Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1471–1475

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Multivariate analysis of paracetamol, propiphenazone, caffeine and thiamine in quaternary mixtures by PCR, PLS and ANN calibrations applied on wavelet transform data

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ARTICLE INFO

Article history:
Received 24 June 2008
Received in revised form
11 September 2008
Accepted 18 September 2008
Available online 30 September 2008

Keywords:
Fractional wavelet transform
Spectrophotometry
Quaternary drug mixture
Multivariate analysis
Artificial neural networks

ABSTRACT

The quantitative resolution of a quaternary pharmaceutical mixture consisting of paracetamol, propiphenazone, caffeine and thiamine was performed by the simultaneous use of fractional wavelet transform (FWT) with principal component regression (PCR), partial least squares (PLS) and artificial neural networks (ANN) methods. A calibration set consisting of 22 mixture solutions was prepared by means of an orthogonal experimental design and their absorption spectra were recorded in the spectral range of 210.0–312.3 nm and then transferred into the fractional wavelet domain and processed by FWT. The chemometric calibrations FWT–PCR, FWT–PLS and FWT–ANN were computed by using the relationship between the coefficients provided by FWT method and the concentration data from calibration set. An external validation was carried out by applying the developed methods to the analysis of synthetic mixtures of the related compounds, obtaining successful results. The models were finally used to assay the studied drugs in the commercial pharmaceutical formulations.

1. Introduction

The conventional spectrophotometric methods use a discrete number of wavelengths that frequently are not enough to furnish the necessary information to resolve a system with severe spectra overlapping [1,2]. Several approaches for the elaboration of spectrophotometric data have been proposed in order to extract a largest analytical information from spectra composed of unresolved bands. In recent years chemometrics–spectrophotometry is rapidly attracting analysts' attention and is playing a very important role in the multicomponent analysis of complex drug mixtures [3–6].

Multivariate chemometric methods allow to extract analytical information from the full-spectra, providing so to use simultaneously an elevated number of signals. Moreover, these techniques allow a rapid analytical response with minimum sample preparation, reasonable accuracy and precision and without the need of lengthy or tedious separations. For these reasons, these modern methods should be strictly considered for routine analysis of the drugs in their formulations.

The present work aims at the simultaneous determination of a quaternary pharmaceutical mixture containing paracetamol (PAR), propiphenazone (PRO), caffeine (CAF) and thiamine (THI), combined in analgesic and antipyretic pharmaceutical specialties. A number of analytical methods have been reported for the simultaneous determination of the studied drugs in mixture. The most recent methods are based on spectrophotometric [7–10] or chromatographic techniques [11,12].

The spectral analysis of standard mixtures as well as pharmaceutical samples containing the above drugs by means of conventional methods gave resulted inaccurate and highly imprecise. Very recently a new technique based on the fractional wavelet analysis [13–17] was introduced in the field of the analytical chemistry [6–9]. The combined use of the wavelet method with chemometric methods such as principal component analysis (PCR), partial least squares (PLS) and artificial neuron network (ANN) has been applied successfully to the quantitative resolution of various mixtures [18–21].

The mathematical tools described in this paper are based on the application of the fractional wavelet transform (FWT) technique combined with multivariate regression methods as PCR, PLS and

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doi:10.1016/j.jpba.2008.09.035

ANN to the assay of the above described quaternary mixtures. The calibration models were built by using a novel experimental design on data from a calibration set of 22 reference mixtures. The defined models were optimized and submitted to an external validation test by assaying a prediction set of 11 synthetic mixtures, in order to verify their prediction ability in terms of accuracy and precision. The proposed approaches were finally applied to the quantitative analysis of the commercial samples containing the four drugs to confirm their effectiveness in the routine analysis of real samples.

2. Experimental

2.1. Apparatus

Absorption spectra were recorded on a λ range of 190–340 nm in a 10 mm quartz cell, by a PerkinElmer Lambda 40P Spectrophotometer at the following conditions: scan rate 1 nm s^{-1}; time response 1 s; spectral band 1 nm; data density 1 point nm^{-1}. The software UV Winlab 2.79.01 (PerkinElmer) was used for spectral acquisition and elaboration.

