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Simultaneous multivariate spectrophotometric analysis of paracetamol and minor components (diphenhydramine or phenylpropanolamine) in tablet preparations

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Abstract

The use of multivariate spectrophotometric calibration is reported for the analysis of two decongestant tablets, where paracetamol is the principal component and diphenhydramine or phenylpropanolamine are the minor components. The resolution of these mixtures has been accomplished without prior separation or derivatisation, by using partial least-squares (PLS-1) regression analysis of electronic absorption spectral data. Although the molar ratios of paracetamol to the minor components were 38:1 and 25:1 respectively, the latter have been determined with high accuracy and precision, and with no interference from tablet excipients. PLS is able to take into account small deviations of paracetamol from linearity in the studied concentration range. The application of classical least-squares (CLS) analysis yields unsatisfactory results, due to the low absorbances of the minor components within the range where all components obey Beer's law.

Keywords: Multivariate PLS analysis; Electronic absorption; Decongestant tablets; Paracetamol; Phenylpropanolamine; Diphenydramine

1. Introduction

N-[2-Diphenylmethoxyethyl]-*N*,*N*-dimethylamine (diphenhydramine) is an effective antihistaminic, and has been used for the treatment of motion sickness and extrapyramidal symptoms, as well as an antitussive and night time sleep-aid [1]. Recently, its use has been reported, in combination with other drugs, as antiemetic for the prevention of cisplatin-induced emesis in chemotherapy treatment [2]. It has also been used as sedative in dentistry for children [3] and in local anaesthesia [4]. On the other hand, 2-amino-

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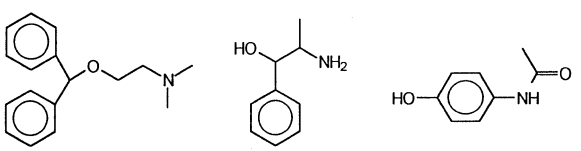
1-phenyl-1-propanol (phenylpropanolamine) is an effective sympathomimetic agent for treating the symptoms of the common cold, such as congestion. However, when excessive doses are given, it produces serious adverse effects on the central nervous and cardiovascular systems [1].

4-Acetamidophenol (paracetamol) is an effective analgesic and antipyretic for the treatment of minor, noninflammatory conditions in patients who are prone to gastric symptoms. Due to the lack of platelet inhibition, it is preferable to aspirin in patients who receive oral anticoagulants, have coagulation disorders, or have a history of peptic ulcer disease [1].



Diphenhydramine Phenylpropanolamine Paracetamol

Phenylpropanolamine, diphenhydramine and paracetamol have been determined both in pharmaceutical mixtures and biological fluids using chromatography [5–12], visible spectrophotometry [13–15] and first and second derivative UV absorption spectroscopy [16,17]. A method for quantitating phenylpropanolamine by electron impact MS has recently been reported [18]. Diphenhydramine has been determined by indirect atomic absorption spectroscopy [19] and by fluorometrically detected flow injection analysis [20]. A mixture of phenylpropanolamine and paracetamol has been resolved using Raman spectra [21] and their first derivative [22].

Multivariate calibration methods [23–25] are being widely used for biomedical and pharmaceutical analyses [26–34]. In conjunction with spectroscopic data (both electronic absorption and fluorescence emission), they offer an interesting alternative to chromatographic techniques. We have recently reported the simultaneous determination of mixtures of urinary metabolites of aspirin [32] and styrene [33] using classical least-squares (CLS) analysis, and also the resolution of a binary mixture of antiepileptics in pharmaceutical preparations by partial least-squares (PLS) regression using the PLS-1 formalism [34]. In the present report, we discuss the possibility of quantitating diphenhydramine and phenylpropanolamine as minor components in two antialergic tablets containing paracetamol as major component, by applying electronic absorption measurements together with multivariate calibration analysis. Since the molar ratios of the minor components to paracetamol are very low, it is necessary to work in a range of concentrations where the absorbances of paracetamol deviate from Beer's law. The results show that PLS-1 allows simultaneous quantitation of the components in both studied mixtures, and also to takes into account small deviations of the major component from linearity.

2. Experimental

2.1. Apparatus

Electronic absorption measurements were carried out on a Beckman DU-640 spectrophotometer, using 1.00 cm quartz cells. All spectra were saved in ASCII format, and transferred to a microcomputer for subsequent manipulation by either CLS or PLS programs. CLS analysis was performed by importing the spectral files from Sigmaplot (version 2.0) and processing them with the standard curve fit package. PLS was applied with an in-house program written in Quick Basic according to the algorithm described in [23].

