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Simultaneous spectrophotometric determination of cyproterone acetate and ethinyl estradiol in tablets using continuous wavelet and derivative transform

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ABSTRACT

In this study a zero crossing technique based on continuous wavelet transform (CWT) as well as classical derivative spectrophotometry (CDS) is presented for simultaneous determination of cyproterone acetate and ethinyl estradiol in binary mixtures and commercial dosage of drug, without using prior chemical pre-treatment. Absorption spectra were recorded in the wavelength range 200–400 nm. Absorbance data were subjected to various mother wavelets from continuous wavelet transform family to find the optimum point of the wavelet signal processing (Matlab 7.5) gaus 15 and morl wavelet functions with scaling factor, $a = 70$ and 3rd derivative with $\Delta\lambda = 10$ nm, were selected. Optimum value of scaling factor was chosen to obtain an appropriate calibration for each method. The validation of proposed methods was investigated by several synthetic mixtures and obtained results were successfully compared among each other. Mean recovery values were found between 96.93% and 101.7% for CWT and 95.55% and 104.22% for DS, respectively for the determination of cyproterone acetate and ethinyl estradiol in synthetic mixtures. The developed methods are rapid, precise and easy to apply for the analysis of overlapping signals of the components in the mixtures. Obtained results from the CWT were compared to those yielded by CDS which were in good agreement and therefore led to a successful determination.

1. Introduction

The combination of ethinyl estradiol (19-norpregna-1,3,5 (10)-trien-20-yne 3,17diol,(17α)) with cyproterone acetate (17-acetoxy-6-chloro-1α,2α-methylrnr 4,6-pregnadien-3,20-dion) is mainly used in hormone therapies for androgen dependent disorders, acne, hirsutism, seborrhea [1] and due to the similarities to contraceptive medicines, sometimes as an oral contraceptive for female patients with androgenic symptoms [2] . Therefore determination of the components in this drug is relatively important and frequently is an analytical problem in quality control industry. Detailed survey of literature revealed several analytical techniques for simultaneous determination of investigated drug; HPLC [3], HPLC/ion-trap mass spectrometry [4], capillary GC chromatography [5], planar chromatography [6] have been used to resolve the complex mixture of ethinyl estradiol and cyproterone acetate in pharmaceutical compounds.

The above-mentioned hyphenated methods bring high cost and time consumption and require expensive and rather complicated devices at the same time there is a need for prior separation steps during analysis. Hence the two proposed drugs in this study exhibit a very strong overlapping between their absorption spectra, direct spectrophotometry is not suitable and cannot be employed. However, the higher derivative process reduces the peak of amplitude which results in decreasing signal to noise ratio (S/N). This decrease follows from the fact that noise always contains the sharpest features in the spectrum.

Wavelet transform (WT) has been developed rapidly during last decade and was applied in various branches of chemistry owing to its efficiency and large number of basis functions which helps to overcome drawbacks such as noisy or incomplete data [7,8]. Also this method has widely been used in signal processing domain, spectral quantitative analysis, analysis of electrochemical noise data, resolving simulated overlapped spectra in various mixtures and flow injection analysis [9–18]. The basis idea of WT is representing of a function by wavelets. The wavelets are scaled and translated copies of a finite-length oscillating waveform [19]. In some respects, WT is simply an analog to Fourier transform (FT). The only difference is the basis of functions. In FT, the trigonometric (sine and cosine) functions are the basis functions, while the basis function in WT is the wavelet. Therefore, a large number of basis functions are available as compared with FT [20]. Wavelet transform contains two distinct parts, discrete and continuous. Recently, continuous Wavelet transform (CWT) plays an important role in

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signal processing technique for overlapping peak resolution and for the significant peak identification in analytical chemistry and neighborhood branches. It was also successfully applied for simultaneous spectrophotometric determination of multi-components in binary and ternary mixtures [21–30].

The main aim of this study was to apply CWT as well as CDS using zero cross-point technique for quantitative spectrophotometric multi-resolution of cyproterone acetate and ethinyl estradiol in their pharmaceutical combination without using any prior separation procedures. The developed analytical techniques were tested by analyzing synthetic mixtures consisting proposed drugs. Comparison between investigated approaches shows a good agreement between results.

