APPLICATION OF CONTINOUS WAVELET TRANSFORM FOR DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF BINARY MIXTURE IN PHARMACEUTICAL DOSAGE FORM

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Derivative calculation is a powerful technique used in analytical chemistry to resolve spectra, sharpen peaks and carry out quantitative analysis. Three different derivative calculation methods have been used in this paper for the simultaneous determination of ambroxol hydrochloride (AMB) and doxycycline (DOX) in their binary mixture. These methods are numerical differentiation (ND), Savitzky–Golay (SG) and continuous wavelet transform methods (CWT). In these methods, calibration curves were linear in the range of 6-40 μg ml $^{-1}$ for AMB and 4-32 μg ml $^{-1}$ for DOX (r in all methods not less than 0.9996). The measurements were carried out at 245, 246, 247 and 259 nm for AMB and 372, 373 and 376 nm for DOX. The proposed three methods are rapid, simple, sensitive and accurate. No preliminary separation steps or resolution equations are required; thus they can be applied for the simultaneous determination of AMB and DOX in ambrodoxy capsules in quality control laboratories.

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1. Introduction

Ambroxol hydrochloride (AMB, figure 1) chemically is 4-[[(2-Amino-3,5-dibromophenyl) methyl]amino]cyclohexanol hydrochloride, is a metabolite of bromhexine and used as a mucolytic agent in treatment of respiratory disorders associated with viscid or excessive [1].

Doxycycline hyclate (DOX, figure 1) is alpha-6-deoxy-5-hydroxytetracycline hydrochloride hemiethanolate hemihydrate, is a tetracycline with bacteriostatic properties against a broad spectrum of bacteria, and also some antiprotozoal properties. It is used in the treatment of chlamydial, rickettsial, mycoplasmal and some spirochaetal infections, as well as in infections due to Gram-positive and Gram-negative pathogens. It has been given long term in the management of moderate to severe acne and has been used for the prophylaxis of malaria [1]. AMB and DOX were determined simultaneously in dosage form by reversed-phase sequential injection chromatography(SIC) technique[2] , HPLC method [3], derivative ratio spectrophotometric method [3] and chemometric methods (CLS, PCR and PLS methods) [3]. Both drugs are formulated together in the local Egyptian market in the form of capsules for the treatment of infections caused by susceptible strains of pathogens in acute and chronic diseases of upper and / or lower respiratory tract concomitant with formation of viscous and hardly separated expectoration [3].

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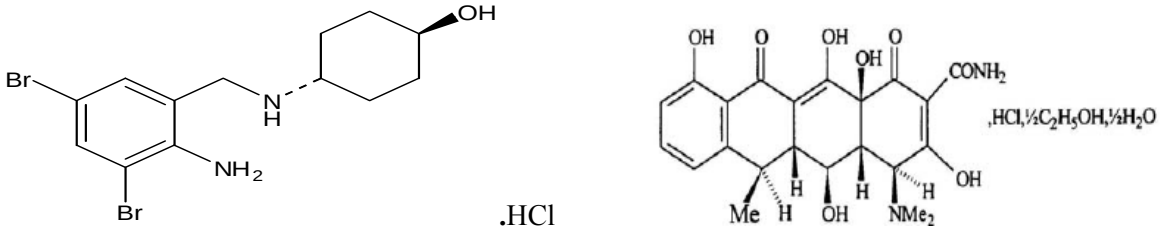
Derivative calculation is a powerful technique used in analytical chemistry to resolve spectra, sharpen peaks, determine potentiometric titration end-points, carry out quantitative analysis and perform similar procedures. There are several methods for derivative calculation presented by El-sayed et al [4]. In this paper, we present the application of three different derivative calculation methods for the simultaneous determination of AMB and DOX in their binary mixture. These methods are numerical differentiation (ND), Savitzky–Golay (SG) and continuous wavelet transform methods (CWT). ND is the simplest method of derivative calculation. This method has a major drawback in increasing the noise level in higher-order derivative calculation. In order to improve the signal to noise ratio (SNR) of higher order derivative calculation, noise reduction is usually performed before calculating the successive order derivative[5] SG method [6] was presented as an alternative and simplified method of derivative calculation depending on calculating polynomial coefficients. Wavelet Transform (WT) is one of the most important methods used for derivative calculation. In practice, both continuous wavelet transform (CWT) and discrete wavelet transform (DWT) can be used for derivative calculation. However, it is preferred to use CWT for derivative calculation and resolution enhancement while DWT is used for data compression and de-noising due to the limitations of DWT in derivative calculation. DWT cannot be used for signals with low SNR since at lower decomposition levels some noise may be retained. Also it requires the number of data not to be small as 50% reduction in data points for each derivative order computation [7]. In this paper, a comparative study between the conventional numerical differentiation (ND), Savitsky-Golay (SG) and continuous wavelet transform (CWT) methods for derivative calculation is done using a pharmaceutical binary mixture of AMB and DOX.



