ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 81–90

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of triamterene and leucovorin in biological fluids by UV derivative-spectrophotometry and partial least-squares (PLS-1) calibration

I. Duran Merás *, A. Espinosa Mansilla, F. Salinas López, M.J. Rodríguez Gómez

Department of Analytical Chemistry, Faculty of Sciences, University of Extremadura, 06071 Badajoz, Spain

Received 28 February 2001; received in revised form 11 May 2001; accepted 26 May 2001

Abstract

The resolution of binary mixtures of triamterene (TAT) and leucovorin (LV) by application of first-derivative spectrophotometry and by application of Partial Least Squares calibration (PLS-1) was performed. Triamterene is determined in presence of leucovorin directly in the absorption spectra at 358 nm, and leucovorin is determined in the first-derivative spectra at 305.6 nm, zero-crossing of the triamterene. The mean recovery values in urine samples were 102 and 97% for TAT and LV, respectively. Partial Least Squares calibration (PLS-1) multivariate calibration of spectrophotometric data, have been applied to the determination of these compounds in serum and in urine without pretreatment of the samples. The absorption spectra of samples of serum or urine, spiked with triamterene and/or leucovorin, were used to perform the optimization of the calibration matrices by PLS-1 method. Mean recovery values were of 107 and 108% for TAT and LV in serum samples, and 98 and 91% for TAT and LV in urine samples.

Keywords: Triamterene; Leucovorin; Derivative spectrophotometry; Multivariate calibration; PLS-1; Serum; Urine

1. Introduction

Triamterene (2,4,7-triamino-6-phenylpteridine) (TAT) (Scheme 1), is a natriuretic agent which is much used in the treatment of several diseases. It can also be applied as doping substance. In sports, this diuretic is abused mainly for two reasons: to obtain a rapid diminution of corporal weight, important in sports which are divided in different weight categories, and to reduce the concentration of medical drugs in urine by dilution by means of a rapid production of an elevated quantity of urine, thus trying to diminish the possibility to detect other doping substances. No medical reason can justify in any sports a rapid decrease of weight, whereas on the other hand this abuse causes grave dangers for health because of possible serious secondary effects. Triamterene is rapidly, but incompleted absorbed

* Corresponding author.
E-mail address: iduran@unex.es (I. Duran Merás).

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00512-X

after oral administration. Once the drug is in the body, ~30–70% of an oral dose is excreted in the urine [1]. Variable amounts are excreted in the bile.

First measurements of triamterene have been direct1y made in pharmaceutical preparations by perchloric acid titrations [2,3]. Mixtures of triamterene and amiloride hydrochloride in pure and pharmaceutical preparations have been analysed by fluorimetry [4]. Also a polarographic method [5] has been described for triamterene and its analogues in biological fluids. But usually, the specific determination of this natriuretic agent in biological fluids involves liquid chromatography [6–9].

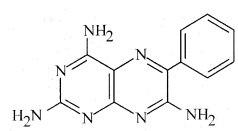
Leucovorin (N^5-formyl-5,6,7,8-tetra-hydropteroyl-L-glutamic acid; hydropteroil-L-glutamic acid) (LV) or folinic acid (Scheme 1), has become as part of antineoplasic combination regimens for several malignant disorders. Also, it has been used for the prevention in the anaemias. Hence, it is used for recue after high and intermediate-dose methotrexate therapy and also LV it is used as a modulator of the pyrimidine antagonist 5-fluoruracil [10].

LV originated from a chemical reduction, which is not stereospecific and therefore results in equal amounts of the diastereoisomers (6R)-LV and (6S)-LV [11]. The biological activity of LV is apparently restricted to the (6S)-LV diastereoisomer which is rapid1y converted to its active metabolite (6 S)-5-methyltetrahydrofolate while the (6R)-LV diastereoisomer is not metabolized and is excreted unchanged [12,13]. Liquid chromatography with UV detection is the most frequently used technique for determined this compound in serum and in urine. Schleyer et al. proposed the separation of the diastereoisomers of LV and 5-methyltetrahydrofolate in serum and urine [14]. There are several HPLC methods for the determination of LV in mixed folates and antifolates by a combination of UV and fluorimetric detection [15,16]. Capillary electrophoresis methods have been developed [17,18]. In one of these, cyclodextrins are used for the chiral separation of LV and its majors metabolite, 5-methyltetrahydrofolate [18]. Birmingham and Col determined LV in serum by HPLC with amperometric detection [19]. Also, LV has been determined, with fluorouracil, in plasma [20].