2. Experimental

2.1. Materials and methods

Pharmaceutical grade cyproterone acetate and ethinyl estradiol were from Bayer Scherng Pharma, Barghemen, Germany (Alborz Bulk Pharm. Co., Tehran, Iran) and were obtained from Abouraihan Pharmaceutical Company (Tehran, Iran). Methanol (analytical grade) was purchased from ROMIL (Cambridge, England). Cyproterone compound tablets containing 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol were used in this study (Iran Hormone, Tehran, Iran).

2.2. Apparatus

Absorption spectra were recorded by Bio-TEK Kon 922 double beam UV–vis spectrophotometer with 1-cm quartz cells. Calculations and signal transforms were obtained by Microsoft Office EXCEL (Ver. 2007), MATLAB (Ver. 7.5).

2.3. Standard solutions

Stock solution of cyproterone acetate and ethinyl estradiol was prepared by dissolving 100 mg of cyproterone acetate and 100 mg of ethinyl estradiol in 100 ml methanol. Standard solutions were prepared by diluting accurate volumes of stock solutions in methanol to reach concentration ranges of 5–60 $\mu g\,ml^{-1}$ and 1–8 $\mu g\,ml^{-1}$ for cyproterone acetate and ethinyl estradiol, respectively.

2.4. Analysis of tablet formulation

For evaluation the validity of proposed methods commercial dosage of this drug was used. Ten tablets were weighted and finely powdered. Appropriate portion of powder equivalent to median mass of one tablet was weighted and dissolved in 50 ml of methanol. Sonicated for 30 min and filtered into a 100 ml volumetric flask and then adjusted to the mark with the same solvent. The general procedure was followed and the concentration of cyproterone acetate and ethinyl estradiol was calculated.

3. Wavelet transform

Wavelet transform is a theory based on signal processing and developed from the basis of Fourier transform and is expressed as a series of functions that relate to each other by simple scaling and translation.

The original WT function is known as mother wavelet [31,32] and is used to generate all basis functions. By scaling and shifting $\psi(\lambda)$ (mother wavelet) a set of functions are generated as:

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

where subspace of scale a is positive and defines the scale parameter and b can be any real number and represents translation parameter used for shifting.

The original signal can be reconstructed by suitable integration after projecting the given signal on a continuous family of frequency bands. CWT of the signal $f(t)$ is defined as:

$$cwt = \{f(t); a, b\} = \int_{-\infty}^{+\infty} f(t)\psi^*_{a,b}(t)dt = < f(t), \psi_{a,b} >, \tag{2}$$

Superscript * represents the complex conjugate and $\psi^*_{a,b}$ is a translated and scaled complex conjugated mother wavelet and $<f(t)\psi_{a,b}>$denote the inner product of function $f(t)$ on the wavelet function $\psi_{a,b}(t)$.

The wavelet ψ is considered invertible if it satisfies the admissibility condition.

$$\int_{-\infty}^{+\infty} \frac{|\psi(\omega)|}{\omega} d\omega < \infty, \tag{3}$$

Morlet is one of the mother wavelets in mathematics, which was originally formulated as a constant k_σ subtracted from a plane wave and then localized by Gaussian.

Morlet is defined as:

$$\psi(t) = c_\sigma \pi^{1/4}\, e^{-t^2/2}(e^{i\sigma t} - k_\sigma), \tag{4}$$

where $k_\sigma = e^{-1/2\sigma^2}$ and the constant c_σ is used for normalization in view of reconstruction and is defined as:

$$C_\sigma = (1 + e^{\sigma^2} - 2e^{-3/4\sigma^2})^{-1/2}, \tag{5}$$

Gaussian is built starting from Gaussian function, by taking the pth derivative of f:

$$f(x) = C_p\ e^{-x^2/2} \quad \text{or} \quad \psi(t) = \left(-\frac{2}{\sqrt{3}}\pi^{-1/4}\right) e^{(-t^2/2)}, \tag{6}$$

The integer p is the parameter of this family and C_P in the previous formula, is such that $||f(p)||^2 = 1$. Where $f(p)$ is the pth derivative of f.

