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Simultaneous spectrophotometric determination of lidocaine and hydrocortisone acetate in pharmaceutical preparations by multivariate calibration methods

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Abstract

The multivariate calibration method, partial least square regression (PLS) was applied for the simultaneous spectrophotometric determination of Lidocaine (LID) and Hydrocortisone acetate (HCA) in their mixtures. The parameters of chemometric technique were optimized and the proposed method was validated with synthetic samples and applied to analyze these drugs in pharmaceutical products with good accuracy and precision. The results were compared with the HPLC method. The squares of correlation coefficients (R^2) for predicted LID and HCA with the proposed method in test samples were 0.9970 and 0.9964, respectively. The relative standard deviations for commercial products were less than 1%.

Keywords: Lidocaine; Hydrocortisone acetate; Spectrophotometric; Multivariate calibration.

1. Introduction

Lidocaine **(**2–(diethylamino)–N– (2, 6–dimethylphenyl) acetamide) (LID) is used commonly in combination with Hydrocortisone acetate (11β)–21–(acetyloxy)–11, 17–dihyroxypregn–4–ene –3, 20–dione (HCA). The mixture of these two drugs is used in treatment for anorectic region disease. Therefore, the determination of these drugs is a frequent analytical problem in quality control of the pharmaceutical industries. The two drugs studied in this work show a strong overlap between their absorption spectra. Hence, their simultaneous determination using conventional spectrophotometric techniques would be hard. Mainly, HPLC is used to resolve a complex mixture of these drugs [1-4].

Partial Least Squares regression (PLS) was originally developed by Wold [5], and the use of PLS method for chemical analysis was pioneered by Wold et al [6-7]. A detailed description on the mathematical principles of the PLS algorithms have been reported by Martens et al [8].

In recent years multivariate calibration methods have been used to resolve mixtures of two or more compounds with similar spectra characteristics. In most cases, multivariate methods for evaluation of spectroscopic data have more advantages as simplicity and cheapness. These

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methods have been applied for determination of drugs [9-12], because HPLC methods and conventional spectroscopic methods were slow, expensive and complex.

The aim of this paper is to investigate the ability of PLS method for quantifying binary mixtures of Lidocaine and Hydrocortisone acetate without prior separation and to apply the optimized method in pharmaceutical preparations.

2. Experimental

2.1. Materials

Commercial samples were bought from pharmacies. Analytical grade LID and HCA were obtained from Aburaihan pharmaceutical company (Tehran, Iran). All other chemical and solvents were of analytical reagent grade.

2.2 Apparatus and software

A Bio-Tek 922 scanning spectrophotometer connected to PC fitted data software and Shimadzu HPLC 10A were used for all the measurements and treatment of data. The Matlab 7.1 and Unscrambler 9.1 Software were used for the statistical treatment of the data and application of various multivariate methods.

2.3. Procedure

The 1500 mg L^{-1} LID and 250 mg/L HCA stock solutions were prepared by dissolving accurately weighed amounts of finely powdered pure LID and HCA in methanol. The calibration and test mixtures prepared by mixing these two solutions with different ratios.

For real sample (Lidocaine-H ointment), 0.25 g of the ointment was weighed accurately and transferred to a 50 mL volumetric flask and dissolved with about 40 mL of methanol using an ultrasonic bath for 45 minutes, diluted to volume with methanol and filtered. 2 mL of this solution was diluted to 25 mL with methanol. The absorption spectra between 190 and 350nm against methanol were recorded for all solutions. The stability of LID, HCA and commercial sample solutions were checked for 4 h, and the UV–Vis absorption spectra of all sample solutions were found to be stable for this period of time. It is also to be noted that the simultaneous determination of the aforementioned drugs with the proposed method can be carried out in less than 1h. Commercial Lidocaine-H ointments were also analyzed using HPLC method [4].

3. Results and discussion

3.1. UV-VIS spectra of LID and HCA

In Fig. 1, the absorption spectra of LID and HCA solutions in methanol recorded between 190 and 300 nm are shown. The two drugs studied show a strong overlap in their absorption spectra, and the conventional spectrophotometric methods can not be applied for resolving this mixture.

3.2. Experimental design of sample sets

Calibration and test sets for two component systems were designed according to factorial principle. Solutions containing drug concentrations in the range 0.0-60.0 mg L^{-1} for LID and 0.0-6.0 mg L^{-1} for HCA were produced by dilution of the stock solutions. A five-level factorial design was used to produce a full set of 25 samples. A three-level set was derived to produce a

calibration set of nine samples, with the remaining 16 samples used for an independent test set [13]. The compositions of the used calibration and test sets are summarized in Table 1 and Table 2, respectively.