Ambroxol HCl (AMB) Doxycycline (DOX)

Fig. 1. Chemical structures of Ambroxol HCl (AMB) and Doxycycline (DOX)

2. Experimental

2.1. Apparatus

A dual-beam Shimadzu UV-visible spectrophotometer 1601 PC connected to an IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 200-400 nm and computations were done using Matlab 7 software and wavelet toolbox.

2.2. Reagents and chemicals

Methanol: spectroscopic grade (Merck).

Reference AMB and DOX certified to contain 99.69% and 99.79 respectively by the manufacturer purity methods were kindly provided by Adwia Pharmaceuticals and Chemical Industries Company, Cairo, Egypt. Ambrodoxy capsules (batch number 060643) were purchased from local market. Each capsule is claimed to contain 75 mg of AMB and 100 mg of DOX.

Ambrodoxy capsules are manufactured by ADWIA Pharmaceuticals and Chemical Industries Company, Cairo, Egypt.

2.3. Standard stock and working solutions

a. AMB standard stock solution; 1mg ml $^{-1}$ in methanol
a.1. AMB working solution; 0.1 mg ml $^{-1}$ in methanol
b. DOX standard stock solution; 1mg ml $^{-1}$ in methanol
b.1. DOX working solution; 0.1 mg ml $^{-1}$ in methanol

2.4. Procedure:

2.4.1. Spectral characteristics of AMB and DOX

Transfer separately aliquots equivalent to 300 µg of AMB and DOX from their stock solutions (1mg.ml^{-1}) into two 25-ml volumetric flasks then complete to volume with methanol. Record the zero order spectra of the prepared solutions from 200 to 400 nm. (figure 2)

2.4.2. Linearity

Transfer accurately measured portions of each of AMB and DOX solutions equivalent to 6-40 µg ml $^{-1}$ and 4-32 µg ml $^{-1}$ respectively into a series of 25-ml volumetric flasks then complete to volume with methanol. Measure the peak amplitude of first, second, third and fourth derivative spectra obtained by ND, SG and CWT methods at selected wavelengths to the corresponding concentrations of AMB or DOX as shown in table (1). Construct the calibration curves relating the peak amplitude of first, second, third and fourth derivative spectra obtained by the three methods at selected wavelengths to the corresponding concentrations of AMB or DOX. Compute the regression equations. The calibration equation data are listed in table (2).

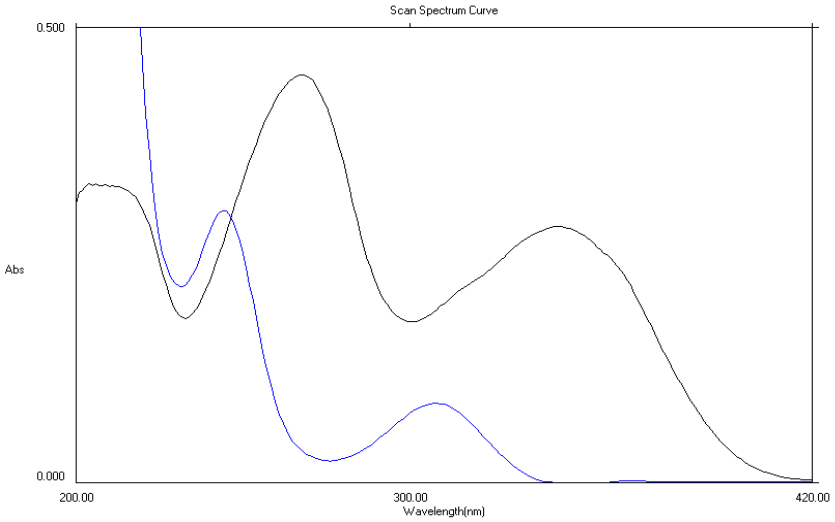


Fig. 2. Zero order absorption spectra of 12 µg ml^{-1} of AMB (——) and 12 µg ml^{-1} of DOX (—) using methanol as a solvent.

