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International Journal of Pharmaceutics 250 (2003) 339–350
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Dissolution and assaying of multicomponent tablets by chemometric methods using computer-aided spectrophotometer

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Received 14 May 2002; received in revised form 4 October 2002; accepted 7 October 2002

Abstract

Dissolution of three component tablets containing paracetamol (APAP), propyphenazone (PP), and caffeine (CAF) was carried out by USP paddle method. Three chemometric methods; inverse least square (ILS), principal component regression (PCR) and partial least squares (PLS) were applied to simultaneous assay of APAP, PP and CAF in tablets. The PCR, PLS and ILS methods were applied to simultaneous dissolution APAP, PP and CAF in tablets using a double beam UV–Vis spectrophotometer without any chemical separation and any graphical treatment of the overlapping spectra of three drugs. Twentytwo mixture solutions in different concentrations were prepared in simulated gastric juice (SGJ, USP) for the chemometric calibrations as training set. The absorbance data matrix was obtained by measuring the absorbance at 14 wavelength points (from 222.5 to 292.5 nm) with the intervals of 5 nm ($\Delta\lambda = 5$ nm) in the spectral region between 200 and 310 nm. Training set and absorbance data were used for the calibrations of chemometric methods. The developed calibrations were tested for the previously prepared solutions of mixture of three drugs for the validation of the assay method. The chemometric calculations were performed by using the 'MAPLE V' software. The results of three chemometric methods were statistically compared with each other. These chemometric calibrations were successfully applied to the content uniformity and dissolution of the multicomponent tablets without any separation procedure. The synthetic mixtures of three drugs were used for the validity of the calibrations. Means recoveries (percent) and relative standard deviation of PLS, PCR and ILS methods were found to be 100.1 ± 0.6, 101.4 ± 1.6 and 100.1 ± 0.6 for APAP; 100.9 ± 3.2, 102.0 ± 3.3 and 100.9 ± 3.2 for PP; 99.9 ± 3.5, 101.6 ± 3.3 and 99.9 ± 3.2 for CAF, respectively. Dissolution profiles of three component tablets were performed. More than 95% of drugs were dissolved within 15 min. All of the three-chemometric methods in this study can be satisfactorily used for the quantitative analysis and for dissolutions test of multicomponent dosage form.

Keywords: Chemometric method; Dissolution of multicomponent tablets; Paracetamol; Propyphenazone; Caffeine

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0378-5173/02/$ - see front matter
PII: S0378-5173(02)00558-6

1. Introduction

One of the fundamental problems of the dissolution testing of pharmaceutical preparations, which contain two or more drugs, is the simultaneous quantitative analysis without any chemical separation step. Several spectrophotometric methods such as classical derivative spectrophotometry, ratio spectra derivative spectrophotometry are frequently made on the basis of zero-crossing measurements and signals corresponding to the maximum point of wavelength for the simultaneous quantitative analysis of binary mixtures of drugs with overlapped spectra, respectively (O'Haver and Green, 1976; Levillain and Fompeydie, 1986; Dinç and Onur, 1997a,b, 1998a,b). In general, these methods are not suitable for ternary mixtures of drugs with overlapped spectra.

In recent years, the chemometric methods, such as classical least squares (CLS), inverse least square (ILS), principal component regression (PCR) and partial least squares (PLS) based on the computer-aided instrumentation are playing a very important role for the multi-component analysis of mixtures (Haaland and Thomas, 1988; Beebe and Kowalski, 1987; Massart et al., 1988; Martens and Naes, 1991; Lavine, 2000; Bautista et al., 1996; Vidal et al., 1997). The chemometric calibration methods were used for the quantitative determination of drugs in multi-component pharmaceutical preparations (Dinç et al., 2001a,b, 2000; Dinç and Üstündağ, 2002; Dinç and Baeanu, 2002). All of the chemometric methods were found suitable for the resolution of the overlapping spectral bands in quantitative determination.

HPLC and other spectroscopic methods were applied to the simultaneous determination of drugs in pharmaceutical formulations containing paracetamol (APAP), propyphenazone (PP) and caffeine (CAF) (Dinç, 1999a,b; Dinç et al., 2000), and also metamizol–APAP–CAF (Dinç and Onur, 1998a,b) and ascorbic acid–asetylsalicylic acid–APAP (Dinç, 1999a,b) combinations were studied.

In this study, three chemometric methods were successfully applied to the simultaneous determination of APAP, PP and CAF in a commercial tablet formulation, tablets without any separation procedure. Dissolution and content uniformity tests of these tablets were carried out by these suggested methods.

The chemometric calibrations were carried out by using the mixtures of these drugs in suitable and possible compositions and concentrations. Means recoveries (%) and relative standard deviation of PLS, PCR and ILS methods were calculated for the validation of the methods. Amounts of the dissolved drugs were calculated by these chemometric methods and dissolution profiles were revealed. The obtained results were statistically compared each other.