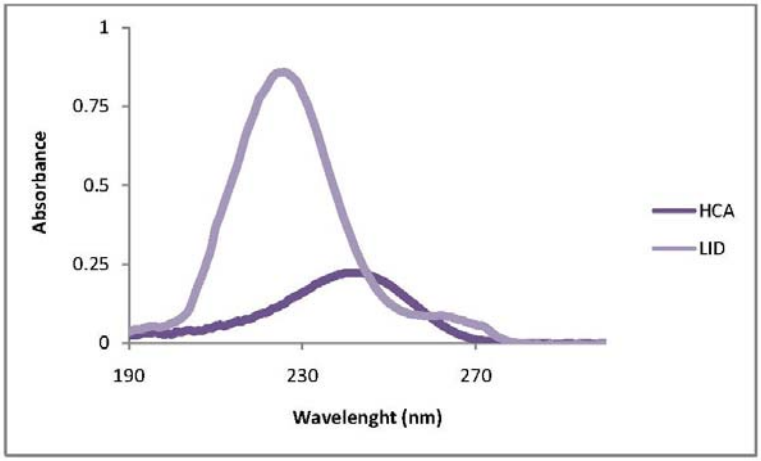

Fig. 1 The UV-Vis absorption spectra LID and HCA standard solutions.

Table 1
Calibration set composition.

Standard	LID (mg L^{-1})	HCA (mg L^{-1})
C_1	0.0	0.0
C_2	0.0	3.0
C_3	0.0	6.0
C_4	30.0	3.0
C_5	30.0	6.0
C_6	60.0	3.0
C_7	60.0	6.0
C_8	30.0	0.0
C_9	60.0	0.0

3.3. Selection of the optimum number of factors and the spectral region

To select the correct number of factors in the PLS algorithm, a cross-validation method, leaving out one sample at a time, was employed. The predicted concentrations $\widehat{Xij}(k)$ of the compounds in each I sample, obtained with k factors, were compared with the already known concentrations Xij and the root mean squared error of cross validation (RMSECV) was calculated for each of the k factor levels as follows:

$$RMSECV(k) = \sqrt{\frac{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(\widehat{Xij}(k) - Xij\right)^2}{I}}$$

The optional value for k is the level that yields the smallest $RMSECV(k)$ value. To check the selected factor number, the external validation method was also used by simply computing the root mean squared error of predication (RMSEP) for L objects in test set for each of the k factor levels as follows:

$$RMSEP(k) = \sqrt{\frac{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(\widehat{Zij}(k) - Zij\right)^2}{L}}$$

where Zij is the known concentration in the test set and $\widehat{Zij}(k)$ is predicted for concentrations with k factors [9]. This showed that the external test set validation and the internal cross-validation indicated about the same number of factors. To select the spectral region, all of the above steps used repeatedly and the spectral region that lead to the lowest values of RMSEP was selected [9]. The optimal number of factors, RMSEP and RMSECV values and optimum spectral regions obtained by PLS-1 and PLS-2 algorithms are summarized in Table 3. The proposed PLS-1 and PLS-2 calibration models were evaluated by prediction of drug concentrations in their own designed calibration set. Recoveries values were between 95.42 and 105.44% for LID and between 97.40 and 101.77% for HCA.

Table 2
Test set composition

Sample	LID (mg L^{-1})	HCA (mg L^{-1})
T_1	0.0	1.5
T_2	0.0	4.5
T_3	15.0	1.5
T_4	15.0	3.0
T_5	15.0	4.5
T_6	15.0	6.0
T_7	30.0	1.5
T_8	30.0	4.5
T_9	45.0	1.5
T_{10}	45.0	3.0
T_{11}	45.0	4.5
T_{12}	45.0	6.0
T_{13}	60.0	1.5
T_{14}	60.0	3.0
T_{15}	15.0	0.0
T_{16}	45.0	0.0

Table 3
Optimal number of factors, RMSEP and RMSECV values.

		Factor No.	RMSEP	Factor No.	RMSECV
PLS-1	LID	3	1.1248	3	1.1344
	HCA	3	0.1005	3	0.1010
PLS-2	LID	3	1.1248	3	1.1344
	HCA	3	0.1035	3	0.1045

3.4. Statistical parameters for the optimized models

Using the internal validation in their own designed calibration set, the following statistical parameters have been obtained: a) The values of root mean squared error of calibration (RMSEC), which is an indication of the average error in the analysis for each component. b) The

square of correlation coefficients (R^2), which is an indication of the quality of the straight line that fits the data.

Table 4
Statistical parameters of the model optimized.

	PLS-1		PLS-2	
	RMSEC	R^2	RMSEC	R^2
LID	1.3228	0.9971	1.3229	0.9971
HCA	0.0737	0.9991	0.0666	0.9992

In Table 4, the results obtained for these parameters by PLS-1 and PLS-2 are shown. We can see that the R^2 values are in all cases very near to 1 which is an indication of similarity between predicted and known values. On the other hand, in general terms, the statistical parameters obtained by PLS-1 and PLS-2 are good.

