Turkish J. Pharm. Sci. 1 (3), 139-151, 2004

UTILIZATION OF MULTIVARIATE CALIBRATION TECHNIQUES FOR THE SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF PARACETAMOL, ASPIRIN AND CAFFEINE IN A PHARMACEUTICAL FORMULATION

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Abstract

Two chemometric techniques, classical least squares (CLS) and inverse least squares (ILS) were applied to UV zero-order and derivative spectrophotometric determination of paracetamol, aspirin and caffeine in their ternary mixture. These chemometric calibrations for both zero-order and first derivative spectra were constructed by measuring the absorbance and dA/dλ values at full spectral points in the wavelength range 210–305 nm for a training set containing 5–25 μg/ml paracetamol, 5–25 μg/ml aspirin and 4–28 μg/ml caffeine in 0.1M HCL. The chemometric calibrations were validated by using the synthetic mixtures containing these drugs. Proposed techniques were applied to a pharmaceutical preparation containing these three active ingredients and the results were statistically compared with each other.

Keywords: *Chemometric techniques, derivative spectrophotometry, paracetamol, aspirin, caffeine.*

Bir farmasötik preparatta parasetamol, aspirin ve kafeinin aynı anda spektrofotometrik miktar tayinleri için çok değişkenli kalibrasyon tekniklerinin kullanılışı

Bu çalışmada, iki kemometrik teknik, klasik en küçük kareler (CLS) ve ters en küçük kareler (ILS) parasetamol, aspirin ve kafein'in üçlü karışımı halindeyken aynı anda UV sıfırıncı derece ve türev spektrofotometrik miktar tayinleri için uygulanmıştır. Bu kemometrik kalibrasyonlar; hem sıfırıncı derece hem de birinci derece türev spektrumlarında absorbanslar ve dA/dλ değerleri 210-305 nm aralığındaki spektrumda okunarak 0.1M HCL içerisinde 5–25 μg/ml parasetamol, 5–25 μg/ml aspirin and 4–28 μg/ml kafein içeren çalışma seti için hazırlanmıştır. Kemometrik kalibrasyonlar bu etken maddeleri iççeren sentetik karışımlara uygulanarak yöntemin geçerliği gösterilmiştir. Önerilen teknikler bu üç etken maddeyi içeren bir preparata uygulanmış ve elde edilen sonuçlar istatistiksel olarak birbirleriyle karşılaştırılmıştır.

Anahtar Kelimeler: *Kemometrik teknikler, türev spektrofotometri, parasetamol, aspirin, kafein,*

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Introduction

Classical spectrophotometric method due to overlapping spectra is not enough to simultaneous determination of active compounds in pharmaceuticals and biomedical fluids. The analysis of drugs in multicomponent preparations need separation or extraction. The use of sophisticated equipments (HPLC, capillary electrophoresis or LC-MS) brings high cost, time consumption and additional work. One way of elimination of these drawbacks is application of numerical and graphical techniques to original absorption spectra. The use of spectrophotometric techniques combined with mathematical algorithms has brought a new, fast and less expensive methodology for the determination of analytes in samples.

Commercial Excedrine Migraine Tablets containing paracetamol (**PAR**), aspirin (**ASP**) and caffeine (**CAF**) are used for the temporary relief of pain associated with migraine headaches. The simultaneous determination of **PAR**, **ASP** and **CAF** in commercial tablet formulations was carried out by HPLC (1) and HPTLC (2), UV spectrophotometric flow-through multiparameter sensors (3) and a flow-through multioptosensor based on the integration of the retention and UV detection of the analytes on a solid support (C_{18}) bonded beads packed in the flow cell (4) .

Chemometric techniques known as numerical techniques are useful for the spectrophotometric resolution of complex mixtures of analytes without needing a priory separation or extraction. Although both PCR and PLS give successful results, and commonly used techniques, it is hard to understand their abstract mathematical theory behind them.

