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Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration

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Abstract

The use of multivariate spectrophotometric calibration is presented for the simultaneous determination of the active components of tablets used in the treatment of pulmonary tuberculosis. The resolution of ternary mixtures of rifampicin, isoniazid and pyrazinamide has been accomplished by using partial least squares (PLS-1) regression analysis. Although the components show an important degree of spectral overlap, they have been simultaneously determined with high accuracy and precision, rapidly and with no need of nonaqueous solvents for dissolving the samples. No interference has been observed from the tablet excipients. A comparison is presented with the related multivariate method of classical least squares (CLS) analysis, which is shown to yield less reliable results due to the severe spectral overlap among the studied compounds. This is highlighted in the case of isoniazid, due to the small absorbances measured for this component.

Keywords: Multivariate spectrophotometric analysis; Partial least-squares; Rifampicin; Izoniazid; Pyrazinamide

1. Introduction

Rifampicin (3-[4-methylpiperazinyliminomethyl]-rifamycin SV), isoniazid (isonicotinic acid hydrazide) and pyrazinamide (pyrazinecarboxamide) are used during an initial 2-month intensive treatment of pulmonary tuberculosis [1]. This combination is more effective than each of the drugs alone, on account of the known resistance to antituberculosis drugs [2,3].

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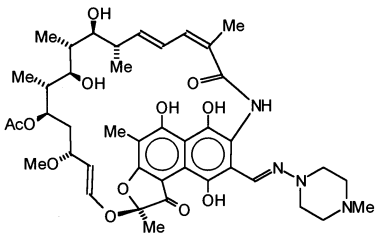
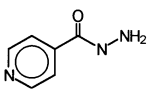
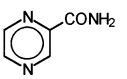


Rifampicin



Isoniazid



Pyrazinamide

Several methods are available for the individual determination of the above compounds, such as spectrophotometry [4–9] and chromatography [10,11]. Rifampicin and isoniazid have been determined in pharmaceutical mixtures using normal- and reversed-phase high-performance liquid chromatography (HPLC) [12,13], classical least squares (CLS) analysis of absorption spectra [14], and two-wavelength spectrophotometry [15]. Recently, rifampicin, isoniazid and pyrazinamide have been determined by high-performance thin-layer chromatography [16] and also by first-derivative UV spectrophotometry [17]. The latter method uses information which is limited to a discrete number of wavelengths in order to solve a system of three equations with three unknowns.

Multivariate calibration methods [18–20] applied to spectral data (both absorptive and emissive) are being increasingly used for biomedical and pharmaceutical analysis [21–25], as a simple and low-cost alternative to chromatography. The former have the advantage of using full spectral information, and are useful for the resolution of complex mixtures of analytes with no need of prior separation or extraction. Full-spectrum methods usually provide significant improvement in precision over methods restricted to a small number of wavelengths, and also make it available the full-spectrum residuals for examination and interpretation. We have recently reported the resolution of mixtures of urinary metabolites of aspirin [26] and styrene [27] using CLS analysis of spectrofluorometric data, and of binary mixtures of antiepileptics [28] and antihistaminics [29] in pharmaceutical preparations, using electronic absorption data and partial least squares (PLS) regression with the PLS-1 formalism.

In the present report, we discuss the possibility of simultaneously quantitating rifampicin, isoniazid and pyrazinamide in antituberculosis tablets, by applying electronic absorption measurements together with multivariate calibration analysis. The results show that PLS-1 regression allows one to accomplish this goal, whereas CLS does not give reliable results.