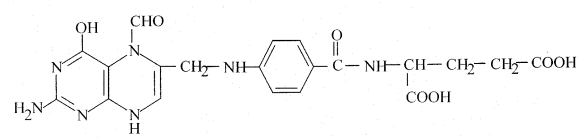
LV and TAT can are present in biological fluids due to diuretic activity of TAT, and their simultaneous determination can be necessary.

The advantage of multicomponent analysis using multivariate calibration is the speed of the proposed procedures methods, as a separation step avoids. No data about the application of chemometric methods have been found in the literature. However, in the recent years, several multicomponent analysis methods based on spectral properties have been demonstrated as powerful tools in diverse analysis fields [21]. Derivative spectrophotometry has been widely applied in pharmaceutical analysis to analyse simple mixtures of drugs. Partial Least Squares methods (PLS) have been applied to resolve complex mixtures of organic compounds by using conventional, derivative and also kinetic signals [22,23].

To taking into account the similar spectral characteristics of LV and TAT, the aim of this paper was to investigate the possibility to simultaneous determination of LV and TAT by using derivative spectrophotometry and PLS methods, in biological fluids such as urine and human serum.



Triamterene (TAT)



Leucovorin (LV, folinic acid)

Scheme 1.

2. Experimental

2.1. Apparatus

A Milton Roy Spectronic 3000 diodes array spectrophotometer, provided with the Rapid Scan Milton Roy software package V 2.2, was used for all acquisition of spectrophotometric data. The Beckman Data Leader software, V 3.0 was used for manipulation of the spectral data. Differentiation was performed according to the simplified least squares procedure of Savitzky and Golay [24]. The GRAMS-386 V 3.0 software package, with the PLS plus/IQ application software [25], was used for statistical treatment of the data and the application of the PLS-1 method.

2.2. Reagents

All experiments were performed with analytical-reagent grade chemicals. Triamterene (TAT) were obtained from Aldrich and Leucovorin (LV; folinic acid) were purchased from Across. Standards solutions were prepared by exact weighing and dissolution in distilled water. A buffer solution of pH 5.0 was prepared from acetic acid and 0.5 M sodium acetate. Serum and urine samples were prepared by spiking blank sera or blank urine, with appropriate amounts of the stock solutions of triamterene and leucovorin.

2.3. Procedures

2.3.1. Determination of triamterene and leucovorin in binary mixtures by spectrophotometry and first-derivative spectrophotometry

Prepare samples in 25 ml volumetric flask containing between 50 and 400 μg of TAT, between 30 and 750 μg of LV, 5 ml of acetate buffer solution (pH 5.0) and dilute to the mark with deionized water. Record the absorption spectra between 250 and 420 mn against reagent-blank prepared in absence of analytes, and obtain the first-derivative spectra with $\Delta\lambda = 11$ nm. Determine TAT content from the absorption spectra measuring the absorbance at 360 nm. Determine LV content from first-derivative spectra by measuring at 305.6 nm, zero-crossing point for TAT.

2.3.2. Procedure for determining triamterene and folinic acid in urine samples by spectrophotometry and first-derivative spectrophotometry

Urine (1-ml aliquots) containing more than 25 g l^{-1} of TAT and LV, were diluted to 100.0 ml. Take 0.5 ml of this solution and applied the procedure described previously.

2.3.3. Procedure for analysis of mixtures of triamterene and folinic acid by PLS-1

In a 25.0 ml calibrated flask, introduce an aliquot of the sample containing between 0 and 300 μg of TAT and between 0 and 375 μg of LV. Add 5.0 ml of acetate buffer solution (pH 5.0) and dilute to, the mark with deionized water. Record the absorption spectrum between 220 and 420 nm against a blank of buffer solution. Apply the optimized calibration matrix, calculated by application of the PLS-1 method, to analyse the spectra of the samples and calculate the concentrations of TAT and LV in the mixture.

