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Determination of the minor component bromhexine in cotrimoxazole-containing tablets by absorption spectrophotometry and partial least-squares (PLS-1) multivariate calibration

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Abstract

The mucolitic bromhexine [*N*-(2-amino-3,5-dibromobenzyl)-*N*-methylcyclohexylamine] has been determined in cotrimoxazole-containing tablets by partial least-squares (PLS-1) multivariate of spectrophotometric calibration data in the spectral range 310-350 nm. In the studied commercial tablets, cotrimoxazole is present in large excess (ca. 100:1 in weight) with respect to bromhexine, and a high degree of spectral overlapping exists among bromhexine and cotrimoxazole components. However, the obtained recoveries are reasonably good with the presently discussed technique.

Keywords: Spectrophotometry; Multivariate calibration; Bromhexine; Cotrimoxazole tablets

1. Introduction

Bromhexine [*N*-(2-amino-3,5-dibromobenzyl)-*N*-methylcyclohexylamine] (BRM) is a mucolitic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. It has been shown to enhance the penetration of erythromycin into bronchial secretions and to improve the symptoms and ventilatory function in elderly patients being treated for acute exacerbations of chronic bronchopulmonary disease [1].

Bromhexine has been determined spectrophotometrically after derivatization with different reagents [2–4]. Recently, first-derivative electronic absorption spectroscopy was used for the determination of both BRM and the codeine-related central cough suppressant dextromethorphan [5] (D-3-methoxy-*N*-methylmorphinan) in pharmaceutical tablets [6]. Other methods, such as high performance liquid chromatography (HPLC), [7–9], preparative thin layer chromatography [10], gas-liquid chromatography [11] and atomic absorption spectrometry [12], have been applied for the simultaneous determination of BRM and other drugs in pharmaceutical preparations.

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Several commercial tablets exist in which BRM is present as a minor component in mixtures with the antibiotic cotrimoxazole, which in turn consists of a 5:1 w/w mixture of sulphamethoxazole (SMZ) and trimethoprim (TMP). In these preparations, the usual ratio of cotrimoxazole to BRM is 100:1 (in weight).

Multivariate calibration methods [13–15] applied to both absorptive and emissive spectral data as well as to electrochemical signals are being increasingly used for the analysis of complex pharmaceutical mixtures [16–22]. They have the advantage of using full spectral information and allow for a rapid determination of mixture components; often with no need for prior separation or sample pre-treatment. For routine pharmaceutical quality control programs, they are more rapid and of lower cost when compared to HPLC. We have recently reported the resolution of mixtures of antiepileptics [23], antihistaminics [24], antibiotics [25,26] and cough-suppressants [27] in pharmaceutical preparations by partial least squares (PLS) regression using the PLS-1 formalism. The latter is one of the simplest multivariate methods and can be performed with easily accessible statistical software. An additional advantage of robust multivariate methods such as PLS, is that calibration can be performed by ignoring the concentrations of all other components except the analyte of interest. This makes these methods especially appealing for the determination of the active components in samples whose components may show absorption spectra which are severely overlapped with those from the analytes.

In the present report, we discuss the possibility of determining bromhexine in cotrimoxazole-containing tablets by applying electronic absorption measurements together with PLS-1 multivariate calibration.

Table 1
Composition of the calibration set for PLS-1 analysis

Calibration sample	SMZ[a] (mg)	TMP (mg l^{-1})	BRM (mg l^{-1})
1	28.1	225	15.0
2	37.5	300	20.0
3	46.9	375	25.0
4	56.2	450	30.0
5	65.6	525	35.0
6	75.0	600	40.0
7	84.4	675	45.0
8	93.7	750	50.0
9	20.9	156	15.0
10	26.2	208	20.0
11	32.8	264	25.0
12	39.3	316	30.0
13	45.9	368	35.0
14	52.5	420	40.0
15	59.1	472	45.0
16	65.6	528	50.0

[a] Weight of SMZ added to 25 ml of each calibration sample.

2. Experimental

2.1. Apparatus

Electronic absorption measurements were carried out on a Beckman DU-640 spectrophotometer, using 1-cm quartz cells. All spectra were saved in ASCII format and transferred to a PC Pentium 166 microcomputer for subsequent manipulation. PLS-1 was applied with an in-house program written in Visual Basic 5.0 according to Ref. [13].

2.2. Reagents

All experiments were performed with analytical-reagent grade chemicals. Stock solutions of bromhexine hydrochloride (250 mg l^{-1}) and TMP (1000 mg l^{-1}) were prepared by dissolving the compounds in HCl 0.1 N.