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 PC 80486 microcomputer for subsequent manipulation by either CLS or PLS programs. CLS

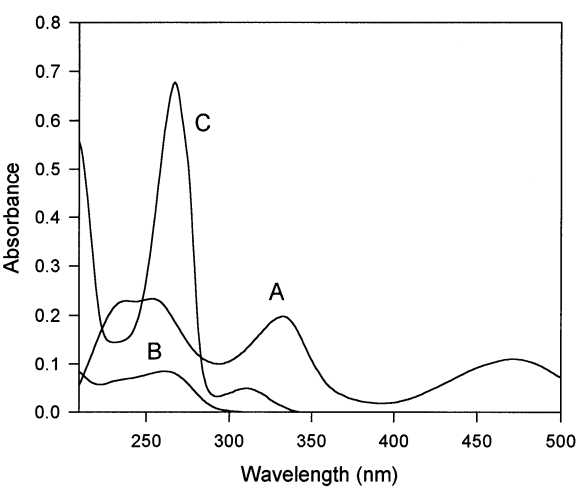


Fig. 1. Electronic absorption spectra of aqueous solutions (pH = 7.0) of: (A) rifampicin (6.80×10^{-6} mol l^{-1}); (B) isoniazid (2.00×10^{-5} mol l^{-1}); and (C) pyrazinamide (1.20×10^{-4} mol l^{-1}). The relative concentrations reflect the typical content of the three drugs in a commercial antituberculosis tablet.

Table 1
Mixture design for the application of PLS-1 analysis

Mixture	Rifampicin	Isoniazid mol $l^{-1} \times 10^6$	Pyrazinamide
M1	1.96	7.82	30.80
M2	1.96	7.82	115.0
M3	1.96	28.90	30.80
M4	1.96	28.90	115.0
M5	7.34	7.82	30.80
M6	7.34	7.82	115.0
M7	7.34	28.90	30.80
M8	7.34	29.90	118.0
M9	0.00	18.30	72.80
M10	9.30	18.30	72.80
M11	4.65	0.00	72.80
M12	4.65	36.60	72.80
M13	4.65	18.30	0.00
M14	4.65	18.30	145.6
M15	4.65	18.30	72.80

analysis was performed by importing the spectral files to 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 ref. [18].

2.2. Reagents

All experiments were performed with analytical-reagent grade chemicals. Stock solutions of rifampicin, isoniazid and pyrazinamide were prepared by dissolving the compounds in doubly distilled water. For the analysis of the active components of an antituberculosis tablet (Richmond Laboratories), the average weight of 20 tablets was obtained. They were then ground and mixed. The amount corresponding to the equivalent to 20 mg of rifampicin was dissolved in 1000 ml of doubly distilled water. The solution was then sonicated for 5 min, filtered, and diluted (1:2). The final pH of the solution should not exceed 7.5, since at higher values the spectrum of rifampicin undergoes a change. In the range 5–7.5, it is possible to work with distilled water, avoiding the use of a phosphate buffer as recommended by [30].

Table 2
Results obtained by applying PLS-1 analysis to the validation set of synthetic ternary mixtures of rifampicin, isoniazid and pyrazinamide

Mixture	Rifampicin[a]	Isoniazid[b] (mol $l^{-1} \times 10^6$)	Pyrazinamide[c]
1	7.06	36.8	157.1
2	7.32	30.6	161.5
3	7.16	37.3	160.2
4	7.01	37.9	161.3
5	6.89	33.0	166.7
6	7.15	37.2	158.7
Average	7.10	35.5	160.9
% Recovery	99.9	104.7	100.4
SD	0.1	2.9	3.3
CV%	2.1	8.7	2.0
REP%	1.90	9.22	2.08

[a] Actual: 7.11×10^{-6} mol l^{-1}.
[b] Actual: 33.9×10^{-6} mol l^{-1}.
[c] Actual: 160.2×10^{-6} mol l^{-1}.

2.3. Solutions for multivariate calibration

2.3.1. CLS method

In order to obtain the calibration matrix for applying CLS analysis, solutions of each of the pure components were prepared, with the concentrations lying in the linear range. The absorbance data (in the range 200–500 nm, digitized every 1.0 nm, 301 points per spectrum) were stored for subsequent application of CLS (see below). Unknown mixtures were prepared either from the studied tablet preparation or by mixing known amounts of each stock solution.

2.3.2. PLS method

A calibration and a validation set of 15 and 6 samples, respectively (in this case mixtures of the studied components) were prepared, with the component concentrations lying in the known linear absorbance–concentration range. The spectral region, interval and number of points for recording the spectra were the same as for CLS. The data were subjected to PLS-1 analysis, selecting an optimum spectral region for each component (see below).