Table 1: Parameters of different derivative calculation methods

Derivative order	AMB				DOX			
	Δλ, j or a	λ (nm)	r (correlation coefficient)	F (scaling factor)	Δλ, j or a	λ (nm)	r (correlation coefficient)	F (scaling factor)
First derivative								
ND	-----	------	----------	--------	9	373	0.9998	100
SG	-----	-----	----------	---------	17	376	0.9999	100
CWT	-----	-----	----------	---------	25	372	1	-------
Second derivative								
ND	9	247	0.9999	100	-----	-----	----------	---------
SG	17	246	0.9998	100	-----	-----	----------	---------
CWT	15	246	0.9999	------	-----	-----	----------	---------
Third derivative								
ND	-----	-----	----------	---------	-----	-----	----------	---------
SG	-----	-----	----------	---------	-----	-----	----------	---------
CWT	-----	-----	----------	---------	34	372	0.9996	------
Fourth derivative								
ND	13	259	0.9999	1000	-----	-----	----------	---------
SG	35	259	0.9999	1000	-----	-----	----------	---------
CWT	21	245	0.9998	-----	-----	-----	----------	---------

Table 2: Calibration equations data of derivative spectra.

Derivative order	AMB		DOX	
	Slope	Intercept	Slope	Intercept
First derivative				
ND	----------	----------	0.0598	0.022
SG	----------	----------	0.0617	0.0073
CWT	----------	----------	0.0435	0.043
Second derivative				
ND	0.0252	-0.0027	----------	----------
SG	0.0106	0.0009	----------	----------
CWT	0.0501	0.006	----------	----------
Third derivative				
ND	----------	----------	----------	----------
SG	----------	----------	----------	----------
CWT	----------	----------	0.0346	0.0113
Fourth derivative				
ND	0.0106	0.0009	----------	----------
SG	0.003	-0.0002	----------	----------
CWT	0.418	0.254	----------	----------

2.4.3. Application of the proposed procedures for the simultaneous determination of AMB and DOX in laboratory prepared mixture

Into a series of 25-ml volumetric flasks, transfer accurately aliquots equivalent to (200-500μg) of AMB and DOX from their working solutions (0.1 mg ml^{-1}) to prepare mixtures

containing different ratios of AMB and DOX as shown in table (3), and then complete to volume with methanol. Proceed as detailed under "Linearity". Calculate the concentrations of DOX and AMB from their corresponding regression equations. The results obtained are shown in table (3).

2.4.4. Application of the proposed procedures for the determination of AMB and DOX in Ambrodoxy capsules

Weigh accurately the content of 10 capsules. Weigh an amount of the powder equivalent to 75 mg AMB and 100 mg DOX into a 250 ml beaker, and then add 50 ml methanol. Stir for 20 minutes using a magnetic stirrer then filter into a 100 ml volumetric flask. Wash the residue three times each with 10 ml methanol and complete to volume with methanol. Make further dilution by taking 10 ml of the above solution in 100 ml volumetric flask.

Table 3: Determination of AMB (a) and DOX (b) in laboratory prepared mixtures by the proposed methods.

a. AMB:

Concentration ($\mu g.ml^{-1}$)		ND		SG		CWT	
		Second derivative	Fourth derivative	Second derivative	Fourth derivative	Second derivative	Fourth derivative
AMB	DOX	R % AMB	R % AMB	R % AMB	R % AMB	R % AMB	R % AMB
12	16	101.76	100.71	101.65	105.55	101.26	102.38
16	12	100.98	99.12	101.03	102.23	100.10	100.81
16	16	100.98	99.12	101.21	102.79	99.77	100.42
12	20	101.55	99.14	100.87	103.99	100.56	101.22
20	12	99.92	98.63	99.74	101.67	99.27	99.58
8	16	101.18	96.82	102.27	103.71	98.26	98.38
16	8	99.32	98.53	99.16	101.06	99.08	99.38
Mean ± S.D.		100.81 ± 0.875	98.86 ± 1.165	100.85 ± 0.846	103.00 ± 1.492	99.76 ± 0.997	100.46 ± 1.381

b.DOX:

Concentration ($\mu g.ml^{-1}$)		ND	SG	CWT	
		First derivative	First derivative	First derivative	Third derivative
AMB	DOX	R % DOX	R % DOX	R % DOX	R % DOX
12	16	102.29	101.60	100.59	98.75
16	12	101.30	100.89	99.87	99.05
16	16	100.59	99.67	99.30	98.20
12	20	99.32	98.86	98.50	98.43
20	12	99.04	97.70	98.11	97.70
8	16	99.53	99.44	98.50	98.57
16	8	98.90	98.68	98.01	99.30
Mean ± S.D.		100.14 ± 1.288	99.55 ± 1.334	98.98 ± 0.968	98.57 ± 0.534

Transfer accurately 3 ml of this solution into a 25-ml volumetric flask, complete to volume with methanol and continue as under "Linearity". Calculate the concentrations of DOX and AMB from their corresponding regression equations. The results obtained are shown in table (4).

3. Results and discussion

3.1. Spectral features

The overlap shown in figure (2) prevents direct determination of AMB and DOX in their binary mixture by ordinary spectrophotometry. This mixture can be resolved by derivative spectrophotometry.

