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Simultaneous spectrophotometric determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric methods

M.R. Khoshayand [a], H. Abdollahi [b,*], M. Shariatpanahi [a], A. Saadatfard [a], A. Mohammadi [a]

[a] Department of Food and Drug Control, School of Pharmacy, Medical Sciences/University of Tehran, Tehran, Iran
[b] Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), P.O. Box: 45195-159, Zanjan, Iran

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Abstract

In this study, the simultaneous determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric approaches using UV spectrophotometry has been reported as a simple alternative to using separate models for each component. Spectra of paracetamol, ibuprofen and caffeine were recorded at several concentrations within their linear ranges and were used to compute the calibration mixture between wavelengths 200 and 400 nm at an interval of 1 nm in methanol:0.1 HCl (3:1). Partial least squares regression (PLS), genetic algorithm coupled with PLS (GA-PLS), and principal component-artificial neural network (PC-ANN) were used for chemometric analysis of data and the parameters of the chemometric procedures were optimized. The analytical performances of these chemometric methods were characterized by relative prediction errors and recoveries (%) and were compared with each other. The GA-PLS shows superiority over other applied multivariate methods due to the wavelength selection in PLS calibration using a genetic algorithm without loss of prediction capacity. Although the components show an important degree of spectral overlap, they have been determined simultaneously and rapidly requiring no separation step. These three methods were successfully applied to pharmaceutical formulation, capsule, with no interference from excipients as indicated by the recovery study results. The proposed methods are simple and rapid and can be easily used in the quality control of drugs as alternative analysis tools.

Keywords: Paracetamol; Ibuprofen; Caffeine; Simultaneous spectrophotometric determination; Chemometrics

1. Introduction

Paracetamol (PCT), ibuprofen (IB) and caffeine (CAF) are active principles widely used and frequently combined in pharmaceutical preparations. PCT is a popular antipyretic and analgesic agent [1]. In several countries, it is one of the most used medicines as an alternative to aspirin (acetylsalicylic acid). IB is a non-steroidal anti-inflammatory drug with good analgesic, anti-inflammatory and antipyretic effects [2]. CAF, a methylated xanthine and potent stimulant of the central nervous system, has been added to PRT and IB in various combinations. This addition seems to be aimed at improving the analgesic efficacy [3,4].

Various methods, including official methods [5–7], spectrophotometry [8–13] and chromatography [14–19] are available for the determination of above compounds, whether alone or in combination with other drugs.

The quality control of dosage form preparations of drug requires reliable and quick analytical methods. UV/vis spectrophotometry is by far the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity, often demanding low cost equipment. Simultaneous quantitative analysis of pharmaceuticals containing multi-active compounds is difficult to perform by classical spectrophotometric method due to overlapping spectra.

Recently, multivariate calibration methods seem to be the proper techniques that show the best performance in terms of complex mixture resolution [20–27]. The same methods and their algorithms have been applied to the simultaneous spectrophotometric determination of drugs in the pharmaceutical

* Corresponding author. Tel.: +98 2414153122; fax: +98 2414153232.
E-mail address: abd@iasbs.ac.ir (H. Abdollahi).

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Table 1
Parameters of linear regression equations for each drug compound

Parameter	PCT	IB	CAF
Sample number	12	25	19
Linear range ($\mu g ml^{-1}$)	0.60–11.00	1.00–24.00	1.00–18.00
Intercept of calibration curve	-7.00×10^{-3}	-2.30×10^{-3}	6.56×10^{-3}
Slope of calibration curve	1.57×10^{-3}	6.69×10^{-2}	9.49×10^{-2}
Standard error of intercept	6.00×10^{-3}	4.30×10^{-3}	9.40×10^{-3}
Standard error of slope	9.30×10^{-4}	2.87×10^{-4}	8.91×10^{-4}
Correlation coefficient	0.9996	0.9995	0.9985
Limit of detection ($\mu g\, ml^{-1}$)[a]	0.21	0.52	0.67

[a] Limit of detection for all compounds was calculated according to the method described in Miller and Miller [59].