2.3.4. Procedure for determining triamterene and folinic acid in serum or urine samples by PLS-1

The calibration set was designed with solutions, that in a final volume of 10 ml, containing 2 ml of serum (or 2 ml or urine), variable amounts of TAT and LV between 15 and 90 and between 10 and 100 μg, respectively, 2.0 ml of acetic/acetate buffer solution, pH 5.0, and desionized water to the mark. Record the absorption spectrum between 250 and 450 nm against a blank of buffer solution. Apply the optimized calibration matrix, calculated by application of the PLS-1 method, to analyse the spectra of the serum or urine samples and calculate the concentrations of TAT and LV.

3. Results and discussion

Both, TAT and LV, are highly absorbing substances in the UV region of the spectrum, TAT shows an absorption maximum at 360 nm, and LV exhibits an absorption maximum at 297 nm (Fig. 1(A)). In this figure, we can see that it is possible the determination of TAT in presence of LV, but it is not possible the determination of LV in presence of TAT. Conventional spectrophoto-

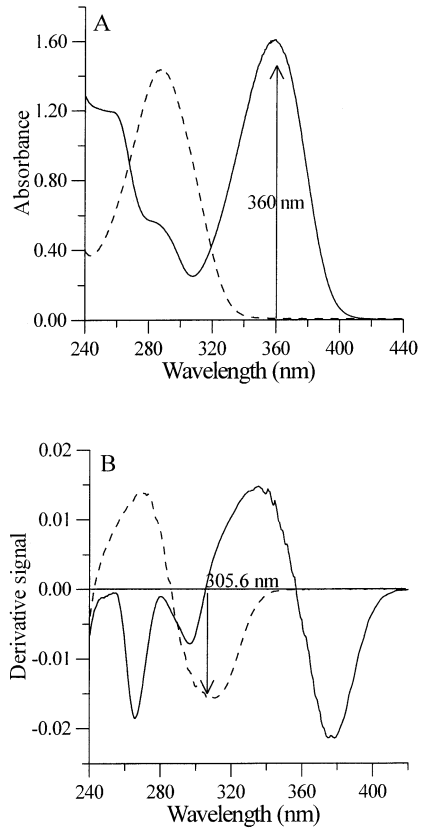


Fig. 1. (A) Absorption; and (B) first-derivative spectra of TAT (—), 20 μg ml^{-1} and LV (- - - -), 30 μg ml^{-1}, at pH 5.0.

metry can be applied to the determination of TAT in presence of LV, but for the determination of LV, in presence of TAT, is necessary to apply the 'zero-crossing' measurement technique over the first derivative spectra. On the other hand, the high overlap between the spectra can be examined by application of the a full spectrum method based in factor analysis, such as partial least squares (PLS) which shows high resolution capacity even in presence of non linearity and/or interactions. Then, we have studied both possibilities for the resolution of the binary mixtures in aqueous medium and in biological fluid.

3.1. Resolution of binary mixtures by applying first-derivative spectra

As aforementioned the resolution of mixtures of TAT and LV can be resolved by spectrophotometry and derivative spectrophotometry. The optimization of the derivative spectra was based on the influence of the number of experimental points for smoothing the spectra and the optimal $\Delta\lambda$ used on the differentiation of the absorption spectra, with the object to optimize the relation signal to noise ratio. In the first derivative mode, when the $\Delta\lambda$ value was increase from 5 to 21 nm, the signal amplitudes decrease slightly. A $\Delta\lambda$ of 11 nm allow us the best signal to noise ratio. The first-derivative spectra of TAT and LV are shown in Fig. 1(B). The wavelength selected, for the determination of LV was 305.6 nm, zero-crossing point for TAT.

Different calibration graphs for the individual determination of TAT and LV were obtained from the absorption spectra, at 360 mn for TAT, and for the first-derivative spectra, by using the peak-height-to-baseline measurements for LV. Ranges of concentration up to 16 μg ml^{-1} for TAT and up to 30 μg ml^{-1} for LV, were tested to obtain calibrations graphs for both compounds. In Table 1, the statistical parameters calculated from the calibration graphs are summarized.