Table 3
Results obtained by applying PLS-1 analysis to three antituberculosis tablets containing rifampicin, isoniazid and pyrazinamide

	Components		
	Rifampicin	Isoniazid	Pyrazinamide
Sample 1 (mg)[a]	165	71	368
Sample 2 (mg)[a]	160	71	386
Sample 3 (mg)[a]	160	75	368
Average of samples (mg)	162	72	374
Amount reported by manufacturing laboratory (mg)	150	75	400
% of the reported content	108	96	94
% of the range accepted by Pharmacopea[b]	90–130	90–110	93–107

[a] Actual concentrations calculated from the content of each component in the tablets, as reported by the manufacturing laboratories.
[b] Ref. [31].

3. Results and discussion

Fig. 1 shows the electronic absorption spectra of the studied compounds. As can be seen, an important degree of spectral overlap occurs in the useful region 200–350 nm. Furthermore, isoniazid presents considerably smaller absorbances than the other two components. A convenient method for resolving mixtures, which can in principle be applied to the present case, is least-squares analysis [18–20]. In the CLS version, a linear relationship between the absorbance 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 $\mathbf{A}$ is the $m \times n$ matrix of calibration spectra, $\mathbf{C}$ is the $m \times l$ matrix of component concentrations, $\mathbf{K}$ is the $l \times n$ matrix of absorbance–concentration proportionality constants, and $\mathbf{E}$ 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 $\mathbf{a}$ is the spectrum of the unknown sample and $\mathbf{K}$ is from Eq. (2). It should be noticed that CLS can be confidently applied provided there is no extensive overlapping between the component spectra. In order to study the effect of spectral overlapping, the following procedure can be adopted. After obtaining the best fit parameters (the unknown component concentrations), CLS programs usually yield the magnitude of the dependency D_i, defined for each of the refined parameters as:

$$D_i = 1 - \frac{\sigma_i^2(\text{marg})}{\sigma_i^2(\text{cond})} = 1 - F_i^{-1} \quad (4)$$

where σ_i^2(marg) and σ_i^2(cond) are the marginal and conditional variances for the parameter c_i, respectively. They are obtained by allowing all parameters to vary in the first case, and fixing all parameters except c_i in the second. Specifically, they are obtained through Eqs. (5) and (6):

$$\sigma_i^2(\text{marg}) = \sigma_{\text{fit}}(\mathbf{B}^{-1})_{ii} \quad (5)$$

$$\sigma_i^2(\text{cond}) = \sigma_{\text{fit}}(\mathbf{B_{ii}})^{-1} \quad (6)$$

where $\mathbf{B}$ is a matrix whose elements are defined by $B_{ij} = [(d\mathbf{a}_{\text{pred}}/dc_i)(d\mathbf{a}_{\text{pred}}/dc_j)^t]$, and

$$\sigma_{\text{fit}} = \sqrt{\sum (a_{\text{act}} - a_{\text{pred}})^2/(n-3)}.$$

As previously discussed in connection with binary mixtures [28], the obtained values of F_i can be compared with the maximum $F(\alpha, \nu_1, \nu_2)$ in order

to judge the reliability of the obtained concentrations (α is the confidence level, and $\nu_{1,2}$ are the degrees of freedom, equal to the number of digitised wavelengths minus two).

Several synthetic ternary mixtures of rifampicin, isoniazid and pirazinamide were subjected to CLS analysis. If the whole spectral region 200–500 nm is used for regression, then the values of F_i are: rifampicin, 1.72; isoniazid, 7.35 and pyrazinamide, 6.54, which can be compared with $F(0.01, 299, 299) = 1.31$, meaning that CLS analysis is to be considered unreliable in order to quantitate the components in the present ternary mixtures. Consequently, whereas the concentrations of rifampicin and pirazinamide could be reasonably fitted by CLS, the recoveries for the most unfavourable component isoniazid were poor (i.e. $\sim 50\%$). In a previous CLS study of binary mixtures of rifampicin and isoniazid [14], it was suggested that the optimum working region involved $\lambda < 300$ nm. However, in the presently studied case, the spectral region 200–300 nm lead to values of F_i as follows: rifampicin, 4.24; isoniazid, 12.7 and pyrazinamide, 7.19, while $F(0.01, 99, 99) = 1.60$. This implies that, particularly for isoniazid, the obtained concentrations may be meaningless.