The chemometric calibration techniques, such as classical least squares (CLS) and inverse least squares (ILS) have widely been applied for the spectophotometric resolution of mixtures containing two or more compounds without any preliminary separation (5–10). Application of these methods bring several advantages including a higher speed of processing data concerning the values of concentrations and absorbance of compounds in the presence of the spectral interference and the errors of calibration model are minimized by measuring the absorbance at many points in the wavelength range of the zero order and derivative spectra. Derivative spectrophotometry gives a satisfactory resolution for the binary mixture systems, eliminate the interference from sample turbidity as well as the effect of excipients placed in the commercial products removes the noise peaks coming from instrument and medium. However, this method has limits for some analytical problems, when the analysis conditions are not appropriate. The simultaneous use of derivative spectrophotometry and chemometric calibration techniques is a power full tool for the quantitative analysis of drugs in samples (11).

In this study, the validation of two chemometric calibration techniques for the absorption spectra and first derivative spectra for comparison purposes were done by analyzing synthetic mixtures containing **PAR, ASP** and **CAF** in different proportions. These developed techniques were applied also to the chemometric determination of three drugs in pharmaceutical preparation.

Experimental

Apparatus

A Shimadzu UV-160 double beam UV-VIS spectrophotometer possessing a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software were used to record the absorption spectra. Data treatments, regressions and statistical analysis were performed by using *EXCEL* and *MATLAB* softwares. The absorption spectra were recorded over the wavelength range of 210–305 nm.

Pharmaceutical Formulation

A commercial tablet formulation (EXCEDRINE® MIGRANE coated tablets produced by Bristol–Myers Squibb Co., USA, and Batch no. 306532) contains 250 mg **PAR**, 250 mg **ASP** and 65 mg **CAF** per tablet was assayed. **PAR, ASP** and **CAF** were kindly donated from Turkish Pharmaceutical Industrial firms.

Standard Solutions

Stock solutions containing 50 mg/100 mL **PAR, ASP** and **CAF** were prepared in 0.1 M HCl. A standard series of the solutions containing 5-25 µg/ mL for both **PAR** and **ASP** and 4-28 µg/mL for **CAF** was obtained from the stock solutions. A training set of 20 standard mixture solutions consisting of 0-25 µg/ mL of both **PAR** and **ASP** and, 0-28 µg/mL **CAF** was made from stock solutions. A validation set consisting of 15 synthetic mixture solutions in the concentration range of 5-20 µg/mL **PAR, ASP** and 4-28 µg/ mL **CAF** were prepared by using the same stock solutions. All the solutions were prepared freshly and protected from light.

Sample Preparation

Twenty tablets were weighted and powdered in a mortar. A tablet amount was transferred to a 100-ml calibrated flask and dissolved in 100 ml 0.1 M HCl. After dissolution process prepared solutions were filtered with 0.2 µm disposable membrane filter (Sartorious, minisart, *f= 0.20 µm)*. The final solution was diluted to the working concentration range for application of the two developed methods.

CLS and ILS Methods for the Zero-order and Derivative Spectra

The constant matrices *K* and *P* for both calibration models were obtained by using the linear equation system based on the use of absorption and derivative absorbance data at full spectral range from 210 nm to 305 nm for the training set (calibration mixture solutions). By inserting the values of *K* and *P* into the multi-linear regression equations, the prediction of unknown concentration of two drugs in samples was done by measuring the zero-order and first derivative absorbance values of full spectral wavelengths in the above range.

Results and Discussion

Figure 1 shows the zero-order absorption spectra of the solutions of **PAR, ASP** and **CAF** in 0.1 M HCl in the 210-300 nm absorption region. The first derivatives of the absorption spectra of the analytes at the same concentrations are displayed in Figure 2. The absorption spectra of compounds show several maxima at the 215, 245, and 260 nm for **PAR, ASP** and **CAF,** respectively. The zero order absorption and first derivative signals of compounds show strong overlap that makes impossible the determination of multicomponent mixtures using classical techniques. For example, the zero-crossing technique is not suitable for the resolution of ternary compounds. There are several graphical methods for the resolution of this kind of ternary systems like simultaneous application of division and derivation of the absorption spectra (12-13) and application of double divisor method that is applied recently by Dinc et *al*. (14). By application of appropriate chemometric methods the analytes can be simultaneously determined in the mixtures. The commercially available ternary mixtures can be analyzed by establishing a well designed concentration set. For the calibration, a twenty set mixtures of the active compounds were prepared as shown in Table 1. The absorption spectra of the training set were recorded in computer and their first derivative was plotted with the interval of 5 nm (scaling factor = 100) as shown in Figure 2. The values of the absorption and first derivative absorbance were measured in the absorbance range of 210–305 nm. The obtained absorbance data were used for the proposed calibration models as explained below. The correlation coefficient (r), which is, indicated the quality of fit of all the data to a straight line is calculated for the checking of each calibration. The standard error of the prediction (SEP) as a second statistical parameter was also computed to control the predictive ability of the estimated calibration model for training set.