The proposed method has been applied to the determination of TAT and LV in eight human

Table 1
Statistical parameters for calibration graphs for the simultaneous determination of triamterene and leucovorin by conventional spectrophotometric and first-derivative spectrophotometry

Component	Scan	Equation	R^a	LOD[b], μg ml^{-1}
Triamterene	Absorption spectra	$Y = 0.0811\ C - 0.00617$	0.9997	0.20
Leucovorin	First-derivative spectra	$Y = 5.07 \times 10^{-4}\ C + 3.32 \times 10^{-5}$	0.9996	0.60

[a] Correlation coefficient.
[b] Limit of detection calculated by Clayton's method ($\alpha = \beta = 0.05$) [27].

Table 2
Recoveries of triamterene and leucovorin in spiked urine by conventional spectrophotometric and first-derivative spectrophotometry

Triamterene, μg ml^{-1}			Leucovorin, μg ml^{-1}		
Added	Found	Recovery, %	Added	Found	Recovery, %
10.00	10.27	103	5.00	4.55	91
10.00	10.19	102	10.00	9.74	97
10.00	10.12	101	15.00	15.13	101
10.00	10.30	103	20.00	19.90	99
10.00	10.17	102	8.00	7.25	91
5.00	5.17	103	10.00	9.94	99
18.00	17.73	99	10.00	9.74	97
12.00	12.38	103	10.00	9.53	95
Mean recovery: 102%			Mean recovery: 97%		

urine samples spiked with different amounts of both compounds. The percentages of recoveries found, are summarized in Table 2. As can be seen the agreement between calculated and experimental values is good. The mean recovery was 102% for TAT and 97% for LV.

3.2. Simultaneous determination of triamterene and leucovorin by PLS-1

With the aim of improving the analyses for these compounds, the multivariate calibration method, PLS-1 was evaluated. The method was evaluated using the absorption spectra for the analyses. A training set of 11 samples was taken. The concentration of TAT was between 0 and 15 μg ml^{-1} and the concentration of LV was varied between 0 and 12 μg ml^{-1} through the calibration matrix. All samples are prepared in a 25 ml volumetric flask and 5.0 ml of buffer acetic/acetate, pH 5.0, are added. In Table 3, the compositions of the binary mixtures used in the calibration matrix, are summarized. The spectra were recorded between 200 and 450 every 0.35 nm. The spectral region between 230 and 403 nm, which implies working with 476 experimental points per spectrum, was selected for the analysis.

3.2.1. Calibration and selection of the optimum number of factors

The optimum number of factors to be used with the PLS-1 algorithms is an important parameter to achieve better performance in prediction. This allows to model the system with the optimum amount of information, avoiding over fitting. The cross-validation procedure was applied, consisting of systematically removing one of the training samples in turn, and using only the remaining ones for construction of the latent factors and regression.

The predicted concentrations were then compared with the actual ones for each of the calibration serum samples, and the predicted error sum of squares [PRESS = [PRESS = $\Sigma(c_i - \hat{c}_i)^2$] ($c_i$ is the data value and $\hat{c}$ is the predicted value) was calculated. The PRESS was computed in the same manner, each time a new factor was added to the PLS model. To select the optimum number of

Table 3
Composition of the calibration samples used for the determination of leucovorin and triamterene by PLS-1

Sample	Triamterene, μg ml^{-1}	Leucovorin, μg ml^{-1}
1	10	2
2	10	10
3	10	15
4	10	8
5	3	10
6	12	10
7	5	0
8	3	0
9	0	10
10	0	2
11	0	0

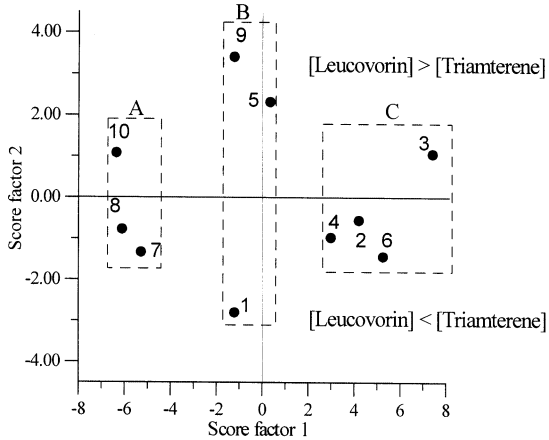


Fig. 2. Plot of scores 2 versus scores 1, showing the position of the 11 calibration mixtures (Table 3).

factors, the criteria proposed by Haaland and Thomas [26] was used. In our case, the value of F corresponding to a probability < 0.75 yield an optimum number of factors of 2 for both components.