2.2. Chemometric software

The calibration set was built by using an experimental design able to define ternary and quaternary mixtures, developed by an algorithm proposed in a previous work [10]. The data treatment was done by using MATLAB 7.0 software (The Math Works, Natick, MA, USA), FWT calculations were performed in MATLAB 7.0 and chemometric multivariate regressions were done in PLS-Toolbox 3.5.

2.3. Chemicals

PAR and CAF were courteously provided from Ogna SpA, Italy; PRO and THI were a generous gift from Montefarmaco SpA, Italy and Comifar SpA, Italy, respectively. Ethanol 95% was of spectrophotometric grade (J.T. Baker, Holland). PTFE 0.45 μm membrane filters were purchased from Supelco (Milan, Italy).

The pharmaceutical specialties Odontalgico Dr. Knapp® (Montefarmaco SpA, Italy), Influrem® (Edmond Pharma s.r.l, Italy), Micranet® (Giovanni Ogna & figli SpA, Italy) and Saridon® (Roche SpA, Italy) were obtained commercially.

2.4. Standard solutions

Stock ethanol solutions of PAR and PRO (4.0 mg ml^{-1}), CAF and THI (0.5 mg ml^{-1}) were used to set up the calibration set with concentration established according to the above described experimental design. Standard mixtures in ethanol with concentration values within the range 0.0–20.4 μg ml^{-1} for PAR, 0.0–19.9 μg ml^{-1} for PRO, 0.0–4.5 μg ml^{-1} for CAF and 0.0–3.8 μg ml^{-1} for THI were prepared from opportune dilution of the stock solutions. The mixture solutions of the calibration set are listed in Table 1.

2.5. Sample solutions

An independent prediction set of quaternary mixtures, randomly established, was prepared to validate the elaborated multivariate models. The concentration values ranged between 5.1–20.4 μg ml^{-1} for PAR, 5.0–19.9 μg ml^{-1} for PRO, 0.6–4.5 μg ml^{-1} for CAF and 0.6–3.8 μg ml^{-1} for THI, respectively.

For the assay of the pharmaceutical formulations, the content of five tablets was weighed and diluted to a volume of 50 ml with ethanol. The suspension was sonicated for 10 min and then filtered through a PTFE 0.45 μm membrane filter. One milliliter of this filtrate was diluted to 250 ml with ethanol and directly analyzed.

Table 1
Calibration set. Concentration values are expressed as μg ml^{-1}.

Sample	PAR	PRO	CAF	THI
1	5.1	0.0	1.1	1.3
2	15.3	9.9	4.5	1.3
3	15.3	0.0	2.3	1.3
4	5.1	14.9	4.5	3.8
5	15.3	19.9	2.3	0.0
6	20.4	5.0	4.5	2.5
7	15.3	9.9	2.3	1.3
8	10.2	0.0	0.6	2.5
9	10.2	14.9	0.6	3.8
10	5.1	5.0	0.0	0.6
11	5.1	19.9	1.1	3.8
12	0.0	14.9	0.6	3.8
13	10.2	5.0	1.1	0.6
14	20.4	9.9	0.0	2.5
15	20.4	9.9	1.1	0.6
16	0.0	9.9	4.5	3.8
17	10.2	19.9	2.3	2.5
18	20.4	18.9	0.6	0.0
19	5.1	5.0	0.6	0.6
20	10.2	14.9	2.3	0.0
21	0.0	5.0	1.1	0.6
22	20.4	19.9	0.6	0.0

3. Results and discussion

3.1. Selection of the method

The original absorption spectra of PAR, PRO, CAF and THI were plotted in the range of 210.0–312.3 nm, as illustrated in Fig. 1A, with concentrations in the same ratio present in commercial formulations. In order to perform an optimal resolution of this quaternary mixture we have used powerful FWT technique in combination with the multivariate PCR, PLS and ANN techniques. These hybrid methods allowed the reduction of the original 1024 data into 126 FWT coefficients.