4. Results and discussion

4.1. Spectral characteristics and selection of appropriate mother wavelets

As Fig. 1 shows, the spectra of cyproterone acetate and ethinyl estradiol show strong overlapping. Due to their mutual interference, the simultaneous determination of two drugs in the same sample is not possible by using classic analytical methodologies.

Application of continuous wavelet transform in combination with zero cross-point technique can perform a successful determination without any preliminary separation steps. Comparison of the spectra of artificial binary mixture of two drugs and real sample (commercial dosage) showed no interference in determination of cyproterone acetate and ethinyl estradiol, caused by excipients placed in commercial preparation. Various wavelet families with different scales were tested to find the optimal signal processing for obtaining desirable calibration graphs and reliable determination of investigated drugs.

Gaussian (gaus 15) (a = 70) and Morlet (morl) (a = 70) were selected for further analysis as optimal mother wavelets for transformation of absorption spectra which gave the highest sensitivity. Absorption spectra of standard solutions of cyproterone acetate and

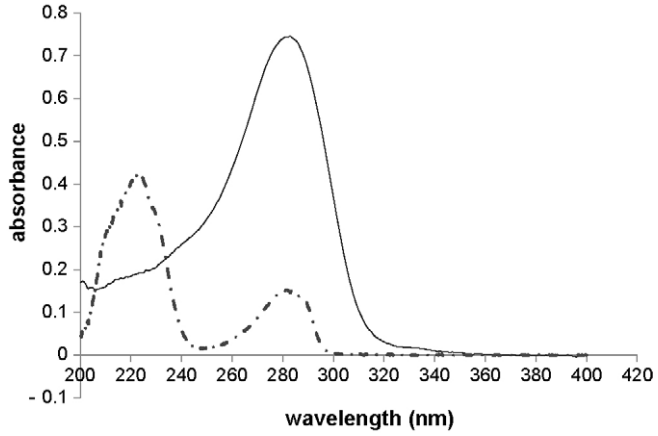

Fig. 1. The absorption spectra of ethinyl estradiol 15 μg/ml (---) and cyproterone acetate 20 μg/ml (—).

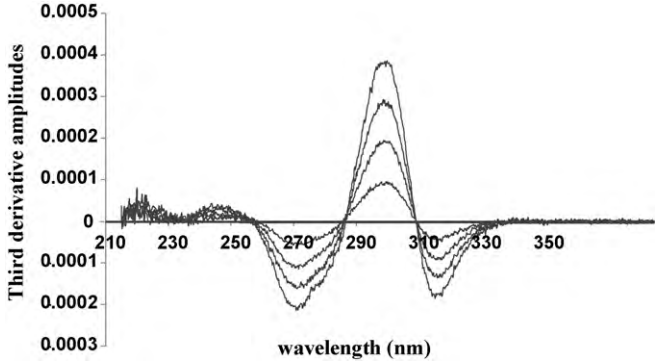

Fig. 2. (a) CWT–gaus 15 spectra of 1, 2, 4, 6, 8 μg/ml ethinyl estradiol (---) and 5, 15, 35, 45, 60 μg/ml cyproterone acetate (—); (b) CWT–morl spectra of 1, 2, 4, 6, 8 μg/ml ethinyl estradiol (---) and 5, 15, 35, 45, 60 μg/ml cyproterone acetate (—); (c) 3rd derivative spectra of 1, 2, 4, 6, 8 μg/ml ethinyl estradiol (---) and 5, 15, 35, 45, 60 μg/ml cyproterone acetate (—).

ethinyl estradiol with different concentrations were recorded in the spectral region 200–400 nm against blank solution. The absorption spectra were transferred from EXCEL to wavelet domain in MATLAB environment. The wavelength (λ) plays the role of parameter t in CWT function, then the $C_{a,b}$ can be plotted versus wavelength number. The optimum value of scaling factor for determination of the two proposed drugs in their binary mixture was found as (a = 70) for both mother wavelets. Fig. 2 shows the graphs of CWT for the calibration sets of cyproterone acetate and ethinyl estradiol which were obtained by (gaus 15) (a = 70) and (morl) (a = 70), respectively, on the absorption spectra at the same wavelength range. In this study we used zero cross-points for simultaneous determination of the drugs. So calibration graphs of each drug were obtained by plotting transformed signals versus the concentration at the zero cross-point of the other drug and vice versa. The created calibration graphs were applied for the prediction of unknown binary mixture of two proposed drugs.