It can be shown from Eqs. (5) and (6) that for a three component mixture:

$$D_i = \frac{S_{ij}^2 + S_{ik}^2 - 2S_{ij}S_{ik}S_{jk}}{1 - S_{jk}^2} \quad (7)$$

where:

$$S_{ij} = \frac{\sum \boldsymbol{\varepsilon}_i \boldsymbol{\varepsilon}_j^t}{\left(\sum \boldsymbol{\varepsilon}_i \boldsymbol{\varepsilon}_i^t \sum \boldsymbol{\varepsilon}_j \boldsymbol{\varepsilon}_j^t\right)^{1/2}}.$$

By analogy with the dot vector product, S_{ij} may be considered as the product between the (normalized) vectors $\boldsymbol{\varepsilon}_i$ and $\boldsymbol{\varepsilon}_j$. For binary mixtures, S_{ij} is a direct measure of the degree of spectral overlap [28]. In the present case, Eq. (7) gives the projection of $\boldsymbol{\varepsilon}_i$ onto the plane defined by $\boldsymbol{\varepsilon}_j$ and $\boldsymbol{\varepsilon}_k$. This shows that $\sqrt{D_i}$ is again a convenient measure of the overlap (notice that D_i only depends on the spectral characteristics of each component). For the presently studied compounds, the most unfavourable case is isoniazid, for which the spectra shown in Fig. 1 lead to $D_i = 0.86$ (in the range 200–500 nm) and $D_i = 0.92$ (in the range 200–300 nm), implying 93 and 96% of spectral overlap, respectively. This also supports the above conclusion on the poor performance of CLS in solving the present ternary mixtures.

One alternative to analysing mixtures when severe spectral overlapping occurs involves the use of PLS methods. Briefly, the data matrix **A** is decomposed into:

$$\mathbf{A} = \mathbf{T}_a\mathbf{B}_a \quad (8)$$

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 calibration mixture matrix **C** is similarly decomposed:

$$\mathbf{C} = \mathbf{T}_c\mathbf{B}_c \quad (9)$$

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

$$\mathbf{T}_c = \mathbf{T}_a\mathbf{V} \quad (10)$$

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 $c = \mathbf{tVb}_c$, 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 optimum h values and spectral regions for each of them.

For the presently studied mixtures, the calibration matrix was designed with concentration ranges $0.00–1.00 \times 10^{-5}$ mol l^{-1} for rifampicin, $0.00–4.00 \times 10^{-5}$ mol l^{-1} for isoniazid and of $0.00–1.58 \times 10^{-4}$ mol l^{-1} for pyrazinamide. The latter concentrations lie within the linear range, as previously verified for CLS. The central composite calibration design used for the analysis is shown in Table 1. For the selection of the optimum number of factors, the cross validation method proposed by Haaland and Thomas was used [18]. Hence, seven factors were used for prediction, with the following spectral regions: rifampicin, 273–500 nm; isoniazid, 220–337 nm and pyrazinamide, 220–347 nm. The square of the correlation coefficient (R^2) and the relative error of prediction (REP), which give an indication of the quality of fit of all the data,

were also calculated as: rifampicin, 0.9990 and 2.06%; isoniazid, 0.9910 and 5.40%, pyrazinamide, 0.9996 and 1.17%, respectively. The predicted component concentrations in the validation set are shown in Table 2, using the previously calculated factors. This latter table also gives statistical parameters for evaluating the method. As can be appreciated, the recoveries are excellent for rifampicin and pyrazinamide, whereas that for isoniazid can be considered as reasonably good in view of the spectral overlap and small relative absorbances for this component. Table 3 summarises the results after applying the PLS method to real samples. The results are highly satisfactory.