Validation of Chemometric Calibrations for Synthetic Ternary Mixtures:

The proposed calibrations are tested by an independent set of validation samples containing **PAR, ASP** and **CAF** in different compositions given in Table 2. The prediction errors of the samples were each calculated with reference to the known concentrations of the analytes.

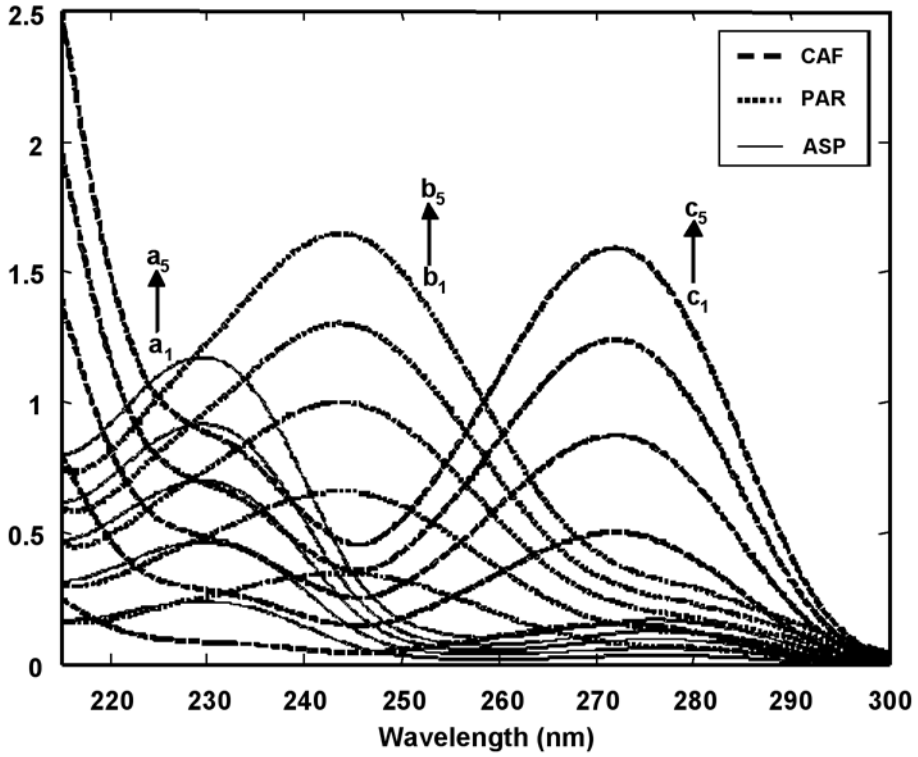

Figure 1. Absorption spectra of **PAR** (·····), **ASP** (——) in the concentration range of (a1=b1) 5 µg/mL, (a2=b2) 10 µg/mL, (a3=b3) 15 µg/mL, (a4=b4) 20 µg/mL, and (a5=b5) 25 µg/mL, and **CAF** (— —) in the concentration of (c1) 4 µg/mL µg/mL, (c2) 10 µg/mL, (c3) 16 µg/mL, (c4) 22 µg/mL and (c5) 28 µg/mL in 0.1 M HCl (scaling factor = 100).