Calibration graphs for cyproterone acetate and ethinyl estradiol with gaus 15 (a = 70) were obtained by measuring the CWT signals at the 257 nm and 281 nm corresponded to zero crosses of ethinyl estradiol and cyproterone acetate, respectively. For prediction of concentration of the drugs in synthetic binary mixtures the same procedure was used except that instead of spectra of standard solutions of drugs, spectra of mixture were used and concentration of each drug was estimated by CWT–gaus 15 calibration equations at zero crosses. Also transformation of calibration spectra of cyproterone acetate and ethinyl estradiol was performed by morl (a = 70). By measuring the amplitude of CWT signals at 281 nm for cyproterone acetate (corresponding to zero cross-point of ethinyl estradiol) and 213 nm for ethinyl estradiol (corresponding to zero cross-point of cyproterone acetate), the calibration graphs for proposed drugs were built. By using calibration equations cyproterone acetate and ethinyl estradiol concentrations were estimated in binary synthetic mixtures. Linear regression analysis and statistical results are shown in Table 1.

4.2. Derivative spectrophotometry

In the CDS approach, 3rd derivative of absorption spectra of ethinyl estradiol and cyproterone acetate was calculated. Linear regression functions for ethinyl estradiol and cyproterone acetate compounds were obtained by measurement of $d^3A/d\lambda^3$ values at 226 and 316 nm, corresponding to zero crossing points, respectively.

The 3rd derivative spectra of ethinyl estradiol and cyproterone acetate using interval $\Delta\lambda = 10$ are shown in Fig. 2(c). Statistical results are represented in Table 1. Obtained regression functions were applied to the simultaneous determination of ethinyl estradiol and cyproterone acetate in their samples, due to the satisfactory statistical data, proposed method found to be acceptable for quantitative evaluation of the samples.

4.3. Method validation

In order to validate the proposed methods, various mixtures containing cyproterone acetate and ethinyl estradiol were used. Mean recoveries and relative standard deviation (R.S.D.) were calculated and are reported in Table 2.

Accuracy and reproducibility were satisfactory and were evident from mean recoveries (close to 100%). Correlation coefficients, limit of detection (LOD) and limit of quantitation (LOQ) were also calculated as shown in Table 1. The good agreement between validation results indicated that CWT method based on different mother wavelets and DS is suitable and effective for estimation of binary mixtures of drugs. Another technique which was applied to investigate the validation of the methods was standard addition.

Table 1
Linear regression analysis and its statistical results.

Parameter	CWT (gaus 15)		CWT (morl)		3rd derivative	
	cyproterone acetate	ethinyl estradiol	cyproterone acetate	ethinyl estradiol	cyproterone acetate	ethinyl estradiol
λ (nm)	257	281	281	213	316	226
Range (μg/ml)	5.0–60.0	1.0–8.0	5.0–60.0	1.0–8.0	5.0–60.0	1.0–8.0
Slope (a)	0.0033	0.0034	−0.0036	0.04	−9.29E−06	2.85E−06
Intercept (b)	−0.0006	0.0288	0.0244	0.0092	6.52E−06	4.09E−07
Corr. coeff. (r)	0.9962	0.9982	0.9931	0.9949	0.998	0.9891
LOD (μg/ml)	5.57	0.07	5.9583	0.06	5.63	0.08
LOQ (μg/ml)	18.57	0.25	18.0555	0.23	18.78	0.29

r, correlation coefficient of the regression function; a, slope of the regression function; b, intercept of the regression function; SE, standard error.

Table 2
Recovery data obtained by application of the developed methods to the synthetic mixtures.