2. Experimental

2.1. Instruments

Shimadzu UV-160 double beam UV–Vis spectrophotometer with a fixed slit width (2 nm) connected to a computer, loaded with Shimadzu UVPC software, equipped with a printer (HP OfficeJet Pro 1150C) was used. The absorption spectra were recorded over the wavelength range 200–310 nm against the reagent blank (simulated gastric juice solution (SGJ)) at 1500 nm/min and stored in the computer. The additional softwares MAPLE V and SPSS 10.0 were used for the computation of the spectral data and the statistical analysis, respectively.

Dissolution tester was Pharmatest type PTWS3C.

2.2. Commercial product

A commercial tablet (Minoset plus® tablet, Roche Pharm, Turkey, Batch no. 10113), contents; 250 mg of APAP, 150 mg of PP, 50 mg of CAF and excipients (Microcrystalline cellulose, formaldehyde–casein, cornstarch, talc, colloidal silica, magnesium stearate and hydroxypropylmethyl cellulose (HPMC)).

APAP, PP and CAF were kindly obtained from Roche Pharm., Turkey.

2.3. Standard solutions

Stock solutions of APAP, PP and CAF, 50 mg/100 ml of each, were prepared in SGJ (USP XXIII). A training set (calibration set) containing 0–12 μg/ml of APAP, 0–12 μg/ml of PP and 0–16 μg/ml of CAF in possible compositions and 15 synthetic mixture solutions as a validation set in the range of 4–12 μg/ml of APAP, 4–12 μg/ml of PP and 1–16 μg/ml of CAF were prepared from the stock solution.

2.4. Tablet content analysis

Thirty tablets were accurately weighed and powdered in a mortar. A sample containing APAP. PP and CAF equivalent to a tablet content was dissolved in 1000 ml SGJ. The content of the flask was mechanically shaken for 30 min and filtrated with 0.20 μm disposable membrane filter (Sartorius, minisart, $\phi = 0.20$ μm) by using an injector. After the resulting solution was diluted 1:25 with the same solvent. All the calibration techniques were applied to the final solution ($n = 10$).

Same procedure was carried out for absorbance of the excipients, which may cause interference, or not.

All of the tablet excipients of which amounts in one tablet were dispersed in 1000 ml SGJ and shaken for 30 min. After filtration and dilution (25 folds) as described above, UV spectra was recorded and absorbances were taken in the range of 222.5–292.5 nm.

Before the filtration of solution, the membrane filters were saturated with the tablet ingredients to avoid possible adsorption on the membrane filter by passing 4 ml of solution as two portions through each membrane filter and ejection. This procedure was applied to all filtration process in the study.

2.5. Content uniformity test

Ten tablets were individually weighed and then each of them were disintegrated and dissolved in SGJ in 1000 ml volumetric flask by shaking for 45 min. After filtration and dilution as explained above spectrophotometric procedure and calibration techniques were applied.

2.6. Dissolution test of tablet

Dissolution test of tablets were carried out by USP (XXIII) method (50 rpm) in SGJ at 37.0 ± 0.1 °C ($n = 6$). The sample was taken by means of an injector with membrane filter (0.20 μm) and diluted with sufficient quantity of dissolution medium. The final solution ($n = 6$) was analyzed by the suggested calibration methods presented in this paper.

3. Chemometric methods

3.1. ILS method

Inverse least-squares (ILS) is the application of multiple linear regression (MLR) to the inverse expression of the Beer–Lambert law of spectroscopy. The mathematical expression is given as:

$$C = P \times A \tag{1}$$

The above equation can be written as a linear equation system as follows:

$$\begin{aligned} C_1 &= P_{11}A_1 + P_{12}A_2 + \ldots P_{1w}A_w \\ C_2 &= P_{21}A_1 + P_{22}A_2 + \ldots P_{2w}A_w \\ &\ldots \quad \ldots \quad \ldots \qquad \ldots \ldots \\ C_c &= P_{c1}A_1 + P_{w2}A_2 + \ldots P_{cw}A_w \end{aligned} \tag{2}$$

where A_w is the absorbance at the wth wavelength. P_{cw} denotes the calibration coefficient for the cth component at the wth wavelength, while C_{cw} is the concentration of the cth component.

3.2. PCR method

In the spectral study, the following steps can explain the fundamental concept of PCR (Martens and Naes, 1991).

The original data obtained in absorbances (A) and concentrations (C) of analytes were reprocessed by mean-centring as $\mathbf{A}_o$ and $\mathbf{C}_o$, respectively. The covariance dispersion matrix of the

centered A_o was computed. The normalized eigenvalues and eigenvectors were calculated starting from square covariance matrix.

The number of the optimal principal components (eigenvectors) is selected by considering only the highest values of the eigenvalues. The other eigenvalues and their corresponding eigenvectors are eliminated.