Fig. 2 shows a plot of score 2 versus score 1. As can be seen score 1 is related to the triamterene plus leucovorin over-all concentrations. Rectangles A, B and C group objects in the calibration matrix with triamterene plus leucovorín over-all concentrations of: (A) 2–5; (B) 10–13; and (C) 18–25 μg ml^{-1}. The increase in the score 1 is consistent with an increase in the over all concentration of both components. Score 2 is concerns with the leucovorin-triamterene concentration ratio and increases with increases this ratio.

3.2.2. Statistical parameters

The values of the root mean square difference (RMSD), which is an indication of the average error in the analysis, when two factors are used, were 0.25 for TAT and 0.15 for LV. The values of the square of the correlation coefficient (R^2), which is an indication of the quality of fit of all data to a straight line, were 0.9967 for TAT and 0.9990 for LV (Table 4).

In order to test the performance of the proposed method, it was applied to the resolution of synthetic mixtures containing various concentrations of both compounds. The recoveries obtained are between 91 and 103% for TAT and between 98 and 103% for LV.

3.2.3. Simultaneous determination of triamterene and leucovorin in biological fluids by PLS-1

With the object to determine these compounds in biological fluids, such as serum and urine, news training set have been prepared, adding the appropriate amounts of TAT and LV to serum or urine samples.

Calibration and selection of the optimum number of factors in a calibration set of serum samples: A training set of eleven samples was taken. In all of them, 2 ml of serum samples, without pretreatment, were spiked with different amounts of the analytes and 2.0 ml of acetic/acetate buffer solution, pH 5.0, were added. The final volume of the solutions was 10 ml. The calibration set, shown in Table 3 was prepared, consisting of basal sera containing TAT in the range between 0 and 12 μg ml^{-1} and the concentration of LV was varied between 0 and 15 μg ml^{-1} through the calibration matrix.

Table 4
Statistical parameters of the PLS-1 methods with the absorption spectra in aqueous medium and with spiked samples of serum or urine

Component	Aqueous medium		Spiked serum		Spiked urine	
	RMSD[a]	R^2	RMSD[a]	R^2	RMSD[a]	R^2
Triamterene	0.2497 (2)	0.9967	0.2944 (3)	0.9958	0.1076 (2)	0.9994
Leucovorin	0.1544 (2)	0.9990	0.3249 (6)	0.9965	0.6617 (4)	0.9837

[a] The number in parentheses correspond to the number of factors used in the prediction.

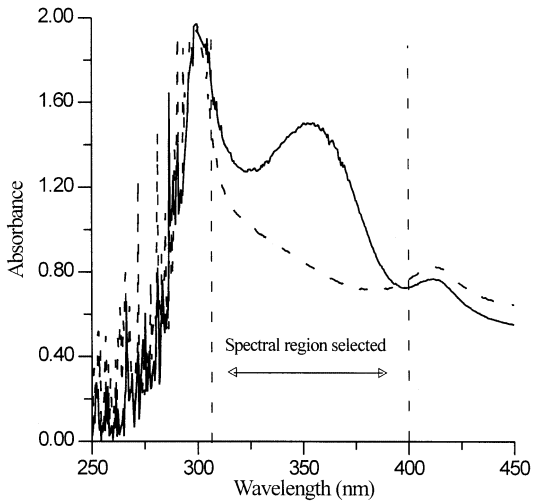


Fig. 3. Absorption spectra of serum sample (- - - -) and serum sample spiked with TAT (10 μg ml^{-1}) and LV (8 μg ml^{-1}) (—).

Then, the absorption spectrum was recorded in the 200–450 nm range, digitized every 0.35 nm. The spectral region between 305 and 400 nm was selected for the analysis, because in this zone the background of the serum is minimum and is maximum the spectra information from the mixture components of interest, as we can see in Fig. 3.