2.2. Reagents and samples

All experiments were performed with analytical–reagent grade chemicals. Stock solutions of diphenhydramine, phenylpropanolamine and paracetamol were prepared by dissolving the compounds in doubly distilled water. For the analysis of the active components of the antialergic tablets Benadryl Day & Night, 20 tablets of each pharmaceutical were ground and mixed. The amounts corresponding to the equivalent of one tablet were dissolved, in each case, in 1000.0 ml of doubly distilled water. The solutions were then stirred for 15 min, filtered and diluted (1 + 9).

2.3. Solutions for multivariate calibration

2.3.1. Classical least squares method

In order to obtain the calibration matrix for applying CLS analysis, five solutions of each of the pure components diphenhydramine, phenylpropanolamine and paracetamol were prepared, with concentrations in the range $5–15 \times 10^{-5}$ mol dm^{-3}. This range was previously verified to obey Beer's law for each of the studied compounds. For paracetamol, a noticeable departure from linearity is observed beyond a concentration of ca. 1.5×10^{-4} mol dm^{-3}. The absorbence data (in the range 205–300 nm, digitised every 1.0 nm, 96 points per spectrum) were subjected to least-squares analysis in order to obtain the calibration **K** matrix, which was subsequently used for prediction within appropriate wavelength ranges for each component (see below). Unknown mixtures were prepared either from the studied tablet preparations or by mixing known amounts of each stock solution.

2.3.2. Partial least squares method

Two training sets of 16 samples were prepared for calibration, one for each of the studied mixtures. They were generated by a four-level full factorial design. The concentrations of both minor components lay within the known linear absorbence–concentration range, but the concentrations for the principal component were outside this linear range (see below). The levels selected were: paracetamol, 1.90, 2.10, 2.30, 2.50×10^{-4} mol dm^{-3}, and diphenhydramine, 5.0, 6.0, 7.0, 8.0×10^{-6} mol dm^{-3} in one case; and paracetamol, 2.00, 2.20, 2.40, 2.60×10^{-4} mol dm^{-3}, and phenylpropanolamine, 6.0, 8.0, 10.0, 12.0×10^{-6} mol dm^{-3} in the other. The unknowns were prepared as described above.

The spectral regions, intervals and number of points were selected in each case in order to increase the performance of the model. Wavelength selection is a critical step for increasing the predictive ability of PLS analyses, and should ideally eliminate both uninformative and/or highly correlated data. A number of selection methods have been proposed in the literature [35–38]. An alternative involves selecting, by trial and error, all wavelengths within spectral regions which are known to contain useful information. For the minor components studied in the present case, we have selected spectral regions containing their corresponding spectral maxima. In comparison, the results are better than those obtained using the full absorbing ranges of the studied mixtures.

3. Results and discussion

Two currently used decongestant tablets (Benadryl Day & Night, Parke–Davis laboratories) consist of the following combinations:

1. diphenhydramine (25 mg) and paracetamol (500 mg) for night use; and
2. phenylpropanolamine (25 mg) and paracetamol (500 mg) for day use.

As seen in Fig. 1, which shows the absorption spectra of the three compounds mentioned above, the extinction coefficients of paracetamol are larger than those corresponding to the minor components in the useful spectral range. This fact, coupled to the unfavourable paracetamol/minor components molar ratios makes the resolution of both binary mixtures a difficult task for absorption spectroscopic techniques.

A simple and convenient method for resolving mixtures, which could in principle be applied to the present case, is least-squares analysis [23–25]. In the classical (CLS) version, a linear relationship between the absorbence and the component concentrations at each wavelength is assumed. In matrix notation, the model for m calibration standards containing l chemical components with spectra at n digitised wavelengths is given by:

$$\mathbf{A} = \mathbf{CK} + \mathbf{E} \quad (1)$$

where **A** is the $m \times n$ matrix of calibration spectra, **C** is the $m \times l$ matrix of component concentrations, **K** is the $l \times n$ matrix of absorbency–concentration proportionality constants, and **E** is the $m \times n$ matrix of spectral errors or residuals not fit by the model. During calibration, the classical least-squares solution to Eq. (1) is:

$$\mathbf{K} = (\mathbf{C}^t\mathbf{C})^{-1}\mathbf{C}^t\mathbf{A} \quad (2)$$

During prediction, the solution for the vector of unknown component concentrations is:

$$\mathbf{c} = (\mathbf{K}\mathbf{K}^t)^{-1}\mathbf{K}_t\mathbf{a} \quad (3)$$

where **a** is the spectrum of the unknown sample and **K** is from Eq. (2).

Several synthetic mixtures were subjected to the present analysis with unsatisfactory results for the minor components. This could be due to at least two reasons:

1. severe spectral overlapping; and/or
2. poor sensitivity towards the minor components.