Using the ordinary linear regression $C = a + b \cdot \mathbf{A}$, we can calculate the coefficients a and b. The coefficient b can be calculated by $b = \mathbf{P} \cdot q$, where $\mathbf{P}$ is the matrix of eigenvectors and q is the C-loadings given by $q = \mathbf{D} \cdot \mathbf{T}^T \ \mathbf{A}_o$. ($\mathbf{T}^T$ is the transpose the score matrix $\mathbf{T}$, $\mathbf{D}$ is a diagonal matrix having on the components the inverse of the selected eigenvalues). a can be easily calculated from the formula: $a = C_{mean} - A^T_{mean}\ b$, by replacing b, where A^T_{mean} represents the transpose of the matrix having the entries the mean absorbances values and C_{mean} is the mean concentration of the calibration set.

3.3. PLS method

The PLS calibration technique, based on the orthogonalized PLS algorithm, was explained and extensively discussed (Massart et al., 1988; Martens and Naes, 1991). The PLS algorithm involves simultaneously the independent and the dependent variables on the data compression and decomposition operations.

In the range of UV–Vis spectra, the absorbance data (A) and concentration data (C) are mean centred to give data matrix $\mathbf{A}_o$ and vector $\mathbf{C}_o$.

The orthogonalized PLS algorithm has the following steps:

a) The loading weight vector $\mathbf{W}$ is given by the following expression:

$$\mathbf{W} = \frac{A'_o C_o}{C'_o C_o} \tag{3}$$

b) The scores and loadings are given by:

$$t_1 = \mathbf{A}_o \mathbf{W}_1 \tag{4}$$

$$\mathbf{P}_1 = \frac{\mathbf{A}^T_o t_1}{t^T_1 t_1} \tag{5}$$

$$q_1 = \frac{\mathbf{C}^T_o t_1}{t^T_1 t_1} \tag{6}$$

c) The matrix and vector of the residuals in $\mathbf{A}_o$ and $\mathbf{C}_o$ are:

$$A_1 = \mathbf{A}_o - t_1 \mathbf{P}^T_1 \tag{7}$$

$$C_1 = \mathbf{C}_o - t_1 \mathbf{q}^T_1 \tag{8}$$

d) From the general linear equation the regression coefficients were calculated by:

$$b = \mathbf{W}(\mathbf{P}^T\mathbf{W})^{-1} q \tag{9}$$

$$a = C_{mean} - A^T_{mean} b \tag{10}$$

As in PCR method, the calibration equation is used for the estimation of the amount of compounds in the samples.

4. Results and discussion

A calibration set was randomly prepared with the mixtures of APAP, PP and CAF in the solvent system (Table 1). The UV–Vis spectra of this calibration set were recorded in the spectral region between 200 and 310 nm as shown in Fig. 1. Their absorbances were measured at 14 wavelength points from 222.5 to 292.5 nm with the intervals of 5 nm ($\Delta\lambda = 5$ nm). The chemometric calibrations were computed with the ILS, PCR and PLS algorithms using the correlation for the training set and its absorbance data. The contents of APAP, PP and CAF in the mixtures and tablets were calculated by the chemometric calibrations. On the other hand, the dissolution profiles of APAP, PP and CAF were recorded by using the same chemometric methods. Mean recoveries (%), relative standard deviation, and other statistical parameters of ILS, PCR and PLS methods were calculated. We observed that these calibration methods gave better results in terms of accuracy and precision (Tables 2–4).

4.1. ILS method

In this method, the coefficient matrix ($\mathbf{P}$) was obtained from the linear equation system using the absorbance data and the training set. Introducing

(**P**) into the linear equation system, we obtain the calibration for ILS as:

$$\begin{bmatrix} C_{APAP} \\ C_{PP} \\ C_{CAF} \end{bmatrix} = \begin{bmatrix} -30.15 & -18.94 & -4.98 & 6.16 & 9.18 & 10.95 & 14.43 & 12.46 \\ 49.66 & 33.59 & 12.29 & -4.19 & -8.12 & -11.47 & -19.22 & -18.82 \\ -8.65 & -6.92 & -3.37 & -1.26 & -1.36 & -0.30 & 3.21 & 5.58 \end{bmatrix}$$

$$\begin{bmatrix} 5.70 & -1.07 & 0.74 & 7.51 & 7.00 & 4.72 \\ -10.68 & -2.08 & -6.22 & -15.75 & -13.28 & -8.19 \\ 6.37 & 6.66 & 8.55 & 8.47 & 5.75 & 2.28 \end{bmatrix} \begin{bmatrix} A_1 \\ A_2 \\ A_3 \\ A_4 \\ A_5 \\ A_6 \\ A_7 \\ A_8 \\ A_9 \\ A_{10} \\ A_{11} \\ A_{12} \\ A_{13} \\ A_{14} \end{bmatrix}$$

In this calibration, C_{APAP}, C_{PP} and C_{CAF} are the concentration of APAP, PP and CAF, respectively. The absorbance values of the samples at the 14 wavelengths in the spectral region from 210 to 290 nm were placed in the above equation and in the amounts of APAP, PP and CAF in samples were calculated.

