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Comparison of the predictive ability of several second-order multivariate methods in the simultaneous determination of two therapeutic drugs in human urine

M.C. Hurtado-Sánchez [a], I. Durán-Merás [a,*], M.I. Rodríguez-Cáceres [a], A. Jiménez-Girón [a], A.C. Olivieri [b]

[a] Department of Analytical Chemistry, University of Extremadura, 06006 Badajoz, Spain
[b] Departamento de Química Analítica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, Rosario S2002LRK, Argentina

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ABSTRACT

The aim of this paper is to study the applicability of second-order multivariate methods in the simultaneous determination of two therapeutic drugs in human urine samples. The studied drugs, irinotecan and thalidomide, are used in the treatment of malignant tumours. Irinotecan (CPT-11) is used to treat colon cancer; recent studies have shown the benefits of using thalidomide in combination with CPT-11 in the treatment of this disease. CPT-11 is highly fluorescent, but the native fluorescence of thalidomide is very weak. The second-order methods assayed were parallel factor analysis (PARAFAC), unfolded partial least-squares (U-PLS) and multidimensional partial least-squares (N-PLS), both combined with the residual bilinearization procedure (RBL). The excitation-emission matrices (EEMs) of the samples were recorded as analytical signal. The accuracy and precision of the algorithms were evaluated through the root mean square error of prediction (RMSEP) and the elliptical joint confidence region test (EJCR), obtaining better results with PARAFAC, which was successfully applied to the determination of thalidomide and CPT-11 in human urine samples, after a previous liquid–liquid extraction with chloroform.

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1. Introduction

Thalidomide (α-phthalimidoglutarimide) is a sedative, hypnotic and anti-inflammatory drug. Many years ago, it was prescribed to pregnant women as an antiemetic to prevent morning sickness and to help them to sleep, until its teratogenic effects were discovered. Afterwards, thalidomide was neither prescribed nor sold for decades. However, in recent years, it has been shown to be a valuable drug for a variety of illnesses, and there is an increased use of oral thalidomide for the treatment of a variety of autoimmune-related diseases such as erythema nodusum leprosum [1], Behecet's syndrome [2,3], Crohn's disease [4,5], and graft-versus-host disease [6–8], infectious diseases such as mycobacterial infections and HIV [9], and cancers such as colorectal, renal or prostate carcinoma [10,11].

Recently, thalidomide has been administered with irinotecan (CPT-11) for the treatment of colorectal carcinoma. CPT-11, a water-soluble semi-synthetic derivative of CPT (camptothecin), is reportedly effective for the treatment of various types of cancer, mainly colorectum cancer. However, it presents side effects, mostly leucopenia and late diarrhea [12], and sometimes it is necessary to stop the chemotherapy. A number of studies have demonstrated that simultaneous administration of both compounds decrease these effects. For example, Govindarajan et al. showed in an interim analysis of nine patients with metastatic colorectal cancer, that thalidomide had almost eliminated the dose-limiting gastrointestinal toxic effects of CPT-11, especially diarrhea and nausea, and eight of nine patients were able to complete the chemotherapy course [13].

Although the exact antitumor mechanism is unknown, thalidomide exhibits both immuno-modulating and anti-angiogenic effects. Based on potentially synergistic mechanisms of action, thalidomide has the potential to enhance the activity of conventional chemotherapy.

An exhaustive bibliographic revision showed that there are no published methods for the simultaneous determination of thalidomide and CPT-11. Normally, due to its low fluorescence intensity, thalidomide has been analyzed by high-performance liquid chromatography (HPLC) with UV-detection at 220 nm [14–16], mass spectrometry (MS), or tandem MS detection [17] in different types of biological samples. Other techniques such as chiral stationary phase (CSP)-HPLC, capillary electrophoresis (CE) or

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

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electrochromatography (CEC) are aimed to separate the (+)-(R)- and (−)-(S)-enantiomers [18]. A single work was reported on a fluorescence method to determine thalidomide, achieving a limit of detection of 1.2 $\mu g L^{-1}$ [19]. Also, thalidomide is a synthetic derivative of glutamine and at physiologic pH can undergo hydrolysis to form this compound again. Li et al. have been developed a HPLC method with indirect UV detection, for study the purity of thalidomide and determine glutamine in thalidomide drug substances and products [20].

On the other hand, CPT-11 has been usually determined by HPLC with fluorimetric detection [21–23]. So, Sparreboom et al. have developed a method for simultaneous determination of CPT-11 and their mains metabolites in human plasma, urine and feces with a previous simple pretreatment of the biological samples consisted in rapid protein precipitation with a mixed of solvent extraction of methanol – 5% (w/v) and aqueous perchloric acid (1:1, v/v) [24]. Some authors have proposed their determination by molecular fluorescence: Rodríguez-Cáceres et al. have studied the fluorescent characteristics of CPT-11 in acidic and basic media, developing two methods for its determination in acidic media, using as oxidants I_2/I^- and Ce (IV) in the presence of Fe^{3+}. These methods were successfully applied to human urine samples and pharmaceuticals [25,26].