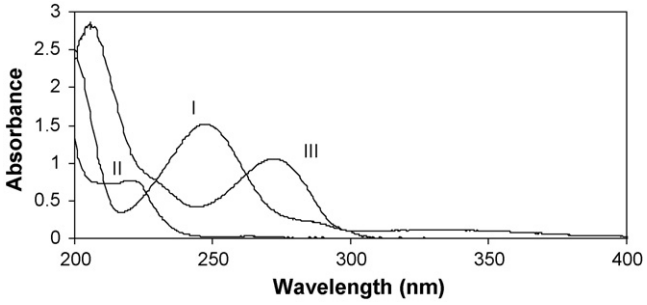

Fig. 1. Absorbance spectra of (I) 10 $\mu g\, ml^{-1}$ paracetamol; (II) 10 $\mu g\, ml^{-1}$ ibuprofen; (III) 10 $\mu g\, ml^{-1}$ caffeine in methanol:0.1 M HCl (3:1).

formulations containing two or more compounds with overlapping spectra.

Partial least squares regression (PLS) has become the most frequently used method for multivariate calibration because high-performance calibration models are obtained, while the software is not only available but also easily implemented [28–32]. Also, PLS has been successfully adopted in many quantitative assays of pharmaceutical preparations [33–37].

It has been shown that PLS is a reasonable choice for the resolution of overlapping signals and quantitative analysis over a wide range of conditions [38]. Although PLS is usually considered as a full spectrum method, literature shows a growing tendency to perform variable selection before multivariate regression, in order to improve its predicting ability. Consequently, in practice, wavelength selection continues to be the process of interest because a selection procedure that optimizes the prediction capacity will lead to those wavelengths for which the analyte of interest absorbs while its absorbance is different from other analytes. Different strategies for wavelength selection in multivariate calibration models have been proposed in the literature, including the use of artificial neural network (ANN) and genetic algorithms (GAs) [39–45].

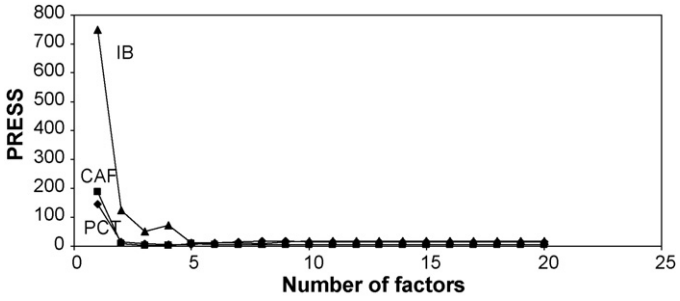

Fig. 2. Plot of PRESS against the number of factors for PCT, IB and CAF.

Among the different variable selection strategies, genetic algorithms are an interesting, flexible and widely used alternative. GAs are a guided random search technique inspired by natural selection mechanisms, which explore the solution space in an efficient manner and are suitable for parallel processing implementations.

Genetic algorithms [46–49] have been used to solve difficult problems with objective functions that do not possess 'nice' properties such as continuity, differentiability, etc. [50–53]. GAs search the solution space of a function through the use of simulated evolution, i.e. the survival of the fittest strategy. GAs have been shown to solve the optimization problem by exploring all regions of the potential solutions and exponentially exploit-

Table 2
Composition of the calibration set for applying PLS, GA-PLS and ANN methods

Number of calibration sample	PCT ($\mu g\, ml^{-1}$)	IB ($\mu g\, ml^{-1}$)	CAF ($\mu g\, ml^{-1}$)
1	1.00	15.60	1.84
2	4.72	3.21	7.74
3	5.02	10.32	13.03
4	7.76	15.25	3.69
5	0.92	3.55	3.05
6	0.94	13.79	5.67
7	8.86	14.37	4.13
8	6.77	18.34	1.92
9	7.35	1.20	1.50
10	9.72	9.07	4.41
11	4.28	18.26	4.78
12	8.68	15.83	1.62
13	7.03	5.54	4.24
14	4.81	5.75	3.61
15	5.31	17.30	1.40
16	0.88	15.42	4.99
17	6.82	4.78	11.74
18	4.50	9.52	11.24
19	9.42	15.02	14.15
20	7.43	14.77	6.84
21	0.60	10.50	14.96
22	1.63	17.14	8.17
23	3.59	9.38	10.05
24	7.60	5.23	9.65
25	3.64	2.94	3.29

Table 3
Composition of synthetic samples, their predictions by PLS model and statistical parameters for the system