To select the number of factors, the same procedure has been applied and the optimum number of factors was found to be 6 for LV and 3 for TAT.

Known concentrations of all tested samples, included in the calibration matrix, were compared with the predicted concentrations by cross-validation. The values of RMSD (CV) and R^2, obtained when plots of actual versus predicted concentrations were constructed, are summarized in Table 4. The actual versus predicted concentration for each component has been calculated for this model, Fig. 4(A). In addition the residual analysis permits us to determine an amount that is not explained by the model for each data point,

$$\varepsilon_i = y_i - \hat{y}_i \text{ being } \hat{y}_i = b_i x_i,$$

where y_i, is the data value and $\hat{y}_i$ is the predicted value by the model; b_i is a regression parameter and x_i, is the independent variable. In Fig. 4(B), the residual concentration values for all components are represented.

Once upon optimized the PLS-1 method for serum, several mixtures were analysed in accordance with the foregoing procedures. In Table 5, the composition of the binary mixtures assayed is shown. The recovery values obtained using the model are also included in Table 5. Satisfactory recovery values are obtained in the samples analysed. The recovery values were 102–106% for LV and 104–112% for TAT.

Calibration and selection of the optimum number of factors in a calibration set of urine samples: To establish a quantitative model, eleven calibration samples containing 2 ml of urine, without pretreatment, different amounts of TAT and LV and 2 ml of acetic/acetate buffer solution were prepared in a final volume of 10 ml. The final concentrations of TAT were varied between 0 and 12 μg ml^{-1} and the concentrations of LV between 0 and 15 μg ml^{-1} through the calibration matrix. The absorption spectra were registered between 250 and 450 nm, Fig. 5. The spectral region between 296 and 400 nm was selected for the analysis, because in this zone the background of the urine is minimum and is maximum the spectral information from the mixture components of interest.

The optimum number of factors selected, in urine samples, are four for LV and two for TAT. The statistical parameters have been summarized in Table 4.

Once upon optimized the PLS-1 method for urine, several mixtures were analysed in accordance with the foregoing procedures. In Table 6, the composition of the binary mixtures assayed is shown. The recovery values obtained using the model are also included in Table 6. Satisfactory recovery values are obtained in most of the samples analysed. The recovery values were 82–103% for LV and 94–101% for TAT.

4. Conclusions

The derivative spectrophotometry allow us a simple procedure to determine LV and TAT in