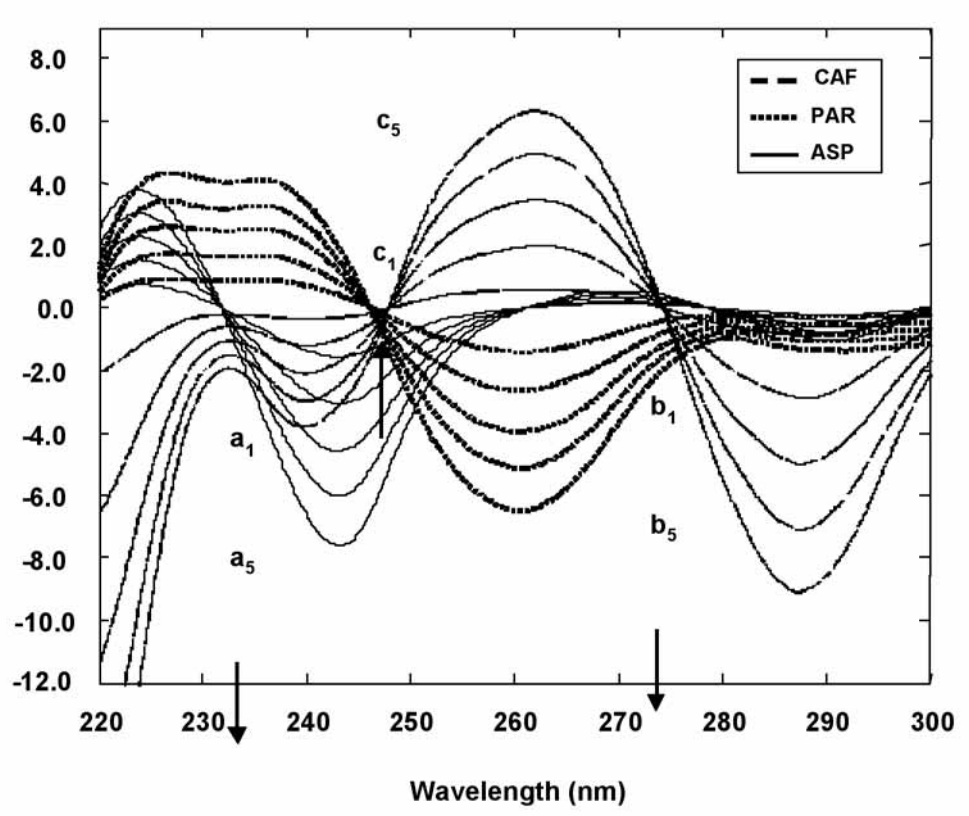

Figure 2. First derivative spectrum of **PAR** (····), **ASP** (—) in the concentration range of (a1=b1) 5 µg/mL, (a2=b2)10 µg/mL, (a3=b3) 15 µg/mL, (a4=b4) 20 µg/mL, and (a5=b5) 25 µg/mL, and **CAF** (— —) in the concentration of (c1) 4 µg/mL µg/mL, (c2) 10 µg/mL, (c3) 16 µg/mL, (c4) 22 µg/mL and (c5) 28 µg/mL in 0.1M HCl.

This parameter is a measure of the predictive ability of the model calculated for each method and then, the statistical values of developed methods were compared. The methods performances were measured by calculating their important statistical parameters including SEP, r, n and m values (Table 3). While r evaluates the goodness of fit of the predicted concentrations to their actual values, SEP is a measure of the average error in the prediction step. From the results, the application of CLS and ILS to the first derivative spectra produces better results than the application of CLS and ILS to the zero spectra of analytes. For the derivative results, r values closer to unity and smaller SEP values shows the better method performance for prediction of analytes (Table 3).

TABLE 1. The training set of three active compounds.

	PAR µg/ml	**ASP** µg/ml	**CAF** µg/ml
1	5.0	20.0	5.2
2	10.0	20.0	5.2
3	15.0	20.0	5.2
4	20.0	20.0	5.2
5	25.0	20.0	5.2
6	0.0	20.0	0.0
7	0.0	20.0	5.2
8	0.0	0.0	5.2
9	20.0	5.0	5.2
10	20.0	10.0	5.2
11	20.0	15.0	5.2
12	20.0	20.0	5.2
13	20.0	25.0	5.2
14	20.0	20.0	0.0
15	20.0	0.0	0.0
16	20.0	20.0	4.0
17	20.0	20.0	10.0
18	20.0	20.0	16.0
19	20.0	20.0	22.0
20	20.0	20.0	28.0

For the comparison, the indicated wavelength region is included to all the proposed calibrations and tested by an independent set of validation samples containing **PAR, ASP** and **CAF** in different compositions given in Table 2. Table 3 summarizes the statistical results of the CLS and ILS algorithms applied to absorption data and first derivative data that represent the resolving power of each application for these ternary combinations. It is ob-viously seen that derivative applications of chemometric techniques (CLS, ILS) show better performance than the zero order applications according to the lower SEP values and n values (Table 3).

