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Talanta 53 (2000) 627–637

Talanta

www.elsevier.com/locate/talanta

Simultaneous kinetic-spectrophotometric determination of levodopa and benserazide by bi- and three-way partial least squares calibration

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Received 8 June 2000; received in revised form 26 July 2000; accepted 3 August 2000

Abstract

A procedure for the simultaneous kinetic-spectrophotometric determination of levodopa (I) and benserazide (II), from their oxidation reaction with KIO_4 in an acidic medium, is described. Both species instantly oxidize, giving rise to compounds which present maximum values of absorbance close to 400 nm. In the presence of an excess of the oxidizing agent, the levodopa derivative evolves to form the corresponding aminochrome ($\lambda_m = 480$ nm), while the benserazide derivative decomposes to yield colorless compounds. The appearance of new compounds, with absorption bands in the region of 500–700 nm, is additionally seen upon adding the oxidizing agent to a mixture of I and II. These compounds also evolve decomposing and forming colorless products. In spite of the complexity of the system studied, the calibration by bi-linear partial least squares (PLS) as well as by three-way partial least squares (nPLS) permit the quantification of both analytes with a precision on the order of 0.7% for levodopa and of 1.5% for benserazide. nPLS also allows for the qualitative interpretation of the phenomena which occur. The proposed method is applied to the quantification of I and II in the commercial, pharmaceutical preparation Madopar, using high performance liquid chromatography (HPLC) as the analytical reference technique.

Keywords: Levodopa; Benserazide; Simultaneous kinetic determinations; nPLS

1. Introduction

The determination of mixtures of chemical compounds with similar structures and properties is one of the topics of chemical analysis which has typically been addressed using separation techniques. Nevertheless, in the last decade differential kinetic methods are re-emerging and being considered as truly alternative methods for mixtures with a small number of analytes to be quantified. Common characteristics of all of these modern differential kinetic methods are the record of a multi-parametric signal which varies with time,

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0039-9140/00/$ - see front matter
PII: S0039-9140(00)00539-7

habitually the absorbance of the mixture or its fluorescence intensity, which are measured at various wavelengths, and a chemometric treatment of the recorded signal [1]. In this way, by treating all of the information together, it is possible to discriminate amongst mixtures on the basis of spectral differences as well as on the basis of differences in the reaction rate with a common reagent, both sources of discrimination having a synergetic effect which makes the quantification possible of compounds which are very similar chemically, with the case limit of similarity being the resolution of mixtures of enantiomers [2].

From among the distinct procedures of multivariable calibration, partial least squares (PLS) regression [3] stands out due to its versatility and predictive capacity. Widely used in diverse spectroscopic techniques, it has been adapted with great success to kinetic analysis, where it has shown itself to be relatively insensitive to noise [4], and it has been applied to the determination of an analyte in the presence of a complex matrix [5], resolution of mixtures which follow a kinetics of first order [4,6], second order [7], in the presence of an interaction between analytes [4], etc.

Its advantages can be summarized in that it is capable of modeling a system without the necessity of any previous knowledge of the implicated kinetics. Its limits arise from the fact of essentially being a linear system which, although capable of modeling non-linearities, such capacity is limited. Thus, it has been found that it cannot solve, with the desirable precision, kinetic systems, which are of pseudo-first order with respect to the reagent [8].

The habitual PLS algorithms are bi-linear, that is, they correlate the data of an absorbances matrix with the data of a concentrations matrix. The usual procedure for its application to an essentially three-dimensional system such as the kinetics data (mixtures, λ, t) is that of unfolding. What is done with this system is to transform the matrix of three-dimensional data into a two-dimensional one, where each row corresponds to the spectral values of the same sample ordered sequentially accordingly to time ($A_{\lambda 1,t1}$, $A_{\lambda 2,t1}$,..., $A_{\lambda n,t1}$, $A_{\lambda 1,t2}$, $A_{\lambda 2,t2}$, ..., $A_{\lambda n,t2}$, ..., $A_{\lambda 1,tk}$, $A_{\lambda 2,tk}$, ..., $A_{\lambda n,tk}$). Although this system has been shown to be highly efficient in terms of its capacity of prediction, it is evident that some information is lost since all of the absorbance values are considered as different variables, when it is obvious that the information contained in the absorbance measured at $\lambda_j t_j$ must be related with the information contained in $\lambda_j t_{j+1}$.