A new algorithm to generate the calibration set, proposed in a previous paper [10], was used. This experimental design allowed to obtain a set of samples characterized by ternary and quaternary mixtures, with a number of samples maintained at the lower value as possible and a frequency as similar as possible for all the concentration levels.

3.2. Fractional wavelet analysis

Fractional wavelet analysis is a generalization of the classical wavelet technique and it was introduced recently by Unser and Blu. The details on the theory about FWT and its application on drug matrices are described in a series of published articles [13–17]. FWT offers the functions of data compression and elimination of noises and fluctuations coming from instrumentation recording, providing so to extract the important information from the complex original spectra.

The FTW spectra are illustrated in Fig. 1B. FWT performed a considerable amplitude of all transformed signals as well as a relevant data reduction. The original absorption spectra from the calibration set were firstly recorded in the spectral range of 210.0–312.3 and then processed by FWT technique in the fractional domain obtaining the spectra shown in Fig. 2. Signals possessing higher amplitudes and shrinking wavelength interval were obtained from the original absorption spectra. In the new FWT graph the main

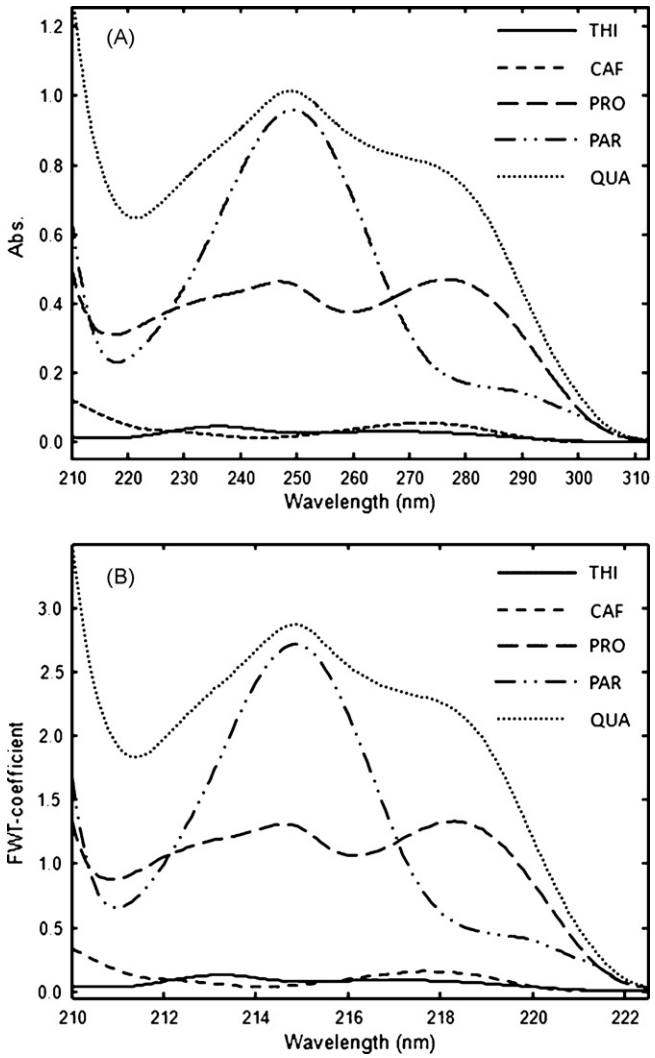

Fig. 1. (A) Absorption spectra and (B) FWT spectra of 1.26 μg ml^{-1} THI (——), 1.10 μg ml^{-1} CAF (- - - -), 9.90 μg ml PRO (— —), 10.20 μg ml^{-1} PAR (— ·· —) and their quaternary mixture (·····) in ethanol solutions.

