ORIGINAL ARTICLE

Simultaneous spectrophotometric determination of overlapping spectra of paracetamol and caffeine in laboratory prepared mixtures and pharmaceutical preparations using continuous wavelet and derivative transform

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Abstract In the present paper, two spectrophotometric methods were used for the simultaneous analysis of paracetamol (PCT) and caffeine (CAF) in their laboratory prepared mixtures and pharmaceutical preparations. Simple spectrophotometric analysis of PCT and CAF is not possible due to their complete spectral overlap. The proposed methods are based on the application of continuous wavelet transform (CWT) and derivative transform (using Savitsky–Golay filters) on the ratio spectra to predict each of CAF and PCT. Several wavelet families were tested. Coif1 and Sym2 were found to give best results under optimum conditions. The transformed signals of ratio spectra were used to plot the calibration curves for both components. The predictability of the built calibrations was validated through their application on several synthetic mixtures of both drugs. The proposed methods were used for the prediction of CAF and PCT in pharmaceutical preparation. The obtained results were statistically compared to a reference HPLC method. No significant differences

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were found between the obtained results and those from the reference method. Being simple, rapid, cheap and sensitive, the proposed methods are recommended for the routine daily analysis of these two drugs in their mixtures in quality control laboratories.

1. Introduction

PCT (N-(4-hydroxyphenyl)acetamide) usually used in combination with CAF (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) for the temporary relief of pain and discomfort associated with a number of conditions.

The pharmaceutical mixture of [PCT-CAF] had been analyzed with several techniques including: HPLC (Zhang et al., 2010; Lotfy et al., 2010; Hashem, 2010; Li et al., 2009; Mo et al., 2008; Crevar et al., 2008), gas chromatography (GC) Guo et al., 2010, liquid chromatography- mass spectrometry (LC/MS) (Feng et al., 2009; Di et al., 2008), thin layer chromatography (TLC) Soponar et al., 2009 and voltametric (Sanghavi and Srivastava, 2010; Lourencao et al., 2009). The mixture was also analyzed spectrophotometrically using second derivative zero crossing point (Tavallali and Salami, 2009) and chemometrically by genetic algorithm optimized methods (GCLS and GILS) (Ozdemir et al., 2010), PLS (Mot et al., 2010) and PCR (Mot et al., 2010).

In this paper, two signal processing techniques called derivative spectrophotometry using Savitsky–Golay filters and continuous wavelet transform are proposed for the simultaneous analysis of this drug mixture without need for any preceding extraction procedures or use of a time consuming, expensive instrumental technique like HPLC.

2. Theory

2.1. Savitsky–Golay method

In higher order derivatives, polynomials can be used to approximate spectra. This aims to improve SNR. The polynomial is moved point by point along spectra. Computation is repeated each time, which is time consuming.

Savitsky and Golay (1964) presented an alternative and simplified method of determining the new value of each data point. Where, instead of calculating each time, Savitsky and Golay tabulated polynomial coefficients. The values of the coefficients depend on the number of data points to be approximated, and the order of polynomial function.

To calculate the approximated spectral values, the absorbance data in certain window widths are multiplied by the corresponding coefficients and the sum of multiplications is divided by the normalization constant. According to the polynomial order used, the level of noise removed is determined.

2.2. Wavelet transform

Wavelet transform is a very powerful recent tool in signal processing. It is similar to Fourier transform with the advantage of having many basic functions called wavelets while the basic functions in Fourier transform are the trigonometric functions (sine and cosine). A wavelet transform is the representation of a function by wavelets. A wavelet is defined as a number of scaled and dilated functions $\Psi_{a,b}(\lambda)$ derived from a basic function $\Psi(\lambda)$. Therefore the basic function is often called a mother wavelet since it gives birth to a family of wavelets.

$$\psi_{a,b} = \frac{1}{\sqrt{|a|}} \psi\left(\frac{\lambda - b}{a}\right), \quad a \neq 0 \quad a, b, \in R \tag{1}$$

Where a is the scale parameter and b is called translation parameter.