Recently, Bro [9] has developed an algorithm of multi-dimensional PLS (nPLS) which maintains the three-dimensional structure of the data, with which it should be capable of extracting more information of a kinetic system than the conventional PLS. However, up to now it has been used very little in kinetic systems, always with inorganic analytes and with the fundamental objective of comparing the predictive capabilities of both algorithms. Thus, there are various examples applied to the resolution of metallic ions on the basis of their reaction with 2-pyridylazoresorcinol (PAR) as the chromogenic reagent [10–12] in diverse experimental situations although always in conditions of pseudo-first order with respect to the analyte. Karlberg et al. studied the resolution of mixtures of phosphate and arsenate [13]. In all of these cases the predictive capacity of PLS and nPLS has turned out to be practically the same. Another procedure of three-dimensional calibration, comparable to nPLS, is the PARAFAC, also applied to the reaction of metallic ions with PAR [10] or PAR/EDTA [14]. Nevertheless, on PARAFAC being a strictly lineal method, its general applicability to kinetic systems is doubtful, having demonstrated, besides, being much more sensitive to experimental noise than nPLS [10].

In this work, nPLS is applied to the simultaneous kinetic-spectrophotometric determination of levodopa and benserazide, from the variation of absorbance over time of their products of reaction with potassium periodate. In the experimental working conditions both compounds instantly oxidize, giving rise to products, which present maximum values of absorbance close to 400 nm. In the presence of an excess of the oxidizing agent, the levodopa derivative evolves according to a kinetics of first order to form a colored compound ($\lambda_m = 480$ nm), while the benserazide derivative decomposes to yield color-

less compounds, following an unknown mechanism. On the other hand, a crossed reaction is observed in the mixtures of both compounds, giving way to the appearance of new products which absorb in the region of 500–700 nm, and which also decompose in the presence of an excess of the oxidizing reagent.

The results found upon applying the kinetic methods proposed are compared with those obtained by high performance liquid chromatography (HPLC), used as the method of reference.

2. Experimental

2.1. Apparatus and software

UV–visible spectra were recorded on a diode-array UV–visible spectrophotometer Hewlett–Packard model HP-8451A, which has a system of internal agitation, HP 89055A, which permits the mixing of the reagents in the measuring cell using a Teflon coated magnet. Quartz cuvettes of 1 cm pathlength were used. All measurements were performed in a thermostated cell of 25.0 ± 0.1°C by means of a Frigiterm S-382 thermostatic bath, and the laboratory temperature was kept constant at 24 ± 1°C.

A system composed of some Kontron model HPLC System 600 pumps with a Hewlett–Packard model 1040A HPLC UV–visible diode-array detector and a Hewlett–Packard 9153 C data station were used for chromatographic determinations. The column was a reverse-phase C18 Spherisorb ODS-2 (15 cm long × 0.46 cm i.d., 5 μm particle size, Tracer). A Selecta ultrasound bath, a SBS A05 magnetic agitator and a micropH 2002 Crison high-resolution pH-meter were also used.

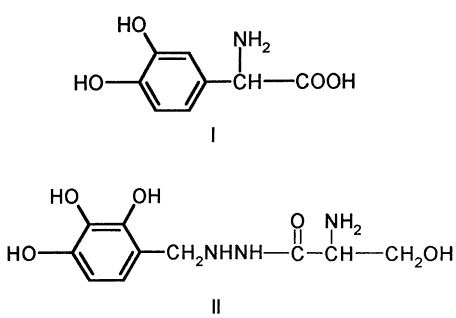

Fig. 1. Formulas of levodopa (I) and benserazide (II).

The PLS algorithm incorporated in The UNSCRAMBLER software package (CAMO A/S, Trondheim, Norway), version 6.1, and the nPLS algorithm developed by Bro, which is written in Matlab (MathWorks; Sherborn, MA; USA) and is available from World Wide Web: http://newton.foodsci.kvl.dk/foodtech, are used for the construction of the different models. First derivative spectra were obtained by using the Savitsky–Golay algorithm with a second-order polynomial and an overall window size of 11 points.