2.3. Calibration set

A training set of 16 samples (mixtures of BRM with different levels of cotrimoxazole) were prepared for calibration, with the concentrations of BRM lying in the known linear absorbance–concentration range (Table 1). According to the content of commercial tablets, when the concentrations of BRM lie in the latter range, SMZ does not dissolve completely. Therefore, the calibration samples were prepared by mixing appropriate amounts of the stock solutions of BRM and TMP with the corresponding weight of solid SMZ, sonicating for 30 min and centrifuging. The

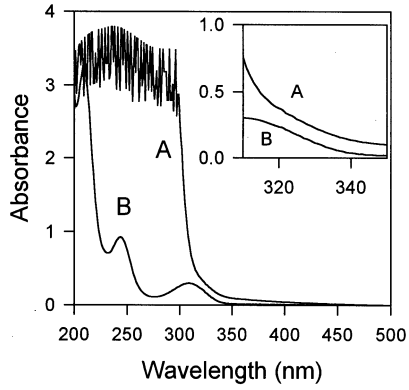

Fig. 1. Electronic absorption spectra of: (A) Bromhexine 30 mg l^{-1} in HCl 0.1 N; and (B) a commercial tablet processed according to Section 2. The insert shows the spectra in the relevant spectral region 310–350 nm.

volumes of both stock solutions, as well as the weight of SMZ, were selected in order to resemble the composition of typical commercial samples.

2.4. Unknown samples

Several synthetic samples were prepared. In one case, they consisted of mixtures of appropriate amounts of the stock solutions and of solid SMZ, as described above. Other synthetic samples were prepared by grounding and mixing solid BRM, SMZ and TMP in relative amounts which were similar to those present in tablets. The obtained solid was then sonicated in HCl 0.1 N for 30 min and centrifuged. The analyzed commercial samples were obtained from Bago Laboratories (Neumobacticel) and Labinca Laboratories (Dosulfin) and were prepared by the following procedure. Ten tablets were ground and mixed in order to determine the average tablet weight. A portion of the obtained solid was treated with 100 ml of HCl 0.1 N, sonicated for 30 min and centrifuged.

2.5. Theory

In the PLS method, the $m \times n$ data matrix $\mathbf{A}$ (m = number of calibration samples; n = number of wavelengths) is decomposed into:

$$\mathbf{A} = T_a B_a \quad (1)$$

where B_a and T_a are the $h \times n$ loading and $m \times h$ scores matrix, respectively; and h is the number of PLS factors. The $m \times l$ (l = number of components) calibration mixture matrix $\mathbf{C}$ is similarly decomposed:

$$\mathbf{C} = T_c B_c \quad (2)$$

where T_c ($m \times h$) and B_c ($h \times l$) are the concentration loading and scores matrixes, respectively. During calibration, the following equation holds:

$$T_c = T_a \mathbf{V} \quad (3)$$

where $\mathbf{V}$ is an $h \times h$ matrix which establishes the internal relation between T_c and T_a. During prediction, the component score is obtained from the unknown spectrum a as $t = B_a a$, and the unknown concentration from $c_i(\text{pred}) = t^t \mathbf{V} b_{c_i}$, where b_{c_i} is the appropriate $h \times 1$ vector associated with the component of interest. Notice that individual components are independently modeled by PLS-1 using an optimum h value for each of them. This method offers the additional advantage of ignoring the concentrations of all other components except i during calibration, provided the former are present and can be adequately modeled.

3. Results and discussion

Fig. 1 shows the electronic absorption spectra of BRM (30 mg l^{-1} in HCl 0.1 N) and the solution obtained after processing a commercial tablet (according to the tablet content, the concentration of BRM is also 30 mg l^{-1}). As can be seen, the regions $\lambda = 200$–310 nm, $\lambda > 350$ nm are rather uninformative. The analysis of the spectra suggests that a convenient working region is 310–350 nm (Fig. 1), although a rather high degree of spectral overlapping between BRM and cotrimoxazole (and presumably other tablet excipients) persists. The usual method for resolving complex mixtures, which can be applied to the present case, is partial least squares analysis (PLS).

Electronic absorption spectra for the samples shown in Table 1 were recorded in the range 310–350 nm and subjected to PLS-1 analysis. For the selection of the optimum number of factors, the cross validation method proposed by Haaland

Table 2
Results obtained by applying PLS-1 analysis to both synthetic and commercial samples of BRM and cotrimoxazole

Sample[a]	SMZ (mg)[c]	TMP (mg l^{-1})	BRM		
			Nominal	Found[b] (mg l^{-1})	Recovery (%)
Synthetic 1	40.8	270	18.0	17.5 (4)	97.2
Synthetic 2	50.6	405	27.0	26.0 (4)	96.3
Synthetic 3	59.2	570	38.0	36.6 (3)	96.3
Synthetic 4	94.4	705	47.0	47.4 (3)	100.8
	(mg)	(mg)		(mg)	
Synthetic 5	600	120	8.0	8.5 (2)	106.2
Synthetic 6	600	120	0.0	0.8 (1)	
Neumobacticel[d]	600	120	8	7.7 (4)	96.3
Dosulfin[d]	800	160	12	12.2 (4)	101.7

[a] Three replicates. See Section 2 on the preparation of synthetic samples.
[b] S.D. in parenthesis.
[c] Amount of solid SMZ added to 25 ml of sample.
[d] The nominal contents of BRM correspond to those reported by the manufacturing laboratories.

and Thomas was used in both cases [13]. Hence, four factors were used for prediction, leading to a squared correlation coefficient (R^2) of 0.997 and a relative error of prediction (REP) of 0.63%. These statistical indicators are given by:

$$R^2 = 1 - \frac{\sum_1^m (c_{act} - c_{pred})^2}{\sum_1^m (c_{act} - \bar{c})^2} \quad (4)$$

$$REP(\%) = \frac{100}{\bar{c}} \left[\frac{1}{m} \sum_1^m (c_{act} - c_{pred})^2 \right]^{1/2} \quad (5)$$

where $\bar{c}$ is the average component concentration in the m calibration mixtures.