Table 1
Calibration mixture containing three drugs

Standard number	Composition of training set		
	APAP (μg/ml)	PP (μg/ml)	CAF (μg/ml)
1	4.0	6.0	2.0
2	6.0	6.0	2.0
3	8.0	6.0	2.0
4	10.0	6.0	2.0
5	12.0	6.0	2.0
6	8.0	0.0	0.0
7	10.0	4.0	0.0
8	10.0	6.0	2.0
9	10.0	8.0	4.0
10	10.0	10.0	6.0
11	10.0	12.0	6.0
12	0.0	8.0	0.0
13	10.0	6.0	1.0
14	10.0	6.0	2.0
15	10.0	6.0	4.0
16	10.0	6.0	8.0
17	10.0	6.0	16.0
18	0.0	0.0	8.0
19	10.0	6.0	0.0
20	0.0	6.0	2.0
21	10.0	0.0	2.0
22	0.0	0.0	2.0

4.2. PCR method

The PCR calibration was constructed using the PCR algorithm as explained in Section 3.2. The concentration of three drugs were obtained in the following equations:

$$\begin{aligned} C_{APAP} = {} & -0.08 - 29.95A_1 - 18.93A_2 - 4.92A_3 \\ & + 6.16A_4 + 9.19A_5 + 10.93A_6 + 14.44A_7 \\ & + 12.42A_8 + 5.67A_9 - 1.04A_{10} + 0.81A_{11} \\ & + 7.44A_{12} + 6.89A_{13} + 4.59A_{14} \end{aligned}$$

$$\begin{aligned} C_{PP} = {} & -0.07 + 49.59A_1 + 33.63A_2 + 12.23A_3 \\ & - 4.17A_4 - 8.11A_5 - 11.42A_6 - 19.24A_7 \\ & - 18.76A_8 - 10.64A_9 - 2.14A_{10} - 6.34A_{11} \\ & - 15.65A_{12} - 13.13A_{13} - 7.96A_{14} \end{aligned}$$

$$\begin{aligned} C_{CAF} = {} & 0.04 - 8.63A_1 - 6.91A_2 - 3.35A_3 - 1.25A_4 \\ & - 1.37A_5 - 0.31A_6 + 3.21A_7 - 5.57A_8 \\ & + 6.36A_9 + 6.68A_{10} + 8.57A_{11} + 8.44A_{12} \\ & + 5.72A_{13} + 2.72A_{14} \end{aligned}$$

Here, C_{APAP}, C_{PP} and C_{CA} are the concentrations of APAP, PP and CAF, respectively. The absorbance values, measured at 14 points between 222.5 and 292.5 nm for every 5 nm were introduced in the equations and the amount of each drug in mixtures and tablets was determined.

4.3. PLS method

The corresponding calibration was obtained using PLS algorithm and as shown in followings:

$$\begin{aligned} C_{APAP} = {} & -0.08 - 29.94A_1 - 18.88A_2 - 4.94A_3 \\ & + 6.15A_4 + 9.16A_5 + 10.94A_6 + 14.43A_7 \\ & + 12.44A_8 + 5.71A_9 - 1.08A_{10} + 0.76A_{11} \\ & + 7.47A_{12} + 6.92A_{13} + 4.64A_{14} \end{aligned}$$

$$\begin{aligned} C_{PP} = {} & -0.07 + 49.58A_1 + 33.57A_2 + 12.26A_3 \\ & - 4.15A_4 - 8.08A_5 - 11.44A_6 - 19.21A_7 \\ & - 18.79A_8 - 10.69A_9 - 2.06A_{10} - 6.25A_{11} \\ & - 15.70A_{12} - 13.16A_{13} - 8.06A_{14} \end{aligned}$$