2.2. Reagents and solutions

Stock solutions of levodopa ((-)-3-(3,4-dihydroxyphenyl)-L-alanine) and of benserazide HCl (DL-serine 2-((2,3,4-trihydroxyphenyl) methyl) hydrazide) (Fig. 1) of concentrations of 2.10×10^{-2} and 8.15×10^{-3} M, respectively, were prepared in HCl 0.1 M, previously deaerated by purging with nitrogen for some minutes, by direct weighing of the solid products (Sigma). The stock solutions were protected from light and stored at 4–5°C, verifying that in these conditions levodopa is stable for 15 days and benserazide is stable for 2 days. Working solutions were prepared daily by adequate dilution of the stock solutions in HCl 0.1 M. The 0.21 M KIO_4 dissolution was prepared every 2 days by direct weighing of the solid product. A 0.2 M (pH 4.5) acetic acid–potassium acetate buffer was prepared from potassium acetate (Panreac) and acetic acid (Probus). All reagents were of analytical grade and their solutions were prepared in deaerated, bi-distilled water.

Samples, acquired in a pharmacy, of two different production batches of the pharmaceutical preparation Madopar® 250 (Roche Laboratories; Madrid, Spain) were analyzed. This preparative is presented in the form of tablets, with a nominal content of 200 mg of levodopa, 50 mg of benser-

azide and non-specified excipients until arriving at the weight of approximately 560 mg per tablet.

2.3. Procedure

The different work solutions were added directly to the spectrophotometric cuvette by means of the use of micro-pipettes. At first, 1 ml of the 0.2 M (pH 4.5) acetic acid–potassium acetate tampon was poured, immediately followed by an appropriate volume of the solutions of levodopa and benserazide and the volume was completed to 2 ml with bi-distilled water. The final concentrations of levodopa and benserazide were comprised of between $(2.4–4.0) \times 10^{-4}$ and $(4.6–7.8) \times 10^{-5}$ M, respectively. The cuvette was placed in the spectrophotometer, and the stirring was begun. After 1 min, a spectrum was recorded which will be used as reference, and 0.1 ml of the potassium periodate solution was added, which represents a concentration on the cuvette of 1×10^{-2} M. The addition of the last drop of reagent was taken as the starting time of the reaction. UV–visible spectra of the reaction mixture were recorded in the wavelength range 300–600 nm each 2 s during the first minute, and each 4 s in superior times. The spectral resolution was of 2 nm and the integration time of 0.3 s.

2.3.1. Preparation of laboratory mixtures

The calibration mixtures were prepared following a 5^2 factorial design, choosing as the central point a mixture with a relation of levodopa/benserazide concentrations equal to that found in the formulation and which yielded an absorbance of approximately 1 at 480 nm 2 min after the reaction start. The extreme levels of the design correspond to $\pm 25\%$ of the concentration of the central level for each analyte. The intervals of the concentrations chosen were of $(2.4–4.0) \times 10^{-4}$ mol l^{-1} for levodopa and $(4.6–7.8) \times 10^{-5}$ mol l^{-1} for benserazide.

To evaluate the predictive capacity of the distinct models of calibration tested, another 15 mixtures were prepared with concentrations comprised within those of calibration. All mixtures were prepared in duplicate, on distinct days and in a random order.

2.3.2. Sample treatment

To determine the average content per batch, 20 tablets of Madopar 250 were weighed from each batch, ground up and homogenized. Distinct portions of approximately 0.38 g, weighed with precision, were taken and treated with 0.1 M HCl. The insoluble excipients were filtered and the filtered solution was make up to a final volume of 200 ml (solution A). This solution (10 ml) were diluted up to 100 ml with 0.1 M HCl (solution B). The simultaneous kinetic-spectrophotometric determination of both analytes was performed as described above for the laboratory mixtures, but now adding a volume of 1 ml of solution B in the spectrophotometric cuvette. For their chromatographic determination, and given the difference of concentrations, which exist between both analytes, benserazide is determined by injecting solution A into the chromatograph, while levodopa is quantified by injecting solution B.

The analysis was performed on five portions of each batch, weighed and treated on distinct days.

2.3.3. HPLC procedure

A 20-μl aliquot of the sample solution, previously filtered with a 0.22 μm filter coupled to the syringe, was injected, and eluted with a mobile phase of a 0.05 mol l^{-1} phosphate buffer, at pH 2.5, at a flow rate of 1.0 ml min^{-1}. In these conditions the peaks were well resolved and tailless, with a retention time for benserazide of 2.8 and of 4.5 min for levodopa. The samples were injected in triplicate and the chromatograms recorded at the wavelengths of the absorption maxima, 278 nm for levodopa, and 268 nm for benserazide.

The linearity of the relation between peak area and concentration, in the concentration range studied, was confirmed. As has been indicated above, the two analytes were quantified separately from two injections since benserazide is found in significantly smaller concentrations than levodopa.