In the last decades, multivariate calibration methods have been introduced for the analysis of complex samples [27]. The advantage of using data involving high-dimensional structured information is the higher stability towards interferents and matrix effects, in comparison with first-order methodologies. In some situations, multiway analysis allows for a direct separation of the measured signals into the underlying contributions from individual analytes. Moreover, these methods present the benefit of short time analysis due to the avoidance of long and tedious pretreatment of samples to eliminate interferences. Parallel factor analysis (PARAFAC) [28] is especially useful when the data follow the so-called trilinear model, and achieves the second-order advantage (analyte quantitation in the presence of uncalibrated interferents). An alternative to working with second-order data is to rearrange them in vectors and then apply a first-order algorithm such as unfolded partial-squares (U-PLS) or the multi-dimensional variant N-PLS. However, N-PLS and U-PLS do not provide the second-order advantage, unless they are complemented with the additional procedure of residual bilinearization (RBL) [29–31]. Examples of application of second-order calibration methods to the analysis of different mixtures in biological samples include the determination of naproxen and salicylic acid in serum and naproxen, salicylic acid and salicyluric acid mixtures in urine [32], fluoroquinolone antibiotics in human serum [33], methotrexate and leucovorin in human urine [34] or flufenamic and meclofenamic acids in human urine samples [35], among others. Recently, several reviews about the applications of second-order calibration methods have been published [36,37].

In the present work, we have resolved a complex mixture of two analytes, CPT-11 and thalidomide, which presents several difficulties. On one hand, CPT-11 is highly fluorescent while thalidomide presents poor native fluorescence. On the other, urine presents a strong native fluorescence, and this signal is overlapped with the fluorescent signal of thalidomide. Three-way fluorescence data recorded in the form of excitation-emission fluorescence matrices (EEMs) were processed with PARAFAC and with N-PLS/RBL and U-PLS/RBL, and the advantages and disadvantages of these chemometric methods are discussed. Good recoveries were obtained in synthetic binary samples with all the multivariate methods employed, but in urine samples only PARAFAC yields good results, because N-PLS/RBL and U-PLS/RBL are unable to differentiate the signal due to thalidomide from the native fluorescence of urine.

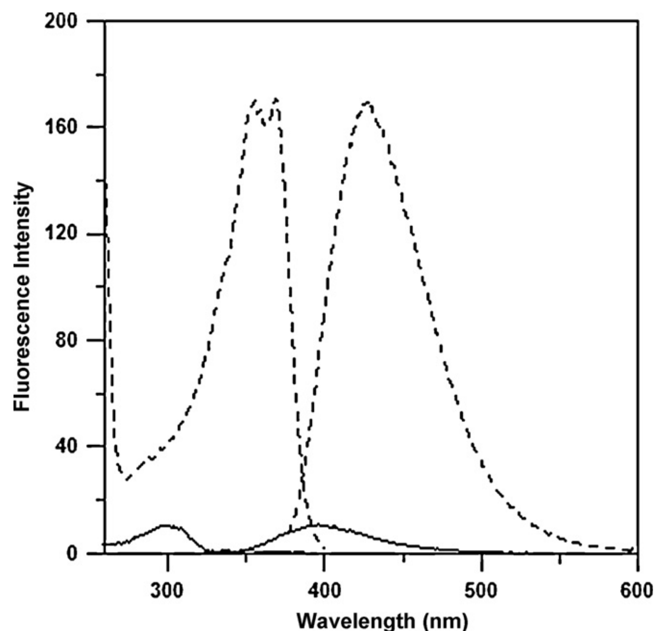

Fig. 1. Excitation and emission spectrum of 25.0 $\mu g mL^{-1}$ thalidomide (—) and 0.11 $\mu g mL^{-1}$ CPT-11 (-----).

2. Experimental procedure

2.1. Reagents

All experiments were performed with analytical reagent grade chemicals. (+)-Thalidomide and CPT-11 were obtained from Sigma–Aldrich (Steinhein, Germany). Ultrapure water was obtained from a Milli-Q Integral A10 system. A stock standard solution of thalidomide was prepared by dissolving 10.0 mg in 100 mL of acetonitrile:ultrapure water (20:80, v/v), while a stock standard solutions of CPT-11 containing 126 $\mu g mL^{-1}$ was prepared in methanol. A buffer solution (pH 5.0, C_t = 0.1 M) was prepared by