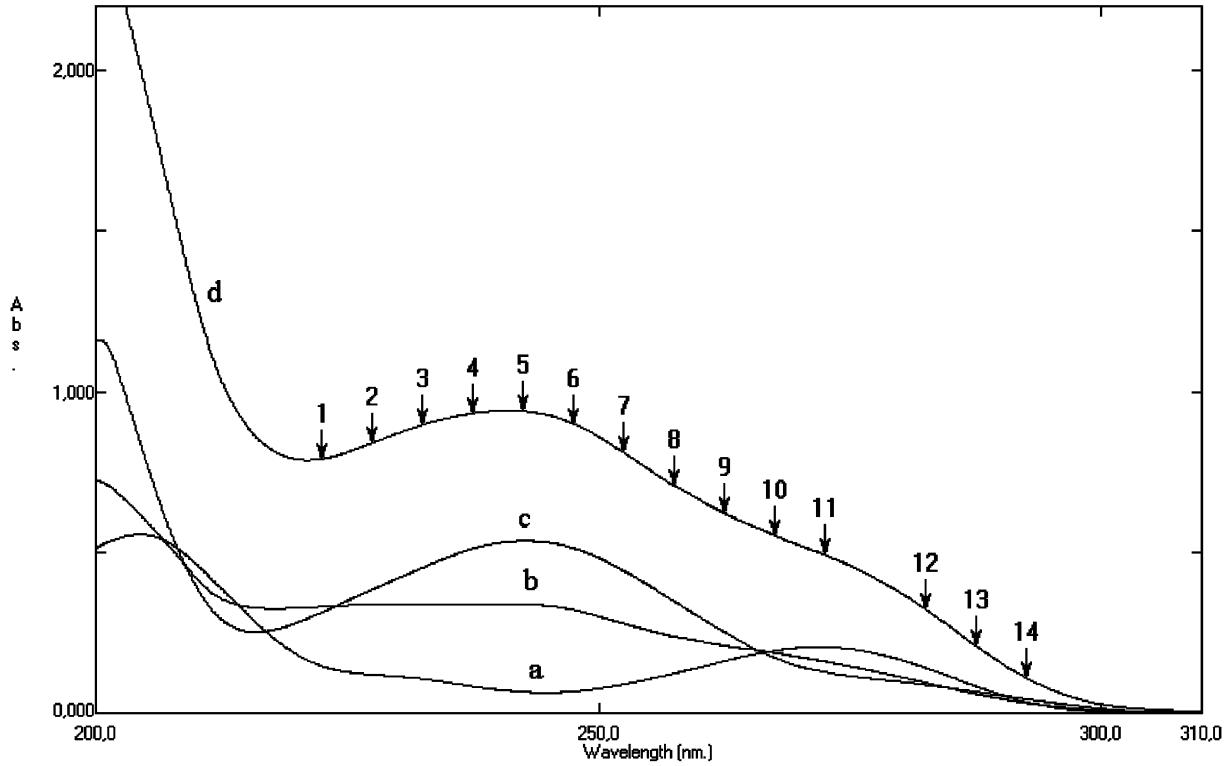

Fig. 1. Absorption spectra of (a) 8 μg /ml APAP (b) 8 μg/ml PP. (c) 4 μg/ml CAF and (d) their mixture in SGJ. $\downarrow^1, \downarrow^2, \ldots, \downarrow^{14}$ corresponding to λ_1, $\lambda_2 \ldots \lambda_{14}$ (from 222.5 to 292.5 nm)).

$$C_{\mathrm{CAF}} = 0.04 - 8.61A_1 - 6.91A_2 - 3.36A_3 - 1.26A_4 - 1.37A_5 - 0.30A_6 + 3.21A_7 - 5.58A_8 + 6.37A_9 + 6.66A_{10} + 8.56A_{11} + 8.46A_{12} + 5.73A_{13} + 2.75A_{14}$$

where C_{APAP}, C_{PP} and C_{CAF} are the concentration of APAP, PP and CAF, respectively, and the absorbance values measured in the same range and the same samples as in PCR method.

4.4. Statistical analysis

We can define the ability of a calibration in several ways. In this subsection we calculated the estimations of the standard variation of the chemometric calibrations in the case of the investigated mixtures.

The standard error of calibration (SEC) and prediction (SEP) are given by the following expression:

$$\mathrm{SEC(SEP)} = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{\mathrm{Added}} - C_i^{\mathrm{Found}})^2}{n-1}} \quad (11)$$

where C_i^{Added} represents the added concentration. C_i^{Found} denotes the determined concentration and n is the total number of samples. The numerical values of SEC were given in Table 2. We concluded that SEC was minimum for PCR method for both drugs. The standard errors of prediction (SEP) of the same mixtures are shown in Table 3 and the similar behavior of the values was also observed for SEC.

For PCR and PLS methods, a number of 14 calibration spectra were used for the selection of the optimum number of factors by using the cross-validation technique.

Table 2
Statistical results of chemometric methods in the calibration step

Drugs	ILS	PCR		PLS	
	SEC	SEC	PRESS	SEC	PRESS
APAP	0.059	0.128	0.349	0.097	0.196
PP	0.055	0.115	0.279	0.089	0.164
CAF	0.179	0.209	0.092	0.066	0.091

Table 3
Statistical parameters of synthetic mixtures

Drugs	ILS				PCR				PLS			
	SEP	*a*	*b*	*r*	SEP	*a*	*b*	*r*	SEP	*a*	*b*	*r*
APAP	0.082	−0.089	1.009	0.999	0.151	−0.038	0.990	0.998	0.124	0.051	0.995	0.999
PP	0.091	−0.394	1.054	0.998	0.148	−0.452	1.052	0.999	0.112	−0.381	1.052	0.997
CAF	0.250	−0.004	1.001	0.999	0.076	−0.036	0.999	0.999	0.965	−0.002	1.000	0.999

The prediction residual error sum-of-squares (PRESS) of the calibration step was calculated as:

$$\mathrm{PRESS} = \sum_{i=1}^{n} (C_i^{\mathrm{Added}} - C_i^{\mathrm{Found}})^2 \quad (12)$$

The values of (PRESS) were indicated in Table 2. By using the cross validation-procedure we found that its numerical values were minimized in the case of first three factors for PCR and one factor for PLS, respectively (Vidal et al., 1997).