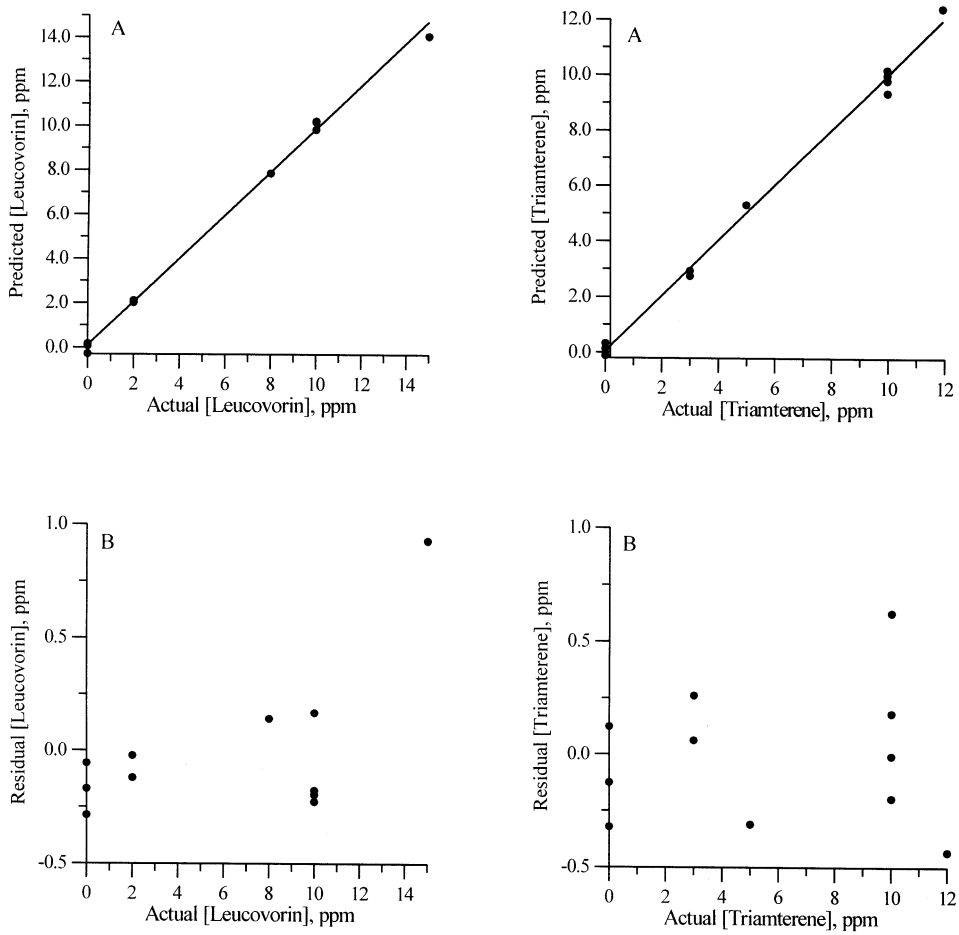


Fig. 4. (A) Actual versus predicted concentration; and (B) Residual versus actual concentration plots for the serum samples spiked with TAT and LV used in the calibration set for the evaluation of the predictive capacity of the method.

Table 5
Results obtained by applying the PLS-1 to a prediction test composed by serum samples spiked with different amounts of triamterene and leucovorin

Triamterene, μg ml^{-1}			Leucovorin, μg ml^{-1}		
Added	Found	Recovery, %	Added	Found	Recovery, %
10.00	10.47	105	5.00	5.22	105
10.00	10.55	105	20.00	20.38	102
5.00	5.62	112	10.00	10.60	106
18.00	18.66	104	10.00	10.25	103
10.00	10.88	109	0.00	0.42	–
10.00	10.47	105	5.00	5.18	104
Mean recovery: 107%			Mean recovery: 108%		

Table 6
Results obtained by applying the PLS-1 to a prediction test composed by urine samples spiked with different amounts of triamterene and leucovorin

Triamterene, μg ml^{-1}			Leucovorin, μg ml^{-1}		
Added	Found	Recovery, %	Added	Found	Recovery, %
10.00	9.94	99	5.00	5.12	103
10.00	9.69	97	20.00	16.44	82
5.00	5.05	101	10.00	9.62	96
18.00	16.97	94	10.00	8.68	87
10.00	10.01	100	0.00	0.00	–
0.00	0.01	–	5.00	4.34	87
Mean recovery: 98%			Mean recovery: 91%		

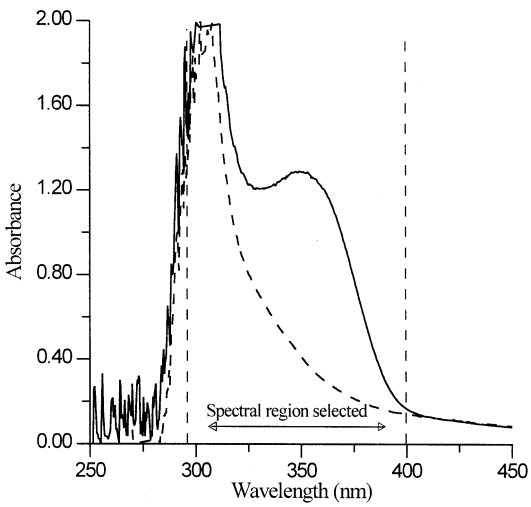


Fig. 5. Absorption spectra of urine sample (- - - -) and urine sample spiked with TAT (10 μg ml^{-1}) and LV (8 μg ml^{-1}) (—).

urine, however the more complex matrix of the human serum needs a more selective and robust procedure to analyse the mentioned compounds. On the other hand the great dilution to which it is necessary to subject the urine to eliminate the background of the urine (1:150) does not allow to determine small quantities of these compounds in human urine.

