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Detection of verapamil drug by fluorescence and trilinear decomposition techniques

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Abstract

A three-way analytical methodology experimentally based on fluorescence excitation emission matrix (EEM) and in PARAFAC and TLD chemometric analysis was assessed for the quantification of verapamil drug in a tablet formulation. A standard addition procedure generates experimental information compatible with the chemometric data analysis model allowing the estimation of verapamil with a detection limit of about 0.04 mg/l using methanol as solvent. The structure of the verapamil EEM follows a trilinear model, but background signals (first- and second-order scatter bands) did not—a trilinear three-factor model is necessary to describe experimental datasets. The comparison of a three-factor PARAFAC model with a United States Pharmacopoeia (USP) standard chromatographic method showed similar results.

Keywords: Verapamil drug; PARAFAC; Fluorescence excitation emission matrix

1. Introduction

Verapamil is a calcium channel blocker widely used as an antihipertensive, antianginal and antiarhythmic drug [1,2]. In pharmaceutical formulations of verapamil, a variety of chemical compounds are used as excipients for its correct administration. Consequently, from the analytical chemistry point of view, the determination of verapamil in pharmaceutical formulations is a mixture analysis problem. Chemical analysis is usually done by liquid chromatography [2–15]. Spectroscopic measurements (molecular fluorescence or UV–VIS) have been proposed after solvent extraction [16–19].

Rapid, robust and inexpensive analytical methodologies are preferred for routine quality control of chemical species. Usually, methods that involved a chromatographic separation or extraction step are relatively time consuming. Alternatively, direct spectroscopic analysis of samples is relatively fast. However, if a mixture of several chemical species, which is almost the case for real cases constitutes a sample, direct spectroscopic analysis of samples may be biased due to the presence of unknown interferences.

Advances in chemometric methods allow quantitative analysis in the presence of unidentified interferents if a three-way experimental data matrix is available per sample. This property is the so called "second-order advantage" [20], and it is based on the earlier work done in the psychometrics field [21,22]. The use of this advantage in analytical chemistry

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was formerly proposed by Ho et al. [23] for the multicomponent analysis of fluorescent mixtures using excitation emission matrix (EEM). Later, Lorber [24] and Sanchez and Kowalski [25], proposed new and simple solutions for the method of Ho et al., and now it is called the generalized rank annihilation method (GRAM). Meanwhile, several other methods have been proposed that try to implement the "second-order advantage".

Methods that try to implement the "second-order advantage" belong to two main groups: (i) the direct solution methods; (ii) the iterative methods. In the first group, besides GRAM, Kowalski group proposed the trilinear decomposition (TLD) method [26,27]. Iterative methods are most common, and some examples are the following: parallel factor analysis (PARAFAC) [28,29]; positive matrix factorization [30]; multilinear engine [31]; curve resolution [32,33]. Besides the existence of several chemometric methods that implement the "second-order advantage", and to the increasing number of analytical applications [34–50], it is still impossible to define a general propose method that fit all the analytical problems because experimental data structures have particularities that have to be understood prior to data analysis.

At present, analytical chemistry laboratories have instrumentation that easily generates multidimensional data structures of experimental data per sample. Molecular fluorescence is a particularly interesting technique because it allows, in a very straightforward manner, the acquisition of this type of information, of which the most typical example is the EEM.

In this paper, a direct analysis method for verapamil in pharmaceutical formulations is proposed. The methodology consists in the acquisition of fluorescence EEM and in the chemometric analysis of experimental data using trilinear decomposition procedures: trilinear decomposition (TLD) and parallel factor analysis (PARAFAC). In order to validate the predicting ability of this new methodology, the results now obtained were compared with a standard high performance liquid chromatography with UV detection (HPLC-UV) United States Pharmacopoeia (USP) method for verapamil determination in tablet formulations [2].

The objective of this work is to assess the performance of the direct EEM analysis plus trilinear decomposition methodology for the quantitative determination of verapamil in pharmaceutical formulations and to evaluate the potential for the determination of the drug in biological fluids.

2. Experimental

2.1. Reagents

The (±)verapamil hydrochloride reference standard 99% purity was obtained from SIGMA–ALDRICH CHEMIE Gmbh (Steinheim, Germany). Deionized water with resistivity higher than 4 MΩ/cm was always used. Spectroscopic grade methanol from Merck (Darmstadt, Germany) was used for spectroscopic verapamil measurements. Methanol and acetonitrile chromatographic grades from Merck (Darmstadt, Germany), 2-amino heptane from Fluka (Neu-Ulm, Switzerland), sodium acetate and glacial acetic acid p.a. from Merck (Darmstadt, Germany) were used for chromatographic verapamil determination.

