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Application of two chemometric methods for the determination of imipramine, amitriptyline and perphenazine in content uniformity and drug dissolution studies

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Abstract

Double-divisor spectra derivative and partial least squares methods were developed for content uniformity and dissolution tests in binary or ternary mixtures. The simultaneous determinations of perphenazine (PER) combined with amitriptyline hydrochloride (AMI) and/or imipramine hydrochloride (IMI) have been accomplished using the information of the absorption spectra of appropriate solutions. The double-divisor method is based on the use of the first derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture PER–AMI–IMI by a standard spectrum resulted from the addition of two of the three analytes in equal concentrations. The concentration of each component is then determined from their respective calibration graphs established by measuring the ratio derivative analytical signal at a specific wavelength. In this method, the linear determination ranges were of 3.65–18.24 μg/mL for PER, 4.32–21.60 μg/mL for AMI, and 4.83–24.19 μg/mL for IMI. The results were compared with those obtained by partial least squares multivariate calibration (PLS) method pre-treated by a wavelet compression-orthogonal signal correction (W-OSC) filter in zero-order derivative spectra. The calibration model was evaluated by internal validation (cross-validation) and by external validation over synthetic mixtures, content uniformity and dissolution tests. According to the dissolution profile test more than 95% of the three substances were dissolved within 10 min. The results from both techniques were statistically compared with each other and can be satisfactorily used for quantitative analysis and dissolution tests of multicomponent tablets.

Keywords: Double-divisor ratio spectra derivative; Partial least squares; Perphenazine; Amitriptyline; Imipramine; Dissolution test; Content uniformity

1. Introduction

Imipramine hydrochloride (IMI) and amitriptyline hydrochloride (AMI) are commonly used to treat the depressive disorders owing to their efficiency in elevating the mood of patients by interfering to the reuptake of norepinephrine or serotonin [1]. These compounds are amines with a common structure formed by two aromatic rings fused with a seven-atom cycle. They are generally named as tricyclic antidepressants and when combined with phenothiazines such as perphenazine (PER) they are widely used in treatment of some psychosis.

Courses of treatment with identical doses of AMI or IMI may present large differences in the concentration of the drug in the plasma most of which may imply a sub-optimum or even mo therapeutic effect [2]. This problem becomes more complicated in the presence of PER. Hence, the possibilities of quantification of these analytes in a triplicate mixture are significant in a combined therapy [3].

The simultaneous determination of the above drugs in pharmaceutical dosage forms, as a quality control test, remains of great interest. Moreover, the dissolution profile of a pharmaceutical formulation is another established mandatory test in International Pharmacopoeias. The test is not only

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valid for measuring the availability of the active ingredients but it is also an assay for testing the reproducibility of the manufacturing process.

Routine analysis methods have been usually developed for the determination of AMI, IMI and PER in either binary or multi-component mixtures by HPLC [4–8], gas chromatography [9,10] whereas the United States Pharmacopoeia procedure involves HPLC determination of AMI-PER in pharmaceutical formulations [11].

Among the various analytical techniques, UV spectrophotometric method remains one of the most popular methods for quantitive analysis of binary or ternary mixtures of tricyclic antidepressants combined with neuroleptics drugs [12–14]. UV–vis spectra usually contain non-specific data, which can be converted into useful information by derivative or multivariate calibration methods [15–19]. In order to achieve this, chemometric and derivative techniques have been proved interesting in analytical molecular spectroscopy. Clear explanations of these approaches and properly designed software could provide a bridge among chemometrics, mathematics and spectroscopic techniques.

Two methods have been reported for the resolution of two or more compounds in mixtures by ratio spectra derivative specrophotometry and derivative ratio spectra zero-crossing method [20–25]. According to these methods, the simultaneous determination of three compounds in ternary mixtures was based on the measurements of the amplitude at the zero crossing points in the derivative spectrum of the ratio spectra. However, in experimental practice there are some mixtures that cannot be treated using the above methodology because they do not present repeatability in zero crossing points during the analysis.

In this work, a new spectrophotometric method (double-divisor ratio spectra derivative) [26,27] and a chemometric PLS (partial least squares) have been successfully applied for the simultaneous determination of PER combined with AMI and/or IMI. The two methods were applied to mixtures of these drugs in pharmaceutical formulations. Tablet analysis and dissolution tests were also carried out with satisfactory sensitivity, accuracy and precision.

2. Theoretical background

2.1. Double-divisor ratio spectra derivative method

Consider a mixture of three compounds M, N, P. If Beer's Law is obeyed for all compounds over the whole wavelength range used and the path length is 1 cm, the absorption spectrum of the mixture is defined by the equation:

$$A_{\lambda i} = \alpha_{M,\lambda i} C_M + \alpha_{N,\lambda i} C_N + \alpha_{P,\lambda i} C_P \quad (1)$$

where $A_{\lambda i}$ is the absorbance of the mixture, $\alpha_{M,\lambda i}$ and $\alpha_{P,\lambda i}$ are the absorptivities of M, N and P at a wavelength λ_i, and C_M, C_N and C_P are their concentrations, respectively.