Table 4: Applying standard addition technique for determination of AMB (a) and DOX (b) in Ambrodoxy capsules (Batch No. 060643) by the proposed methods.

a.AMB:

Sample No.	Authentic added µg ml $^{-1}$		ND		SG		CWT	
	AMB	DOX	Second derivative	Fourth derivative	Second derivative	Fourth derivative	Second derivative	Fourth derivative
			R % AMB	R % AMB	R % AMB	R % AMB	R % AMB	R % AMB
1	6	8	99.62	99.69	98.94	97.92	99.00	100.65
2	8	12	101.30	100.71	100.57	98.63	99.75	100.93
3	12	16	101.90	99.37	98.26	96.08	98.67	99.25
Mean ± R.S.D.			100.94± 1.184	99.92± 0.699	99.26± 1.187	97.54± 1.312	99.14± 0.555	100.27± 0.900
Found of AMB in Ambrodoxy capsules * (%± S.D.)			98.54± 2.036	96.78± 0.535	100.51± 1.883	98.67± 0.871	100.70± 1.411	99.98± 1.653

* The average of 5 experiments

b. DOX:

Sample No.	Authentic added µg ml $^{-1}$		ND	SG	CWT	
	AMB	DOX	First derivative	First derivative	Firstderivative	Third derivative
			R % DOX	R % DOX	R % DOX	R % DOX
1	6	8	99.04	99.00	99.00	100.10
2	8	12	101.25	98.94	99.75	100.13
3	12	16	97.45	97.56	98.67	99.19
Mean ± R.S.D.			99.25.94± 1.911	98.50± 0.815	99.14± 0.555	99.80± 0.534
Found of DOX in Ambrodoxy capsules * (%± S.D.)			102.94± 0.663	101.481± 1.638	102.99± 0.867	99.98± 1.653

* The average of 5 experiments

3.2. Derivative calculation

3.2. a. Numerical Differentiation method (ND)

ND is the simplest method of derivative calculation. The major problem is the choice of $\Delta\lambda$. Wide $\Delta\lambda$ values can be used to suppress noise and increase SNR, but they degrade resolution as well. Finding the optimum $\Delta\lambda$ to compromise between the high resolution and good SNR is considered a challenge. Also the values obtained by ND are very small, so they are usually multiplied by scaling factor (F) to be considerable. Upon examining the first derivative spectra of the two drugs

(figure 3), it was noticed that DOX could be determined at 373 nm where AMB shows zero crossing while AMB could be determined by second and fourth derivative calculation at 247 and 259 nm respectively, where DOX shows zero crossing(figures 4 and 5). Linearity of the peak amplitudes of the first, second and fourth derivative curves to the corresponding concentrations of DOX and AMB respectively was examined at the selected wavelength. The calibration equation data are listed in table (2). The correlation coefficient (r), Δ λ, wavelengths used and F values are listed in table (1).In order to validate the above method, the former signal analyzing procedures were applied to the determination of both drugs in laboratory prepared mixtures. The mean recoveries and standard deviation (S.D.) are summarized in table (3).

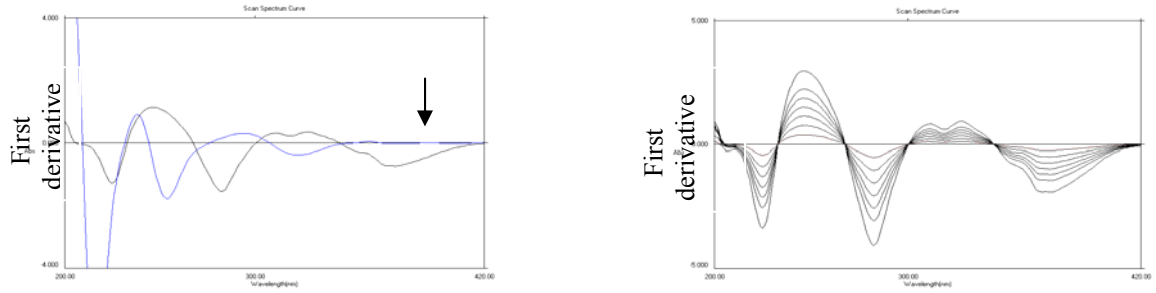


Fig. 3. First derivative absorption spectra of (a) 12 µg ml^{-1} of AMB (——)and 12 µg ml^{-1} of DOX (—) and (b) different concentrations of DOX using ND method showing zero-crossing point.