2.3.4. Data acquisition and processing

The recorded spectra were transferred to a compatible IBM PC-486 computer by means of a series RS232C/HP-829339A inter-phase for their

subsequent mathematical treatment. Only the absorbance values measured every 4 nm have been used, in order to facilitate the calculations and the data manipulation.

For the construction of the different PLS models the cross-validation method, leaving out one sample at a time [3], was used, always leaving out the two replicates of each sample. For each model, the number of significant factors has been chosen as the lower number whose mean squared error of prediction by cross validation (MSECV) [15] was not significantly different from the lowest MSECV value.

$$\mathrm{MSECV} = \frac{\sum_{i=1}^{I_c} (c_i - \hat{c}_i)^2}{I_c} \quad (1)$$

where c_i and $\hat{c}_i$ are the experimental and calculated concentrations, respectively, and I_c the total number of calibration samples.

The nPLS algorithm used does not have a cross-validation sub-routine available, so that the optimum number of factors was empirically chosen from a Scree plot [16], such as that from which the explained variance of the concentration matrix of the calibration samples did not significantly increase.

For an easier comparison and interpretation of the results obtained in each one of the models tested, the relative standard error of prediction (RSEP) for the calibration and predictions sets of each analyte j was calculated.

$$\mathrm{RSEP}_j\% = \sqrt{\frac{\sum_{i=1} (c_i - \hat{c}_i)^2}{\sum_{i=1}^{I} c_i^2}}\, 100 \quad (2)$$

Where c_i and $\hat{c}_i$ have the same meaning as in the previous equation, and I is the total number of samples included in the different sets.

3. Results and discussion

3.1. Oxidation of levodopa and benserazide

Both compounds oxidize in the presence of KIO_4, but in spite of their structural similarity, the reaction mechanism is quite different for the two species.

The oxidation reaction of levodopa ($\lambda_m = 280$ nm) follows a well-established mechanism and has been widely applied for analytical ends, using a great variety of oxidizing agents [17–20]. Aminochrome is the final product resulting from the sequence of three reactions. Initially, levodopa oxidizes to give rise to the corresponding *o*-benzoquinone ($\lambda_m = 395$ nm) and if the pH of the work medium is higher than 4, this specie is cycled and oxidizes again to finally transform itself into aminochrome ($\lambda_m = 300$, 480 nm). Two moles of potassium periodate for each mol of levodopa are necessary to complete the process. In the presence of an excess of the oxidizing agent, formation of the *o*-benzoquinone is practically instantaneous and the determinant step of the reaction rate is that of cycling. In the experimental conditions of this work (pH 4.5, concentration of $KIO_4 = 10^{-2}$ M), the global reaction followed a kinetics of first order with respect to the concentration of levodopa, with an apparent half-life reaction time of 83 ± 11 s, calculated from six pure solutions of levodopa in the interval of concentrations comprised between $0.5–5 \times 10^{-4}$ M.

The mechanism of the oxidation reaction of benserazide ($\lambda_m = 270$) is not known. By adding KIO_4 to one of its solutions, benserazide also oxidizes in a practically instantaneous fashion to give rise to the corresponding quinone ($\lambda_m = 406$ nm), needing 2 moles of potassium periodate for each mol of benserazide to complete its oxidation. When the oxidizing agent is added in a slight excess with respect to the concentration of benserazide, the appearance of one or several colored species, with absorption maxima at 490 and 524 nm is also observed. But all of these reaction products decompose to transform themselves into colorless species. In the presence of an excess of the oxidizing agent, the oxidation of benserazide to the quinone is practically instantaneous, but the decomposition also occurs very rapidly, so only a decrease of absorbance over time can be observed in the studied time and wavelength ranges. This kinetic behavior is shown in Fig. 2 where the variation of absorbance over time, at the wavelength of the quinone absorption maxi-