Both CWT and DWT can be used for derivative calculation. However, CWT is preferred to be used for approximate derivative calculation. This is because of two major limitations of DWT in derivative calculations. DWT cannot be used for signals with low SNR as some noise may be retained at lower decomposition levels. Also it requires the number of data points not to be small because of the 50% reduction in data points for each derivative order computation (Nie et al., 2002).

Due to the presence of large number of basic wavelets, wavelet transform provides a solution for almost all chemistry problems with one or more of its wavelets by choosing the suitable scaling parameter.

Wavelet transform had been used over the past decade in many areas like de-noising, data compression and quantitative analysis of multicomponent systems. It had been used associated with zero crossing point in some papers in pharmaceutical

Table 1 Concentrations of caffeine and paracetamol in the 25 laboratory prepared mixtures.

Sample no.	Caffeine (μg/mL)	Paracetamol (μg/mL)
1	3.2	25.6
2	3.2	20.48
3	2.56	20.48
4	2.56	30.72
5	3.84	23.04
6	2.88	30.72
7	3.84	25.6
8	3.2	23.04
9	2.88	23.04
10	2.88	28.16
11	3.52	30.72
12	3.84	28.16
13	3.52	25.6
14	3.2	30.72
15	3.84	30.72
16	3.84	20.48
17	2.56	28.16
18	3.52	20.48
19	2.56	25.6
20	3.2	28.16
21	3.52	28.16
22	3.52	23.04
23	2.88	20.48
24	2.56	23.04
25	2.88	25.6

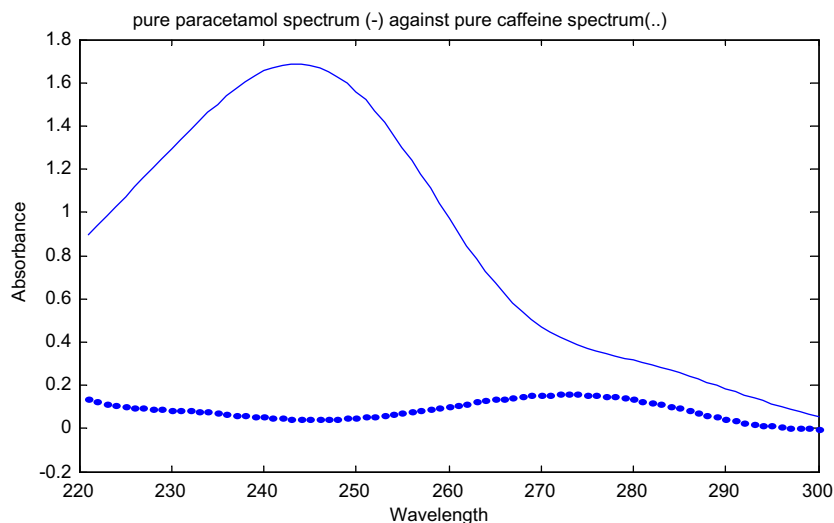pure paracetamol spectrum (-) against pure caffeine spectrum(..)

Figure 1   The pure spectra of caffeine (….) and paracetamol in solid line in their ratio in the pharmaceutical preparation.

drug mixture analysis (Sohrabi et al., 2010; Dinc and Baleanu, 2010; Dinc and Baleanu, 2009a; Dinc and Baleanu, 2010b; Zhang and Qi, 2007; Ugurlu et al., 2008; Dinc et al., 2007).

In this work, both derivative transform using Savitsky–Golay filters and wavelet transform are used to analyze paracetamol and caffeine mixture by their application on the ratio spectra. Several wavelet families were tested at different scales to obtain the best family for the analysis.