4.5. Validation set

The proposed chemometric methods were applied to the determination of artificial mixtures containing various concentrations of APAP, PP and CAF, to check the validity of the calibration models. The mentioned calibration models were used to predict the concentration of the drugs in 15 synthetic mixtures. Results were summarized in Table 4. The tested mixtures were compared in respect to the amount of drug added and found. The obtained results can be considered satisfactory in condition of spectral overlapping between APAP, PP and CAF. The means recoveries and the relative standard deviations of our proposed methods were computed and indicated in Table 4. Their numerical values were found satisfactory for the validity of all calibration methods.

4.6. Tablet analysis

The experimental results of commercial tablet were given in Table 5. The results of all methods were very close to each other as well as to the label value of commercial tablet. We observed that all the excipients of tablet do not interfere in the analysis of three drugs. The numerical values of all statistic parameters indicated that the investigated techniques are suitable for the determination of three drugs in the tablet formulation.

The mean tablet weight was 647.5 mg, standard deviation and relative standard deviation were 1.02 mg and 0.16, respectively. Very low weight variation of the tablets we used encourages us to determine the content uniformity test.

4.7. Content uniformity

Three chemometric methods were applied for the content uniformity test of tablets for the comparison of mean value and each tablet contents (S.D. and R.S.D.). Standard dose error

Table 4
Recovery data for the determination of APAP, PP and CAF in different synthetic mixtures by using the chemometric techniques

Mixture Added (μg)			ILS Recovery (%)			PCR Recovery (%)			PLS Recovery (%)		
APAP	PP	CAF	APAP	PP	CAF	APAP	PP	CAF	APAP	PP	CAF
4.0	6.0	2.0	100.5	105.0	98.5	102.0	105.5	100.0	100.0	104.5	98.0
6.0	6.0	2.0	100.8	102.3	99.0	101.8	103.0	100.5	100.5	102.0	98.5
8.0	6.0	2.0	101.5	101.5	101.5	102.4	102.5	103.5	101.4	101.3	102.0
10.0	6.0	2.0	100.5	102.2	102.0	101.3	103.2	104.0	100.5	102.2	102.5
12.0	6.0	2.0	100.1	104.8	103.0	100.8	106.2	105.0	100.2	105.0	103.0
10.0	4.0	0.0	99.7	107.8	101.0	103.0	109.5	102.5	99.5	107.8	100.5
10.0	6.0	2.0	100.2	102.2	108.0	101.0	103.3	110.0	100.2	102.2	108.0
10.0	8.0	4.0	98.9	98.4	98.5	99.7	99.3	100.0	98.9	98.5	98.5
10.0	10.0	6.0	99.7	98.5	97.5	106.0	99.3	99.5	99.9	98.6	98.0
10.0	12.0	6.0	100.0	98.2	101.0	101.0	98.8	103.5	100.2	98.3	101.5
10.0	6.0	1.0	99.2	98.3	95.0	100.0	99.5	97.0	99.2	98.3	95.0
10.0	6.0	2.0	100.1	95.5	95.5	100.0	96.7	97.5	100.1	95.5	95.5
10.0	6.0	4.0	100.2	98.5	99.5	101.0	99.5	100.5	100.2	98.5	99.5
10.0	6.0	8.0	99.6	100.3	99.5	100.6	101.7	100.0	99.8	100.7	99.5
10.0	6.0	16.0	99.8	99.8	99.9	101.0	101.5	100.3	100.2	100.3	100.1
Mean			100.1	100.9	100.0	101.4	102.0	101.6	100.0	100.9	100.0
R.S.D.			0.64	3.24	3.15	1.55	3.34	3.27	0.58	3.18	3.21

R.S.D., relative standard deviation.

(S.D.E.) between label value and experimental value S.D.E. was calculated by the following formula:

$$\mathrm{SDE} = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{\text{Label value}} - C_i^{\text{Exp. value}})^2}{n-1}} \qquad (13)$$

where $C^{\text{Label value}}$ and $C^{\text{Exp. value}}$ are the concentrations of the drug label and experimental values in a tablet formulation, respectively. Result of content uniformity test of the tablet is shown in Table 6.