If Eq. (1) is divided by a corresponding equation resulted from the addition of spectra, for two of the three components (e.g., $A_{\lambda i} = \alpha_{M,\lambda i} C^0_M + \alpha_{N,\lambda i} C^0_N$) and if their concentrations are equal $C^0_M = C^0_N$, the following equation can be written:

$$\frac{A_{\lambda i}}{C^0(\alpha_{M,\lambda i} + \alpha_{N,\lambda i})} = \frac{\alpha_{M,\lambda i} C_M + a_{N,\lambda i} C_N}{C^0(a_{M,\lambda i} + a_{N,\lambda i})} + \frac{a_{P,\lambda i} C_P}{C^0(a_{M,\lambda i} + a_{N,\lambda i})} \quad (2)$$

The first factor of the second part of Eq. (2) can be equal to a constant (K) with respect to λ in a certain region or point of wavelength. Theoretically, this can be obtained in wavelengths corresponding to isoabsortivity values ($\alpha_{M,\lambda i} = \alpha_{N,\lambda i}$) for the two components M, N. Practically, these points can be found comparing the elaborated final spectra of mixtures with the corresponding final spectra of the analyte P (Fig. 1).

When the first factor of Eq. (2) can be substituted to a constant (K) Eq. (3) is derived:

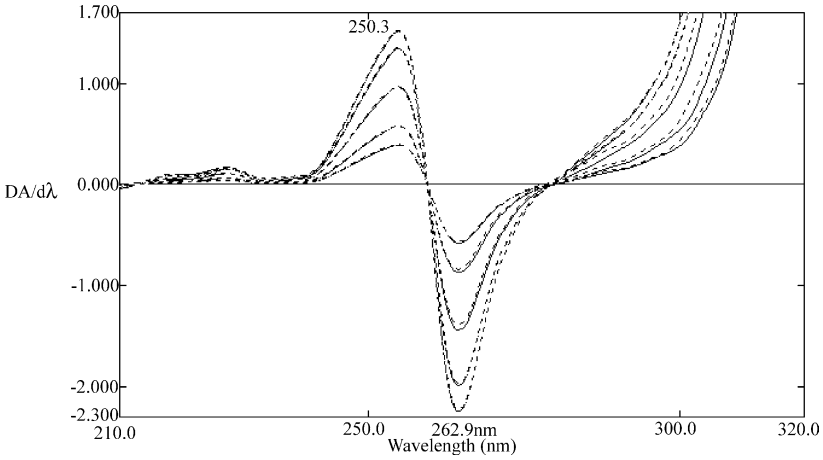

Fig. 1. The coincident spectra of the first derivative of the ratio spectra of 3.65–14.59 μg/mL pure PER (—) and ternary mixture AMI–IMI–PER (- - -) in 0.1N HCl.

$$\frac{A_{\lambda i}}{C^0(\alpha_{M,\lambda i}+\alpha_{N,\lambda i})} = K + \frac{a_{P,\lambda i}C_P}{C^0(a_{M,\lambda i}+a_{N,\lambda i})} \quad (3)$$

If the first derivative of Eq. (3) was taken, since the derivative of a constant is zero, Eq. (4) would be obtained:

$$\frac{d}{d\lambda}\left[\frac{A_{\lambda i}}{[a_M+\alpha_{N,\lambda i}]C^0}\right] = \frac{d}{d\lambda}\left[\frac{\alpha_P}{[a_{M,\lambda i}+\alpha_{N,\lambda i}]}\right]\frac{C_P}{C^0} \quad (4)$$

Eq. (4) is the mathematical foundation of multicomponent analysis, which permits the determination of the concentration of each of the active compounds in solution without interference from the other compounds of the ternary system.

In practice, the derivative signal of the ratio spectrum of the ternary mixture is dependent only on the concentration values C_P and C^0, but it is independent of the concentration values of the two other components M, N in the ternary mixture.

If $C_N = 0$, the Eq. (1) becomes:

$$\frac{d}{d\lambda} = \left[\frac{A_{\lambda 1}}{a_{M,\lambda 1}C^0_M}\right] = \frac{C_P}{C^0_M}\frac{d}{d\lambda}\left[\frac{\alpha_{P,\lambda 1}}{\alpha_{M,\lambda 1}}\right] \quad (5)$$

Eq. (5) indicates that in the binary mixtures, the concentration of P is independent of the values of CM.