3. Experimental work

3.1. Apparatus and software

A Shimadzu UV 1800 double-beam spectrophotometer connected to a computer loaded with Shimadzu software UV probe 2.32 was used (Hiroshima, Japan). UV spectra were recorded using a 1-cm quartz cell; the scan range was
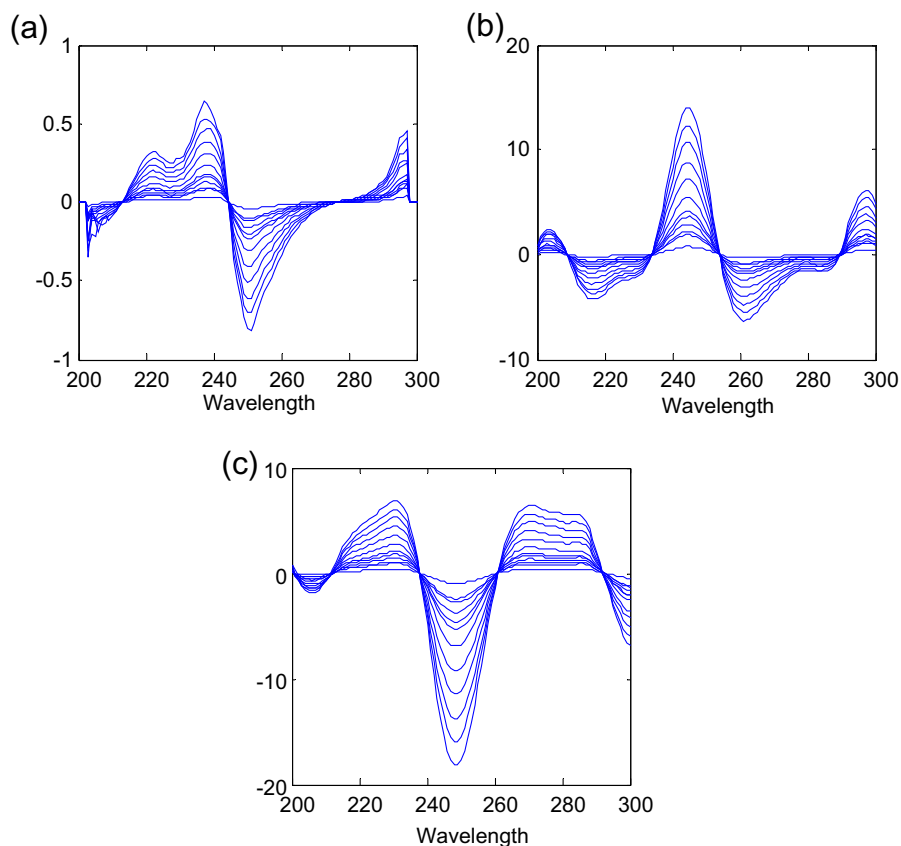
Figure 2   Plot of (a) Savitsky Golay, (b) Coif1 and (c) Sym2 application on the ratio spectra of paracetamol (2–40 μg/ml).

200–400 nm with 1 nm intervals. The computations were done using the Matlab 7.1 software, Matlab wavelet toolbox for continuous wavelet transform analysis and Microsoft Excel.

3.2. Samples and reagents

- Pure samples:
  (a) Paracetamol: was kindly supplied by October Pharma S.A.E certified to contain 99.8% according to the manufacturer method.
  (b) Caffeine: was kindly supplied by October Pharma S.A.E certified to contain 100.2% according to the manufacturer method.
  - Market samples:

Commercial pharmaceutical formulation (Prontoplus®) tablets manufactured by October Pharma S.A.E Batch No. B0510111 labeled to contain 400 mg paracetamol and 50 mg caffeine per tablet and was obtained from the local market.

- Reagents:

Hydrochloric acid was of analytical spectroscopic grade and obtained from El-Nasr Pharmaceutical Chemicals Company.

3.3. Standard solutions

3.3.1. Stock solutions
Stock standard solutions of paracetamol and caffeine were prepared by dissolving 0.1 g of each drug in 0.1 N HCl in 100 mL volumetric flask and the volume was completed to the mark by 0.1 N HCl and so obtaining 1 mg/mL stock standard solution for both components.

3.3.2. Working solutions
Working standard solutions of paracetamol and caffeine were prepared by diluting 32 mL and 4 mL of stock solutions of paracetamol and caffeine, respectively in 250 mL volumetric flask and completing to the mark with 0.1 N HCl to obtain working solutions of 128 μg/mL and 16 μg/mL of paracetamol and caffeine respectively.

