.62		10.0	9.7		1			80.0	76.16		240.0	227.91		10.00	9.33	
1	100.0	92.38		300.0	278.21		12.0	12.1		1			100.0	91.67		300.0	276.39		12.00	11.99	
2	60.0	63.42		180.0	188.48		8.0	7.19		2			60.0	63.43		180.0	188.51		8.0	7.16	
2	80.0	76.01		240.0	227.0		10.0	9.3		2			80.0	75.90		240.0	226.92		10.00	9.23	
2	100.0	92.5		300.0	272.92		12.0	11.88		2			100.0	90.47		300.0	272.16		12.00	11.78	
3	60.0	63.27		180.0	188.75		8.0	7.16		3			60.0	64.86		180.0	193.18		8.0	7.32	
3	80.0	75.62		240.0	225.67		10.0	9.26		3			80.0	78.51		240.0	235.39		10.00	9.74	
3	100.0	90.76		300.0	272.12		12.0	11.79		3			100.0	92.66		300.0	278.44		12.00	12.13	
4	60.0	62.56		180.0	186.46		8.0	7.03													
4	80.0	76.04		240.0	228.10		10.0	9.33													
4	100.0	90.12		300.0	270.0		12.0	11.68													
Recovery%	97.14			97.25			94.31						98.27			98.03			95.22		
RMSE	5.83			17.76			0.60						5.84			16.81			0.53		
REP%	7.28			7.4			6.0						7.3			7.0			5.3		

* $\text{RMSE} = \left[\frac{1}{m} \sum_{i=1}^m (c_{\text{act}} - c_{\text{pred}})^2 \right]^{1/2}$; $\% \text{REP} = \frac{100}{\bar{c}} \left[\frac{1}{m} \sum_{i=1}^m (c_{\text{act}} - c_{\text{pred}})^2 \right]^{1/2}$, $\bar{c}$ is the average component in the m mixtures.

a=Three different levels of each compound analysed in three times (1, 2, 3) within-day.

Table 3
Comparison of results in two method analysis of commercial formulation (Actidem®)*

n ₁ =n ₂ =5	DEX (mg/mL)		PSE (mg/mL)		TRP (mg/mL)	
	PLS-2	Derivative Spectrophotometry	PLS-2	Derivative Spectrophotometry	PLS-2	Derivative Spectrophotometry
1	2.02	1.83	6.05	5.69	0.25	0.24
2	1.95	1.85	5.86	5.79	0.24	0.22
3	2.1	1.94	6.29	5.88	0.27	0.23
4	2.06	1.99	6.18	6.12	0.26	0.26
5	2.09	2.03	6.28	6.08	0.26	0.27
mean ± SD	2.04±0.06	1.93±0.09	6.13±0.18	5.91±0.18	0.26±0.01	0.24±0.02
Recovery%	102.0	96.5	104.6	98.5	96.2	96.0
t test of significance		2.29		1.91		2.0
F test of Significance		2.25		1.0		4.0

$$t_{8}^{0.05} = 2.31 \quad F_{4,4}^{0.05} = 6.39$$

* Amount labeled (mg/mL) DEX (2), PSE (6) and TRP (0.25).

In order to test inter- and intra-assay accuracy and precision of the proposed method, placebo samples including the different concentrations of DEX, PSE and TRP carried out in four consecutive weeks and three times a day (Table 2). As regards the results provided by PLS-2 on the validation set, good recoveries were obtained for DEX, PSE and TRP (Relative error of prediction (REP%) values not exceeded 15%).

The PLS-2 method proposed in this study was compared with the derivative spectrophotometric method. In this method, PSE was determined from the second derivative peak to peak method at 257-260 nm and, DEX and TRP were determined from the first derivative zero-crossing method at 291 and 303 nm, respectively /17,18/. All solutions were prepared as in PLS-2 method. The assay results obtained by both methods were statistically compared at the 5% level. As shown in Table 3 there were no significant differences between the mean values and precisions of the two methods and the recoveries can be considered as reasonably good in view of the usual limits of 90-110% established by regulatory agencies /19,20/.

CONCLUSION

PLS-2 calibration was employed to analyse the ternary mixtures of DEX, PSE and TRP in the syrup samples. The results obtained by PLS-2 were compared with the derivative spectrophotometric method. This rapid, easy and cheap method can be proposed as a viable alternative to the routine analysis methods.

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