3. Experimental

3.1. Instruments

A Shimadzu UV–vis double beam Spectrophotometer model UV-2501 PC consisting of a double monochromator with a high performance double-blazed holographic grating in the aberration corrected Czerny-Turner mounting and a light source of both a 50 W halogen lamp and a D_2 lamp. The optimized operating conditions for spectrophotometric measurements were ^{1}D derivative mode with $\Delta\lambda = 4$ nm, scan speed 210 nm/min, slit width 1.0 nm and sampling interval 0.1 nm. The system was connected to a Pentium III computer fitted with UV-PC Personal Spectroscopy software and a HP DeskJet 940c series printer, which was used for all absorbance measurements. Data pre-treatment methods and evaluation of figures of merit were implemented in a set of reduced routines under MATLAB® software version 6.5 (Multivariate calibration 1, MVC1 data Toolbox) [28] and visualization environment.

A dissolution system (Pharmatest type PT-PT7) complying with the USP Apparatus 2 specification (paddle method) was used in all tests.

3.2. Materials

All chemicals used for spectrophotometric method were of analytical reagent grade, unless otherwise specified. All drugs were obtained from Sigma–Aldrich Company. The diluent was an aqueous solution of 0.1N HCl for dissolution test and a methanolic solution 95% for content uniformity test. Water was redistilled by a “Millipore-Q plus 185”. The analyzed samples were compacted powder mixtures (200 mg) containing (a) 4 mg PER, 10 mg AMI, 10 mg IMI (b) 2 mg PER, 10 mg IMI and (c) 4 mg PER, 25 mg AMI. Excipients were starch maize, lactose monohydrate, talk, alginic acid and magnesium stearate.

Preparation of compacts: physical mixing, for 10 min, was performed in a planetary mixer (WAB Turbula system, T2C-Willy A. Bachofen, AG, Basel, Switzerland). Amounts of the physical mixtures were weighed (±1 mg) and tablets were prepared by compression on a manually operated hydraulic press. Hence, a 9 mm diameter flat face punch and die set were used and pressure in 600 psi was applied for 10 s, resulting in minimal attainable compact porosity (saturated compacts).

3.3. Standard solutions

Solutions of 3.65–18.24 μg/mL PER, 4.32–21.60 μg/mL AMI and 4.83–24.19 μg/mL IMI were prepared, separately, with a solvent mixture.

About 22.0 mg of AMI, IMI, and PER standards were accurately weighed and transferred to separate 50 mL volumetric flasks. About 5 mL of methanol was added to dissolve the analytes by shaking and then made up to volume with diluent 0.1N HCl for dissolution test or 95% methanol for content uniformity test. A portion 6.0 mL of each standard was transferred to four different 50 mL volumetric flasks and diluted with diluent. Thus, three different intermediate stock solutions were obtained. Seven different portions of the latter were transferred into 25 mL volumetric flasks to yield three series of standard solutions, which were used for the construction of the calibration curves. Similarly, a fourth series of binary or triplicate mixed standard solutions, containing AMI–IMI–PER, AMI–PER and IMI–PER were prepared in five different concentrations. These synthetic mixtures were employed for recovery studies. The concentration range for Beer’s law compliance was 3.65–18.24 μg/mL for PER, 4.32–21.60 μg/mL for AMI and 4.83–24.19 μg/mL for IMI.

3.4. Procedure for tablets

3.4.1. Content uniformity

As PER is insoluble in water and soluble in methanol, a solution of methanol 95% was considered as convenient for content uniformity test. The presence of 5% water was necessary to insure tablets disintegration in the first stage of the elaboration according to the following procedure.

Ten tablets were individually weighed and transferred in 1000 mL volumetric flask and each of them was disintegrated and then dissolved by adding 50 mL of water. About 400 mL of methanol was added to each flask and the dispersions

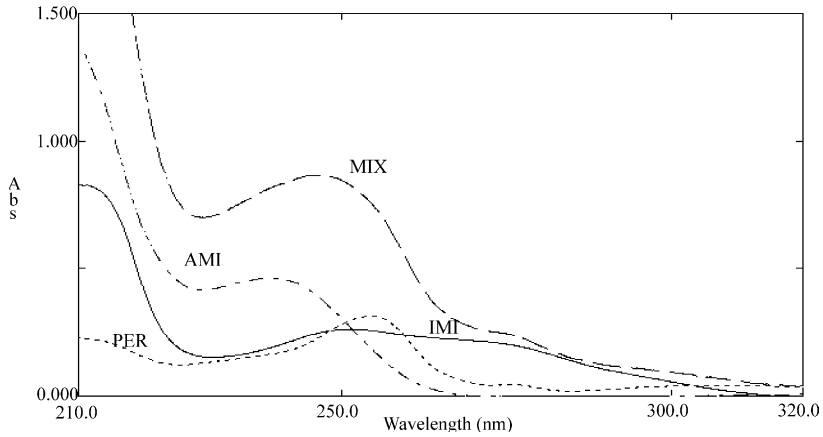

Fig. 2. Zero-order spectra of 11.1 μg/mL IMI(—), 4.4 μg/mL PER (- - -), 11.1 μg/mL AMI (– –) and their mixture (– –) in 0.1N HCl.

were vigorously shaken for 45 min on a mechanical shaker. Ultrasonication followed for 10 min and the solutions were diluted to volume with methanol and left to precipitate Filtration with acrodisc Gelman hydrophilic polypropylene (GHP) membrane was used to ultra clean them of particles 0.45 μm or larger.