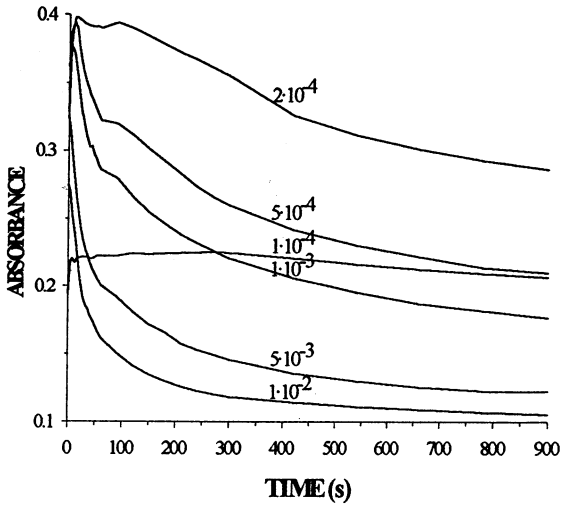

Fig. 2. Kinetics profiles for the oxidation reaction of benserazide at different KIO_4 concentrations (M). Benserazide 1.010^{-4} M; $\lambda = 406$ nm; pH 4.5; 25°C.

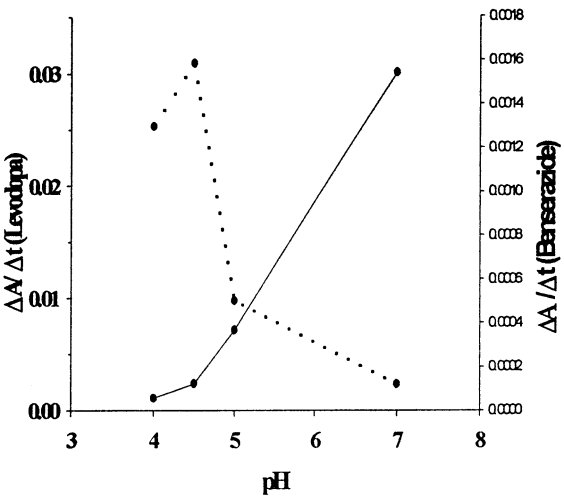

Fig. 3. Effect of pH on the reaction rates. Levodopa (—); benserazide (…).

mum has been represented for distinct concentrations of periodate. It can be seen that when half of the stoichiometric amount of oxidant was added (curve 1×10^{-4} M KIO_4), the absorbance value was approximately half of the maximum, and it remained stable over the measured time. At the stoichiometric addition (2×10^{-4} M KIO_4) the absorbance maximum was reached in a few seconds but the quinone decomposed slowly. At higher periodate concentrations the rate of the decomposition reaction increased and the maximum absorbance was not reached, which means that the quinone had already begun disappearing in a significant extension.

In the working conditions, the kinetics of the decomposition deviated from a pseudo first order model. A plot of the logarithm of initial rate versus the logarithm of benserazide concentration was a straight line with a slope of a 0.85.

As observed in Fig. 3, the pH influences in an important and contrasting fashion upon the rate of both reactions. In the interval of pH 4–7 the reaction rate of levodopa increases continuously with pH, while for benserazide it shows a maximum at 4.5, dropping down at higher pHs. Taking into account that in the formulation, benserazide is found in considerable scarcity opposite levodopa, a pH 4.5 has been chosen, since in these conditions the contribution of benserazide to the spectrum of the mixture will be in its maximum.

3.2. Analysis of levodopa and benserazide mixtures

In Fig. 4A–C, the variation over time of the absorbance spectrum from 300 to 600 nm during the first 2 min of a 2.4×10^{-4} M levodopa (A) solution, of a 7.8×10^{-5} M benserazide (B) solution, and of a mixture of both analytes in the same experimental conditions (C) are shown. As can be seen, levodopa presents a great contribution to total absorbance, and although the contribution of benserazide to the absorbance of the mixture is very small, its different kinetic behavior allows a good discrimination between both species. If the kinetic and spectral profiles of the pure solutions as well as of the mixture are analyzed in detail, it is seen (Fig. 5) that new species appear in the mixture, with absorption bands between 500 and 700 nm, probably the product of a crossed reaction. These new compounds also decompose rapidly to give rise to colorless species.

3.2.1. Calibration

The values of RSEP% found for the calibration and validation sets are shown in Table 1. Only the absorbance values between 328 and 600 nm recorded during the first minute of the reaction has been used for these calculations. At higher reaction times, the oxidation product of benserazide is already practically decomposed, and obviously, these data does not contain discriminating information. PLS and nPLS present the same capacity of prediction, with RSEP values of around 0.7% for levodopa and of 1.3% for benserazide. The fact stands out that the number of factors has been the same for both algorithms, three for levodopa and four for benserazide.