Table 2 collects the prediction results for a set of synthetic and commercial samples. As can be seen, PLS-1 yields excellent recoveries in all cases, despite the high degree of overlapping among the spectra of the mixture components.

Finally, it may be noticed that previous studies on mixtures of SMZ and TMP have demonstrated the feasibility of applying PLS-1 for the simultaneous quantitation of these antibiotics in tablets [26]. It would, therefore, be interesting if all three components, SMZ, TMP and BRM could be determined in the presently studied pharmaceuticals. This is not possible from a single calibration due to the different concentration scale of BRM as compared to the other two components. However, by recording absorption spectra of solutions obtained with different dilutions, it is indeed possible to quantitate BRM with the present calibration scheme, and both SMZ and TMP with an already described methodology [26].

4. Conclusions

Antibiotic-mucolitic tablets were studied as regards the content of bromhexine [*N*-(2-amino-3,5-dibromobenzyl)-*N*-methylcyclohexylamine] with the aid of spectrophotometric measurements, together with the multivariate methods of partial least-squares (PLS-1). Although the spectra of bromhexine and cotrimoxazole are severely overlapped, the recoveries are good.

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References

[1] W. Martindale, The Extra Pharmacopeia, Thirtieth ed., The Pharmaceutical Press, London, UK, 1993.
[2] M.I. Santoro, M.M. Dos Santos, J.F. Magalhaes, J. Assoc. Off. Anal. Chem. 67 (1984) 532.
[3] S.S. Zarapker, R.V. Rele, V.J. Doshi, Ind. Drugs 26 (1988) 38.
[4] B. Gala, A. Gomez Hens, D. Perez Bendito, Anal. Lett. 26 (1993) 2607.
[5] J.L. Benn, R. Peck, Drug Saf. 7 (1992) 190.
[6] V. Tantishaiyakul, J. Pharm. Biomed. Anal. 17 (1998) 237.
[7] N. Badwe, A. Garg, D. Eapem, P.D. Sethi, East. Pharm. 38 (1995) 179.
[8] O. Lau, C. Mok, J. Chromatogr. A 693 (1995) 45.
[9] J. Rauha, H. Salomies, M. Aalto, J. Pharm. Biomed. Anal. 15 (1996) 287.
[10] P. Parimoo, P. Umapathi, J. Samuel, East. Pharm. 36 (1993) 125.
[11] E.V. Rao, R.G. Rao, S. Raghuveer, P. Khadgapathi, Analyst 112 (1987) 871.
[12] C. Nerin, A. Garnica, J. Cacho, Anal. Chem. 58 (1986) 2617.
[13] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1193.
[14] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, UK, 1989.
[15] K.R. Beebe, B.R. Kowalski, Anal. Chem. 59 (1989) 1007A.
[16] A. Guiberteau Cabanillas, T. Galeano Díaz, A. Espinosa-Mansilla, F. Salinas Lopez, Talanta 41 (1994) 1821.
[17] A. Espinosa-Mansilla, A Muñoz de la Peña, F. Salinas, M. Martínez Galera, Anal. Chim. Acta 276 (1993) 141.
[18] M. Sánchez Peña, A. Muñoz de la Peña, F. Salinas, M.C. Mahedero, J.J. Aaron, Analyst 119 (1994) 1177.
[19] A. Espinosa-Mansilla, F. Salinas, A. Zamoro, Analyst 119 (1994) 1183.
[20] A. Guiberteau Cabanillas, T. Galeano Díaz, A. Espinosa-Mansilla, P.L. López de Alba, F. Salinas López, Anal. Chim. Acta 302 (1995) 9.
[21] A. Bozdogan, A.M. Acar, G.K. Kunt, Talanta 39 (1992) 977.
[22] Durán Merás, A. Muñoz de la Peña, M.I. Rodriguez Cáceres, F. Salinas López, Talanta 45 (1998) 899.
[23] H.C. Goicoechea, A.C. Olivieri, Talanta 47 (1998) 103.
[24] H.C. Goicoechea, A.C. Olivieri, J. Pharm. Biomed. Anal. 20 (1999) 255.
[25] H.C. Goicoechea, A.C. Olivieri, J. Pharm. Biomed. Anal. 20 (1999) 681.
[26] M.E. Ribone, A.P. Pagani, A.C. Olivieri, Anal. Lett. 32 (1999) 1389.
[27] H.C. Goicoechea, A.C. Olivieri, Talanta 49 (1999) 793.