3.2. b. Savitzky-Golay method (SG)

Another method for derivative calculation is SG method. As ND, SG parameters should be optimized. These parameters include window width (j), scaling factor (F). DOX can be determined by first derivative calculation and measuring the peak amplitude at 376 nm (figure 6), while AMB can be determined by second and fourth derivative calculation and measuring the peak amplitude at 246 and 259 nm respectively (figures 7 and 8). These points showed zero crossing for AMB and DOX respectively. Linearity of the peak amplitudes of the first, second and fourth derivative curves to the corresponding concentrations of DOX and AMB respectively was examined at the selected wavelength and the calibration equation data are listed in table (2).

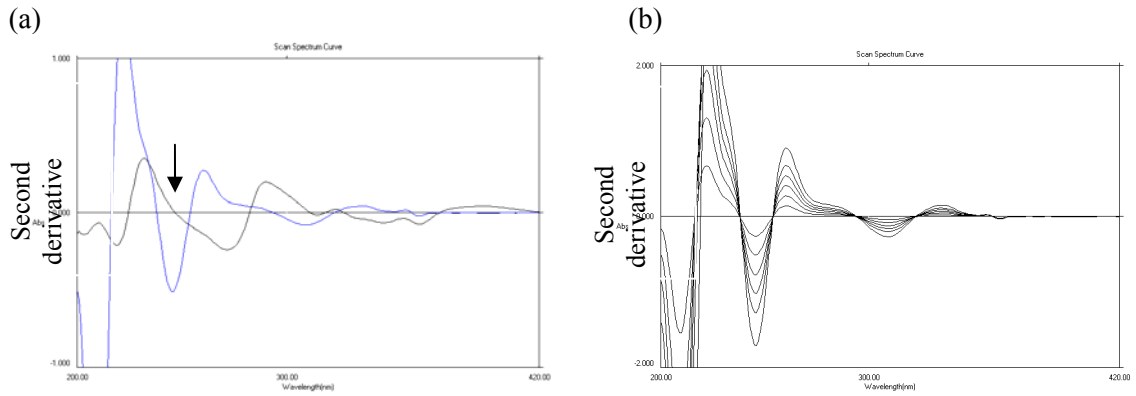


Fig. 4. Second derivative absorption spectra of (a)12 µg ml^{-1} of AMB (——)and 12 µg ml^{-1} of DOX (—) and (b) different concentrations of AMB using ND method showing zero-crossing point

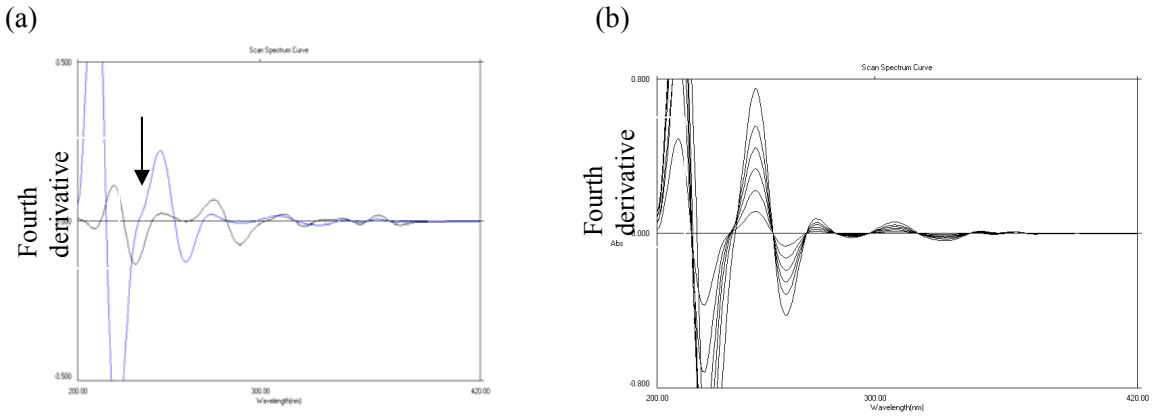


Fig. 5: Fourth derivative absorption spectra of (a) 12 µg ml^{-1} of AMB (——) and 12 µg ml^{-1} of DOX (—) and (b) different concentrations of AMB using ND method showing zero-crossing point