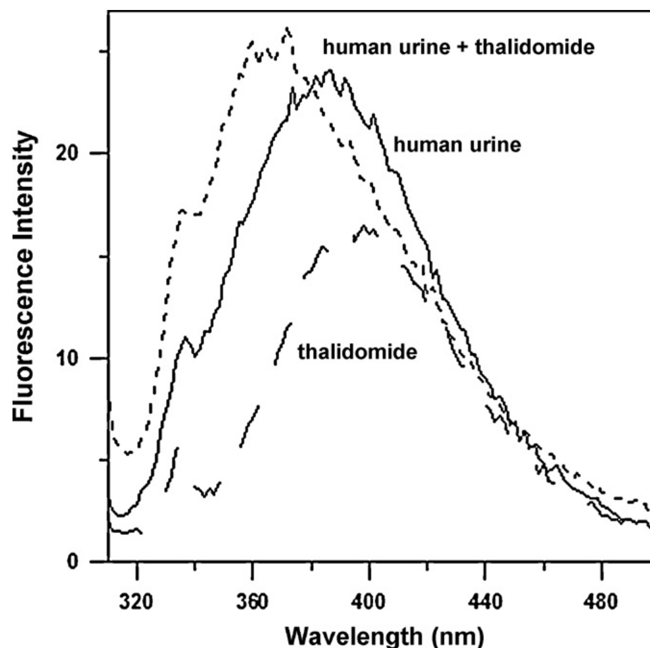

Fig. 2. Emission spectrum of 15 $\mu g mL^{-1}$ thalidomide (– – – – –), human urine diluted 1:5 (—) and human urine diluted 1:5 and spiked with 15 $\mu g mL^{-1}$ of thalidomide (—–-). λ_{exc} = 300 nm.

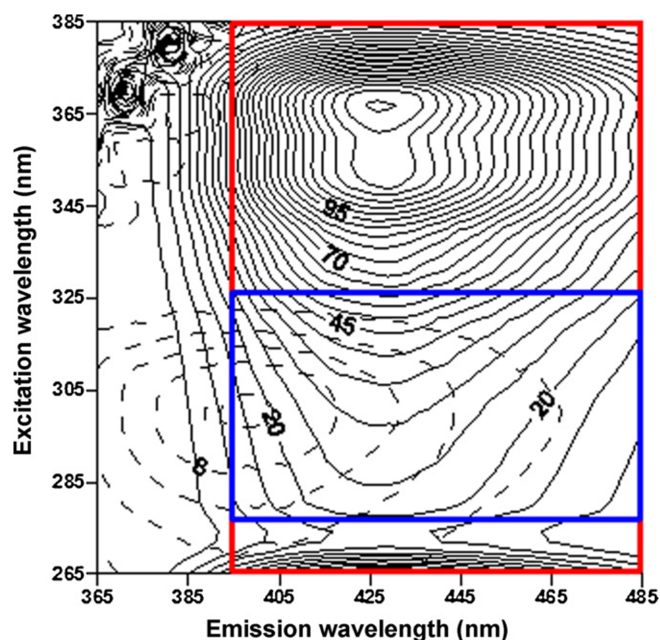

Fig. 3. Contours plots of the total fluorescence spectra of aqueous solution (pH 5.0) of 25 μg mL^{-1} thalidomide (—) and 0.11 μg mL^{-1} CPT-11 (- - - -). The selected area illustrates the spectral excitation and emission ranges of work for the second-order multivariate analysis of thalidomide ( ) and CPT-11 ( ).

dissolving a suitable amount of sodium acetate (Scharlau, Spain) with ultrapure water and adjusting the pH of the resulting solution to the desired value with hydrochloric acid (Scharlau, Spain). Chloroform and methanol were from Panreac (Barcelona, Spain).

2.2. Apparatus and software

Fluorescence measurements were performed on a Varian Model Cary Eclipse fluorescence spectrophotometer, equipped with two Czerny-Turner monochromators, a xenon light source and two photomultiplier tubes as detectors. The Cary Eclipse software was used for data acquisition. The corresponding excitation-emission matrices were registered in the following ranges: excitation, 265–385 nm each 10 nm and emission, 365–485 nm each 2 nm. The instrument was set up as follows: wavelength scanning speed, 1000 nm/min, monochromators band pass exc/em (nm/nm), 5/5 and detector voltage, 600 V. The cell was thermostated at 25 °C.

A Crison MicropH 501 meter (Barcelona, Spain) equipped with a combined glass/saturated calomel electrode was used for pH measurements.

The software package The Unscrambler® v6.11 (CAMO A/S Olav Tryggvasonsgt, N-7011, Trondheim, Norway) was used for the experimental design. All calculations were done using MatLab R2008a, using the MVC2 routine, an integrated MatLab toolbox for second-order calibration developed by Olivieri [38] and the program ACOC, developed by our group [39] for the statistical analysis.

2.3. Calibration, validation and spiked urine samples

A calibration set was constructed using a central composite design with the central point replicated three times. Concentration levels ranging from 2.00 to 18.00 μg mL^{-1} of thalidomide and from 0.02 to 0.20 μg mL^{-1} of CPT-11 were employed. This design provided a total of ten standards. However, six additional standards were added containing only thalidomide with the object of maximizing the information about this analyte. For preparing a given calibration sample, appropriate aliquots of stocks solutions were mixed with 0.6 mL of buffer acetic acid/acetate (0.1 M, pH

Table 1
Composition of the calibration set.