One of the criticisms which is generically made of the kinetic methods is that 'they are difficult to reproduce'. Reproducibility is defined [21] as *the closeness of agreement between the results of the same measure and where the measurements are carried out under reproducible conditions*. Strictly speaking, the reproducibility of an analytical method can only be determined from an exercise of inter-laboratories comparison. Nevertheless, given that the replicates of the distinct samples have been prepared and measured on distinct days, by using distinct solutions we can consider that the differences found in the prediction of the replicates are a good measurement of the reproducibility of the method. This measurement of reproducibility has been defined by ICH [22] as *intermediate precision*.

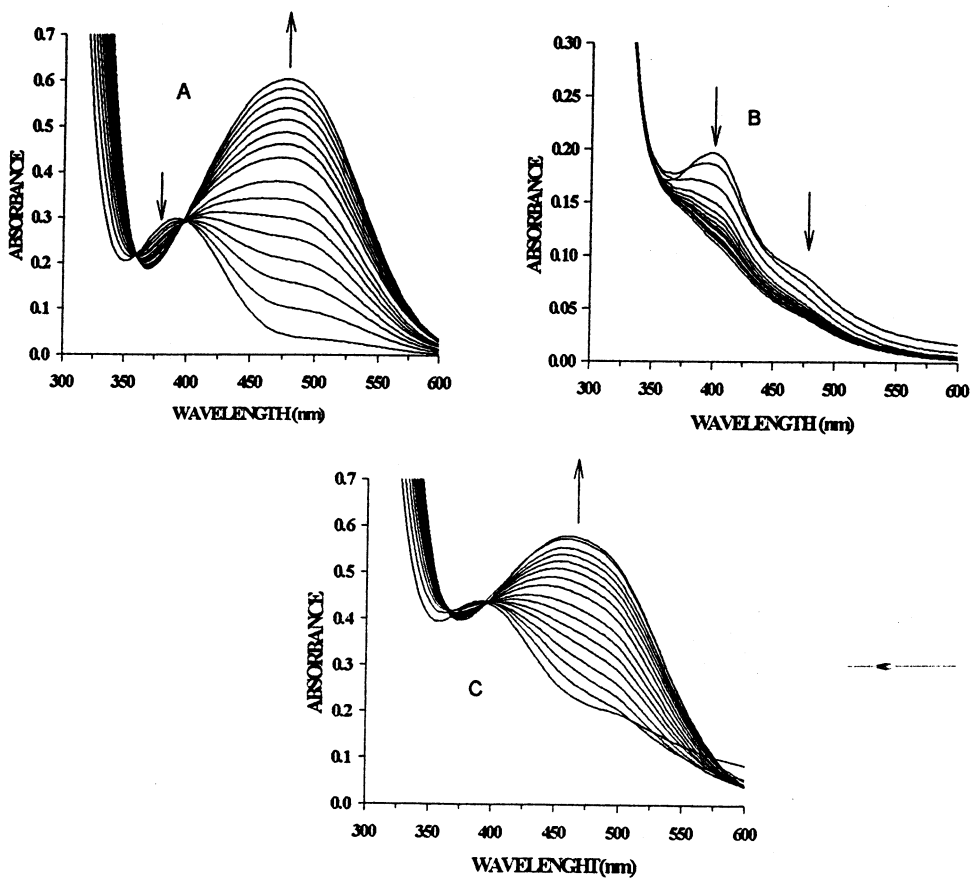

Fig. 4. (A–C) Absorbance-kinetic spectra for the first 2 min of reaction. (A) Levodopa 2.4×10^{-4} M; (B) benserazide 7.8×10^{-5} M; (C) a mixture with the same concentrations. KIO_4 10^{-2} M; pH 4.5; 25°C.

Table 1
Percent RSEP, reproducibility limit (R, mol l^{-1}) and ARSD% found for laboratory mixtures[a]

	PLS						nPLS					
	Levodopa[b]			Benserazide[c]			Levodopa[b]			Benserazide[c]		
	RSEP (%)	R	ARSD (%)	RSEP (%)	R (M)	ARSD (%)	RSEP (%)	R (M)	ARSD (%)	RSEP (%)	R (M)	ARSD (%)
Calibrationn	74	5.4×10^{-6}	60	138	1.6×10^{-6}	92	74	5.4×10^{-6}	60	145	1.7×10^{-6}	96
Prediction	72	5.2×10^{-6}	57	113	2.4×10^{-6}	132	71	5.2×10^{-6}	57	10	2.2×10^{-6}	122

[a] Absorbance values measured in the range 328–600 nm forthe first 60 s.
[b] Number of factors used to build up the calibration model, $a=3$.
[c] Number of factors used to build up the calibration model, $b=4$.