Multilevel multifactor design was used for the construction of 25 binary mixture solutions. A five-level two-factor design was used in which (4, 4.5, 5, 5.5, 6 mL) of each working standard solution was mixed independently and diluted in 25 mL volumetric flasks giving rise to 25 solutions in which the concentrations of both components are changed in a mutually independent manner. The concentrations of paracetamol are ranging from 20.48–30.72 μg/mL and the concentrations of caffeine are ranging from 2.56–3.84 μg/mL. Table 1 shows the concentrations of the two components in the 25 laboratory prepared samples.
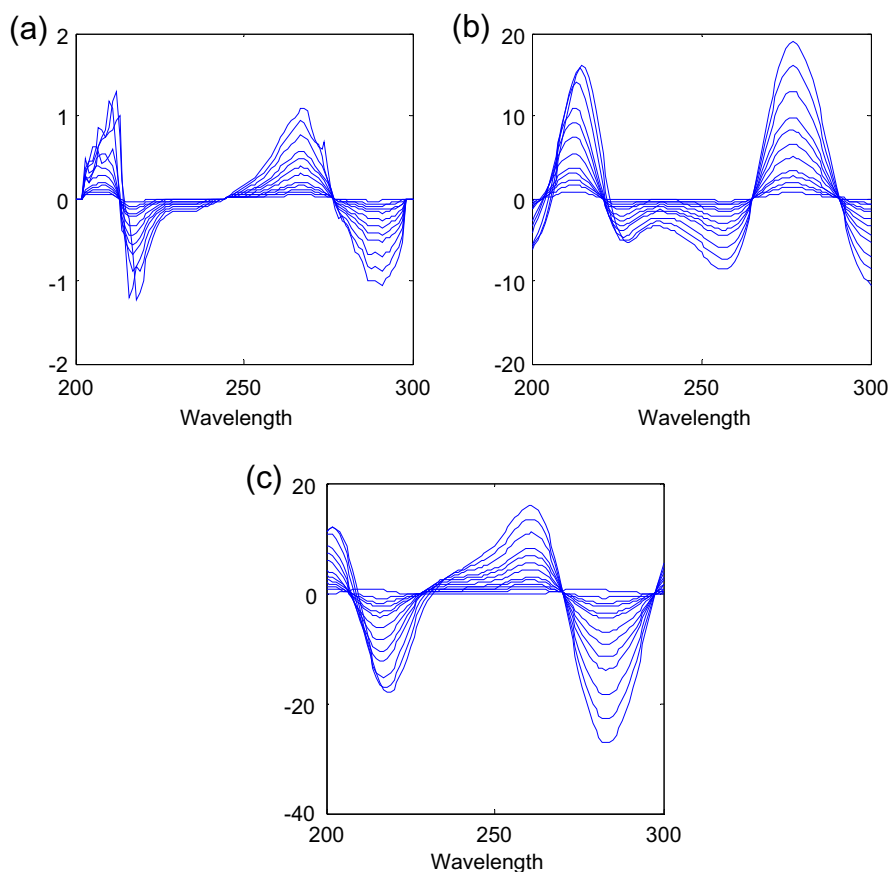

Figure 3 Plot of (a) Savitsky Golay, (b) Coif1 and (c) Sym2 application on the ratio spectra of Caffeine (2–50 μg/mL).

3.4. Pharmaceutical preparations

Ten tablets of prontoplus® were accurately weighed and powdered. An amount equivalent to 400 mg paracetamol was taken and put in contact with 30 mL of 0.1 N HCl and subjected to mechanical shaking for 30 min to ensure complete dissolution of the two active ingredients. The solution was then filtered in 100 mL volumetric flask. The residue was washed three times each with 10 mL 0.1 N HCl. The solution was then completed to 100 mL with 0.1 N HCl. 2 mL of the filtrate was diluted with 0.1 N HCl in 100 mL volumetric flask to obtain working solution. 5 mL of this solution was diluted with 0.1 N HCl in 25 mL volumetric flask and measured spectrophotometrically.

3.5. General procedure

The absorption spectra of calibration set, validation set and pharmaceutical preparation were recorded in the range 200–400 nm. The spectra were transferred to Matlab 7.1 for signal processing and analysis.