Verapamil determinations were made in a tablet formulation of verapamil 40 mg—Rathiopharm®.

2.2. Instrumentation

Molecular fluorescence measurements were made with a Perkin-Elmer LS 50 (Norwalk, CT, USA) luminescence spectrometer using 1 cm quartz cells. The instrument was operated with excitation and emission slits widths of 10 nm in an excitation range of 200–310 nm with 10 nm increments and in a emission range of 290–800 nm with a 200 nm/min scanning speed. The data was obtained with a default data interval of 0.5 nm.

HPLC-UV determinations were made with the following system: Hewlett-Packard HP1100 isocratic pump; injection valve Rheodyne (model 7752i); Rheodyne 20 μl loop; pre-column Agilent (20 mm × 4 mm); Supelco column (100 mm × 4.6 mm) Hypersil® ODS of 3 μm particle diameter; diode array detector Ati Unicam Crystal 250 model.

2.3. Solutions

For spectroscopic determinations standard stock solutions of (±)verapamil hydrochloride were prepared in water or methanol by rigorous weighing to a

final concentration of 100 mg/l. Synthetic standard solutions of lower concentrations were obtained by rigorous dilution of these stock solutions.

Samples of one tablet solutions in water and methanol were prepared by dilution of one tablet of verapamil 40 mg—Rathiopharm® in the respective solvent with the help of an ultrasonic bath. Centrifugation separates the solution from the non-soluble material and, by dilution, a tablet solution is obtained with a concentration of 1.6 mg/l.

Mixtures of 20 tablets were prepared by grounding and homogenization following USP specifications. A sample of this mixture with a mass equivalent to a tablet was prepared for spectroscopic (dissolved in methanol) and USP chromatographic analysis (dissolved in the chromatographic mobile phase). Mobile phase was prepared accordingly USP [2].

2.4. Spectroscopic EEM calibration

A standard addition method with four experimental points was used for the calibration and prediction of verapamil in synthetic solutions of the drug and in sample solutions. Typical conditions for the standard addition method consists in the following sequence of operations: (i) acquisition of the EEM of 3 ml of a verapamil solution (tested solution) contained in a 1 cm quartz cell; (ii) addition of 6 μl of the standard stock solution of verapamil hydrochloride 100 mg/l to the solution contained in the quartz cell, mixing with a micro magnetic bar (Hewlett-Packard 9301-1161) followed by EEM acquisition; (iii) two repetitions of step (ii), but the added volume of the standard stock solution is increased to 10 and 15 μl, respectively. Tested solutions were the following: (i) pure water and methanol (i.e. solutions having a zero concentration of verapamil); (ii) synthetic standard solutions of verapamil in water and methanol (1 mg/l); (iii) sample solutions in water and methanol (the expected verapamil concentration was 1.6 mg/l).

Several independent standard addition sequences were made using different added amounts of the verapamil standard stock solution—besides the typical conditions previously described other sequences were used: (i) 6, 9 and 15 μl; (ii) 15, 15 and 15 μl. Total verapamil concentrations in the solutions inside quartz cell were in the range of 0.2–2 mg/l. This range of concentration was selected according to the probable concentration of verapamil in the different tested solutions.

2.5. USP chromatographic calibration

Following the USP recommendations an external standard method of comparison was used [2].

2.6. Chemometric analysis

Sets of experimental data (EEM) (11 excitation wavelengths × 103 emission wavelengths × 4 samples) were analyzed by TLD and PARAFAC as implemented in the PLS-toolbox for MATLAB (Eigenvector Research Inc., USA). Moreover, PARAFAC program from Ramus Bro (www.models.kvl.dk/source/nway-toolbox) was used for weight PARAFAC calculations and for three-way decomposition model validation. The original 0.5 nm data interval of the emission spectra was reduced to a 5 nm data interval to be used in the data analysis.

The number of factors in the PARAFAC model was assessed by the analysis of the fit test (%), defined by [51]

$$\text{fit (\%)} = 100 \times \left(1 - \frac{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} e_{ijk}^2}{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} x_{ijk}^2}\right) \quad (1)$$

where e_{ijk} is the residual of the ijkth element and x_{ijk} the corresponding data element. Also, PARAFAC decomposition was assessed by the core consistency test (%) defined by [52]

$$\text{core consistency (\%)} = 100 \times \left(\frac{1-\sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F}(g_{\text{def}}-t_{\text{def}})^2}{\sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F} t_{\text{def}}^2}\right) \quad (2)$$

where g_{def} and t_{def} denote the elements of the calculated core and of the intrinsic super-diagonal core, respectively, and F the number of factors of the model. If g_{def} is equal to t_{def}, the core consistency is perfect and has a value of unity (100%). The appropriate number of factors is accessed by the model with the highest number of factors and a valid value of core consistency diagnostic test. The analysis of the results of these tests should allow the development of criteria for the selection of the number of factors.