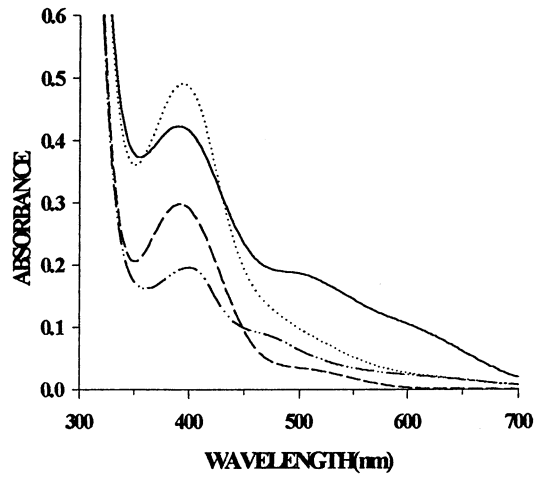

Fig. 5. Spectra at 20 s of the reaction start. 2.4×10^{-4} M levodopa (---); 7.8×10^{-5} M benserazide (-..-); theoretical spectrum obtained by addition of the individual contributions (….); experimental spectra of the mixture at the same concentrations than above (—);10^{-2} M KIO_4; pH 4.5; 25°C.

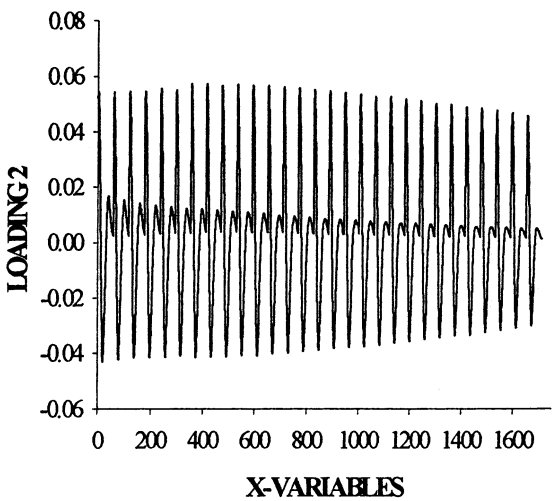

Fig. 6. Plot of the loading of the second factor for PLS model of benserazide. It accounts for the 88% of the concentration variance.

From the standard deviation of these replicated (s_R), the reproducibility limit (R) can be expressed with a degree of certainty of 95% as the maximum difference which can be expected between two measurements, which can be calculated according to Eq. (3):

$$R = 2.83 s_R \quad (3)$$

A more conventional manner of expressing it is to consider the average relative standard deviation (ARSD), defined by means of Eq. (4):

$$\text{ARSD}\% = \frac{s_R}{\bar{c}} 100 \quad (4)$$

where $\bar{c}$is the average concentration of the group of samples I.

The reproducibility of the method proposed being very elevated, on the order of 0.6% for levodopa and 1.2% for benserazide (Table 1), stands out.

3.2.2. Comparison of PLS and nPLS models

From the data seen in Table 1 it is quite clear that the reproducibility and predictive capacity of both calculation methods is absolutely analogous. It is in the interpretation of the loadings, especially in that of kinetic loadings, where the greatest possibilities of nPLS are manifested.

The principal characteristic of the nPLS algorithm is that it maintains the three-dimensional structure of the data matrix. Upon the matrices' decomposition, instead of a score vector and a loading vector, as what happens with the bi-lineal PLS, the tri-lineal model consists of a score vector and two loadings vectors, one for the second order of measurement (in this case the spectral) and the other for the third order of measurement (the reaction kinetics). In this fashion, upon separating the information, its interpretation is more immediate and more sure.

Fig. 6 shows, as a way of example, the loading for the second factor of the PLS model for benserazide. Although conclusions can be drawn from a detailed study, by simultaneously containing spectral and kinetic information, it is difficult to draw useful information about the system from a simple visual observation of the figure. Fig. 7, shows the kinetic loadings of the components which explain the major part of the variance of the concentrations matrix for levodopa and benserazide in the nPLS models. It is evident that these loadings are explaining the kinetic evolution of both species to us, with a growing absorbance in one case, and a decreasing one in the other. On

the other hand, the third loading (Fig. 8) is practically the same for the two analytes. One possible interpretation is that this third component is explaining the evolution of the species produced by the crossed reaction to us. These species are quantitatively of little importance for the prediction of levodopa (explained variance of the concentration matrix, 2%), but much more for benserazide, which is in considerable scarcity in the system (explained variance, 11.1%).