4. Results and discussions

4.1. Spectral features

The spectra in Fig. 1 show the complete spectral overlap between caffeine and paracetamol in the range 220–300 nm. This spectral overlap prevents the analysis of such mixture by simple direct spectrophotometric method.

4.2. Application of Savitsky–Golay filter on ratio spectra

The ratio spectra had been obtained by dividing the pure spectra of each component on the normalized spectrum of the other. Different window sizes were tested to obtain the optimal window size for analysis of each component using Savitsky–Golay filters on the ratio spectra. Window size of 7 points was found to give optimum results for both caffeine and paracetamol. The calibration graphs were built by measuring the signal at 250 nm and 267 nm for caffeine and paracetamol, respectively. Figs. 2a and Fig. 3a show the application of SG filters on the ratio spectra of paracetamol and caffeine, respectively. The optimal parameters (i.e. window size, slope, intercept, correlation coefficient, range of concentrations and the wavelength of analysis) are presented in (Tables 2 and 3) for paracetamol and caffeine, respectively.

4.3. Application of CWT on ratio spectra

Several wavelet families were tested to obtain the optimal wavelet families (Coiflet1 and Sym2). Different scales were tested to obtain the optimal scale values for the analysis of such mixture. For Coif1, the optimum scale value was found to be 20 ($a = 20$). Calibration graphs were built by measuring the transformed signals at 245 nm and 257 nm for paracetamol and caffeine, respectively. For Sym2, the optimum scale value was found to be 25 ($a = 25$). Calibration graphs were built by measuring the transformed signals at 249 nm and 261 nm for paracetamol and caffeine, respectively. (Figs. 2 and 3) show the application of (b) Coif1 and (c) Sym2 on the ratio spectra of paracetamol and caffeine, respectively. The optimal

Table 2 Optimal parameters for the determination of paracetamol using Savitsky–Golay filters, Coif1 and Sym2 wavelets

Paracetamol			
Parameter	Sav.Gol.	Coif1	Sym2
Wavelength	250	245	249
Window size or scale	7	20	25
Range	2–40	2–40	2–40
Slope	0.0079	0.3598	−0.4678
Intercept	0.0017	0.0733	−0.0869
Correlation coefficient	0.9998	0.9997	0.9997

Table 3 Optimal parameters for the determination of caffeine using Savitsky–Golay filters, Coif1 and Sym2 wavelets.

Caffeine			
Parameter	Sav.Gol.	Coif1	Sym2
Wavelength	267	257	261
Window size or scale	7	20	25
Range(µg/mL)	2–50	2–50	2–50
Slope	0.0199	−0.1545	0.2803
Intercept	0.0036	−0.0366	0.0654
Correlation coefficient	0.9999	0.9997	0.9997

Table 4 The percentages of recoveries, mean and standard deviation of paracetamol in laboratory prepared mixtures for SG, Coif1 and Sym2.

Paracetamol			
Sample	SG	Coif1	Sym2
1	101.90	101.83	101.86
2	99.57	100.50	100.46
3	98.67	99.62	98.53
4	101.31	101.01	101.00
5	99.92	100.53	100.54
6	101.66	101.40	101.44
7	99.28	100.10	100.12
8	99.23	100.01	100.03
9	100.13	100.86	100.86
10	100.48	100.90	100.88
11	101.24	101.21	101.22
12	100.28	100.68	100.70
13	100.34	100.76	100.80
14	100.53	100.85	100.88
15	98.63	99.76	99.76
16	99.37	100.28	100.27
17	100.34	100.56	100.57
18	99.89	100.52	100.52
19	100.37	100.66	100.70
20	100.37	100.38	100.45
21	99.71	100.59	100.53
22	98.73	99.56	99.30
23	98.59	99.14	99.02
24	98.83	99.13	99.03
25	98.93	99.35	99.26
Mean	99.93	100.41	100.35
S.D.	0.962	0.689	0.810

Table 5 The percentages of recoveries, mean and standard deviation of caffeine in laboratory prepared mixtures for SG, Coif1 and Sym2.