3.4.2. Dissolution test for tablets

The dissolution test of tablets was carried out by USP27 method for AMI-PER [11] (paddle sped 50 rpm, dissolution medium 900 mL 0.1N HCl, time 60 min) in 37 ± 0.1 °C ($n = 6$). The samples were taken by means of an injector with membrane filter (0.20 μm) and analyzed by derivative ratio and PLS methods.

4. Results and discussion

4.1. Double-divisor ratio spectra derivative method

In ternary mixtures of AMI, IMI, PER, the absorption spectra for each component of the mixtures overlap sufficiently (Fig. 2) and demonstrate the resolving power of the proposed method.

In order to obtain the best spectra recoveries for PER, AMI and IMI it is necessary to study and optimize parameters as $\Delta\lambda$ to obtain the first derivative, smoothing function, scaling factor and divisor standard concentration.

The absorption spectra of the solutions of PER in 0.1N HCl were recorded in the range 210–320 nm, divided by the double-divisor (15.12 μg/mL IMI and 15.12 μg/mL AMI) and their ratio spectra was obtained. They were smoothened at $\Delta\lambda = 4$ through the use of 17 experimental points (Fig. 3). Fig. 1 indicates the first derivatives, which were calculated with interval of $\Delta\lambda = 4$ and scaling factor 10 from the ratio spectra. The concentration of PER was determined by measuring the amplitude at 250.3 nm corresponding to a maximum point.

Similarly for AMI the divisor was a standard mixture solution of IMI 12.78 μg/mL and PER 12.78 μg/mL (scaling factor = 10, $\Delta\lambda = 4$) while for IMI it was a mixture of AMI 10.8 μg/mL and PER 10.8 μg/mL (scaling factor = 1, $\Delta\lambda = 4$). The regression equations with the confidence limits for the intercept and slope were:

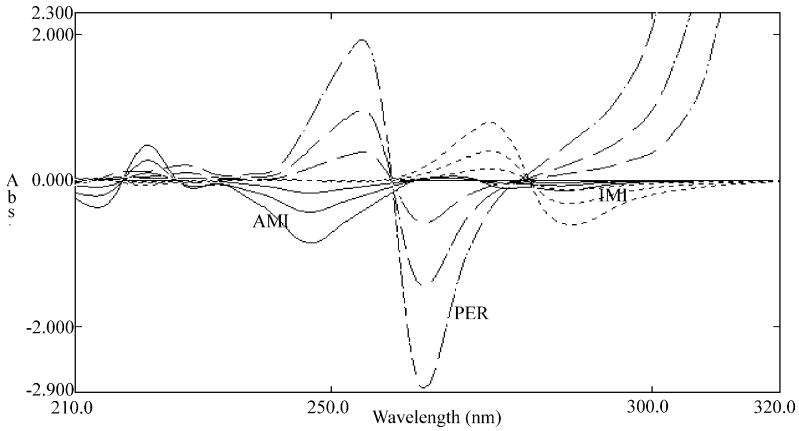

Fig. 3. Ratio spectra of PER (– –) 3.65, 9.12, 18.24 μg/mL (15.12 μg/mL AMI + 15.12 μg/mL IMI as divisor). AMI (—) 4.32, 10.8, 21.6 μg/mL (12.78 μg/mL PER + 12.78 μg/mL IMI as divisor). IMI (- - -) 4.83, 12.1, 24.19 μg/mL (10.8 μg/mL AMI + 10.8 μg/mL PER as divisor) in 0.1N HCl.

Table 1
Recovery results for different concentrations of synthetic mixtures AMI–IMI–PER by applying the derivative ratio and PLS algorithm (W-OSC) methods

AMI			IMI			PER		
Added μg/mL	PLS method %found	Ratio method (246.8 nm) %found	Added μg/mL	PLS method %found	Ratio method (287.2 nm) %found	Added μg/mL	PLS Method %found	Ratio method (250.3 nm) %found
4.32	98.1	98.6	4.83	103.1	102.6	3.65	100.8	101.9
6.48	99.8	100.9	7.26	102.9	100.2	5.47	99.0	99.6
10.8	103.1	101.4	12.1	101.4	99.3	9.12	99.9	99.9
15.12	96.5	98.5	16.93	99.8	102.2	12.77	99.0	100.8
17.28	99.5	99.6	19.35	98.9	100.6	14.57	99.7	99.4
% mean recovery	99.4	99.8	% mean recovery	101.2	101.0	% mean recovery	99.7	100.3
%R.S.D.	2.47	1.3	%R.S.D.	1.8	1.3	%R.S.D.	0.7	1.0

$$\text{PER}: y = (104.8 \pm 2.6) \times 10^{-3}x + (4.9 \pm 3.0) \times 10^{-3}$$

(250.3 nm, $r = 0.9998$)

$$\text{AMI}: y = (38.2 \pm 2.2) \times 10^{-3}x + (11.2 \pm 20.9) \times 10^{-3}$$

(246.8 nm, $r = 0.9995$)

$$\text{IMI}: y = (24.5 \pm 0.6) \times 10^{-3}x + (8.9 \pm 10.1) \times 10^{-3}$$

(287.2 nm, $r = 0.9998$)

where x is the concentration in μg/mL and y is the ^{1}D values.