Calibration set	[THL] (μg mL^{-1})	[CPT-11] (μg mL^{-1})
P1	–	0.11
P2	17.92	0.11
P3	10.08	–
P4	10.08	0.20
P5	2.02	0.02
P6	17.92	0.02
P7	2.02	0.20
P8	17.92	0.20
P9	10.08	0.11
P10	10.08	0.11
P11	6.05	–
P12	6.05	–
P13	8.06	–
P14	8.06	–
P14	14.00	–
P16	14.00	–

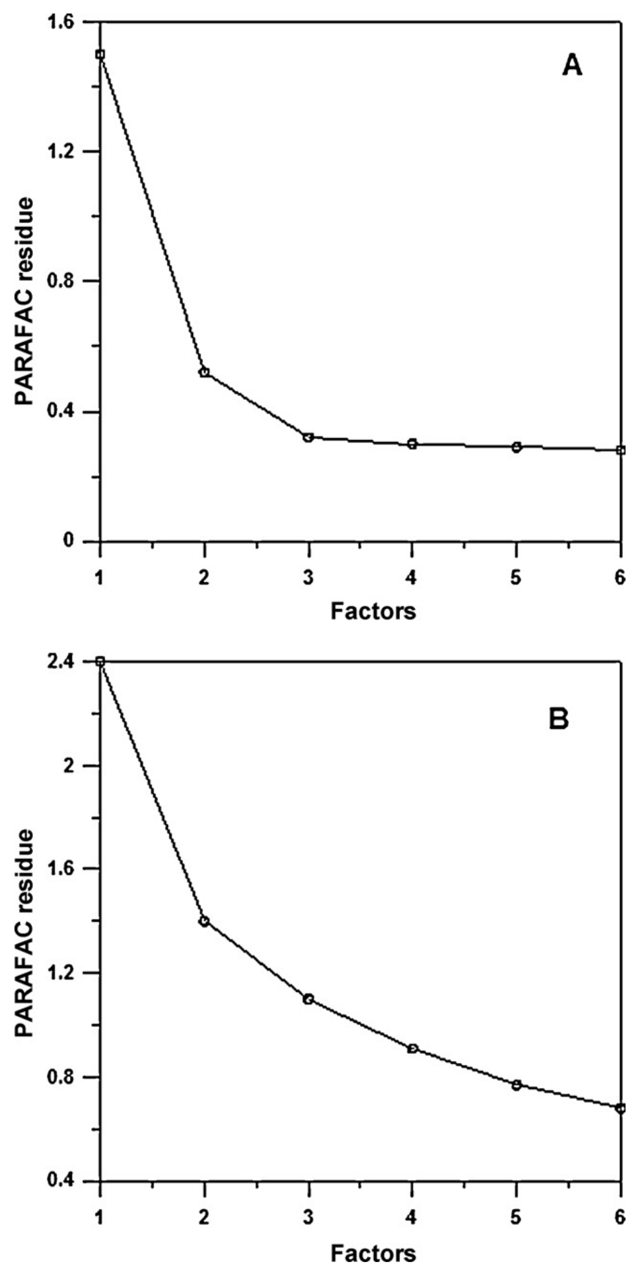

Fig. 4. Plot of the prediction residuals a function of a trial number of factors for test, containing 12.01 μg mL^{-1} thalidomide and 0.120 μg mL^{-1} CPT-11, corresponding to work region of thalidomide (A) and CPT-11 (B).

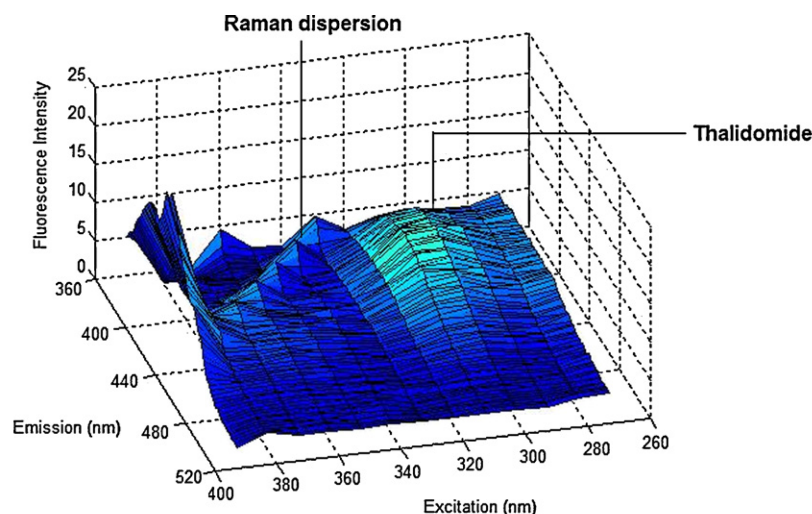

Fig. 5. Excitation-emission matrix of standard containing 14.0 μg mL^{-1} thalidomide.

5) and with ultrapure water to complete to 3.0 mL. Analytes were extracted with 5 mL of chloroform after shaking vigorously for 90 s. The organic phase was evaporated to dryness at 40 °C in a rotatory evaporator and the residue was dissolved in 1.00 mL of methanol and 0.3 mL of acetic/acetate buffer solution 0.1 M and deionised water was added to complete to 5.0 mL. The excitation-emission matrices of these solutions were recorded in the wide spectral excitation range from 265 to 385 nm and emission range from 365 to 485 nm, and the data were subjected to three-way analysis.

A validation set was also prepared, composed of four samples with three replicates each, in the same form as those for calibration, but using a random design, i.e., selecting the target concentrations of both analytes at random from each calibration range (see Table 1 for details on the composition of these samples).