Caffeine			
Sample	SG	Coif1	Sym2
1	98.73	98.86	98.05
2	98.84	96.82	95.78
3	104.30	102.58	102.97
4	99.96	102.09	100.56
5	100.33	98.69	97.79
6	100.92	101.50	100.89
7	102.31	99.76	99.14
8	102.56	100.30	99.47
9	101.82	99.22	97.94
10	100.95	100.00	98.72
11	102.29	101.41	100.14
12	102.66	101.55	100.64
13	100.84	99.14	98.47
14	102.19	101.20	100.58
15	102.15	101.02	100.53
16	97.70	96.81	95.65
17	102.47	102.84	101.51
18	101.00	99.22	97.99
19	100.05	100.15	99.39
20	99.18	99.36	98.66
21	101.11	101.32	101.00
22	101.75	100.02	99.12
23	99.11	97.95	96.61
24	99.80	99.97	98.42
25	96.64	99.25	97.49
Mean	100.79	100.04	99.10
S.D.	1.768	1.587	1.778

parameters (i.e. scale value, slope, intercept, correlation coefficient, range of concentrations and the wavelength of analysis) are presented in (Tables 2 and 3) for paracetamol and caffeine, respectively.

4.4. Method validation

SG filters and the two wavelet families were applied on the ratio spectra of the laboratory prepared mixtures under the optimal conditions stated in (Tables 2 and 3). The recovery

Table 6 The percentages of recoveries, mean and standard deviation of paracetamol in the pharmaceutical preparation for SG, Coif1, Sym2 and reference method.

Paracetamol				
Sample	SG	Coif1	Sym2	Ref
1	102.51	102.21	102.73	102.16
2	98.70	99.01	99.50	100.94
3	100.93	101.20	101.72	99.41
4	99.24	99.58	100.16	100.77
Mean	100.34	100.50	101.03	100.82
S.D.	1.727	1.472	1.467	1.124
F-test (9.266)[*]	2.357	1.705	1.701	
t-test (2.447)[*]	0.461	0.346	0.224	

[*] The values in the parenthesis are the corresponding theoretical values at $P = 0.05$.

Table 7 The percentages of recoveries, mean and standard deviation of caffeine in the pharmaceutical preparation for SG, Coif1, Sym2 and reference method.

Caffeine				
Sample no.	SG	Coif1	Sym2	Ref
1	99.37	97.48	97.44	98.89
2	99.12	98.02	97.63	97.73
3	98.44	96.96	96.88	101.21
4	102.70	100.04	100.49	97.94
Mean	99.91	98.12	98.11	98.94
S.D.	1.902	1.348	1.618	1.595
F-test (9.266)[*]	1.425	1.398	1.031	
t-test (2.447)[*]	0.778	0.783	0.733	

[*] The values in the parenthesis are the corresponding theoretical values at $P = 0.05$.

percentages, mean and standard deviation are given in (Tables 4 and 5) for paracetamol and caffeine, respectively. Accuracy of analysis was good indicated by the mean being close to 100%. The method also showed good precision and repeatability indicated by standard deviation below 2.

4.5. Analysis of commercial formulation

SG filters and the two wavelet families were applied on the ratio spectra of the market samples under the optimal conditions. The recovery percentages, mean and standard deviation are given in (Tables 6 and 7) for paracetamol and caffeine, respectively. The obtained results were statistically compared to the results obtained using a reference HPLC method (Lotfy et al., 2010) using F-test and t-test at 95% confidence level ($P = 0.05$). The calculated values are below tabulated ones. No significant differences were found between the results of proposed methods and those of the reference method.

5. Conclusion

SG first derivative on the ratio spectra along with Coif1 and Sym2 wavelet transform on ratio spectra were applied for the analysis of mixtures of paracetamol and caffeine with overlapping peaks and had shown good accuracy and precision. These methods show ability to solve these mixtures without need of prior separation. The obtained results were not statistically different from the reference method results. Being simple, sensitive and fast, SG derivative ratio and CWT can be used for the analysis of this pharmaceutical mixture in routine laboratory work in quality control laboratories.

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