Recovery studies of the described method were performed in three different series of synthetic mixtures, AMI–IMI–PER prepared by adding accurately weighed amounts of drugs. Results are presented in Table 1.

Furthermore, the ratio spectra derivative zero crossing point method has also been applied unsuccessfully for the resolution of the above mentioned ternary mixtures. The problem for the analysis of these mixtures was that the zero crossing points in most of the samples were not fixed in the same wavelength.

In binary mixtures of PER combined with AMI or IMI the absorption spectra of the solutions prepared at different concentrations of PER were recorded and divided by the spectrum of AMI (10.8 μg/mL) standard solution, or IMI (12.1 μg/mL). The first derivatives of the ratio spectra were calculated with $\Delta\lambda = 4$ nm. In the binary mixtures, the concentration of PER was determined by measuring the first derivative signals at 256.6 and 266.8 nm for PER–AMI (scaling factor 1) and 253.7 and 263.7 nm for the PER–IMI (scaling factor 1) (Fig. 4) mixtures, respectively.

Similarly, for AMI (Fig. 4) and IMI, the stored UV absorption spectra of standard solutions were divided wavelength-by-wavelength by a standard spectrum of PER (9.1 μg/mL). The content of the substances was determined by selecting the first derivative of the ratio spectrum with a scaling factor 5 for AMI and 1 for IMI, respectively. The regression equations with the confidence limits for the intercept and slope were as follows:

PER–AMI mixture

$$\text{PER}: y = (52.2 \pm 1.2) \times 10^{-3}x + (0.8 \pm 1.5) \times 10^{-3}$$

(256.6 nm, $r = 0.9999$)

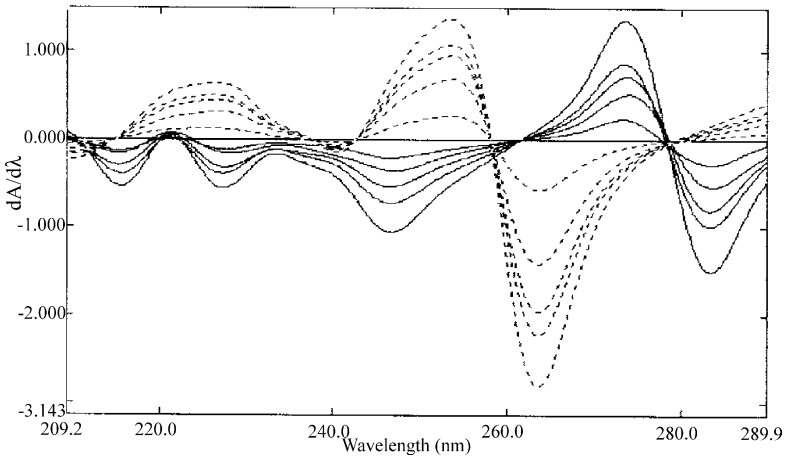

Fig. 4. First derivative of the ratio-spectra for binary mixtures of (a) AMI (—) 4.32–21.60 μg/mL; divisor PER (9.10 μg/mL). (b) PER (- - -) 3.65-18.24 μg/mL; divisor IMI (12.10 μg/mL) in 95% MeOH.

$y = (51.9 \pm 2.2) \times 10^{-3}x + (20.6 \pm 25.9) \times 10^{-3}$

(266.8 nm, $r = 0.9995$)

AMI : $y = (48.4 \pm 1.8) \times 10^{-3}x + (4.2 \pm 2.5) \times 10^{-3}$

(246.6 nm, $r = 0.9996$)

PER–IMI mixture

PER : $y = (74.8 \pm 1.6) \times 10^{-3}x + (7.0 \pm 12.2) \times 10^{-3}$

(253.7 nm, $r = 0.9999$)

$y = (152.1 \pm 3.0) \times 10^{-3}x + (18.4 \pm 27.4) \times 10^{-3}$

(263.7 nm, $r = 0.9999$)

IMI : $y = (110.1 \pm 3.2) \times 10^{-3}x + (22.7 \pm 29.6) \times 10^{-3}$

(275.2 nm,$r = 0.9998$)

$y = (93.7 \pm 2.6) \times 10^{-3}x + (26.3 \pm 20.6) \times 10^{-3}$

(283.9 nm, $r = 0.9998$)

Limit of detection (LOD) and limit of quantitation (LOQ) values, for the ratio derivative procedure (Table 2) were calculated according to the following criterions:

$$\text{LOD} = \frac{3S_{y/x}}{m}, \qquad \text{LOQ} = \frac{10S_{y/x}}{m}$$

where $S_{y/x}$, is the residual standard deviation and m is the calculated slope of the corresponding calibration [29,30].