4. Conclusions

The contents of rifampicin, isoniazid and pyrazinamide, a combination currently used in tablet formulations for the treatment of tuberculosis, were simultaneously determined using electronic absorption measurements, together with PLS-1 multivariate calibration analysis. Synthetic ternary mixtures as well as commercial tablets were studied, with excellent recoveries in all cases. A related multivariate method (CLS) has been shown to be unreliable in quantitating the studied compounds in their mixtures, due to extensive spectral overlapping.

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References

[1] Medical Sciences Bulletin, Pharmaceutical Information Associates Ltd., Levittwon, PA, June, 1994.
[2] A. Goodman-Hilman, T. Rall, A. Nier, P. Taylor, The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, 1996.
[3] A. Tomasz, N. Engl. J. Med. 330 (1994) 1247.
[4] S.M. Galal, S.M. Blaih, M.E. Abdel-Hamid, Anal. Lett. 25 (1992) 725.
[5] C.S.P. Sastry, T.E. Divakar, U.V. Prasad, Indian J. Pharm. Sci. 47 (1985) 45.
[6] A.M. El-Brashy, L.A. El-Hussein, Anal. Lett. 30 (1997) 609.
[7] P. Nagaraja, K. Murthy, C. Srinivasa, H.S. Yathirajan, Talanta 43 (1996) 1075.
[8] N.M. Mahfouz, K.M. Emara, Talanta 40 (1993) 1023.
[9] R.G. Ramana, S.S.N. Murty, P. Khadgapathi, R.E. Venkata, Indian Drugs 26 (1989) 362.
[10] W. Von Sassen, M. Castro-Parra, E. Musch, M. Eichelbaum, J. Chromatogr. 338 (1985) 113.
[11] M. Ishii, H. Ogata, J. Chromatogr. 426 (1988) 412.
[12] Y. Shah, S. Khanna, K. Jindal, V.S. Dighe, Drug Dev. Ind. Pharm. 18 (1992) 1589.
[13] S.V. Erram, S.M. Doshi, V.M. Kulkarni, Indian J. Pharm. Sci. 54 (1992) 116.
[14] K.K. Mahalanabis, D. Basu, B. Roy, Analyst 114 (1989) 1311.
[15] C.S. Sharma, S. Das, S.K. Talwar, J. Assoc. Off. Anal. Chem. 70 (1987) 679.
[16] A.P. Argekar, S.S. Kunjir, K.S. Purandare, J. Pharm. Biomed. Anal. 14 (1996) 1645.
[17] A.R. Rote, A.K. Sharma, Indian J. Pharm. Sci. 59 (1997) 119.
[18] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1193.
[19] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, 1989.
[20] K.R. Beebe, B.R. Kowalski, Anal. Chem. 59 (1989) 1007A.
[21] A. Espinosa-Mansilla, A. Muñoz de la Peña, F. Salinas, M. Martínez Galera, Anal. Chim. Acta 276 (1993) 141.
[22] M. Sánchez Peña, A. Muñoz de la Peña, F. Salinas, M.C. Mahedero, J.J. Aaron, Analyst 119 (1994) 1177.
[23] A. Espinosa-Mansilla, F. Salinas, A. Zamoro, Analyst 119 (1994) 1183.
[24] A. Guiberteau Cabanillas, T. Galeano Díaz, A. Espinosa-Mansilla, P.L. López de Alba, F. Salinas López, Anal. Chim. Acta 302 (1995) 9.
[25] A. Bozdogan, A.M. Acar, G.K. Kunt, Talanta 39 (1992) 977.
[26] P. Damiani, G. Ibáñez, M.E. Ribone, A.C. Olivieri, Analyst 120 (1995) 443.
[27] M E. Ribone, A.P. Pagani, A.C. Olivieri, Anal. Chim. Acta, submitted for publication.
[28] H.C. Goicoechea, A.C. Olivieri, Talanta 47 (1998) 103.
[29] H.C. Goicoechea, A.C. Olivieri, J. Pharm. Biomed. Anal, in press.
[30] British Pharmacopoeia, H.M. Stationery Office, London, 1993, pp. 1089–1090.
[31] United States Pharmacopeia XXII, United States Pharmacopeial Convention, Rockville, MD, 1990, pp. 729–730; 1227–1228.