A cross-validation of the PARAFAC models under analysis was also performed in a way that all the elements are left out once and models are fitted to the remaining data. A fit test (%) (Eq. (1)) is performed in the residuals between fitted and left out elements. Cross-validation calculations were performed with function ncrossdecomp from www.models.kvl.dk/source/nwaytoolbox.

Because a standard addition method was adopted in this work to estimate the concentration of verapamil in unknown concentration solutions (synthetic standard solutions used as validation sets and samples) the concentrations of verapamil estimated from PARAFAC and TLD decomposition (C_e, in abstract units) must follow the model [53]:

$$C_e = (\Delta C + C_0)k = \Delta Ck + C_0k \quad (3)$$

where ΔC is the known change in total verapamil concentration, C_0 the unknown verapamil concentration in the solution under analysis, and k the constant coefficient (slope) in the linear relation between C_e and ΔC.

3. Results and discussion

3.1. Preliminary analysis of spectra

Fig. 1 shows EEM of verapamil standard (1 ppm) (Fig. 1a) and diluted tablet of verapamil (1.6 ppm) (Fig. 1b) in methanol. The analysis of these EEM shows that verapamil has two well-resolved bands in the excitation (maximum of the bands at 230 and 280 nm) and emission (maximum of the bands at 312 and between 609 and 615 nm) fluorescence spectra. When water is used as solvent the shape of the spectra and the maximum of the bands are similar to those obtained in methanol.

The detailed analysis of Fig. 1 shows the existence of second-order scatter bands. These bands are clearly visually detected in the EEM of pure methanol shown in Fig. 2 (the EEM of water is similar) together with first-order scatter bands in the EEM top left corner.

3.2. Number of factors of PARAFAC model

Due to the presence in the experimental EEM of scatter bands and signal noise, besides the verapamil fluorescence signal, several models containing varying

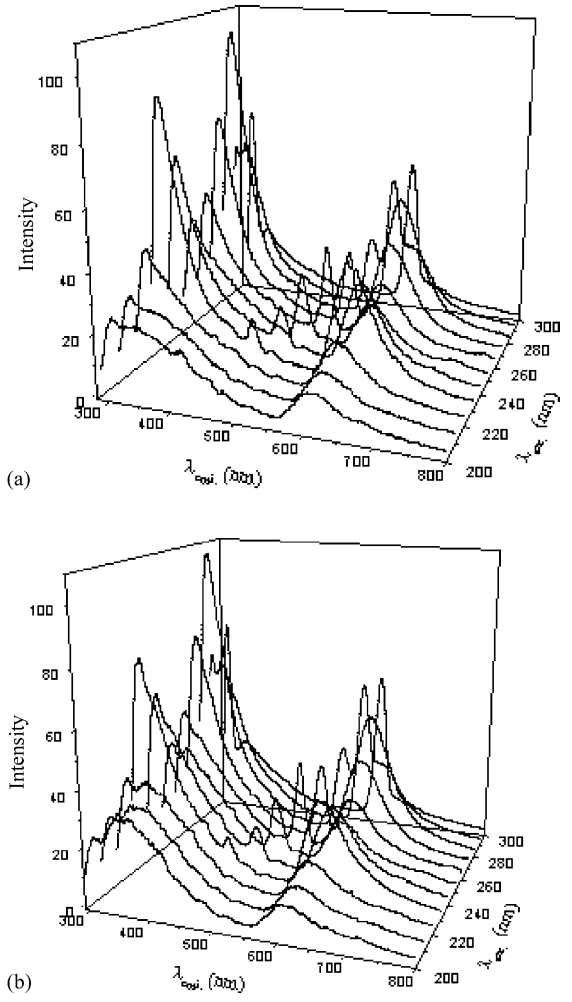

Fig. 1. Fluorescence excitation emission matrix of 1 ppm verapamil standard (a) and of a diluted tablet of 1.6 ppm verapamil Rathiopharm® in methanol (b).

number of factors (up to five factors) were tried for PARAFAC and TLD decomposition. For all the tested models PARAFAC converged to a solution—models with higher number of factors usually show unrealistic solutions.