4.2. Partial least squares

4.2.1. Calibration and validation of PLS models

Partial least square method (PLS) was evaluated by using the same spectra of the samples which were employed in the previous derivative method in their zero and first order. The spectral region between 210 and 350 nm was selected by using 141 points as (X) variables for the analysis. To model the system with the optimum amount of information, cross validation was applied obtaining statistical parameters that show the efficiency for a calibration fit mode. Then in order to select the optimum number of significant PLS components, the criteria proposed by Haaland and Thomas [31] were used.

Adopting the cross-validation method useful magnitudes have been calculated:

- Q^2 is the fractions of the total variation of the Y's that can be predicted by a component. Values of Q^2 close to 1.0 indicate an excellent model.

$$Q^2 = 1 - \frac{\text{PRESS}}{\text{SS}}$$

  where SS is the residual sum of squares and PRESS the prediction error sum of squares
- The root mean square error of prediction (RMSEP), which is the standard deviation of the predicted residuals (error), is given by

$$\text{RMSEP} = \sqrt{\frac{\sum (\text{obs} - \text{pred})^2}{N}}$$

  where N is the total number of calibration samples.
- The root mean square error of estimation (RMSEE), which is the standard deviation of the estimated residuals (error), is computed as

$$\text{RMSEE} = \sqrt{\frac{\sum (\hat{c}_i - c_i)^2}{N}}$$

  where $\hat{c}_i$ represents the estimated concentration and c_i the reference concentration
- The square of the correlation coefficient (r^2), which is an indication of the quality of the fit of all data to a straight line, is presented by

$$\frac{r^2 = \sum_{i=1}^{N} (\hat{c}_i - c_i)^2}{\sum_{i=1}^{N} (c_i - \bar{c}_i)^2}$$

  where $\bar{c}_i$ represents the means of the true concentrations in the predictor set.

These statistical parameters will be used to evaluate and optimize the performance of multivariate calibration models using different pre-treatment data method.

Table 2
Limit of detection and limit of quantitation for the ternary and binary mixtures of the analytes by the derivative ratio method

Compounds	Derivative ratio method			
	LOD (μg/mL)		LOQ (μg/mL)	
AMI	0.924 (246.8 nm)		3.082 (246.8 nm)	
IMI	0.459 (287.2 nm)		1.531 (287.2 nm)	
PER	0.328 (266.9 nm)		1.094 (266.9 nm)	
AMI	0.604 (246.6 nm)		2.013 (246.6 nm)	
PER	0.324 (256.6 nm)	0.572 (266.8 nm)	1.079 (256.6 nm)	1.907(266.8 nm)
IMI	0.156 (275.2 nm)	0.496 (283.9 nm)	1.718 (275.2 nm)	1.652 (283.9 nm)
PER	0.294 (253.7 nm)	0.206 (263.7 nm)	0.981 (253.7 nm)	0.688 (263.7 nm)

Hence, a comparative study of the prediction capabilities of PLS approach, was undertaken by using different kind of filters such as orthogonal signal correction (OSC) [32], multiple scatter correction (MSC) [33], standard normal deviate (SNV) [34] and different systems for wavelet compression or de-noising of spectra. The optimal number of components was selected in each case by considering the values giving minimal RMSEE/RMSEP values and Q^2 values close to 1.0. When there was no agreement between them, the number of component giving minimal RMSEP values was finally selected.

The most appropriate results were received in zero-order derivative spectra by using a wavelet compressor spectral (WCS) and orthogonal signal correction (OSC) filter, since the number of components calculated for the other filters was >7, the RMSEE values between 1.17 and 2.16 and the RMSEP values from 2.609 to 32.74.

In the first order derivative spectra the values obtained for the three analytes are respectively higher in all cases than those described previously.

OSC is a PLS-related filter, which removes variations from (X) that does not contain any information about (Y), i.e., it removes only so much of (X) as is unrelated (orthogonal) to (Y).

In Table 3, a summary of prediction errors for AMI, IMI and PER, in binary or triplicate mixtures, using PLS and PLS (W-OSC) filter pre-treatment methods are given. According to the Table 3, the statistical parameters using PLS (W-OSC) in both binary and ternary mixtures are better than these without filter pretreatment. However, slightly better results by using PLS method in RMSEE values for AMI and IMI in triplicate mixtures are presented. On other hand, the negative Q^2 value of PER in the same mixture and the generally higher RMSEP values confirm the use of W-OSC filter before PLS method.