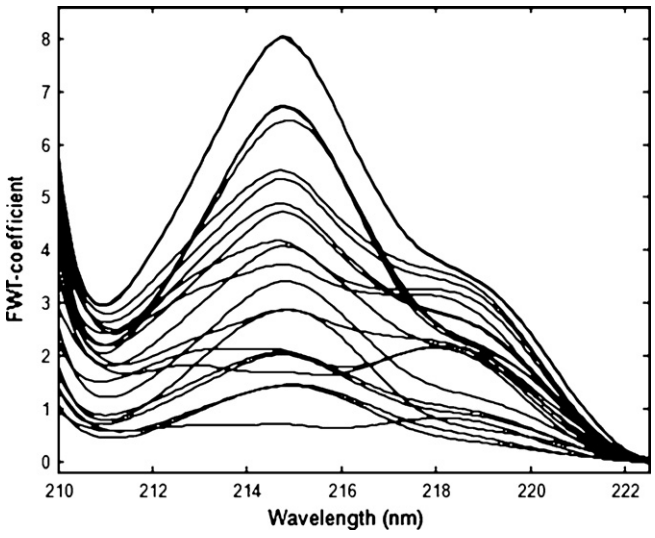

Fig. 2. FWT spectra of the reference solutions of calibration set.

peak increased from 1.0 to 2.8, while the abscissa scale decreased from the 210.0–312.3 nm wavelength range to 210.0–222.6 nm. The same treatment was applied on the spectra recorded on prediction set and commercial samples.

3.3. Multivariate techniques

The multivariate regression methods allow to define models describing a system by using a series of predictors representing the same system. In spectrophotometry, the predictors are represented by the absorbance data correlated with the concentration values. These methods are also called "factor methods" because they transform the original variables into a smaller number of orthogonal variables called factors or principal components (PCs), which are linear combinations of the original variables. This first phase is called calibration step and is followed by a prediction step in which the built model is used to estimate unknown concentrations of a mixture from its spectrum. The most used regression methods are PCR and PLS. The theory of such techniques has been fully described by several authors [22–26].

Artificial neural networks (ANN) represents a non-linear calibration model and it is among the most widely used mathematical algorithms for overcoming non-linearity [27,28]. This technique has demonstrated to be a very powerful data modeling tool able to capture and represent complex input/output relationships [29]. In the last time, ANN has demonstrated high ability in acquiring useful information from complex systems, in presence of noise or instrumental fluctuations, providing robust models.

3.4. FWT–PCR, FWT–PLS and FWT–ANN calibrations

PCR, PLS and ANN algorithms were applied to the FWT coefficients to obtain FWT–PCR, FWT–PLS and a back propagation FWT–ANN models. Calculation was performed on the reference solutions of the calibration set in the fractional wavelet domain. The input for the topological neural network consisted of 42 FWT points and 22 concentration values from the calibration set. An FWT–ANN model, which consisted of 42 neurons in input layer, 7 neurons in hidden layer and 4 neurons in output layer, was chosen as the optimal configuration.

3.5. Statistical analysis

Prediction ability of the chemometric calibrations was evaluated by the standard error of calibration (SEC), calculated from difference between nominal and predicted concentrations of all the components in the calibration set:

$$\mathrm{SEC} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{c} - c_i)^2}{n}}$$

SEC values for all compounds were summarized in Table 2. A cross-validation procedure was performed by using a different number of factors to obtain the best recovery results. An optimal number of five factors was established for both PCR and PLS calibrations. Analogously, the standard error of prediction (SEP) was calculated in the validation step and listed in Table 2. The validation process was enriched by perform a classical linear regression analysis between the nominal values versus the found values, obtaining the regression parameters slope (m), intercept (n) and correlation coefficient (r), reported in Table 2.

Table 2
Statistical parameters from FTW–PCR, FTW–PLS and FTW–ANN applied on calibration set. *m*, *n* and *r* are the regression parameters slope, intercept and correlation coefficient.