Spiked urine samples were prepared as follows: 1.00 mL of human urine spiked with CPT-11 and thalidomide was placed in a separating funnel, the pH was fixed to 5 by adding acetic/acetate buffer solution 0.1 M, and finally deionised water was added to complete to 3.00 mL. Analytes were extracted with 5 mL of chloroform after shaking vigorously for 90 s. The organic phase was evaporated to dryness at 40 °C in a rotatory evaporator and the residue was dissolved in 1.00 mL of methanol and 0.3 mL of acetic/acetate buffer solution 0.1 M and deionised water was added to complete to 5.0 mL. Each urine sample was prepared in triplicate (details on the nominal analyte concentrations for these samples are provided below). The EEMs were subsequently recorded for all these samples in the same manner as described above.

3. Results and discussion

3.1. Fluorimetric study of the analytes

Thalidomide is weakly fluorescent, while CPT-11 presents a high fluorescence emission, as can be seen in Fig. 1. The excitation spectra show maxima located at 300 and 368 nm for thalidomide and CPT-11 respectively, and the emission spectra show maxima at 400 nm for thalidomide and 427 nm for CPT-11. As is shown in Fig. 1, the quantification of thalidomide in the presence of CPT-11 is challenging, due to the poor fluorescence intensity of the former analyte and to the fact that in the working wavelength regions the signal of this analyte is overlapped with that for urine. Fig. 2 shows the emission spectrum of thalidomide, typical urine diluted 1:5 and the same urine diluted 1:5 but spiked with thalidomide. As can be seen, the thalidomide emission spectrum is completely

Table 2
Recovery of thalidomide and CPT-11 in artificial mixtures.

Added (μg mL^{-1})		Found (μg mL^{-1})											
		PARAFAC				*N-PLS*				*U-PLS*			
THL	*CPT-11*	*THL*	*% Rec*	*CPT-11*	*% Rec*	*THL*	*% Rec*	*CPT-11*	*% Rec*	*THL*	*% Rec*	*CPT-11*	*% Rec*
		10.48	130.0	0.083	102.5	10.90	135.2	0.083	97.6	11.06	137.2	0.083	97.6
8.06	0.081	9.43	117.0	0.089	109.9	9.19	114.0	0.087	93.1	10.15	125.9	0.087	93.1
		11.17	138.6	0.087	107.4	11.01	136.6	0.083	97.6	11.27	139.8	0.084	96.4
		13.54	112.7	0.122	101.7	13.08	108.9	0.120	100.0	13.30	110.7	0.120	100.0
12.01	0.120	13.44	111.9	0.124	103.3	13.17	109.7	0.122	98.4	13.82	115.1	0.122	98.4
		13.50	112.4	0.123	102.5	13.22	110.1	0.123	97.6	13.33	111.0	0.123	97.6
		17.36	115.7	0.160	100.6	17.30	115.3	0.149	106.7	16.08	107.1	0.149	106.7
15.01	0.159	16.55	110.3	0.162	101.9	16.73	111.5	0.158	100.6	15.77	105.1	0.158	100.6
		15.98	106.5	0.157	98.7	15.91	106.0	0.160	99.4	15.33	102.1	0.160	99.4
		20.00	111.6	0.192	95.5	20.17	112.6	0.190	105.8	19.14	106.8	0.190	105.8
17.92	0.201	18.69	104.3	0.198	98.5	20.41	113.9	0.196	102.6	19.21	107.2	0.196	102.6
		18.93	105.6	0.197	98.0	20.08	112.1	0.195	103.1	19.14	106.8	0.195	103.1
% Rec[a] ± SD[b]		115 ± 10		102 ± 4		115 ± 10		100 ± 4		115 ± 13		100 ± 4	
RMSEP[c]		1.9		5.0×10^{-3}		2.1		5.6×10^{-3}		1.8		5.6×10^{-3}	
% REP[d]		12.6		3.4		13.7		4.0		13.4		4.0	

[a] Rec: average recovery.
[b] SD: Standard Deviation.
[c] RMSEP: Root Mean Square Error Prediction.
[d] REP: Relative Error of Prediction.

overlapped with a typical human urine spectrum, making necessary the use advanced multi-way modeling techniques for quantifying thalidomide in the presence of the urine background. We propose a fluorescence method in combination with second-order multivariate calibration algorithms, which have proved to be very powerful in the resolution of complex mixtures. Hence, PARAFAC, U-PLS/RBL and N-PLS/RBL performances have been evaluated regarding the quantitation of both analytes in urine, since all of them have the interesting second-order advantage (the possibility to determine one or several analytes in the presence of unexpected components) [40,41].

On the other hand, the fluorescence signal of a typical urine sample without any pretreatment is significantly higher than the signal of THL, precluding the direct determination of this analyte. With the purpose of reducing the matrix signal, a previous liquid–liquid extraction process with chloroform is necessary. According to previous studies [22,23], the pH of the aqueous phase was fixed at 5.0 by the addition of 0.3 mL of 0.1 M of acetic acid/acetate buffer solution in a final volume of aqueous phase of 3.0 mL. The shaking time and phase volume ratio were optimized; a shaking time of 90 s and a phase ratio aqueous/organic of 0.6 were selected as optimum values.