Mixtures (μg/ml)		Recoveries (%) cyproterone acetate			Recoveries (%) ethinyl estradiol		
		CWT (gaus 15)	CWT (morl)	3rd derivative	CWT (gaus 15)	CWT (morl)	3rd derivative
cyproterone Acetate	ethinyl estradiol	λ = 257	λ = 281	λ = 316	λ = 281	λ = 213	λ = 226
5	35	100.06	98.2	102.4	99.97	99.26	99.71
20	35	99.65	99.4	95.55	99.94	99.34	99.51
40	35	97	99.65	94.7	100.01	99.47	100.57
60	35	96.93	101.7	101	100	99.27	101.51
20	10	99.65	99.4	97.2	100.2	97.5	99.3
20	40	100	98.75	95.55	98.7	97.55	99.65
20	60	100.08	100.5	96.85	101.16	100.28	104.22
20	80	99.9	99.4	96.65	98.9	98.15	99
Mean		99.16	99.625	97.49	99.86	98.85	100.43
R.S.D.		1.3758	1.0744	2.82	0.7695	1.0128	1.72

R.S.D.: relative standard deviation.

Table 3
Determination results obtained by applying proposed methods to the real sample.

Method	cyproterone acetate			ethinyl estradiol		
	CWT (gaus 15)	CWT (morl)	3rd derivative	CWT (gaus 15)	CWT (morl)	3rd derivative
λ	257	281	316	281	213	226
Mean	1.9633	1.9883	1.9733	0.0343	0.03435	0.0346
Standard error	0.0033	0.0083	0.0133	0.0001	0.00007	0.0002
Standard deviation	0.0058	0.0144	0.0231	0.0002	0.00013	0.0003
Confidence	0.0143	0.0358	0.0574	0.0005	0.00033	0.0007
Level (95.0%)						
R.S.D.	0.2941	0.7259	1.1706	0.6149	0.38512	0.867

Table 4
The ANOVA results by applying three methods to the real samples.

	Source of variation	SS	df	MS	*F*	*F* crit
cyproterone acetate	Between groups	9.50E−04	2	4.70E−04	1.8387	5.1432
	Within groups	1.50E−03	6	2.50E−04		
	Total	2.50E−03	8			
ethinyl estradiol	Between groups	1.22E−07	2	6.09E−08	1.2004	5.1432
	Within groups	3.04E−07	6	5.07E−08		
	Total	4.26E−07	8			

Table 5
Recovery results obtained from the standard addition technique by application of the proposed methods.

Method	cyproterone acetate			ethinyl estradiol		
	CWT (gaus 15)	CWT (morl)	3rd derivative	CWT (gaus 15)	CWT (morl)	3rd derivative
λ	257	281	316	281	213	226
Mean	98.54	99.56	99.1	99.95	98.54	100.53
R.S.D.	1.29	1.08	1.97	0.87	1.23	1.86

Pure components of the drug were mixed up with the commercial form. Prepared solutions were analyzed and results are shown in Table 5. According to the results proposed methods have no interference or systematic error with the determination.

4.4. Commercial sample analysis

To demonstrate the applicability of the proposed method, commercial cyproterone compound tablet produced by Iran Hormone Pharmaceutical Co. was analyzed using gaus 15 and morl transforms and also CDS. Experimental results are summarized in Table 3. Obtained results of both families were compared with each other and with the DS data using one-way ANOVA test, the calculated *F*-values were less than the tabulated *F*-values which confirms no significant errors were observed in determination of drugs by approached methods at 95% confidence level. No interference was observed from the sample matrix or excipients in the tablets, and the results were in a good agreement with the labeled content (Table 4). In these statistical tests CDS was used as a reference method.

5. Conclusion

Despite the strong spectral overlapping, zero cross-point technique based on CWT and DS was successfully applied for analysis of overlapping absorption spectra of ethinyl estradiol and cyproterone acetate in pharmaceutical formulation. The methods were validated by quantitative determination of components in different complex mixtures. Obtained results were satisfactory for simultaneous determination of binary mixture of investigated drugs. The proposed methods offer several advantages over the common procedure including simplicity, sensitivity, being precise, speci-

ficity without the need of any time consuming pre-treatment steps. Furthermore they are easy to understand and easy in application assessment. High amplitude in CWT method improves the sensitivity and having several zero cross-points can be used to eliminate probable interferences and diminishing the noise. Reliable and satisfactory results suggest that CWT can be a suitable tool for quality control and routine analysis of binary mixtures and various commercial products [24–26].

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