A simple multivariate calibration spectrophotometric methods, for the determination of triamterene and leucovorin in blood serum and in urine, have been satisfactorily developed. In any case is necessary a pretreatment of the samples, only a 1:5 dilution for the both, serum and urine samples, must be made. A PLS calibration model for each type of samples has been specifically optimized. The optimum number of factors have been calculated for each case, and in accordance with the complexity of the samples, the highest number of factors is calculated to optimize the model in presence of human serum. The robust optimized PLS models can be applied to the routine analysis of urine and serum in toxicity control analysis. These methods allow us to determine small quantities of TAT and LV in biological fluids.

Acknowledgements

The authors wish to express their gratitude to the DGESIC of the Ministry of Sciences and Technology of Spain (Project BQU2000-0215) and to the Consejería de Educación, Ciencia y Tecnología de la Junta de Extremadura (Project IPR00A036) for financial support of this work.

References

[1] Clarke's Isolation and identification of Drugs, second ed., Pharmaceutical Press, London, 1986, p. 1037
[2] The British Pharmacopoeia, Her Majesty's Stationery Office, London, 1973, p. 481.
[3] The United States Pharmacopoeia, XIX, US Pharmacopeia, Rockville, 1975, p. 517.
[4] C.S.P. Sastry, M.V. Suryanarayana, A.S.R. Prasad-Tipirneni, T. Satyanarayana, Indian Drugs 26 (1989) 651.

[5] E. González, R. Montes, J.J. Laserna, Anal. Chim. Acta 282 (1993) 687–693.
[6] S. Sved, J.A.A. Sertie, L.J. McGilveray, J. Chromatogr. 162 (1979) 474–479.
[7] R.R. Brodie, L.F. Chasseaud, T. Taylor, L.M. Walmsley, J. Chromatogr. 164 (1979) 527–533.
[8] G.J. Yakatan, J.E. Cruz, J. Pharm. Sci. 70 (1981) 949–951.
[9] E.T. Lin, Clin. Liq. Chromatogr. 1 (1984) 123–127.
[10] J.R. Bertino, Cancer Treat. Rep. 65 (1981) 131.
[11] J.A. Straw, E.M. Newman, J.H. Doroshow, NCI Monogr. 5 (1987) 41.
[12] E.M. Newman, J.A. Straw, J.H. Doroshow, Cancer Res. 49 (1989) 5755.
[13] J.A. Straw, D. Szapary, W.T. Wynn, Cancer Res. 44 (1984) 3114–3119.
[14] E. Schleyer, J. Reinhardt, M. Unterhalt, W. Hiddemann, J. Chromatogr. B 669 (1995) 319–330.
[15] S. Belz, C. Frickel, C. Wolfrom, H. Nau, G. Henze, J. Chromatogr. B 661 (1994) 109–118.
[16] A. Mandl, W. Lindner, Chromatographia 43 (1996) 327–330.
[17] F. Sczesny, G. Hempel, J. Boos, G. Blaschke, J. Chromatogr. B 718 (1998) 177–185.
[18] A. Shibukawa, D.K. Lloyd, I.W. Wainer, Chromatographia 35 (1993) 419–429.
[19] B.K. Birmingham, D.S. Greene, J. Pharm. Sci. 72 (1983) 1306–1309.
[20] C. Vandenbosch, S.V.an Belle, M. De Smet, G. Taton, V. Bruynseels, G. Vandenhoven, D.L. Massart, J. Chromatogr. B 612 (1993) 77–85.
[21] T.C. O'Haver, G.L. Green, Anal. Chem. 48 (1976) 312–318.
[22] H.C. Goicoechea, A.C. Olivieri, A. Muñoz de la Peña, Anal. Chim. Acta 384 (1999) 95–103.
[23] A. Espinosa Mansilla, I. Durán Merás, F. Salinas López, J. Pharm. Biom. Anal. 17 (1998) 1325–1334.
[24] A. Savitzky, M. Golay, Anal. Chem. 36 (1964) 1627–1639.
[25] GRAMS-386 Software package, Version 3.0 and PLSplus/IQ application, Galactic Industries, Salem NH.
[26] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1193–1202.
[27] C.A. Clayton, J.W. Hines, P.D. Elkins, Anal. Chem. 59 (1987) 2506–2514.