3.2. Validation data

To establish a quantitative model for the system, 16 calibration samples containing different amounts of THL and CPT-11 were prepared using a central composite design with three levels. The levels correspond to values in the range 0.00–0.20 mg L^{-1} for CPT-11and 0.00–18 mg L^{-1} for THL. The composition of the calibration samples is summarized in Table 1. As can be seen, six samples containing only THL are included with the aim of improving the robustness of the calibration, due to the low fluorescence of this compound. In all cases, samples were previously extracted with chloroform and the organic phase was evaporated to dryness under vacuum at 40 °C. The residue was re-dissolved with 1.00 mL of methanol and 0.3 mL of 0.1 M acetic acid/acetate buffer solution (to fix the pH to 5.0), and deionized water to complete to 5.0 mL.

Fig. 3 shows the contour plot of an EEM for a mixture containing THL and CPT-11. It was recorded in wide spectral excitation and emission ranges, 265–385 and 365–485 nm, respectively, and shows Rayleigh scattering. This latter signal is undesirable because it is not correlated with the target concentration of the studied analytes. Therefore, for both calibration and prediction purposes, an optimal region was selected for each analyte: for THL, 275–325 nm for excitation and 397–485 nm for emission, and for CPT-11, 265–385 nm for excitation and 397–485 nm for emission. The region selected for THL is restricted in order to avoid the interference due to the signal of CPT-11, and to obtain the best statistical indicators in the calibration step.

A first phase in the data processing with different second-order algorithms is the estimation of the number of responsive components. With N-PLS and U-PLS the usual procedure is the well-known leave-one-sample cross-validation procedure, according to Haaland and Thomas' criterion [42,43]. In both of these methods the number of components was 3 for thalidomide and 2 for CPT-11. In the case of these validation samples, there is no need to assess the number of interferents (required by the RBL procedure), because the composition of these samples is similar to those employed for calibration, and thus RBL is not employed.

In the case of PARAFAC, several procedures are available for estimating the number of responsive components: (1) the core consistency diagnostic test (CORCONDIA) [44], (2) the consideration of the residuals of the PARAFAC least-squares fit models for an increasing trial number of components [28], and (3) the visual examination of the recovered profiles. For the presently discussed analytical problem, the best results were obtained by assessing the number of PARAFAC components using a combination of (2) and (3), as discussed in detail below.

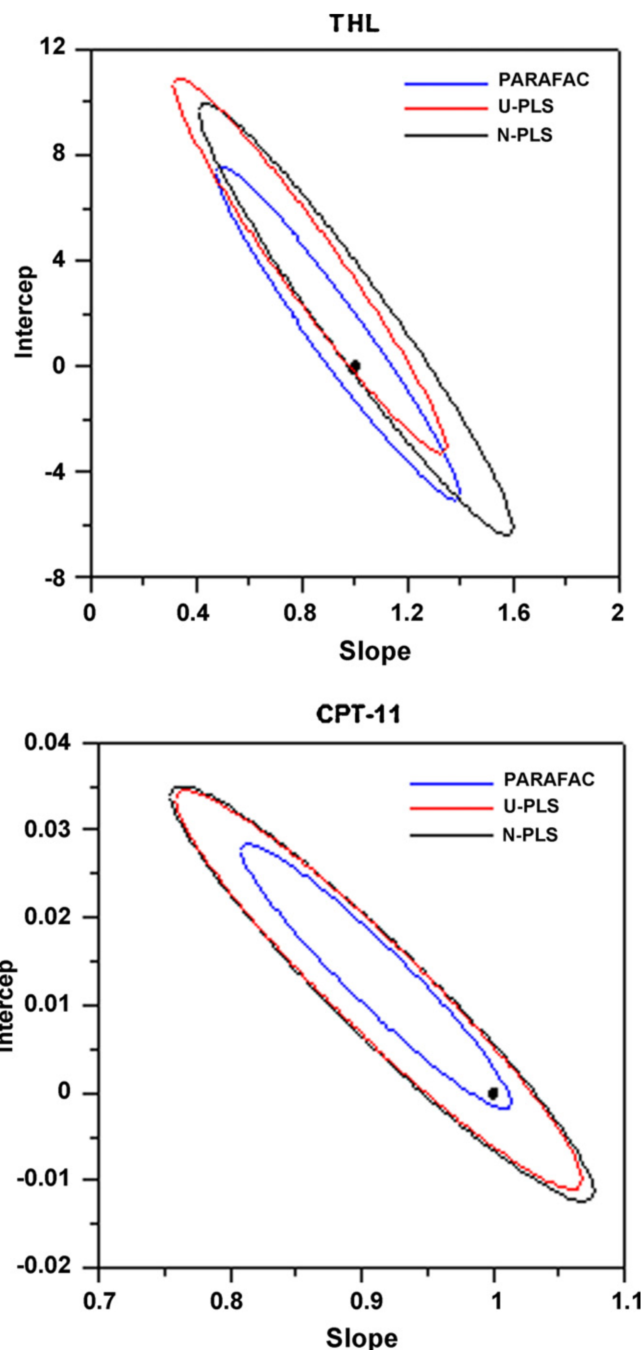

Fig. 6. EJCR (95% confidence level) for the slope and intercept of the regressions of the theoretical versus predicted concentrations of thalidomide and CPT-11.