Typical values for the percentage of fit and core consistency obtained with PARAFAC for all experimental datasets are shown in Table 1. A global analysis of the fit values shows that generally the percentage value increase quite rapidly up to about 98% and then levels of about this value. This information shows that three

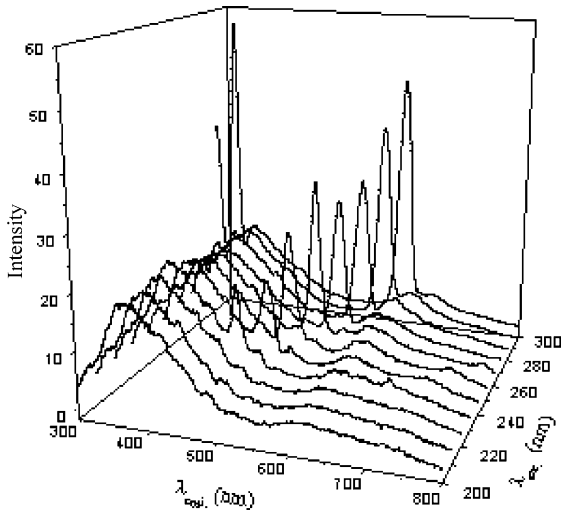

Fig. 2. Fluorescence excitation emission matrix of the solvent methanol.

factors are required to achieve 99% for all datasets, but for the methanol pure solvent where five factors are required to achieve that fit.

The analysis of the core consistency supports that three factors are necessary (core consistency in the range 50–80% and adding a new factor to the models resulted in a minimum value for the core consistency test) for all experimental datasets, except for the synthetic standard in methanol and one tablet sample in water where only a two-factor PARAFAC model is necessary (three-factor models resulted in a core consistency about 0%).

Cross-validation fit values for all the PARAFAC models are similar to the fit values presented in Table 1 obtained with the experimental datasets without missing elements.

3.3. Prediction of verapamil concentration in standard pure solutions

Typical results obtained by the standard addition method (Eq. (3)) using the PARAFAC decomposition results of the experimental data of the pure solvents and 1 ppm synthetic standard are summarized in Tables 2 and 3, respectively—similar results were obtained with TLD. These tables present the linear regression parameters of the estimated concentrations of verapamil (C_e of Eq. (3), in abstract units) versus the true concentrations (ΔC of Eq. (3), in mg/l) as well as the correlation coefficient of the estimated excitation and emission spectra versus the corresponding experimental spectra for the component recognized as corresponding to verapamil (the recognition was based in the calculated and experimental spectral comparison) (Figs. 3 and 4). The detection limit is also presented in these tables.

A good linear fit was observed between C_e versus ΔC (a correlation coefficient higher than 0.999 and a relatively small standard deviation of the residuals)—this condition is necessary for the successful application of the standard addition method [53]. The estimated excitation and emission spectra that corresponds to the verapamil factor reproduce well the experimental spectra—correlation coefficients about

Table 1
Fit values and core consistency diagnostic values in percentages versus The number of components in the PARAFAC model

Dataset	Number of factors									
	One		Two		Three		Four		Five	
	Fit	Core	Fit	Core	Fit	Core	Fit	Core	Fit	Core
Methanol										
Pure solvent	91.01	100	94.96	100	97.78	60.66	98.27	−0.14	98.86	99.95
Synthetic standard	93.91	100	97.98	100	98.86	−2.42	99.36	−0.25	99.59	99.97
Sample (one tablet)	95.58	100	98.00	100	98.88	46.30	99.39	−0.08	99.61	99.99
Sample (20 tablets)	96.92	100	98.38	100	99.05	54.90	99.43	44.51	99.62	99.89
Water										
Pure solvent	94.40	100	97.82	100	98.83	80.61	99.29	1.77	99.51	99.48
Synthetic standard	96.75	100	98.44	100	99.16	63.59	99.54	40.21	99.70	99.98
Sample (one tablet)	97.28	100	99.01	100	99.44	2.22	99.67	2.92	99.77	99.87

Table 2
Comparison of the results obtained for the solvents (methanol and water) by standard addition method after PARAFAC decomposition with two, three and four factors[a]