These two factors are reflected in the equation for the standard deviation for the refined parameters in a binary mixture:

$$s_{\mathrm{MARG}} = s_{\mathrm{fit}} \times \sqrt{(B^{-1})_{ii}} \quad (4)$$

where $S_{\mathrm{fit}} = \sqrt{\Sigma[a_{\mathrm{pred}} - a_{\mathrm{exp}}]^2 / (N-2)}$, N is the number of experimental data, and $(B^{-1})_{ii}$ are the diagonal elements of the inverse of the matrix **B**, defined by $B_{ij} = [(d\mathbf{a}_{\mathrm{pred}}/dc_i)(d\mathbf{a}_{\mathrm{pred}}/dc_j)^t]$, i.e. $B_{ij} = \Sigma\varepsilon_i\varepsilon_j^t$ (assuming the optical path is 1.00 cm) [34].

We have previously reported that the degree of spectral overlapping can be obtained from the fitting parameter D_i (the so-called parameter dependency) [34]:

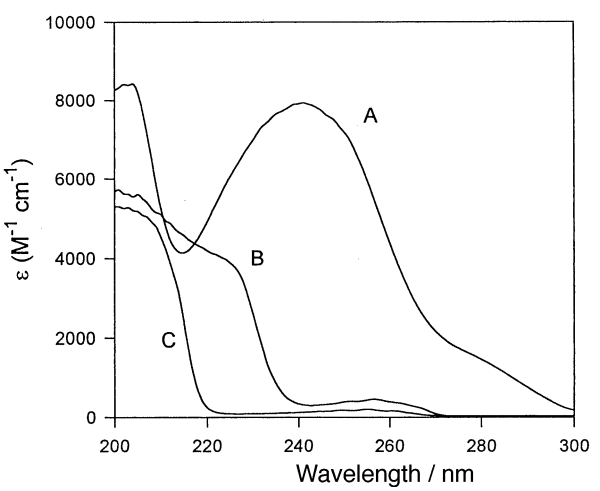


Fig. 1. Electronic absorption spectra in aqueous solution of: A) paracetamol, B) diphenhydramine and C) phenylpropanolamine.

$$\text{Overlap } (\%) = \sqrt{Di} \times 100 \quad (5)$$

In the useful range for quantitating the minor components (210–240 nm, see Fig. 1) we obtained: for paracetamol/phenylpropanolamine, $D_i = 0.17$, Overlap = 41%; and for paracetamol/diphenhydramine, $D_i = 0.60$, Overlap = 77%. On the other hand, when the unknown mixtures were subjected to CLS, s_{fit} was ca. 0.01–0.02, so that the computation of $(B^{-1})_{ii}$ led to values of s_{MARG} which were of the order of $1\text{–}2 \times 10^{-6}$ mol dm^{-3}. Since the concentrations of the minor components were ca. 3×10^{-6} mol dm^{-3} in the unknown mixtures (where paracetamol obeys Beer's law), one may not expect CLS to yield reliable results for the quantitation of phenylpropanolamine or diphenydramine.

One alternative to the above problem is to work in a range of concentrations where the minor components give larger absorbences, although causing the major component (paracetamol) to deviate from linearity (see Section 2). Small degrees of nonlinearity could be taken into account by multivariate factor–base methods such as PLS. Briefly, in the latter method the data matrix **A** is decomposed into:

$$\mathbf{A} = \mathbf{T}_a\mathbf{B}_a \quad (6)$$

where $\mathbf{B}_a$ and $\mathbf{T}_a$ are the $h \times n$ loading and $m \times h$ scores matrix respectively, and h is the number of PLS factors. The matrix **C** of calibration mixtures is similarly decomposed:

$$\mathbf{C} = \mathbf{T}_c\mathbf{B}_c \quad (7)$$

During calibration, the following equations is solved by least-squares:

$$\mathbf{T}_c = \mathbf{T}_a\mathbf{V} \quad (8)$$

where **V** is the $h \times h$ calibration matrix.

During prediction, the component score is obtained from the unknown spectrum **a** as $\mathbf{t} = \mathbf{a}(\mathbf{B}_a)^t$, and the unknown concentration from $c = \mathbf{t}\mathbf{V}\mathbf{b}_c$, where $\mathbf{b}_c$ is the appropriate $h \times 1$ vector associated with the component of interest. Notice that individual components are independently modelled by PLS-1, using an optimum h value for each of them.