Fig. 4A shows the fitting residuals as a function of increasing components for thalidomide. The optimum number of factors for this analyte is 3, where the residuals stabilized at a value compatible with the instrumental noise. Although two components might be expected for these samples in view of their composition, the need of three components can be explained considering the very small contribution of this analyte to the total signal of the sample (even the Raman solvent scattering shows a signal comparable to that of thalidomide, as shown in Fig. 5). Consequently, a small background fluorescent signal would compete with that of the analyte.

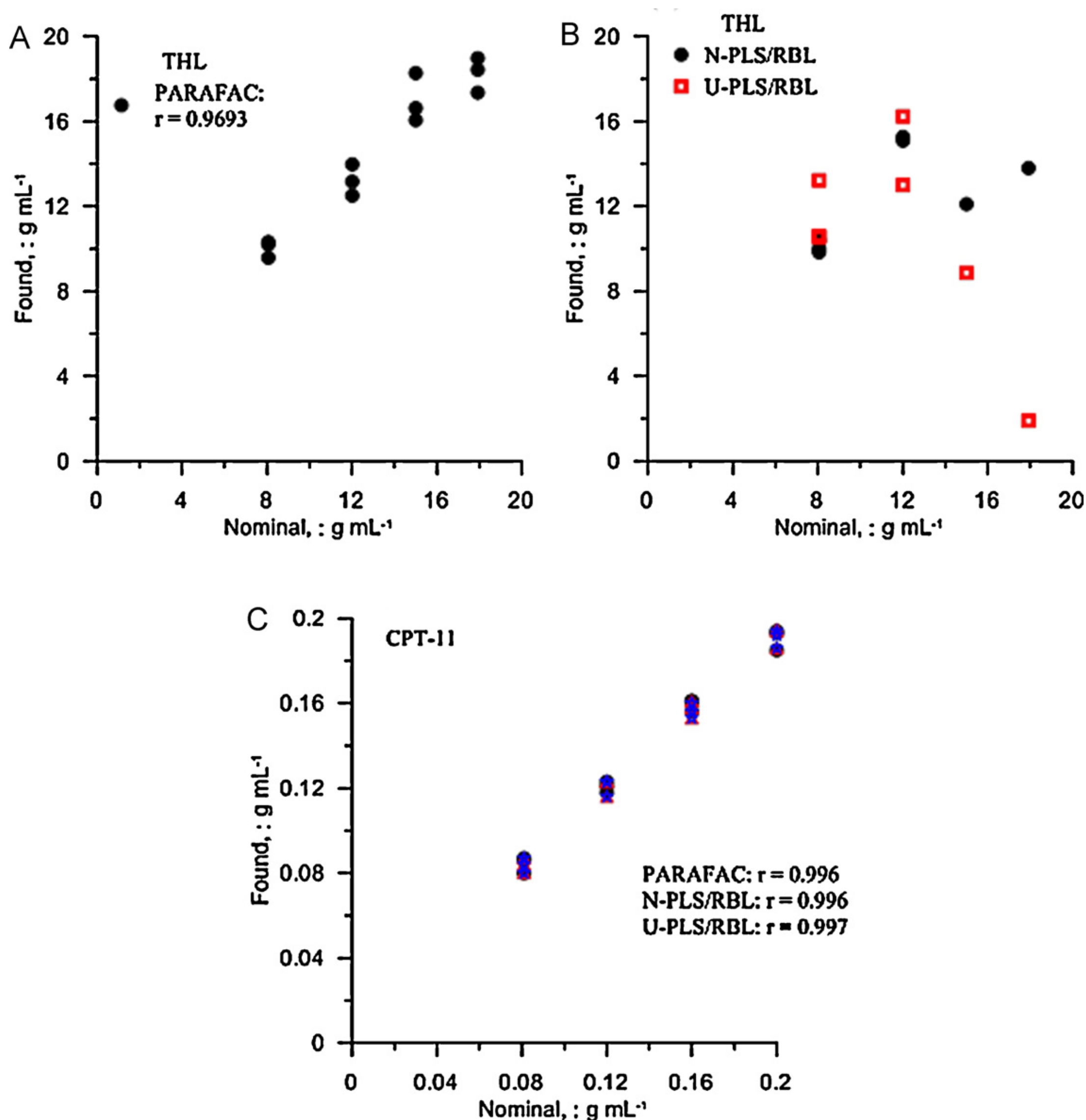

Fig. 7. Plots of THL (A and B) and CPT-11 (C) predicted concentrations in urine spiked samples as a function of the nominal values using PARAFAC, U-PLS/RBL and N-PLS/RBL.

These facts could explain the three responsive components for this analyte.

For CPT-11, on the other hand, the fitting residuals did not appear to stabilize on increasing the number of components (Fig. 4B). Nevertheless, this number for CPT-11 was selected after visual examination the profiles extracted by the PARAFAC model, the results obtained in the analysis of the validation set of samples, and on the basis of their chemical composition. The optimal number of factors selected for CPT-11 was 2.