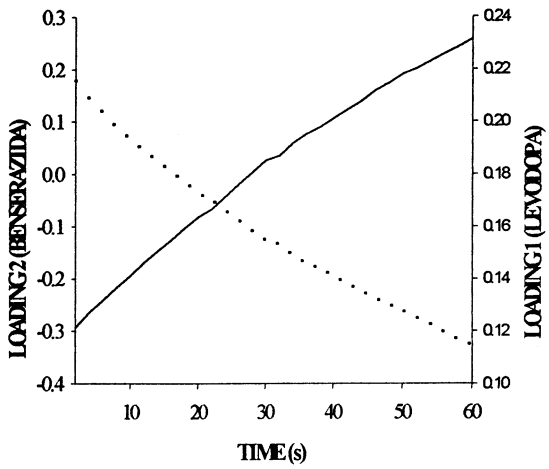

Fig. 7. Plot of the kinetics loading for nPLS models. Levodopa (—), 93% of concentration variance; benserazide (…), 76% of concentration variance.

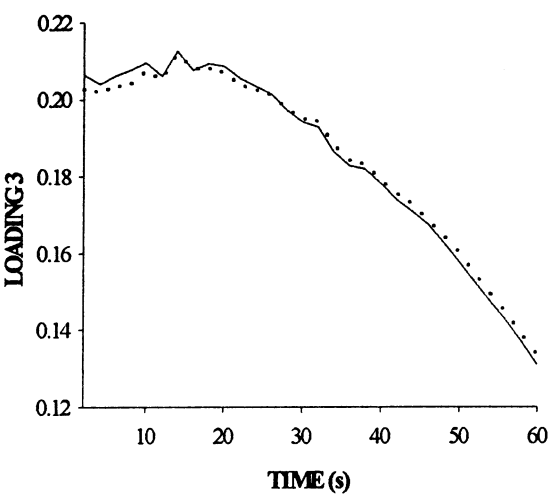

Fig. 8. Plots of the loading of the third factor for nPLS models. Levodopa (—); benserazide (…).

3.3. Analysis of levodopa and benserazide in Madopar®

The average values (mg per tablet) and confidence interval found for five replicates in the analysis of two batches of Madopar by the proposed kinetic method and by HPLC are compared in Table 2.

The comparison of the mean values and their precision by means of the Student's t-test and the variance ratio F-test shows that for a 95% degree of significance there is no significant difference between the kinetic and the chromatographic results.

4. Conclusions

It has been clearly shown that a differential kinetic method based upon the recording of a multi-parametric signal and a calibration by PLS can compete in precision and accuracy with HPLC in the determination of a mixture of two active compounds in a commercial pharmaceutical preparation. On the other hand, although the predictive capabilities of the bi-lineal and tri-lineal PLS are analogous, the latter possesses a much greater potential in the qualitative interpretation of the measured phenomena. Consequently, nPLS should be preferred. However, since PLS has been used for more than 20 years, it is very well-known and many complementary routines to help in the construction of the calibration model (detection of outliers, etc.) are available. That is not the case for nPLS, which is in the first steps of its development, and its performances in front of different situations, as non-linearities for instance, is still being evaluated. It can be expected that in a few years powerful commercial nPLS software will be available and used in a routinary way.

Acknowledgements

The authors are grateful to the Spanish Dirección General de Investigatión Científica y Técnica (DGICyT) for financial support received through Project PB94-0693 y PB97-0213. Núria Villegas

Table 2
Average content (mg per tablet) and confidence intervals ($\alpha = 0.05$, $n = 5$)[a]

	Levodopa			Benserazida		
	HPLC	Kinetic method		HPLC	Kinetic method	
		PLS1	n-PLS1		PLS1	n-PLS1
Batch 1	204 ± 2	201 ± 1	201 ± 1	49 ± 2	52 ± 2	52 ± 2
Batch 2	202 ± 2	200 ± 1	200 ± 1	50 ± 3	51 ± 3	51 ± 2

[a] Label claim for levodopa = 200 mg per tablet and benserazide = 50 mg per tablet.

also wishes to acknowledge additional support from the DGU of the Generalitat de Catalunya in the form of an FPI scholarship.

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