3.5. External validation of PLS-1 and PLS-2 calibration models

16 synthetic mixtures in test set were predicted by applying PLS-1 and PLS-2 methods. The square of correlation coefficients (R^2) and recovery range for these synthetic solutions are summarized in Table 5. Satisfactory values are obtained in most of mixtures analyzed by the methods. Limits of detection (LOD's) were calculated as three times of standard error of estimation (SEE) values [9]. LODs of 4.2093 mg/L for LID, 0.2346 mg/L for HAC, 4.2093 mg/L for LID, and 0.2115 mg/L for HCA were obtained in PLS-1 and PLS-2 models, respectively.

Table 5
Recovery range and R^2 for synthetic mixtures in test set.

	LID		HCA	
	%Rec. range	R^2	%Rec. range	R^2
PLS-1	95.42-104.9	0.9970	96.42-103.34	0.9966
PLS-2	95.42-104.9	0.9970	95.20-102.64	0.9964

3.6. Analysis of commercial samples

Two commercial Lidocaine-H ointments produced by the Aburaihan and Sinadaru factories were analyzed using two methods: the proposed spectrophotometric method and HPLC method [4]. Results are summarized in Table 6. As can be seen, satisfactory results were obtained in all cases by the proposed methods. Statistical parameters obtained by replicate analysis, for the commercial ointments with proposed PLS-1, PLS-2 and HPLC standard methods are also given in Table 6. These results show that the precision of the proposed methods is better than HPLC method.

Table 6
Analysis of commercial ointments.

	Aburaihan		Sinadaru	
	LID±%RSD	HCA±%RSD	LID±%RSD	HCA±%RSD
Declared contents*	5	0.5	5	0.5
PLS-1 Results	4.89±0.73	0.55±0.95	4.62±0.88	0.54±0.59
PLS-2 Results	4.89±0.72	0.56±0.98	4.62±0.88	0.56±0.91
HPLC Results	4.76±2.72	0.52±1.25	4.60±2.63	0.51±1.22

*Results presented as grams per 100 grams of ointment.

4. Conclusion

A comparative study of the use of PLS-1 and PLS-2 for the resolution and simultaneous determination of LID and HCA in a binary mixture has been accomplished, showing that this method provide a clear example of the high resolving power of these techniques. In several terms, similar results were obtained for these two drugs in both synthetic and commercial applications by PLS-1 and PLS-2. The results obtained confirm the suitability of the proposed method for accurate and precise analysis of Lidocaine and Hydrocortisone acetate in pharmaceutical preparations. These methods were applied directly to the commercial preparations without previous treatment. Also no expensive (dissolution and injection) laboratory technique is needed. In addition the proposed methods are suitable for application without interference of the excipients.

References

[1] L. Zivanovic, M. Zecevic, S. Markovic, S. Petrovic, I. Ivanovic, J. Chromatogr. A 1088 (2005) 182-186.
[2] S. Liawruangrath, B. Liawruangrath, P. Piobool, J. Pharm. Biomed. Anal. 26 (2001) 865-872.
[3] R. Hajkova, P. Solich, J. Dvorak, J. Sicha, J. Pharm. Biomed. Anal. 32 (2003) 921-927.
[4] J. M. Lemus Gallego, J. Perez Arroyo, Anal. Bioanal. Chem. 374 (2002) 282-288.
[5] H. Wold, in Research Papers in Statistics, John Wiley, New York, 1996.
[6] H. Wold, Systems under Indirect Observations, North-Holland, Amesterdam, 1982.
[7] S. Wold, H. Martens, H. Wold, The Multivariate Calibration Problem in Chemistry solved by PLS, Matrix Pencils (Lecture Notes in Mathematics), Springer, Heidelberg, 1992.
[8] H. Martens, T. Naes, Multivariate Calibration, John Wiley, Chichester, 1992.
[9] K. Kargosha, A.H.M. Sarrafi, J. Pharm. Biomed. Anal. 26 (2001) 273-279.
[10] O. Aksu, A. Bozdogan, G. Kunt, Anal. Lett. 39 (2006) 751-761.
[11] G. M. Hadad, A. El.-Gindy, W. M. M. Mahmoud, Spectrochim. Acta, Part A 70 (2008) 655-663.
[12] M.R. Khoshayand, H. Abdollahi, M. Shariatpanahi, A. Saadatfard, A. Mohammadi, Spectrochim. Acta, Part A 70 (2008) 491-499.
[13] C. K. Bayane, I. B. Rubin, Exprimental Design, VCH, New York, 1986.