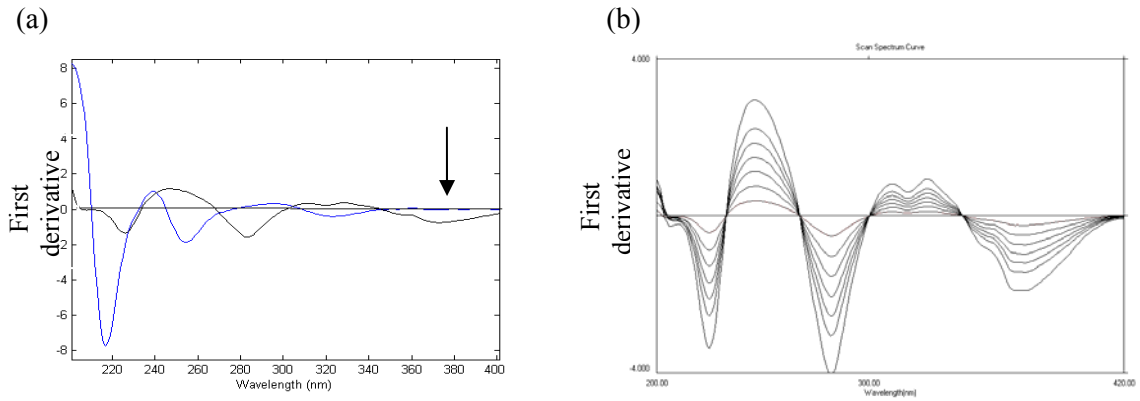


Fig. 6: First derivative absorption spectra of (a)12 µg ml^{-1} of AMB (——)and 12 µg ml^{-1}of DOX (—) and (b) different concentrations of DOX using SG method showing zero-crossing point

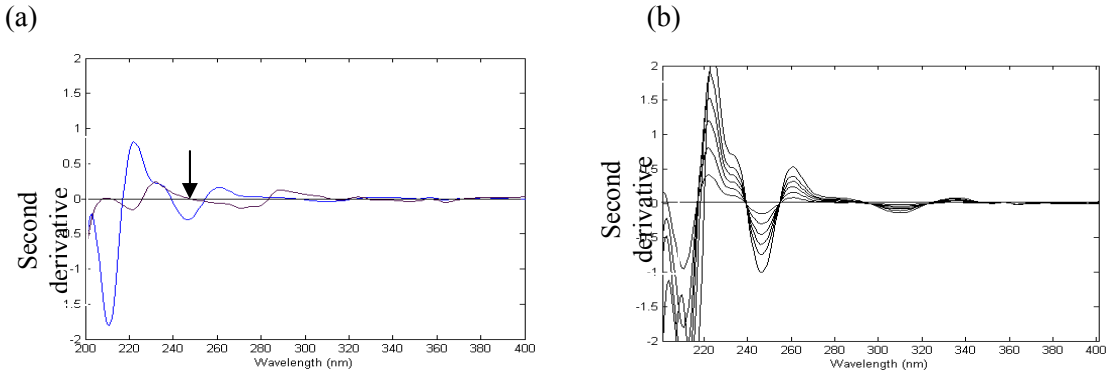


Fig. 7: Second derivative absorption spectra of (a)12 µg ml^{-1} of AMB (——)and 12 µg ml^{-1} of DOX (—) and (b) different concentrations of AMB using SG method showing zero-crossing point

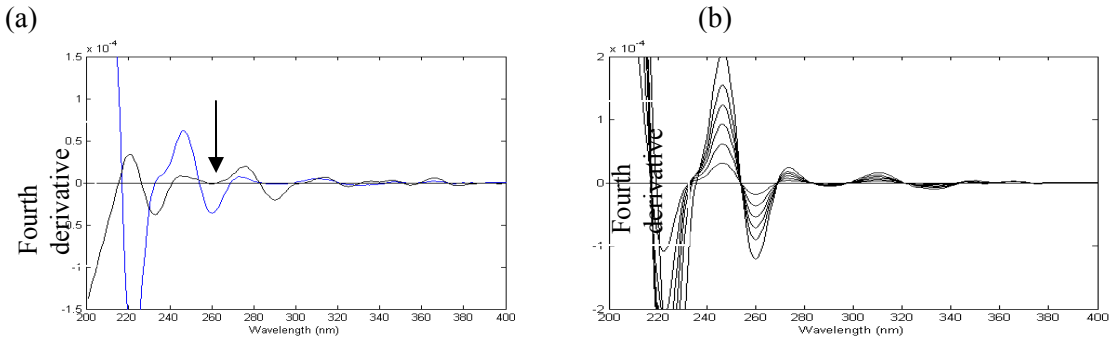


Fig. 8: Fourth derivative absorption spectra of (a)12 µg ml^{-1} of AMB (——)and 12 µg ml^{-1} of DOX (—) and (b) different concentrations of AMB using SG method showing zero-crossing point

The correlation coefficient (r), window width (j), wavelengths used and F values are listed in table (1). In order to validate the above method, the former signal analyzing procedures were applied to the determination of both drugs in laboratory prepared mixtures. The mean recoveries and standard deviation (S.D.) are summarized in table (3).