The validity of the calibration models was also tested evaluating the standard addition method. Thus, the developed models were employed to calculate the concentration of the three analytes in the five samples and results are shown in Table 4 in terms of overall means, their standard deviations, relative standard deviations, standard errors and 95% confidential limit arranged for each method and component. The represented results also show that the derivative results produce better results when we compare standard errors, relative standard deviations and other statistical results.

Assay procedure:

The obtained results by applying CLS and ILS to the absorption data and same methods to the derivative data of commercial tablet preparation were shown in Table 5. A good coincidence was observed between the experimental results and label claim of the commercial tablet formulation in this study. The numerical values of all statistical parameters calculated in Table 5 are in acceptable determination limits in application of two methods to the tablets. In the comparison of two methods, application of CLS and ILS to derivative spectra have produced better statistical results as in standard addition method.

The calculated results showed that the observed errors have proper repeatability of the measurements. In order to select the most appropriate procedure for this multiple determination, their performances were evaluated. In order to compare the results obtained by applying the proposed techniques to the above mentioned pharmaceutical preparation, Snedecor's *F* values for the proposed techniques, zero order spectra and first derivative spectra, were separately computed by using one-way ANOVA of four set of ten sub-sample for each drug, respectively. Although the *F*-values for the zero-order spectra and first derivative spectra were found as F_{PAR} = 1.658, F_{ASP} = 2.847 and F_{CAF} = 2.084, respectively. The experimental or calculated *F*-values did not exceed the tabulated values of $F_{critical}$ = 3.2389 in the ANOVA, indicating that there was no significant difference between the techniques. Therefore, it was concluded that they were suitable for the quantification purposes.

TABLE 2. Recovery results obtained for the determination of three drugs in synthetic mixtures by the chemometric techniques.

Recovery (%)															
				Zero-order spectra						First derivative spectra					
	Added (mg/mL)			**CLS**			**ILS**			**CLS**			**ILS**		
	PAR	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**
1	5	20	5.2	103.3	101.0	103.3	102.7	99.7	96.6	100.2	100.2	100.0	103.3	100.2	97.2
2	10	20	5.2	103.8	101.2	103.5	101.1	99.8	99.2	101.0	100.6	101.9	100.8	101.3	98.1
3	15	20	5.2	102.6	101.9	103.8	101.2	100.9	99.9	101.9	101.5	103.4	101.0	102.5	98.5
4	20	20	5.2	98.2	99.6	98.0	99.7	101.0	109.3	100.1	100.1	101.3	100.4	98.2	98.7
5	25	20	5.2	99.3	99.8	97.6	100.7	98.4	99.5	101.0	100.2	102.0	99.9	101.9	98.1
6	20	5	5.2	102.6	102.7	103.8	99.8	94.6	101.5	100.4	101.7	102.8	98.8	103.0	97.9
7	20	10	5.2	101.7	102.5	99.3	99.4	99.7	100.6	100.0	101.4	102.9	98.9	101.4	99.8
8	20	15	5.2	101.0	101.4	103.0	100.2	99.6	101.1	100.3	101.1	102.4	100.0	100.2	101.5
9	20	20	5.2	99.8	100.2	99.0	101.5	99.0	99.5	100.3	100.2	100.1	101.3	98.9	101.2
10	20	25	5.2	98.9	99.5	98.0	101.2	99.2	99.7	100.6	99.9	98.2	100.7	101.0	99.5
11	20	20	4	99.0	99.9	98.9	100.2	99.8	97.7	100.7	100.2	96.1	100.3	100.0	99.2
12	20	20	10	103.5	101.9	103.1	98.8	99.2	97.6	98.9	100.4	99.7	99.0	99.2	97.6
13	20	20	16	102.4	103.4	104.2	100.7	101.3	100.6	98.8	100.9	101.0	99.9	100.0	99.9
14	20	20	22	101.0	101.8	101.7	100.3	97.4	99.6	99.0	101.2	100.0	101.7	99.3	100.1
15	20	20	28	98.0	97.4	98.0	96.9	94.8	98.3	100.6	97.3	98.2	98.0	102.4	99.3
		Mean		101.0	100.9	101.0	100.3	99.0	100.0	100.2	100.5	100.7	100.3	100.6	99.1
		SD		2.02	1.55	2.60	1.34	1.97	2.90	0.83	1.06	2.05	1.32	1.43	1.26
		RSD %		2.00	1.53	2.58	1.34	1.99	2.90	0.83	1.06	2.04	1.32	1.42	1.27

SD: Standard deviation

RSD: Relative standard deviation

TABLE 3. Statistical results for the optimized chemometric techniques in the calibration step.