Synthetic ($\mu g\,ml^{-1}$)			Prediction ($\mu g\,ml^{-1}$)			Recovery (%)		
PCT	IB	CAF	PCT	IB	CAF	PCT	IB	CAF
4.58	9.18	1.61	4.71	10.21	1.31	102.8	111.2	81.4
4.96	19.16	11.44	5.00	19.22	11.23	100.8	100.3	98.1
0.67	18.19	1.77	0.69	19.00	2.10	102.5	104.4	118.4
2.77	16.82	11.41	2.90	16.61	11.53	104.8	98.8	101.1
7.27	4.12	4.22	7.20	4.10	4.55	99.1	99.4	107.9
9.05	11.94	13.63	9.51	12.10	13.50	105.1	101.4	99.0
8.58	5.23	9.53	8.65	5.98	10.76	100.8	114.3	112.8
3.62	4.15	5.49	4.12	4.21	5.44	113.9	101.4	99.0
3.08	2.16	8.29	3.00	2.44	8.41	97.3	113.2	101.4
1.80	16.10	14.60	1.99	18.43	14.80	110.3	114.5	101.4
0.96	8.61	6.79	1.00	9.99	7.10	103.9	116.0	104.5
5.09	17.76	7.90	5.10	19.12	8.43	100.2	107.7	106.7
5.29	8.88	7.78	5.62	9.34	8.40	106.2	105.1	107.9
0.74	14.79	7.60	0.88	14.10	8.23	119.2	95.3	108.3
8.72	3.44	12.09	9.10	3.55	12.00	104.3	103.3	99.2
8.75	13.44	6.31	8.97	14.21	6.45	102.5	105.7	102.3
1.12	19.22	8.52	1.33	19.70	9.23	119.1	102.5	108.3
1.46	18.54	4.12	1.55	19.54	3.80	105.9	105.4	92.2
4.90	15.05	3.57	5.20	15.10	3.43	106.2	100.3	96.1
3.44	15.45	2.89	3.81	16.30	2.80	110.7	105.5	97.0
Mean recovery						105.8	105.3	102.2
R.S.E. (%) single[a]						4.5	6.2	5.1
R.S.E. (%) total[b]							5.8	

[a] Calculated by Eq. (1).
[b] Calculated by Eq. (2).

Table 4
Composition of synthetic samples, their predictions by GA-PLS model and statistical parameters for the system

Synthetic ($\mu g\,ml^{-1}$)			Prediction ($\mu g\,ml^{-1}$)			Recovery (%)		
PCT	IB	CAF	PCT	IB	CAF	PCT	IB	CAF
4.58	9.18	1.61	4.41	9.22	1.65	96.2	100.5	102.5
4.96	19.16	11.44	5.12	19.12	11.65	103.2	99.8	101.8
0.67	18.19	1.77	0.68	17.98	1.67	101.0	98.8	94.1
2.77	16.82	11.41	2.71	16.76	11.54	97.9	99.7	101.1
7.27	4.12	4.22	7.25	4.23	4.21	99.8	102.6	99.8
9.05	11.94	13.63	9.11	11.67	13.13	100.7	97.8	96.3
8.58	5.23	9.53	8.55	5.43	9.14	99.7	103.8	95.9
3.62	4.15	5.49	3.53	4.21	5.23	97.6	101.4	95.2
3.08	2.16	8.29	3.12	2.19	8.34	101.2	101.6	100.6
1.80	16.10	14.60	1.92	16.54	14.74	106.4	102.7	101.0
0.96	8.61	6.79	0.95	9.10	6.67	98.7	105.7	98.2
5.09	17.76	7.90	5.23	18.12	7.58	102.8	102.1	95.9
5.29	8.88	7.78	5.32	8.65	7.83	100.5	97.4	100.6
0.74	14.79	7.60	0.76	15.11	7.56	102.9	102.1	99.5
8.72	3.44	12.09	8.81	3.55	12.15	101.0	103.3	100.5
8.75	13.44	6.31	8.89	13.49	6.33	101.6	100.3	100.4
1.12	19.22	8.52	1.15	18.97	8.45	103.0	98.7	99.1
1.46	18.54	4.12	1.33	19.17	4.45	90.8	103.4	108.0
4.90	15.05	3.57	5.10	16.01	3.50	104.1	106.4	98.1
3.44	15.45	2.89	3.75	15.12	2.85	108.9	97.9	98.7
Mean recovery						100.9	101.3	99.4
R.S.E. (%) single[a]						2.3	2.6	2.4
R.S.E. (%) total[b]							2.5	

[a] Calculated by Eq. (1).
[b] Calculated by Eq. (2).