Table 1
PLS analysis of paracetamol–diphenhydramine mixtures: statistical parameters for the calibration

Parameter[a]	Paracetamol		Diphenhydramine	
Spectral region (nm)	205–300	205–235 and 250–300	205–300	205–235
RMSD	0.050	0.014	0.283	0.240
r^2	0.945	0.996	0.939	0.955
REP(%)	2.29	0.64	4.35	3.69
Number of factors	1	1	5	3

[a] $RMSD = \left[\frac{1}{m}\Sigma_1^m(c_{act}-c_{pred})^2\right]^{1/2}$; $r^2 = 1-\frac{\Sigma_1^m(c_{act}-c_{pred})^2}{\Sigma_1^m(c_{act}-\bar{c})^2}$, $\bar{c}$ is the average component concentration in the m calibration mixtures;

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

Table 2
PLS analysis of paracetamol–phenylpropanolamine mixtures: statistical parameters for the calibration

Parameter[a]	Paracetamol		Phenylpropanolamine	
Spectral region (nm)	205–300	210–220	205–300	250–300
RMSD	0.019	0.011	0.656	0.408
r^2	0.992	0.998	0.914	0.966
REP(%)	0.81	0.47	7.28	4.53
Number of factors	1	1	4	3

[a] For the definition of parameters, see Table 1.

For the selection of the optimum number of factors, the cross validation method proposed by Haaland and Thomas was used. It involves computing the PRESS (prediction error sum of squares) for each value of h, selecting the one which yields the minimum PRESS (h^*), and computing the following F ratio:

$$F(h) = \frac{\text{PRESS}(h)}{\text{PRESS}(h^*)} \quad (9)$$

where $h < h^*$. The optimum number of factors is suggested to correspond to a probability of less than 75%.

Tables 1 and 2 summarise the results obtained for both analysed mixtures. These tables give the values of the root mean square difference (RMSD), square of the correlation coefficient (r^2) and relative error of prediction (REP), which provide an indication of the quality of fit of all the data. The spectral regions used for calibrating the model are also shown (Tables 1 and 2). Acceptable statistical indicators are obtained on using the PLS-1 method even when full spectral data (i.e. the range 205–300 nm) are used for calibration. However, these indicators are significantly improved by selecting appropriate wavelength ranges for each component (see above). Another noticeable fact is that a single factor is needed to calibrate for paracetamol, in agreement with the fact that this is by far the main component, whereas three factors are needed in the case of the minor constituents (within their corresponding optimum spectral ranges, see Tables 1 and 2).

The above PLS calibration was applied to the prediction of the concentrations of the components in both real and synthetic samples, with the results collected in Table 3 for paracetamol–diphenhydramine and in Table 4 for paracetamol–phenylpropanolamine. As can be appreciated, the recoveries for the major component in the synthetic samples are excellent. On the

other hand, in view of the unfavourably low concentrations, those for the minor components can be regarded as satisfactory. This means that PLS-1 is able both to account for the nonlinearity of the major component paracetamol, and to quantitate with precision the very minor components present in the studied mixtures.

4. Conclusions

The contents of paracetamol–diphenydramine and paracetamol–phenylpropanolamine, usual components of decongestant tablet formulations, were simultaneously determined using electronic absorption measurements, together with PLS-1 multivariate calibration analysis. Synthetic binary mixtures of both combinations as well as commercial tablets were conveniently studied. A related multivariate method, CLS, has been shown to be unreliable in quantitating the studied compounds in their mixtures.

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Table 3
Results obtained by applying PLS-1 analysis to both synthetic and real binary mixtures of paracetamol and diphenhydramine

Mixture	Component	Actual mol $dm^{-3} \times 10^6$	Found mol $dm^{-3} \times 10^6$	Recovery(%)
Synthetic	Paracetamol	250	251	100.4
	Diphenhydramine	7.0	6.9	98.9
Synthetic	Paracetamol	250	246	98.4
	Diphenhydramine	5.0	4.7	93.8
Benadryl night[a]	Paracetamol	232	221	95.3
	Diphenhydramine	5.2	5.3	101.9

[a] Actual concentrations calculated from the content of each component in the tablets, as reported by the manufacturing laboratory.

Table 4
Results obtained by applying PLS-1 analysis to both synthetic and real binary mixtures of paracetamol and phenylpropanolamine

Mixture	Component	Actual mol $dm^{-3} \times 10^6$	Found mol $dm^{-3} \times 10^6$	Recovery (%)
Synthetic	Paracetamol	260	260	100.0
	Phenylpropanolamine	10.0	9.8	98.0
Synthetic	Paracetamol	260	259	99.6
	Phenylpropanolamine	12.0	11.4	95.0
Benadryl Day[a]	Paracetamol	258	260	100.8
	Phenylpropanolamine	10.4	12.2	117.3

[a] Actual concentrations calculated from the content of each component in the tablets, as reported by the manufacturing laboratory.

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