One-way ANOVA test was applied to four sets of ten samples for each drug in tablet formulation for the comparison the differences between methods. For this reason, Snedecor's F-values were computed and compared with the standard tabulated value ($P = 0.05$). The same computation

Table 5
Quantitative results of tablet (mg per tablet) by three calibration techniques

	Chemometric methods								
	ILS			PCR			PLS		
	APAP	PP	CAF	APAP	PP	CAF	APAP	PP	CAF
Mean	251.8	150.5	50.9	250.7	150.8	49.7	249.9	149.9	50.8
S.D.	1.54	1.59	1.13	1.77	2.18	1.81	1.76	1.75	1.36
R.S.D.	0.61	1.05	2.22	0.71	1.45	3.64	0.70	1.17	2.68
S.E.	0.89	0.92	0.65	1.02	1.26	1.04	1.02	1.01	0.79
CL ($P = 0.05$)	1.79	1.85	1.31	2.06	2.54	2.10	2.05	2.04	1.58

S.D., standard deviation; R.S.D., relative standard deviation; S.E., standard error; CL, confidence limit. Obtained results are the average of ten experiments for each methods.

Table 6
Content uniformity results of the tablets by three chemometric techniques

Sample number	Chemometric methods								
	ILS			PCR			PLS		
	APAP (mg)	PP (mg)	CAF (mg)	APAP (mg)	PP (mg)	CAF (mg)	APAP (mg)	PP (mg)	CAF (mg)
1	251.0	151.0	52.3	251.5	148.3	50.1	245.0	147.8	50.1
2	247.5	147.8	52.0	248.3	151.5	51.5	247.5	145.5	51.5
3	252.5	145.5	52.0	250.3	147.9	48.3	256.3	151.0	48.3
4	250.1	140.8	51.1	259.8	148.9	51.5	251.0	140.7	51.5
5	246.5	169.1	49.0	255.7	169.5	49.2	247.3	169.5	49.2
6	243.0	164.4	49.9	242.4	165.3	47.9	243.0	165.0	47.9
7	247.3	148.7	48.1	247.5	148.9	48.0	247.3	148.8	48.0
8	246.3	140.6	49.8	245.9	140.8	49.8	246.3	142.2	49.8
9	249.6	142.5	47.0	249.3	142.5	47.1	249.6	143.1	47.1
10	252.1	151.2	48.0	251.3	148.9	48.9	247.4	151.7	48.9
Mean	248.6	150.2	49.9	250.2	151.3	49.2	248.1	150.5	49.2
S.D.	2.99	9.61	1.42	4.90	9.15	1.50	3.63	9.59	1.50
R.S.D	1.20	6.40	2.84	1.96	6.05	3.04	1.47	6.37	3.04
S.E.	1.73	5.55	0.82	2.83	5.28	0.86	2.10	5.54	0.86
CL($P=0.05$)	3.48	11.17	1.65	5.70	10.64	1.74	4.23	11.16	1.74
S.D.E. ($n=10$)	3.34	9.6	1.89	4.50	9.23	1.70	4.16	9.61	1.71

S.D.E., standard error of dosage.

process was repeated for the drugs. In standard table, for $n_1=2$ and $n_2=27$ ($P=0.05$), the F-value is 3.35. ANOVA test results were calculated as 2.89 for APAP, 1.13 for PP and 2.34 for CAF. The experimental (calculated) F-values did not exceed the F-tabulated value in the variance analysis. It was observed that there was no significant difference among the methods.

4.8. Dissolution test

The proposed methods were applied to the dissolution test of the tablets which contain APAP, PP and CAF, without using any separation procedure. Dissolution data were calculated by three calibration methods (Tables 7–9) and the dissolution profiles of APAP, PP and CAF were shown in Fig. 2a, b and c respectively. We observed that 88% of APAP, 81% of CAF and 65% PP were dissolved within 5 min. In another expression, the percentage and confidence limit ($P=0.05$) of the dissolved drug within 15 min were found as 98.2 ± 1.9, 99.1 ± 2.7 and 98.0 ± 1.8 for APAP; 97.6 ± 6.3, 94.8 ± 10.7 and 97.3 ± 6.1 for PP; 96.7 ± 2.5, 96.3 ± 2.0 and 96.5 ± 2.0 for CAF by PLS, PCR and ILS methods, respectively.

5. Conclusions

In spite of the interference of the original absorption spectra of APAP, PP and CAF in the wavelength range of 200–310 nm, the chemometric calibrations were applied to the simultaneous determination and dissolution test of the tablets. We observed that the quantitative determination results obtained in the chemometric methods proposed by us are comparable with the HPLC (Dinç et al., 2000). In addition, the proposed calibrations do not require any pretreatment; a priory separation step as used in HPLC. Results also showed that these methods are more reliable than ratio spectra-zero crossing spectrophotometry (Dinç et al., 2000). These chemometric methods not contain a derivation and a division of