Component	Method	Calibration				Validation			
		SEC	*r*	*m*	*n*	SEP	*r*	*m*	*n*
PAR	FWT–PCR	0.0583	0.9998	1.0000	−4.41E−07	0.0459	0.9999	1.0040	−0.0167
	FWT–PLS	0.0575	1.0000	1.0000	9.00E−03	0.0513	1.0000	1.0056	−0.0506
	FWT–ANN	0.0096	0.9999	1.0008	2.21E−02	0.0478	1.0000	1.0034	−0.0623
PRO	FWT–PCR	0.2155	0.9995	1.0000	1.61E−07	0.1596	0.9996	1.0031	−0.0303
	FWT–PLS	0.1815	0.9995	1.0000	−8.00E−03	0.2131	0.9996	0.9952	0.0313
	FWT–ANN	0.1448	1.0000	0.9998	1.18E−02	0.1993	0.9999	0.9870	0.0482
CAF	FWT–PCR	0.0454	0.9998	1.0000	−9.86E−08	0.0404	0.9994	1.0091	−0.0015
	FWT–PLS	0.0392	0.9998	1.0000	−1.07E−02	0.0451	0.9997	1.0031	−0.0092
	FWT–ANN	0.0070	1.0000	1.0000	5.00E−03	0.0176	0.9998	1.0050	−0.0148
THI	FWT–PCR	0.1446	0.9994	1.0000	3.35E−07	0.0240	0.9995	1.0029	−0.0157
	FWT–PLS	0.1037	0.9991	1.0000	−1.89E−02	0.0208	0.9998	0.9982	−0.0062
	FWT–ANN	0.0862	1.0000	0.9998	2.70E−02	0.0138	0.9997	0.9990	−0.0004

3.6. Application of the models to the prediction set

An independent prediction set of synthetic mixtures prepared by adding known amount of PAR, PRO, CAF and THI was used for testing the accuracy and precision of the results obtained by the FWT–PCR, FWT–PLS and FWT–ANN calibrations. During the analytical procedures no interference and systematical error were observed. Application of the elaborated models to the prediction set gave successful results summarized in Table 3. PCR, PLS and ANN models, coupled with the FWT data treatment, presented all recovery values between 99.4% and 100.9% and precision, expressed as R.S.D. (%), between 0.60% and 2.68%.

3.7. Analysis of pharmaceutical formulations

Quantitative analysis of the commercial tablets containing PAR, PRO, CAF and THI was performed by the defined FWT–PCR, FWT–PLS and FWT–ANN models. The results for tablets determination are listed in Table 4. A good coincidence between the results from the three proposed methods was observed. The assay results obtained by applying the methods to the tablets were compared by using analysis of variance (ANOVA). The calculated *F*-values ($P = 0.05$, $n_1 = 3$ and $n_2 = 15$) were found lower than the theoretical *F*-value. The ANOVA test results were not statistically significant and the variance values differed only randomly. ANOVA test

Table 3
Results from application of FTW–PCR, FTW–PLS and FTW–ANN models on prediction set. Concentration values are expressed as μg ml^{-1}.

Sample	Nominal				Found											
					FWT–PCR				FWT–PLS				ANN			
	PAR	PRO	CAF	THI	PAR	PRO	CAF	THI	PAR	PRO	CAF	THI	PAR	PRO	CAF	THI
1	5.1	9.9	4.5	1.3	4.99	9.85	4.52	1.32	5.10	9.90	4.54	1.31	5.11	9.90	4.55	1.28
2	15.3	14.9	4.5	3.8	15.27	15.18	4.53	3.85	15.54	15.16	4.55	3.86	15.36	15.13	4.50	3.79
3	20.4	5.0	4.5	2.5	20.32	4.92	4.32	2.53	20.36	4.96	4.39	2.52	20.33	5.03	4.43	2.50
4	10.2	9.9	2.3	0.6	10.14	9.74	2.22	0.60	10.16	9.79	2.24	0.61	10.29	9.96	2.24	0.64
5	10.2	14.9	0.6	3.8	10.21	14.85	0.56	3.74	10.23	14.99	0.57	3.75	10.25	15.12	0.57	3.90
6	5.1	19.9	1.1	3.8	5.08	19.68	1.14	3.79	5.10	19.79	1.15	3.79	5.10	20.09	1.14	3.78
7	10.2	5.0	1.1	0.6	10.26	5.11	1.12	0.66	10.21	5.00	1.14	0.65	10.26	5.01	1.13	0.64
8	20.4	9.9	1.1	0.6	20.35	9.76	1.09	0.64	20.36	9.79	1.10	0.63	20.45	10.10	1.15	0.65
9	10.2	19.9	2.3	2.5	10.21	19.91	2.25	2.50	10.23	20.00	2.26	2.52	10.25	19.99	2.25	2.54
10	5.1	5.0	0.6	0.6	5.20	5.15	0.57	0.65	5.18	5.17	0.58	0.64	5.20	4.99	0.57	0.62
11	10.2	9.9	1.1	1.3	10.15	9.87	1.10	1.29	10.07	9.77	1.14	1.29	10.20	9.84	1.15	1.28