3.2.c. Continuous wavelet transform method (CWT)

CWT method is an important signal processing technique for the overlapping peak resolution and for the significant peak identification[5] .Haar wavelet function was chosen for derivative calculation due to its simplicity and its symmetric property. The only parameter that should be optimized after choosing Haar wavelet is the scaling parameter (a). No need for scaling factor (F) as CWT has the advantage of signal amplification compared to ND and SG methods as shown in the calibration curves slopes in table (2). Upon examining different derivative spectra of the two drugs, it was noticed that DOX could be determined by first and third derivative at same wavelength (373 nm) where AMB shows zero crossing (figures 9 and 10) while AMB could be determined by second and fourth derivative calculation at 246 and 245 nm respectively, where DOX shows zero crossing (figures 11 and 12). Linearity of the peak amplitudes of different derivative curves to the corresponding concentrations of DOX or AMB was examined at the selected wavelength and the calibration equation data are listed in table (2).

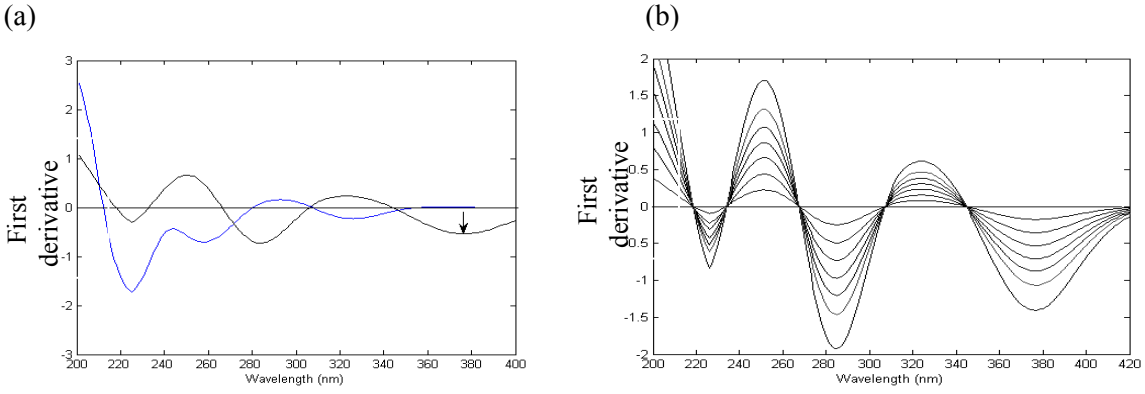


Fig. 9: First derivative absorption spectra of (a)12 µg ml^{-1} of AMB (——)and 12 µg ml^{-1} of DOX (—) and (b) different concentrations of DOX using CWT method showing zero-crossing point

(a) (b)

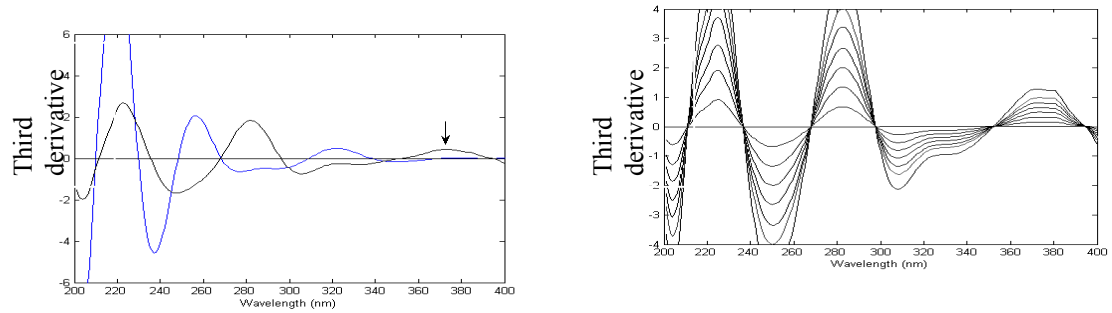


Fig. 10: Third derivative absorption spectra of (a) 12 µg ml−1 of AMB (——)and 12 µg ml−1 of DOX (—) and (b) different concentrations of DOX using CWT method showing zero-crossing point

(a) (b)