Table 7
Dissolution data by ILS method

Time (t)	APAP		PP		CAF	
	Mean (%)	CL* ($P = 0.05$)	Mean (%)	CL* ($P = 0.05$)	Mean (%)	CL* ($P = 0.05$)
1	39.6	5.8	18.1	6.6	27.6	5.8
2	66.0	7.2	32.0	7.1	55.1	8.0
3	77.0	8.0	45.1	6.9	68.3	5.1
4	83.9	4.4	56.4	8.2	75.9	5.1
5	88.1	3.9	64.9	6.9	81.1	3.6
7	92.7	3.7	77.4	7.2	87.9	4.2
10	96.4	3.3	89.3	6.5	93.3	2.7
15	98.0	1.8	97.3	6.1	96.5	2.3
20	98.8	0.8	100.8	6.8	97.9	2.5

Table 8
Dissolution data by PCR method

Time (t)	APAP		PP		CAF	
	Mean (%)	CL* ($P = 0.05$)	Mean (%)	CL* ($P = 0.05$)	Mean (%)	CL* ($P = 0.05$)
1	38.5	9.9	17.6	6.9	28.7	5.5
2	64.5	8.5	31.7	7.4	54.0	7.2
3	76.9	10.1	45.0	7.1	67.6	4.5
4	83.9	5.5	55.4	8.4	76.1	5.4
5	88.6	13.1	65.0	7.2	81.1	3.3
7	93.6	5.1	77.9	7.4	87.4	3.9
10	97.4	4.4	88.6	6.7	93.2	2.5
15	99.1	2.7	94.8	10.7	96.3	2.0
20	100.1	2.1	100.1	7.0	97.5	2.0

Table 9
Dissolution data by PLS method

Time (t)	APAP		PP		CAF	
	Mean (%)	CL* ($P = 0.05$)	Mean (%)	CL* ($P = 0.05$)	Mean (%)	CL* ($P = 0.05$)
1	39.2	6.0	17.6	6.9	26.7	6.1
2	65.8	7.2	31.7	7.4	53.1	8.1
3	77.5	4.8	45.0	7.1	67.5	5.2
4	83.9	4.5	55.2	8.5	75.6	5.3
5	88.1	3.9	65.0	7.2	81.1	3.7
7	93.7	3.8	77.7	7.3	88.0	4.3
10	96.5	3.4	89.5	6.7	93.5	2.7
15	98.1	1.9	97.6	6.3	96.7	2.5
20	99.0	0.9	101.1	7.0	97.8	2.6

*, Confidence limit.

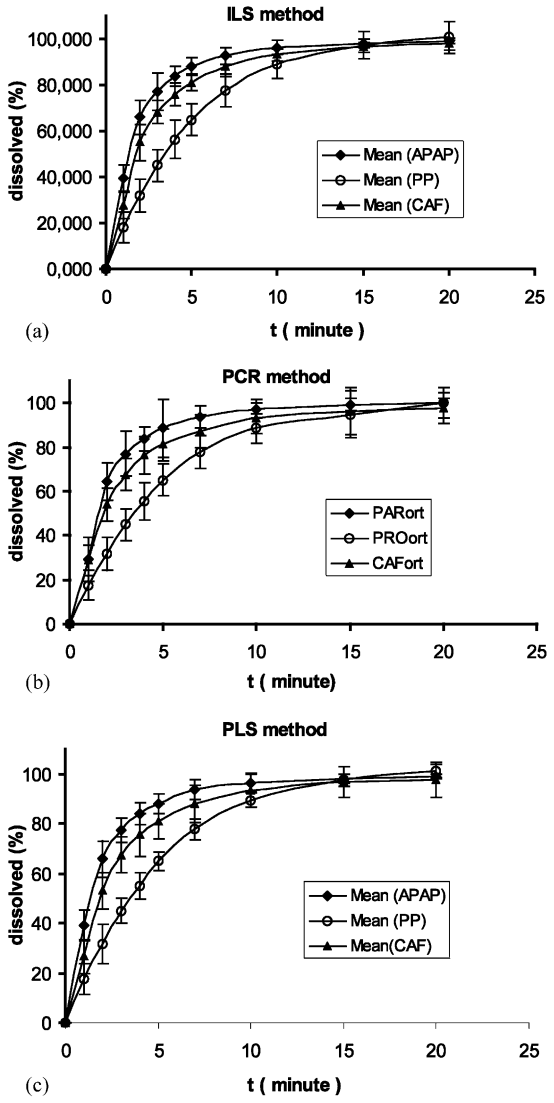

Fig. 2. Dissolution profile of APAP. PP and CAF in the commercial tablets by (a) ILS, (b) PCR and (c) PLS methods.

the spectra as in ratio spectra derivative spectrophotometry described in the literature.

Although the methods suggested by us for the determination of three drugs are more rapid, easy, cheap, precise and accurate than the methods given in the literature. However, the multivariate calibrations are required only the data processing with powerful software as well as the manipulations of the abstract vector space and its applications to the regression analysis.

The results obtained in this paper strongly encourage us to apply these calibration models for quality control such as dissolution test and content uniformity test of the commercial tablet containing three drugs.

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