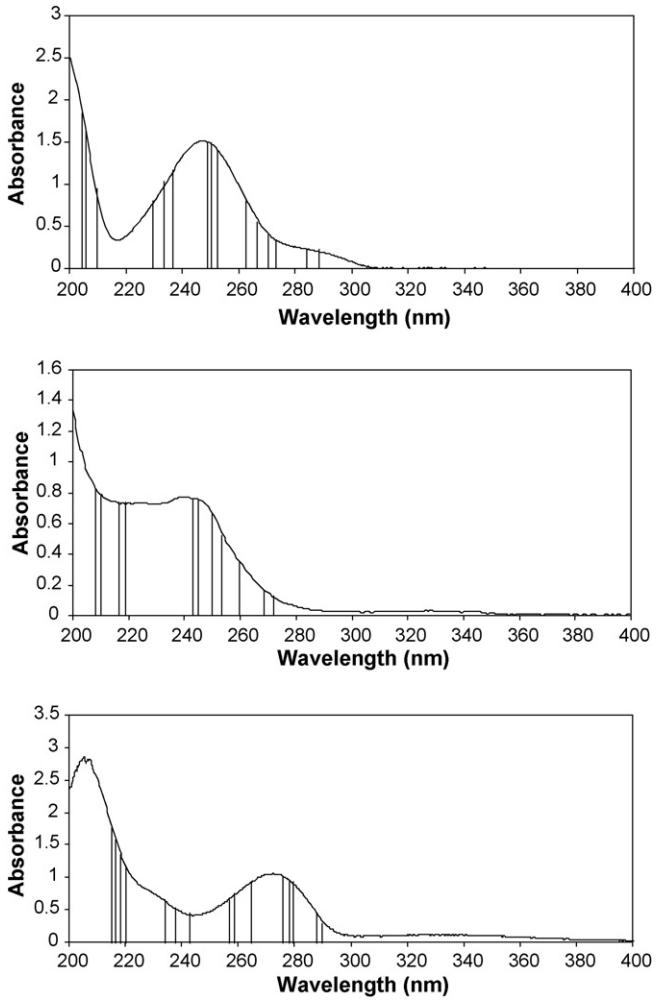

Fig. 3. Selected wavelengths by the GA-PLS for PCT (top), IB (middle) and CAF (bottom).

ing promising area through mutation, crossover and selection operation applied to individuals in the populations. A complete discussion of genetic algorithms can be found in references [50–53].

Although PLS method assumes a linear relationship between the measured samples parameters and the intensity of its absorption bands, small deviation from linearity is acceptable and can be readily suppressed by including additional modelling factors [54]. However, in the presence of substantial non-linearity, PLS tends to give large prediction errors and calls for more suitable models. Intrinsically non-linear calibration methods such as artificial neural networks are applicable in the later cases.

Artificial neural network [55] is a multivariate calibration method used mainly for modelling non-linear data, although, some applications use the neural network for modelling linear data. It is important to state that this method is computationally more complex than linear methods, they have limitation of being prone to over fitting and they heavily depend on amount and quality of data available. In many cases the principal disadvantage of the neural network is the time required for its training.

In this study, three chemometric methods are proposed for the simultaneous spectrophotometric determination of PCT, IB and CAF in their mixtures and pharmaceutical preparation, i.e. capsule. To the best of our knowledge, there is no other previous report for the simultaneous spectrophotometric determination by chemometric methods of these three compounds in synthetic samples or pharmaceutical compounds.

Table 5
Optimized parameter value for GA-PLS

Component type	Crossover type	Mutation rate	Population size	Max. generation
Paracetamol	Double point	0.005	32	35
Ibuprofen	Double point	0.005	32	30
Caffeine	Double point	0.005	32	20

The predictive abilities of multivariate calibration methods, including Partial least squares regression, genetic algorithm-partial least squares regression (GA-PLS) and principal component-artificial neural networks (PC-ANN) were investigated and successfully compared with each other.

2. Experimental

2.1. Apparatus and software

Digitized UV/vis absorbency spectra were collected using a GBC Cintra 40 spectrophotometer, with 1 cm quartz cells, a scan rate of 1000 nm min^{-1} and the slit width of 2 nm. The UV spectra of mixtures were recorded over the wavelength 200–400 nm with one data point per nanometer. All spectral measurements were performed using blank solution as a reference. Measurements of pH were made with a Metrohm 691 pH meter using a combined glass electrode.

Partial least squares regression, genetic algorithm coupled with PLS and principal component-artificial neural network were used for chemometric analysis of data. For all calculations Matlab for windows (Version 7.0) [56] was used. GA-PLS and ANN methods were carried out with the PLS-Toolbox [57] and Neural Network Toolbox [58] for using with Matlab, respectively. All programs were run on a Pentium (IV) microcomputer, with windows XP home edition.

2.2. Chemicals

Paracetamol, ibuprofen and caffeine were kindly donated by the pharmaceutical industries and were used without further purification. All solvents and reagents were of analytical reagent grade (Merck Chem. Ind.).