Table 4
Results obtained by applying the proposed FWT chemometric calibrations to the commercial pharmaceutical formulations. Concentrations are expressed as mg sample^{-1}.

Nominal				Found											
				FWT–PCR				FWT–PLS				FWT–ANN			
PAR	PRO	CAF	THI	PAR	PRO	CAF	THI	PAR	PRO	CAF	THI	PAR	PRO	CAF	THI
Odontalgico Dr. Knapp															
300	150	25	15	310.8	153.6	24.7	15.2	310.8	152.4	24.6	15.4	310.0	154.3	25.0	15.6
Influrem															
100	300	25	–	102.7	211.9	25.2	–	99.4	304.4	24.7	–	105.3	292.7	23.7	–
Micranet															
175	250	25	–	181.6	253.1	24.6	–	172.0	252.9	23.8	–	180.9	254.8	24.6	–
Saridon															
250	150	25	–	253.5	153.8	24.7	–	252.4	148.5	24.6	–	250.1	148.6	24.4	–
R.S.D.				1.26	2.32	1.54	2.52	0.98	1.78	2.11	1.69	071	1.52	2.84	1.31
Recovery (%)				103.63	101.89	98.32	103.56	102.07	101.54	99.54	104.62	103.36	102.90	101.23	103.61

Table 5
ANOVA test. SS, d.f. and MS are the parameters of sum of squares, degree of freedom and mean squares, respectively.

Component	Source of variation	Between groups	Within groups	Total
PAR	SS	2.46	75.42	77.88
	d.f.	2	15	17
	MS	1.23	5.03	
	F-calculated	0.24		
PRO	SS	12.09	203.19	215.28
	d.f.	2	15	17
	MS	6.04	13.55	
	F-calculated	0.45		
CAF	SS	0.44	5.99	6.43
	d.f.	2	15	17
	MS	0.22	0.40	
	F-calculated	0.55		
THI	SS	0.14	1.58	1.72
	d.f.	2	15	17
	MS	0.07	0.11	
	F-calculated	0.65		
F-tabulated		3.68	($P = 0.05$)	

indicated that there are no significant differences between the results of FWT–PCR, FWT–PLS and FWT–ANN. The results of ANOVA test are shown in Table 5.

4. Conclusions

The proposed study on a complex quaternary mixture containing PAR, PRO, CAF and THI presents its own interest because of the very closely overlapping spectra of the components and the very low content of two components in commercial formulations. Three chemometric methods, based on the combination of FWT approach with PCR, PLS and ANN methods were elaborated to solve the system. The fractional analysis changed the small amplitudes of the initial absorbance values into bigger amplitude of the transformed signals. The successful results from application of all the models to synthetic samples and pharmaceutical formulations demonstrated that these chemometric approaches were suitable to the simultaneous quantitative determination of the investigated mixture. FWT–ANN method was found above all suitable because of elimination of the non-linear effects due to the presence of both high and small level concentrations of compounds.

Being the cost, the analysis time, the accuracy and the precision of the proposed methods very good, this new hybrid approach was found suitable for both analytical and economical reasons and it seems very promising to describe the quantitative resolution of complex multicomponent mixtures.

Acknowledgement

The authors thank the Ministry of University and Research (MIUR) of Italy for financial support of this work.

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