Chemometric analysis	PARAFAC					
	Methanol			Water		
	Two factors	Three factors	Four factors	Two factors	Three factors	Four factors
C_0 (ppm)	0.25 ± 0.14	0.10 ± 0.13	0.10 ± 0.13	0.06 ± 0.05	0.04 ± 0.05	0.05 ± 0.05
DL (ppm)	0.095	0.096	0.096	0.044	0.042	0.044
Calibration						
Intercept	12.00 ± 5.54	5.02 ± 5.67	5.01 ± 5.65	2.36 ± 2.00	1.48 ± 1.88	1.83 ± 2.00
Slope	48.55 ± 5.90	49.01 ± 6.04	49.06 ± 6.02	40.64 ± 1.75	40.69 ± 1.64	40.82 ± 1.75
$S_{y/x}$	1.539	1.575	1.570	0.601	0.563	0.601
R	0.9992	0.9992	0.9992	0.9999	0.9999	0.9999
Spectra comparison						
R_{exc}	0.9789	0.9883	0.9879	0.9956	0.9964	0.9959
R_{emi}	0.9883	0.9991	0.9898	0.9967	0.9967	0.9967

[a] C_0, standard addition estimated concentration of verapamil plus 95% confidence interval; DL, detection limit (=$3S_{y/x}$/slope); $S_{y/x}$, standard deviation of the residuals; R, correlation coefficient between the estimated and expected concentrations; R_{exc}, R_{emi}, correlation coefficient between the estimated and experimental excitation or emission spectrum, respectively.

0.99 or higher. The only exception, with a correlation coefficient lower than 0.99, is for the estimated emission spectra of verapamil 1 ppm standard solution in water. The detection limit evaluated is in the range of 0.04 and 0.1 ppm.

The possibility of detection of verapamil by the standard additions method in the presence of the scatter bands and signal noise was initially performed analyzing the solvent and the synthetic standard solution of verapamil 1 ppm. For the experiments with the pure solvent (Table 2), the results show that at least three factors are necessary to obtain feasible results—using as critical criteria, the estimated concentration of verapamil, which was zero ($C_0 = 0$ mg/l). This result is consistent with the above discussion about the right number of factors of the PARAFAC model,

Table 3
Comparison of the results obtained for a standard solution of verapamil (=1 ppm) in methanol and water by standard addition method after PARAFAC decomposition with two, three and four factors[a]

Chemometric analysis	PARAFAC					
	Methanol			Water		
	Two factors	Three factors	Four factors	Two factors	Three factors	Four factors
C_0 (ppm)	0.99 ± 0.09	0.87 ± 0.10	0.88 ± 0.09	0.84 ± 0.24	0.79 ± 0.24	0.79 ± 0.24
DL (ppm)	0.031	0.037	0.035	0.124	0.129	0.129
Calibration						
Intercept	37.38 ± 1.28	33.49 ± 1.55	33.97 ± 1.45	35.22 ± 6.12	32.77 ± 6.31	32.94 ± 6.26
Slope	37.86 ± 2.25	38.36 ± 2.72	38.49 ± 2.56	42.12 ± 5.22	41.73 ± 5.39	41.71 ± 5.35
$S_{y/x}$	0.393	0.477	0.448	1.745	1.799	1.787
R	0.9998	0.9997	0.9998	0.9992	0.9991	0.9991
Spectra comparison						
R_{exc}	0.9805	0.9900	0.9893	0.9937	0.9962	0.9959
R_{emi}	0.9219	0.9212	0.9196	0.9937	0.9941	0.9935

[a] See footnote of Table 2.