Table 6
Optimized parameters values for ANN

Drug	Number of PCs	Number of epochs	Number of neurons	Momentum constant	Learning rate
PCT	5	2300	4	0.10	0.01
IB	6	1600	4	0.25	0.08
CAF	4	2100	3	0.20	0.05

Table 7
Composition of synthetic samples, their predictions by PC-ANN model and statistical parameters for the system

Synthetic ($\mu g\,ml^{-1}$)			Prediction ($\mu g\,ml^{-1}$)			Recovery (%)		
PCT	IB	CAF	PCT	IB	CAF	PCT	IB	CAF
4.58	9.18	1.61	5.10	9.32	1.77	111.3	101.5	110.0
4.96	19.16	11.44	4.43	18.89	10.98	89.3	98.6	96.0
0.67	18.19	1.77	0.59	17.78	1.62	87.6	97.7	91.3
2.77	16.82	11.41	2.67	16.18	11.56	96.5	96.2	101.3
7.27	4.12	4.22	7.77	4.42	4.43	107.0	107.2	105.0
9.05	11.94	13.63	9.10	12.00	13.56	100.6	100.5	99.5
8.58	5.23	9.53	8.66	5.56	9.67	100.9	106.3	101.4
3.62	4.15	5.49	3.33	4.34	5.89	92.1	104.5	107.2
3.08	2.16	8.29	3.10	2.57	9.11	100.6	119.2	109.8
1.80	16.10	14.60	1.88	17.00	15.10	104.2	105.6	103.4
0.96	8.61	6.79	0.95	8.56	7.01	98.7	99.4	103.2
5.09	17.76	7.90	5.15	17.17	7.89	101.2	96.7	99.8
5.29	8.88	7.78	5.33	9.16	6.90	100.7	103.1	88.7
0.74	14.79	7.60	0.79	14.54	7.56	107.0	98.3	99.5
8.72	3.44	12.09	8.75	3.25	12.66	100.3	94.6	104.7
8.75	13.44	6.31	8.88	13.39	6.56	101.5	99.6	104.0
1.12	19.22	8.52	1.16	19.44	8.19	103.9	101.1	96.1
1.46	18.54	4.12	1.62	18.90	4.44	110.6	101.9	107.7
4.90	15.05	3.57	4.76	15.87	3.65	97.2	105.4	102.3
3.44	15.45	2.89	3.32	15.60	2.50	96.5	101.0	86.6
Mean recovery						100.4	101.9	100.9
R.S.E. (%) single[a]						4.3	3.0	4.6
R.S.E. (%) total[b]							3.6	

[a] Calculated by Eq. (1).
[b] Calculated by Eq. (2).

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (Novafen® capsule BROWN & BURK (UK), batch no: NVK-5015) was purchased from local resources and assayed. Its declared content was as follows: paracetamol BP (325 mg), ibuprofen BP (200 mg) and caffeine anhydrous BP (40 mg), in each capsule.

2.4. Standard solutions

Stock solutions of paracetamol, ibuprofen and caffeine, containing 1000 $\mu g\,ml^{-1}$ were prepared in 100 ml volumetric flasks, by dissolving 100 mg of each compound in methanol:0.1 M HCl (3:1). Working standard solutions were prepared daily by diluting the stock solutions for each drug according to its linear calibration range. Two sets of standard solutions were prepared, the calibration set contained 25 standard solutions and the prediction set contained 20 standard solutions. To a series of 10 ml volumetric flasks, aliquots of paracetamol, ibuprofen or caffeine solutions, containing appropriate amount of these drugs in the range of calibrations, were added and then the solutions were diluted to 10 ml with methanol:0.1 M HCl (3:1). UV spectra of the mixtures were recorded in the wavelength range 200–400 nm versus a solvent blank, and digitized absorbance was sampled at 1 nm intervals. All the solutions were prepared freshly and were protected from light.