In Table 2 the results obtained in the analysis of the validation samples are summarized. In general, the added and found contents were consistent for all of the mixtures tested. The recoveries are better for CPT-11, as expected from the higher fluorescence intensity for this analyte. However, the results obtained for thalidomide can be considered satisfactory taking into account its low signal and the overlapping with CPT-11.

In order to get further insight into the accuracy and precision of the algorithms analyzed, nominal versus found concentration values were compared by application of the EJCR (Elliptical Joint Confidence Region) test [45,46]. The corresponding plots are shown in Fig. 6. For both analytes, all confidence regions contain the ideal point of unit slope and zero intercept (indicating accuracy), but the elliptic size obtained with PARAFAC is smaller, suggesting that this chemometric methodology show better predictive ability than both N-PLS and U-PLS. These results are confirmed with the statistical results shown in Table 2, with very satisfactory values for the root mean square error of prediction (RMSEP) and relative error of prediction (REP) for the two analytes and with the three second-order calibration methods.

3.3. Urine samples

With the purpose of analyzing the potentiality of the evaluated second-order algorithms, the determination of the analytes in urine was carried out. A set of twelve urine samples was investigated with the aid of PARAFAC, N-PLS/RBL and U-PLS/RBL, all potentially achieving the second-order advantage (the RBL procedure is required to complement the PLS methods because of the presence of the urine signal in the test samples). When applying N-PLS/RBL and U-PLS/RBL to the spiked urine samples, it was necessary to assess the number of unexpected components (N_{unx}) to be employed in the RBL procedure. This can be done by analysing the sample modeling residuals, s_u, as a function of a trial number of unexpected components, as has already been described [47]. The

result was the same for all urine samples, and showed that a single new factor, besides those required for calibration (see above in connection with the validation samples). Therefore, in addition to the calibration factors (3 for thalidomide and 2 for CPT-11), one RBL component was needed for all test urine samples. The new factor is required to model the urine background. The spectrum of this factor is similar to the urine spectrum and all correlation coefficients between the new factors and the corresponding urine background were found to be close to 1.

When applying PARAFAC, the assessment of the number of components was made by analyzing the least-squares residues as a function of the number of components, as well as visually inspecting the profiles obtained with different number of components for each urine sample. All urine samples required the consideration of four factors for THL and three factors for CPT-11.

The prediction results obtained for the urine set are represented in Fig. 7. Three spectra of three different samples were used for each concentration level. As can be seen, the agreement between calculated and experimental values for CPT-11 is reasonable with the three procedures. However, in the presence of urine, unsatisfactory results were obtained for thalidomide when N-PLS/RBL and U-PLS/RBL were applied. With both of these multivariate methods, the relative errors of prediction for THL are higher in comparison with those of obtained the synthetic validation samples. Better results are obtained when the PARAFAC algorithm was applied: all predictions are reasonable and the relative error of prediction is significantly smaller than those for the PLS/RBL models. The poor results obtained when N-PLS/RBL and U-PLS/RBL were applied can be attributed to the extensive spectral overlapping between the analyte THL and the urine background, which apparently cannot be handled by the RBL procedure. In the presently studied case, only thalidomide predictions are affected, which is logical if we consider that the urine background mainly affects the signal of this particular analyte (see Fig. 2). The behavior is more pronounced with increasing concentrations of THL; N-PLS/RBL and U-PLS/RBL provide very poor recoveries for concentrations higher than 12 $\mu g\ mL^{-1}$ (Fig. 7B). Further studies should be made in order to gain a deeper insight into this interesting aspect of these second-order multivariate calibration.

For CPT-11, good results are obtained in all cases, see Fig. 7C. In this case, when nominal versus found concentration values were compared, all the slopes calculated are near 1 and the regression coefficients of the three procedures are all excellent.

4. Conclusions

The resolution of mixtures of two analytes with different fluorescence quantum yields, such as thalidomide and CPT-11 (two anticancer drugs), has been studied by recording three-way data sets, and applying several algorithms such as PARAFAC, N-PLS and U-PLS. The use of three-way data, exploiting the information contained in full fluorescence excitation-emission matrices and second-order algorithms, allowed the successful simultaneous determination of thalidomide and CPT-11 in synthetic samples. The simultaneous determination of thalidomide and CPT-11 in urine samples presents several difficulties. The problems are: (1) the high degree of overlap between the signals of the analytes and the human urine, (2) the absolute intensity of the urine background, which is, on the average, comparable to that for thalidomide in the investigated concentration ranges and (3) thalidomide is weakly fluorescent. In this case, N-PLS and U-PLS, in combination with the separate procedure RBL, give good results for CPT-11, but the recoveries for THL are considerably worse, probably because these algorithms are unable to differentiate the signal due to thalidomide from the native fluorescence of urine. However, when the processing algorithm was PARAFAC, the resolution of mixtures of THL and CPT-11 in human urine samples provided good recoveries. Recoveries for CPT-11 are better than for thalidomide, as expected due to the fact that the fluorescence intensity of thalidomide is lower than that for CPT-11, and the background signal of urine mainly overlaps with the thalidomide signal. A noticeable result from this study is that under such adverse conditions, PARAFAC allows for the successful quantitation of the analytes.

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