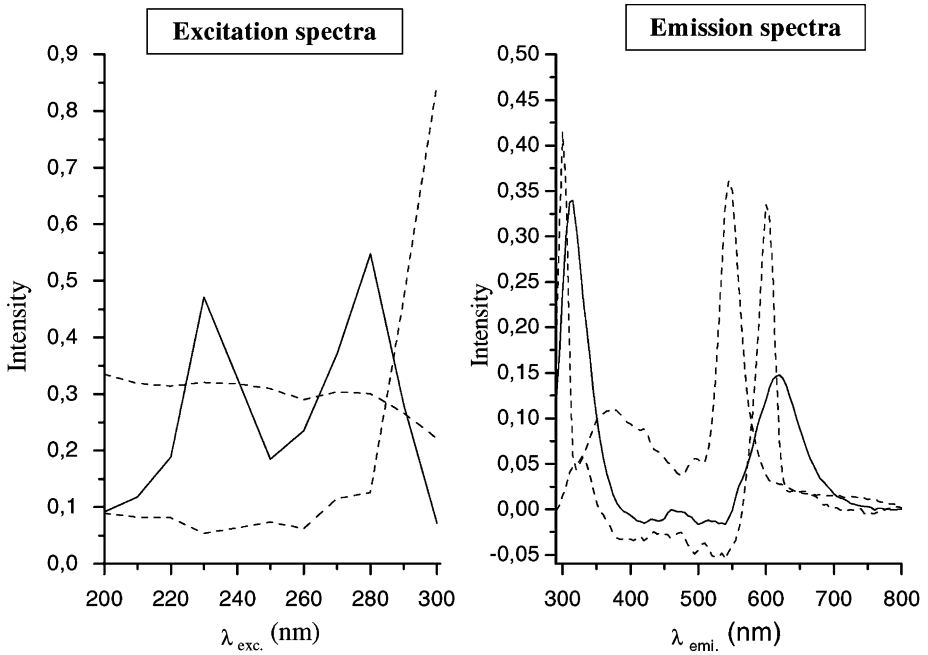

Fig. 3. Excitation and emission spectra calculated with PARAFAC for the three-factor model of the 1 ppm standard solution of verapamil in methanol: scatter spectral bands (- - -); fluorescence spectra of verapamil (—).

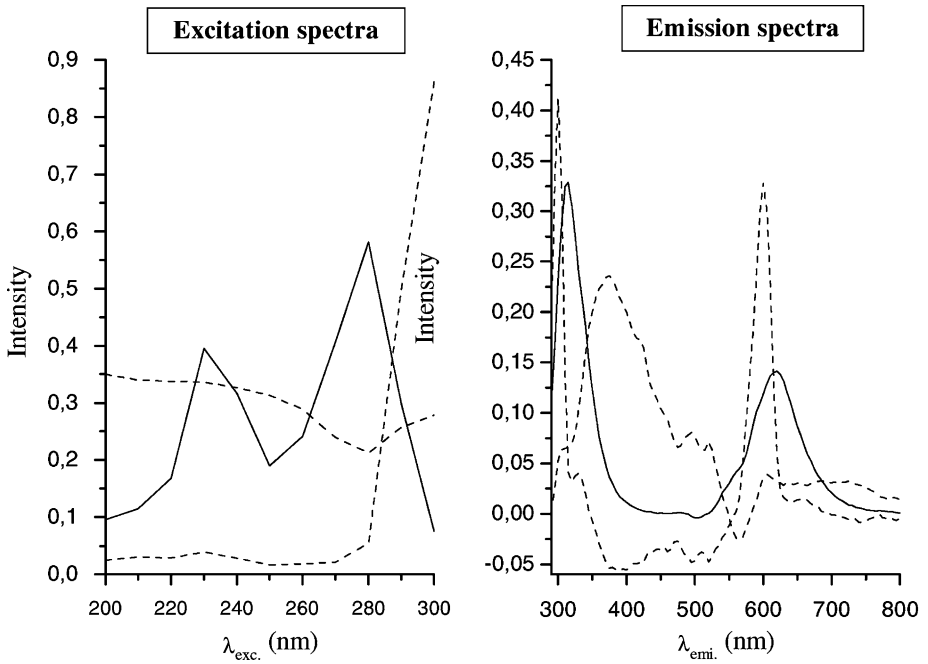

Fig. 4. Excitation and emission spectra calculated with PARAFAC for the three-factor model of the 1.6 ppm verapamil Rathiopharm® in methanol: scatter spectral bands (- - -); fluorescence spectra of verapamil (—).

which was three. Indeed, using a model with less than three factors the estimated concentration is statistically different (higher) from the expected zero. When a four-factor model is used the solution is not different from that obtained with a three-factor model. This result shows that the condition of "zero response for a zero concentration of analyte" necessary for the successful application of the standard addition method [53] is observed when a three-factors model is used for PARAFAC or TLD.

The analysis of the three emission and excitation spectra calculated with PARAFAC for the three-factor model, and shown in Fig. 3, show that only one factor correspond to verapamil and the other two to background signals (mainly scatter bands). Consequently, verapamil fluorescence shows the expected trilinear model while two factors are necessary to model the background signal.

Table 3 shows the results obtained when a 1 ppm synthetic standard solution is used. Contrary to what was observed for the pure solvent experiments, now a model with only two factors is necessary to obtained good results for the estimation of the verapamil concentration ($C_0 = 0.99 \pm 0.09$ and 0.84 ± 0.24 ppm). When more than two factors are included in the model sub estimation of the concentration is observed. This result agrees with the number of factors estimated by the core consistency test.

The reduction on the number of factors needed to obtain good results in the estimation of the verapamil concentration that was observed is probably due to minor spectral variations that are only relatively important when the EEM fluorescence signal is relatively small. This situation occurs, e.g. in the pure solvent experiments, where an extra factor was needed to model that relatively small spectral variation. When the EEM fluorescence signal becomes relatively large, it mixes or overlaps with minor spectral variation resulting in their practical annihilation.

The analysis of Table 3 shows that when the solvent was methanol the estimates of the concentrations were more precise than those obtained with water.