Table 8
Assayed results of simultaneous determination of PCT, IB and CAF in Novafen capsules by the proposed methods

	PCT			IB			CAF		
	PLS	GA-PLS	ANN	PLS	GA-PLS	ANN	PLS	GA-PLS	ANN
Novafen									
Sample 1 (mg)	332.8	327.6	320.8	204.0	200.4	201.6	41.3	40.4	39.5
Sample 2 (mg)	330.5	327.3	322.4	201.8	199.6	202.8	41.4	40.2	39.2
Sample 3 (mg)	334.1	324.6	320.4	203.6	198.8	201.4	41.6	40.3	39.6
Sample 4 (mg)	331.1	324.7	323.3	203.0	199.8	202.6	41.2	40.4	39.3
Sample 5 (mg)	329.2	326.3	324.7	203.8	199.0	203.4	41.4	40.3	39.4
Amount on the label (mg)	325.0	325.0	325.0	200.0	200.0	200.0	40.0	40.0	40.0
Mean % recovery	102.1	100.3	99.1	101.6	99.8	101.2	103.5	100.8	98.5
S.D. % recovery	0.62	0.43	0.55	0.45	0.32	0.42	0.37	0.21	0.40

Table 9
Recovery studies of PCT, IB and CAF in commercial capsules (Novafen) using PLS method

Spiked sample	PCT			IB			CAF		
	Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery (%)
1	162.5	162.2	99.8	100	101.0	101.0	20	20.4	102.0
2	162.5	160.2	98.6	100	100.3	100.3	20	20.5	102.5
3	162.5	162.6	100.1	100	99.4	99.4	20	20.3	101.5
4	162.5	161.7	99.5	100	101.0	101.0	20	20.5	102.5
5	162.5	161.6	99.4	100	99.5	99.5	20	20.6	103.0
Mean			99.5			100.2			102.3
S.D.			0.56			0.78			0.57

2.5. Sample preparation

The contents of 20 capsules were emptied and mixed. An amount of powder equivalent to one capsule was accurately weighted and transferred into a 100 ml volumetric flask using methanol:0.1 M HCl (3:1). The flask was half filled with the same solvent, shaken automatically for 15 min and then completed to the mark. Dilution of stock solution quantitatively with methanol:0.1 M HCl (3:1) furnished suitable working sample solutions for UV measurements.

3. Results and discussions

3.1. Individual calibration

Fig. 1 displays the UV absorption spectra for PCT, IB and CAF in the standard solutions recorded between 200 and 400 nm.

Individual calibration curves were constructed with several points as absorbance versus drugs concentrations in the range of 0.60–11.00 $\mu g\,ml^{-1}$ for paracetamol, 1.00–24.00 $\mu g\,ml^{-1}$ for ibuprofen and 1.00–18.00 $\mu g\,ml^{-1}$ for caffeine and were evaluated by linear regression [59]. Characterization parameters for the regression equations of individual calibrations by absorption UV spectra are shown in Table 1.

3.2. Multivariate methods

The first step in simultaneous determination of the ternary mixture of drugs by multivariate calibration methods involves constructing the calibration matrix for ternary mixture of PCT, IB and CAF.

Twenty-five ternary mixtures were selected by random design as the calibration set. The composition of the samples was randomly designed in order to obtain non-correlated concentration profiles (Table 2). In order to minimize the correlation between concentration vectors, the correlation coefficient matrix is considered as a criterion. The calibration model in each chemometric method was validated with 20 synthetic mixtures set containing the drugs under study in different proportions selected randomly. The predictive abilities of PLS, GA-PLS and ANN were examined for simultaneous determination of PCT, IB and CAF in sample mixtures. The common requirement for all the mentioned methods is that the unknown samples and standards be of the same nature.

3.2.1. Partial least squares regression (PLS)

PLS [60] is a method for building regression models based on the latent variable decomposition. The PLS algorithm takes into account the information of responses and concentrations simultaneously. There are two procedures available to solve the system in PLS1; one model is built for each analyte by using its concentration vector while in PLS2, all analyte concentrations are simultaneously considered in constructing the calibration model. In this way, factors from a PLS model are calculated as those variables that describe the maximum amount of information for the concentration matrix. Once the model is built, it can be used to predict the concentration of unknown samples. In this study the cross validation method, leaving out one sample at a time, was used to select the optimum number of factors [61].

The prediction error was calculated for each drug for the prediction set whose samples are not participating in the construction of the model. The error was expressed as the prediction

Table 10
Recovery studies of PCT, IB and CAF in commercial capsules (Novafen) using GA-PLS method

Spiked sample	PCT			IB			CAF		
	Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery (%)
1	162.5	163.0	100.3	100	99.4	99.4	20	20.1	100.5
2	162.5	162.0	99.7	100	100.5	100.5	20	19.9	99.5
3	162.5	161.9	99.6	100	99.9	99.9	20	20.0	100.0
4	162.5	161.1	99.1	100	99.7	99.7	20	20.1	100.5
5	162.5	163.9	100.8	100	100.8	100.8	20	20.1	100.5
Mean			99.9			100.1			100.2
S.D.			0.66			0.58			0.45