Table 3
Statistical parameters using PLS (W-OSC) and PLS algorithm in zero-order derivative spectra

Components	No. of component	Q^2	r^2	RMSEE	RMSEP
PLS (W-OSC)					
AMI	3	0.9920	0.9985	0.308	0.286
IMI		0.9987	0.9993	0.235	0.187
PER		0.9971	0.9999	0.062	0.068
AMI	2	0.9930	0.9997	0.164	0.199
PER		0.9996	0.9999	0.072	0.102
IMI	2	0.9975	0.9997	0.181	0.222
PER		0.9988	0.9998	0.092	0.110
PLS					
AMI	3	0.9948	0.9987	0.275	1.858
IMI		0.9974	0.9997	0.145	0.674
PER		−0.0917	0.9959	0.424	1.045
AMI	2	0.9985	0.9995	0.203	0.437
PER		0.5186	0.9964	0.444	0.632
IMI	2	0.996	0.9999	0.111	0.323
PER		0.8313	0.9990	0.236	0.504

In order to test the accuracy of the proposed method, PLS (W-OSC) was applied for the quantitation of synthetic mixtures containing various concentrations of AMI–IMI–PER. The tested mixtures were compared in respect to the amount of drug added and found. As can be observed from Table 1 all the results are satisfactory with a %R.S.D. <2.5 and %recovery value 100 ± 1.2.

4.2.2. Analytical figures of merit

With regard to traditional single wavelength (univariate) calibrations, figures of merit for multiwavelength calibration have been reported to quantify the quality of a given multivariate model. The method selected relies on net analyte signal calculations (NAS) [35] defined as the part of the measured signal that is unique for the consider analyte.

NAS allow the estimation of the figures of merit in multivariate calibration models, such as sensitivity (SEN), selectivity (SEL), analytical sensitivity (γ) and limit of determination (LOD).

- It is well known that sensitivity gives the ability of a particular method to distinguish between small changes in analyte concentration, due to small changes in instrument response. For multiwavelength zero-order calibration model, the sensitivity is proportional to the regression vector.

$$\mathrm{SEN} = \frac{1}{\|b\|} = \|\mathrm{NAS}\|$$

  where $\|b\|$ is the norm of the regression vector in the response vector r and$\|\mathrm{NAS}\|$ is the norm (defined as the square root of squared elements of the corresponding NAS spectral vector) of the net analyte signal.
- Selectivity gives the ability of a particular method for the determination of a component in a complex sample without the interference of other components. It is defined as:

$$\mathrm{SEL} = \frac{\|s_k^*\|}{\|s_k\|}$$

  where $\|s_k^*\|$ is the pure spectrum norm of the component and $\|s_k\|$ the total spectrum norm of the sample.
- Limit of determination in spectral multicomponent analysis is not so relevant since the amount of the analyte that

Table 4
Analytical figures of merit by PLS (W-OSC) and PLS model

Components	Selectivity	Sensitivity	Analytical sensitivity, γ (mL/μg)	LOD (μg/mL)
PLS (W-OSC)				
AMI	0.350	0.174	13.761	0.218
IMI	0.687	0.092	7.299	0.411
PER	0.779	0.248	19.608	0.153
PLS				
AMI	0.656	0.159	7.672	0.391
IMI	0.892	0.095	4.580	0.655
PER	0.939	0.240	11.628	0.258

Table 5
Content uniformity results for PER–AMI and PER–IMI obtained from compacted powder mixtures (tablets)

No. of tablets	Compounds							
	PER–AMI				PER–IMI			
	4 mg PER		25 mg AMI		2 mg PER		10 mg IMI	
	PLS[a] method	Ratio method	PLS[a] method	Ratio method	PLS[a] method	Ratio method	PLS[a] method	Ratio method
1	101.2	101.9	98.3	99.5	100.7	100.8	101.8	102.3
2	100.1	99.9	101.8	101.4	100.5	100.4	99.3	101.4
3	100.9	100.4	100.4	100.3	99.3	98.9	101.2	103.1
4	99.3	99.6	100.6	101.1	100.1	100.2	101.7	101.8
5	98.1	98.3	104.2	102.9	98.5	98.7	104.4	103.9
6	100.1	100.3	97.2	97.5	100.5	100.7	97.8	97.5
7	99.2	99.4	101.2	101.4	101.5	101.1	100.9	101.4
8	101.2	100.6	100.3	100.3	99.6	100.1	103.6	103.4
9	101.6	102.3	100.4	99.8	99	99.2	101.6	101.7
10	100.4	100.5	98.2	97.2	100.6	100.9	98.9	97.2
% mean recovery	100.2	100.3	100.3	100.1	100.0	100.1	101.1	101.4
%R.S.D.	1.1	1.2	2.0	1.8	0.9	0.9	2.0	2.3

[a] W-OSC filter.

can be detected is a function of the concentration of the interferences. LOD may be expressed as [36]:

$$\mathrm{LOD} = 3\,\|\varepsilon\|\,\|b\|$$

where $\|\varepsilon\|$ is the norm of the instrumental error estimated from the standard deviation of the spectral residuals (for a particular calibration model).