With the objective of model simplification experimental datasets were subtracted for the EEM of the pure solvent. Indeed, if the background (pure background signal plus scatter bands) was stable and constant, a simple subtraction would eliminate the imprecision detected in the selection of the number of factors. In this case, a one-factor model would be sufficient because the verapamil fluorescence follows a trilinear model. However, PARAFAC and TLD of the subtracted EEM resulted in unacceptable concentration estimations, showing that the background EEM signal is marked dependent on the sample composition.

3.4. Analysis of verapamil in a tablet formulation

Sets of EEM of a sample solution (verapamil tablet—1.6 ppm of verapamil) (Fig. 1b) plus the corresponding standard additions were subjected to an analysis with the models evaluated previously. The calculated excitation and emission spectra obtained for the three-factor model are presented in Fig. 4 and a summary of the analysis of the results is presented in Table 5.

The results obtained for the tablet formulation shows that the selection of the adequate number of factors is not straightforward if the previously tests for the selection of the number of factors have not been made. Indeed, models with two to four factors originate good solutions of the concentration of verapamil and spectra. The apparent increase of complexity of the model that is necessary to deal with the experimental tablet datasets is probably due to the effect of the other chemical constituents of the tablet on the background signal. Indeed, the analysis of the calculated excitation and emission spectra due to background signal (Fig. 4) are somewhat different than those obtained with a pure verapamil standard solution (Fig. 3). This result shows that the background signal is particularly sensitive to the chemical composition of the solution and that the trilinear decomposition can easily deal with this problem.

The analysis of Table 4 shows that a three-factor model gives good estimates of the verapamil concentration in the tablet formulation for the experimental settings used in this work—under these experimental conditions, at least two factors are necessary to model background. Also, methanol allows verapamil concentration estimations with greater precision and with a lower detection limit than those obtained using water as solvent.

Similarly to the above-discussed results about EEM background subtraction (end of Section 3.3), the results obtained with the tablet formulation were not

Table 4
Comparison of the results obtained for verapamil Rathiopharm® (≅1.6 ppm) in methanol and water by standard addition method after PARAFAC decomposition with two, three and four factors[a]

Chemometric analysis	PARAFAC					
	Methanol			Water		
	Two factors	Three factors	Four factors	Two factors	Three factors	Four factors
C_0 (ppm)	1.69 ± 0.13	1.51 ± 0.13	1.54 ± 0.13	1.56 ± 0.23	1.53 ± 0.23	1.53 ± 0.23
DL (ppm)	0.040	0.043	0.042	0.092	0.097	0.095
Calibration						
Intercept	59.05 ± 1.66	53.47 ± 1.82	54.51 ± 1.79	27.30 ± 1.79	26.77 ± 1.88	26.77 ± 1.84
Slope	35.04 ± 1.77	35.40 ± 1.94	35.51 ± 1.91	17.52 ± 1.54	17.55 ± 1.62	17.52 ± 1.58
$S_{y/x}$	0.462	0.506	0.497	0.537	0.564	0.553
R	0.9999	0.9998	0.9999	0.9996	0.9995	0.9996
Spectra comparison						
R_{exc}	0.9902	0.9943	0.9931	0.9946	0.9978	0.9978
R_{emi}	0.9917	0.9927	0.9931	0.9940	0.9944	0.9943

[a] See footnote of Table 2.

satisfactory when the subtraction was performed. As an alternative approach a weighted PARAFAC program was tried using a weight matrix in which is elements are calculated by inversion of the elements of the background matrix (pure solvent). This simple operation gives lower weights to the spectral regions were scatter bands appear. Table 6 shows the results of the weight PARAFAC calculations of the tablet formulation data for the two solvents used.

The analysis of Table 5 shows that weighting the data matrix originate statistically similar information to the unweighing data. However, the analysis of the absolute estimates for verapamil concentration in the tablet formulations, particularly for the case of a two-factor model (methanol) and three-factor model (water), shows a probable improvement. This result suggests that weighting strategies should be coupled to the PARAFAC procedure of EEM experimental

Table 5
Comparison of the results obtained for verapamil Rathiopharm® (≅1.6 ppm) in methanol and water without blank subtraction obtained by standard addition method after Weight PARAFAC decomposition with two, three and four factors[a]