Table 11
Recovery studies of PCT, IB and CAF in commercial capsules (Novafen) using ANN method

Spiked sample	PCT			IB			CAF		
	Actual (mg)	Found (mg)	Recovery (%)	Actual (mg)	Found (mg)	Recovery (%)	Actual (mg)	Found mg)	Recovery (%)
1	162.5	162.8	100.2	100	100.1	100.1	20	20.4	102.0
2	162.5	162.2	99.8	100	99.3	99.3	20	20.5	102.5
3	162.5	163.5	100.6	100	101.2	101.2	20	20.4	102.0
4	162.5	162.6	100.1	100	99.0	99.0	20	20.6	103.0
5	162.5	163.3	100.5	100	99.0	99.0	20	20.3	101.5
Mean			100.2			99.7			102.2
S.D.			0.32			0.94			0.57

residual error sum of squares (PRESS). PRESS was calculated for the first variable, which build the PLS modelling in the calibration step. After that, another factor was added for the model building and the PRESS was calculated again. These calculations were repeated for 1–20 factors, which were used in PLS modelling. This procedure was repeated for each drug. Fig. 2 shows a plot of PRESS versus the number of factors. For finding the smallest model with the fewest number of factors, the **F** statistics was used to carry out the significant determination [62]. The optimal number of factors for PCT, IB and CAF was obtained as 5, 6 and 4, respectively.

In this work, 20 synthetic test samples were analysed with the proposed method. The prediction results are given in Table 3. The prediction error of a single component in the mixture was calculated as the relative standard error (R.S.E) of the prediction concentrations [63],

$$\text{R.S.E.}(\%) = 100 \times \left(\frac{\sum_{j=1}^{N} (\hat{C}_j - C_j)^2}{\sum_{j=1}^{N} (C_j)^2} \right)^{1/2} \quad (1)$$

where N is the number of samples, C_j the concentration of the component in the jth mixture and $\hat{C}_j$ is the estimated concentration. The total prediction error of N samples is calculated as follows:

$$\text{R.S.E.}_{\text{t}}(\%) = 100 \times \left(\frac{\sum_{i=1}^{M} \sum_{j=1}^{N} (\hat{C}_{ij} - C_{ij})^2}{\sum_{i=1}^{M} \sum_{j=1}^{N} (C_{ij})^2} \right)^{1/2} \quad (2)$$

where C_{ij} is the concentration of the ith component in the jth sample and $\hat{C}_{ij}$ is its estimation. Table 3 also shows reasonable single and total relative standard error for such a system.

3.2.2. Genetic algorithm-partial least squares regression

Constructing the PLS model after selecting the optimal variables (wavelengths) improves the prediction capacity of the model [64,65]. GA can be used successfully for variable selection in PLS calibration. A p-dimensional vector, **w**, will represent each wavelength subset selected in the spectrum with binary coordinate. If the ith wavelength is selected, then the ith coordinate of **w** is one; in other cases it is zero. Each **w** is a chromosome.

Given a chromosome (**w**), a PLS calibration is constructed using, from each spectrum, only the wavelengths represented by **w**. Each chromosome is evaluated using the PRESS(**w**) value reached in the calibration. The genetic algorithm searches for minimum PRESS(**w**) in the space of all the possible chromosomes without establishing, a priori, the latent structure of the calibration.

The GA was run for 201 variables (in the range 200–400) using a PLS regression method where the maximum number of factors allowed is the optimal number of components determined by cross validation on the model containing all the variables, and the selected variables were used for running of PLS. For obtaining the optimum set of wavelength for determination of each drug, the GA procedure was repeated 10 times. Finally a wavelength was selected if the percentage of selection for that variable exceeded the critical value. The thresholds of 88%, 90% and 82% were obtained for PCT, IB and CAF, respectively, according to minimum errors of prediction for each drug. The selected wavelengths are 204, 205, 210, 230, 235, 237, 249, 250, 253, 261, 264, 271, 273, 285 and 288 for PCT, 208, 210, 216, 219, 243, 245, 251, 254, 260, 268 and 272 for IB and 214, 217, 218, 220, 234, 238, 242, 256, 259, 265, 276, 277, 280, 288 and 290 for CAF as shown in Fig. 3. Also GA reduced the optimal number of factors to 3, 4 and 3 for PCT, IB and CAF, respectively, after suitable variable selection. The predictive ability of the method was determined using 20 ternary drug mixtures the results of which are shown in Table 4. Comparison of the GA-PLS results with those of PLS shows that the GA-PLS is more suitable for simultaneous determination of these drugs. Also, the construction of the optimized GA-PLS model is summarized in Table 5.