Finally, the analytical sensitivity γ allows comparing analytical methods regardless of the specific technique and establishes the minimum concentration difference (γ^{-1}), which is statistically discernible by the method across the dynamic range where it is applicable. This parameter may be defined, in analogy to unvariate calibration as:

$$\gamma = \frac{\mathrm{SEN}}{\|\varepsilon\|}$$

In Table 4 the figures of merit of AMI, IMI, PER for PLS (centered data) and PLS (W-OSC) models have been summarized. These results are given for the optimal number of components chosen for optimal (minimal) prediction errors, RMSEE and RMSEP, previously indicated in Table 3. According to the results of Table 4, a slightly superiority of PLS (W-OSC) model over PLS (centered data) can be observed.

4.3. Content uniformity, dissolution tests

To study the accuracy of the two proposed methods and to check the interference from the excipients used in the dosage forms, recovery experiments in binary and ternary mixtures of AMI, IMI and PER were carried out with content uniformity (Table 5) and dissolution tests (Table 6). The major advantage of these methods is the quick sample analysis without prior separation or purification. From the dissolution profile in Fig. 5 it was observed that more than 95% of all the substances were dissolved within 10 min.

Table 6
Dissolution data for AMI–IMI–PER compacted tablets by PLS (W-OSC) and double-divisor ratio derivative methods

Dissolution %recovery[a]						
Time (min)	AMI (10 mg)		IMI (10 mg)		PER (4 mg)	
	PLS method	Ratio method	PLS method	Ratio method	PLS method	Ratio method
3	63.9	65.1	71.9	70.9	68.2	69.5
6	89.9	87.1	95.3	92.8	93.5	92.8
10	95.7	94.5	96.5	96.5	97.4	97.4
15	97.3	95.4	98.2	97.9	97.6	96.1
30	97.2	96.2	98	98.9	98.5	98.1
45	98.4	97.2	98.5	98.2	96.1	96.2
60	98.5	99.8	101.2	100.1	98.3	98.7

[a] Mean of six tablets.

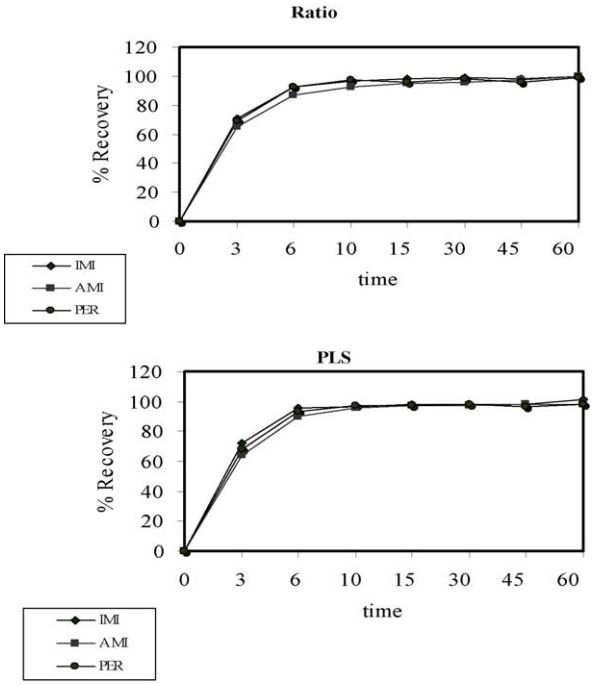

Fig. 5. Dissolution profiles for AMI–IMI–PER in compacted tablets by derivative ratio and PLS (W-OSC) methods.

5. Conclusions

By applying the double-divisor zero crossing spectra derivative method and the PLS method for the analysis of synthetic mixtures in pharmaceutical tablets preparations, successful results were obtained. It was observed that the two proposed methods are more simple and precise than the methods described in the literature, in spite of the fact that the three compounds AMI, IMI, PER produce a complete overlapping spectrum in zero-order spectra. These methods, compared to an alternative method such as HPLC, are quick, less expensive and require neither sophisticated instrumentation nor any prior separation step.

The main advantage during the analysis of the double-divisor ratio spectra derivative method over partial least squares method is the time and standards consuming. The application of the ratio method in routine analysis (content uniformity and dissolution tests) can be accomplished with only one or two external reference standards solutions with approximately the same concentration as the samples solutions. PLS method for the same determination needs the analysis of a sufficient number of synthetic mixtures standards solutions. Moreover, a comparative study of the use of derivative and multivariate calibration method for the resolution of ternary mixtures AMI, IMI and PER has been accomplished showing that the new ratio derivative technique may be equivalent and, in some cases, even superior to PLS.

Finally, it is concluded that the proposed methods have a very promising field in the quality control tests such as dissolution and content uniformity of commercial tablets containing two or three active ingredients.

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