	Zero order						First derivative				
	CLS			**ILS**			**CLS**			**ILS**	
Parameter	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**
SEP	0.3567	0.3027	0.2835	0.2212	0.3428	0.1966	0.1525	0.1943	0.1667	0.1970	0.2597
r	0.9976	0.9984	0.9993	0.9989	0.9979	0.9997	0.9990	0.9992	0.9998	0.9992	0.9987
n	0.4558	0.2911	0.1056	0.1697	0.1393	0.0957	0.0603	0.1784	0.1412	0.2206	0.1058
m	0.9821	0.9916	0.9974	0.9920	0.9992	0.9858	0.9987	0.9935	0.9859	0.9885	0.9992

SEP: Standard error of prediction, r : Regression coefficient , n : Intercept, m : Slope

TABLE 4. Results of standard addition method applied to commercial tablet preparation by the proposed methods

	Zero order						First derivative				
	CLS			**ILS**			**CLS**			**I**	
Parameter	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**A**
Mean(mg)	237.6	251.9	60.1	239.7	256.2	66.6	242.4	253.3	62.2	241.9	24
SD	3.08	2.94	0.95	4.38	3.80	2.76	2.93	2.88	0.84	4.52	3.
RSD	1.30	1.17	1.58	1.83	1.48	4.14	1.21	1.14	1.34	1.87	1.
SE	1.38	1.31	0.42	1.96	1.70	1.23	1.31	1.29	0.37	2.02	1.
CL (P:0.05)	2.70	2.58	0.83	3.84	3.33	2.42	2.57	2.52	0.73	3.97	3.

SE: Standard error, CL: confidence limit

TABLE 5. Assay results of the commercial tablet preparation (Excedrin®) by the proposed methods

(Label claim = 250 mg **PAR**, 250 mg **ASP** and 65 mg **CAF** per tablet)

	Zero order						First derivative				
	CLS			**ILS**			**CLS**			**ILS**	
Parameter	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**	**CAF**	**PAR**	**ASP**
Mean:	241.5	251.4	63.6	244.5	257.1	69.4	247.6	252.7	63.3	247.4	247.5
SD	2.76	2.22	2.44	2.79	3.68	2.36	1.75	2.43	1.37	2.12	3.42
RSD	1.14	0.88	3.84	1.14	1.43	3.40	0.71	0.96	2.16	0.86	1.38
SE	0.87	0.70	0.77	0.88	1.16	0.75	0.55	0.77	0.43	0.67	1.08
CL (0.05)	1.71	1.38	1.51	1.73	2.28	1.46	1.08	1.51	0.85	1.31	2.12

Conclusions

Although the absorption spectra of both drugs were overlapped in the working wavelengths, chemometric calibration techniques (CLS and ILS) using in UV absorption and derivative spectrophotometry were successfully applied to the simultaneous determination of **PAR, ASP** and **CAF** in synthetic mixtures and tablets. It was observed that the concentrations of the active compounds were predicted with acceptable errors and that all of the commercial preparations proved to comply with the manufacturers declared amounts of their active ingredients. Interference due to the excipients was not observed. Comparison of the applied procedures indicated that application of chemometrics methods to the derivative data produced better results than the absorption data. The obtained results were reproducible and proved to be not statistically different in their ability to evaluate the three analytes.

The two statistically equivalent multipurpose calibration models were successfully applied to the absorption and derivative data for the quantitative analysis of synthetic samples and commercial tablet preparations containing mixtures of the **PAR, ASP** and **CAF**. These applications provide an alternative tool for better resolution and rapid determination of active ingredients in preparations containing these multicomponent mixtures.

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received: 04.01.2005
accepted: 10.02.2005