3.2.3. Artificial neural networks (ANN)

Artificial neural networks (ANN) are computer programs that are designed to simulate some functions of the human brain using different algorithms, which can learn from experience. Its base theory and application to chemical problems can be found elsewhere [66,67]. An ANN is formed by series of interconnected nodes (neurons) that receive and/or send number values to the other nodes. In the back-propagation ANN, the network architecture generally comprises several layers: an input layer, in which each node represents an explanatory variable; an output layer, in which a node represents a dependent variable; and in between these two layers, there are one or more 'hidden' layers [68]. The artificial nodes process information based on weighted inputs using their transfer function and send out outputs. The nodes in adjacent layers are fully or partially interconnected with weighted links.

Reducing the number of inputs to a network reduces the training time, repetition and redundancy in the input data and so potentially giving a more accurate network. Principal component analysis (PCA) is often used to reduce the large number of data to much smaller PCs. Therefore, the principal components scores have been used as network input instead of original data.

In this work, the optimum value for learning rate, number of PCs, number of epochs and number of nodes in hidden layers were evaluated by obtaining those that yielded a minimum in the error of prediction. Over fitting is avoided by using two sets of samples, thus weights are calculated from a calibration set (training set) while the concentration of another sample set (test set) is being simultaneously predicted. The neural network models were tested on an external prediction set (validation set) that considered the samples belonging to neither the calibration set nor the test set. The predictive abilities of training set, testing set and validation set were compared by means of the relative standard error (R.S.E.) as defined earlier in Eqs. (1) and (2). The construction of optimized ANN model is summarized in Table 6. Also the prediction results of applying the constructed PC-ANN model are shown in Table 7.

3.3. Applications

In order to assess the applicability of the proposed methods to the analysis of real samples, they were applied to the determination of these drugs in pharmaceutical formulation. Five replicate measurements were made. The results are shown in Table 8. The good agreement between these results and the label claims indicates the successful applicability of the proposed procedure for the simultaneous determination of paracetamol, ibuprofen and caffeine in real sample. To check the validity of the proposed method, after the addition of the known amounts of PCT, IB and CAF to the commercial formulation, we found that the amount of these drugs did not change. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug (standard addition method). These results are shown in Tables 9–11. Moreover, we compare the spectra obtaining from the mixture of PCT, IB and CAF in standard and drug formulation solutions that showed similar patterns in their spectra (Fig. 4.). These findings indicate that excipients placed in commercial preparation did not interfere in the measurement of PCT, IB, and CAF in pharmaceutical formulation.

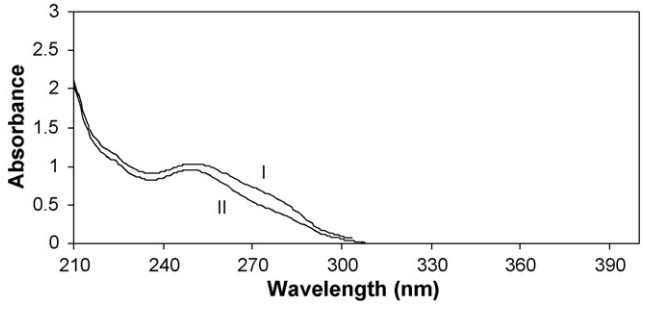

Fig. 4. Absorbance spectra of (I) mixture of PCT, IB and CAF; (II) commercial formulation (Novafen) in methanol:0.1 M HCl (3:1).

4. Conclusions

The most striking features of spectrophotometric method are its simplicity and rapidity without requiring time-consuming sample preparation. Chemometric calibration techniques in spectral analysis are widely used in quality control of drugs in mixtures and multicomponent pharmaceutical formulations with overlapping spectra, as separation procedures in the drug determinations are not required. A comparative study of the use of PLS, GA-PLS and PC-ANN for the simultaneous spectrophotometric determination of paracetamol, ibuprofen and caffeine has been accomplished. In general terms, it was found that the GA-PLS method is more accurate to model the considered system for determination of these drugs. It seems that the superiority of GA-PLS over other applied multivariate methods is due to the wavelength selection in PLS calibration using genetic algorithm without loss of prediction capacity that provides useful information about the multicomponent system. High percentage of recovery shows that the methods are free from interference of the excipients used in the commercial formulation. Results also showed that the developed methods can be applied to a routine analysis, quality control of mixtures and commercial preparations containing these three drugs.

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