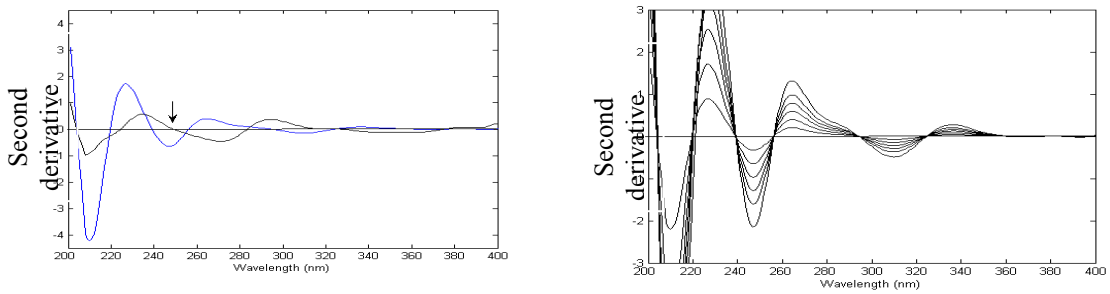


Fig. 11: Second derivative absorption spectra of (a) 12 µg ml^{-1} of AMB (——) and 12 µg ml^{-1} of DOX (—) and (b) different concentrations of AMB using CWT method showing zero-crossing point

(a) (b)

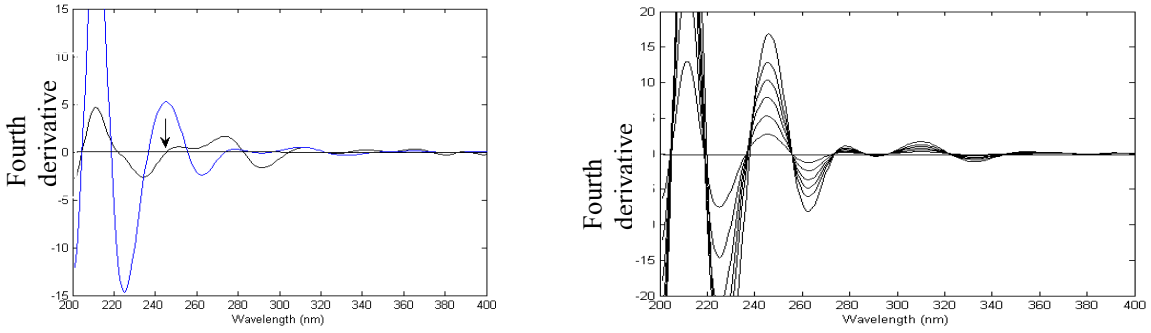


Fig. 12: Fourth derivative absorption spectra of (a) 12 µg ml−1 of AMB (——) and 12 µg ml−1 of DOX (—) and (b) different concentrations of AMB using CWT method showing zero-crossing point

The correlation coefficient (r), scaling parameter (a) and wavelengths used are listed in table (1). This CWT approach was confirmed by analyzing the synthetic mixtures containing the analyzed two active compounds. The mean recoveries and standard deviation (S.D.) are summarized in table (3). The proposed methods were successfully applied for the determination of AMB and DOX in Ambrodoxy capsules. The results are shown in table (4). The validity of the proposed methods was assessed by applying the standard addition technique as shown in table (4). The results obtained for the analysis of AMB and DOX in the pure powdered form by the

proposed methods were statistically compared with those obtained by applying the reported method [3] and no significant difference was obtained as shown in table (5).

4. Conclusion

The proposed methods are simple, sensitive, selective and can be used for the routine analysis of AMB and DOX either in the pure powdered form or their available pharmaceutical dosage forms. Moreover, CWT method gives us many advantages over ND and SG methods as peak amplification, more zero-crossing points and preservation of SNR at higher order derivative calculation

Table 5: Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of AMB (a) and DOX (b) in pure powdered form

Item	ND		SG		CWT		Reported method*
	Second	Fourth	Second	Fourth	Second	Fourth	
Mean	100.09	100.29	100.27	100.43	100.23	99.65	99.98
S.D.	0.964	1.116	0.733	0.615	0.743	1.368	1.191
Variance	0.929	1.246	0.538	0.378	0.553	1.873	1.419
n	6	6	6	6	6	6	7
F test	1.528 (4.95)	1.139 (4.95)	2.639 (4.95)	3.753 (4.95)	2.568 (4.95)	1.319 (5.05)	
Student's t test	0.850 (2.201)	0.631 (2.201)	0.599 (2.201)	0.403 (2.201)	0.651 (2.201)	0.665 (2.201)	

a.AMB

The figures in parenthesis are the corresponding tabulated values at P=0.05[8] .

* PLS method [3].

b.DOX

Item	ND	SG	CWT		Reported method*
	First	First	First	Third	
Mean	101.27	99.76	99.78	100.40	99.96
S.D.	1.078	1.1697	0.933	1.450	1.222
Variance	1.163	1.368	0.871	2.104	1.493
n	7	7	7	7	7
F test	1.330(4.28)	1.131(4.28)	1.775(4.28)	1.360(4.28)	
Student's t test	0.066 (2.179)	0.774 (2.179)	0.777 (2.179)	0.571 (2.179)	

The figures in parenthesis are the corresponding tabulated values at P=0.05 [8].

* PLS method [3].

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