Chemometric analysis	Weight PARAFAC					
	Methanol			Water		
	Two factors	Three factors	Four factors	Two factors	Three factors	Four factors
C_0 (ppm)	1.58 ± 0.14	1.51 ± 0.14	1.50 ± 0.14	1.73 ± 0.33	1.54 ± 0.23	1.51 ± 0.22
DL (ppm)	0.044	0.046	0.045	0.128	0.096	0.090
Calibration						
Intercept	32.91 ± 0.95	32.35 ± 1.18	32.27 ± 1.18	31.72 ± 2.62	30.29 ± 2.09	30.04 ± 1.99
Slope	20.86 ± 1.01	21.48 ± 1.25	21.57 ± 1.25	18.39 ± 2.25	19.68 ± 1.80	19.91 ± 1.71
$S_{y/x}$	0.309	0.326	0.327	0.785	0.627	0.596
R	0.9998	0.9998	0.9998	0.9991	0.9996	0.9996
Spectra comparison						
R_{exc}	0.9975	0.9925	0.9929	0.9995	0.9949	0.9970
R_{emi}	0.9942	0.9937	0.9934	0.9985	0.9951	0.9939

[a] See footnote of Table 2.

Table 6
Results obtained for a sample of a mixture of 20 tablets of verapamil Rathiopharm® in methanol without blank subtraction obtained by standard addition method after PARAFAC and Weight PARAFAC decompositions with two, three and four factors[a,b]

Chemometric analysis	PARAFAC			Weight PARAFAC		
	Two factors	Three factors	Four factors	Two factors	Three factors	Four factors
C_0 (ppm)	2.17 ± 0.18	1.81 ± 0.18	1.71 ± 0.15	2.06 ± 0.20	1.77 ± 0.16	1.71 ± 0.15
DL (ppm)	0.102	0.114	0.099	0.119	0.107	0.100
Calibration						
Intercept	27.87 ± 0.84	26.16 ± 1.05	25.57 ± 0.95	27.40 ± 1.02	15.69 ± 1.00	25.59 ± 0.96
Slope	13.74 ± 0.76	15.47 ± 0.96	16.02 ± 0.86	14.28 ± 0.93	25.92 ± 0.91	16.01 ± 0.87
$S_{y/x}$	0.467	0.587	0.531	0.568	0.557	0.535
R	0.9992	0.9990	0.9993	0.9989	0.9978	0.9992

[a] See footnote of Table 2. USP: United States Pharmacopoeia. In the spectroscopic EEM PARAFAC determinations the precisions correspond to the 95% confidence interval and were calculated with six experimental points.
[b] Standard USP chromatographic procedure: C_0 = 1.761 ± 0.005 ppm (n = 5).

data containing scatter bands and will be subjected of future research.

3.5. Comparison of spectroscopic analysis results with the USP chromatographic procedure

In order to obtain a final experimental validation about the predicting ability of the spectroscopic EEM plus PARAFAC decomposition method, a fraction of a mixture of 20 tablets was analyzed with this new procedure and compared with a standard USP chromatographic method. The result obtained with the standard procedure was 1.761 ± 0.005 ppm (five independent determinations).

A similar strategy to that followed in the previous section for the spectroscopic EEM plus PARAFAC decomposition analysis of this new tablet formulation sample was used, i.e. a two to four-factor PARAFAC model and the unweighed and weighed PARAFAC were tried. The results are presented in Table 6.

The analysis of Table 6 shows that the predictions of verapamil in the sample of the 20 tablet mixture obtained with the three-factor model [C_0(unweighed PARAFAC) = 1.81 ± 0.18 ppm; C_0(weighed PARAFAC) = 1.77 ± 0.16 ppm] are similar to those obtained with the standard method [C_0(USP) = 1.761 ± 0.005 ppm]. This number of factors for the PARAFAC model has also been previously selected from the fit and core consistency tests.

4. Conclusions

The EEM of verapamil, either in pure solvents or in a tablet matrix, presents a trilinear structure that can be successfully decomposed by PARAFAC or TLD. The analysis of verapamil in drug formulation by the spectroscopic EEM plus three-factor model PARAFAC decomposition gives similar results with a standard USP chromatographic procedure.

The development and assessment of a trilinear decomposition method for the analysis of verapamil in drug formulations using EEM should follow the following three steps: (i) selection and validation of the number of components used in the trilinear decomposition method; (ii) adjustment of C_e to the standard addition method model (Eq. (3)) fulfils the two necessary conditions of the standard addition method ("linear relationship" and "zero response"); (iii) estimated emission and excitation fluorescent spectra of the extracted factor that corresponds to verapamil are highly correlated with experimental spectra.

Attending to the trilinear structure of the EEM of verapamil, the composition of the matrix and the detection limit evaluated permit to consider the possibility of using the methodology presented in this work to the quantification of verapamil in biological fluids.

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