Department of Analytical Chemistry[1], Faculty of Pharmacy, Ankara University, Tandoğan, Ankara; Department of Pharmacology and Toxicology[2], Faculty of Veterinary, University of Erciyes, Kayseri; Department of Mathematics and Computer Sciences[3], Faculty of Arts and Sciences, Çankaya University, Ankara, Turkey; National Institute for Laser, Plasma and Radiation Physics[4], Institute of Space Sciences, Magurele – Bucharest, Romania

Comparative application of wavelet approaches to absorption and ratio spectra for the simultaneous determination of diminazene aceturate and phenazone in veterinary granules for injection

E. Dinç[1], M. Kanbur[2], D. Baleanu[3,4]

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Assoc. Prof. Dr. Erdal Dinc, Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, TR-06100 Tandoğan, Ankara, Turkey
dinc@pharmacy.ankara.edu.tr

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A comparison of two wavelet approaches, Daubechies and reverse Biorthogonal, is described for the quantitative resolution of a binary mixture of diminazene aceturate (DIMA) and phenazone (PHE) in veterinary granules for injection without any chemical separation. These two approaches were specified as db4 (a = 180) and rbior3.7 (a = 125) respectively, after testing the signal analysis parameters for the overlapping absorption spectra and ratio spectra. In the first step db4 (a = 180) was applied to the original absorbance data vector of DIMA and PHE. In the second step rbio3.7 (a = 125) was applied to the ratio spectra data vectors of DIMA using the divisor PHE. The same approach was also subjected to the ratio spectra of PHE using the divisor DIMA. The db4 (a = 180) and rbior3.7 (a = 125) calibration graphs were constructed using the transformation values obtained in the wavelet domain. In the method validation, the wavelet calibration functions were tested using synthetic mixtures and the standard addition technique. The simultaneous quantitative analysis of DIMA and PHE in the commercial veterinary preparation was achieved by the elaborated methods. The assay results were compared with each other and good agreement was observed.

1. Introduction

Diminazene aceturate (DIMA) is an antiprotozoan drug, used for treatment of babesiosis, trypanosomiasis and theileriosis in cattle, sheep, goats and horses. It has low toxicity and a wide safety margin as a blood parasite drug. As is known, PHE is an antipyretic drug. A combination of DIMA and PHE has been widely used in veterinary medicine.

Determination of DIMA in samples has been done by HPLC analysis (Atsriku et al. 2002), with related substances identified by LC/MS (Atsriku et al. 2002).

Paired-ion extraction and HPLC analysis has been applied to the determination of DIMA in plasma (Otaru Aliu 1983). Quantitative analysis of PHE in samples with other compounds, with their derivatives and degradation products, has been performed by GC-MS (Koutsouba et al. 2003), by spectrophotometry (Santoni et al. 1990), by GC (Sioufi and Colussi 1978; Sioufi and Marfil 1978), by GC-MS and HPLC (Reddersen et al. 2002) and by HPLC (El Sadek et al. 1991), respectively. So far no method for the simultaneous determination of these drugs in samples is available.

The development of mathematical techniques offers the use of powerful tools such as wavelet transform and other signal analysis methods in analytical determinations. These new approaches for signal processing remove or reduce the disadvantages of classical signal transformation methods such as standard derivative spectrophotometry and ratio-spectra derivative spectrophotometry.

Recently, the mathematical basis and program algorithms of wavelet transform multiresolution were described for signal analysis such as denoising and compressing processes (Walczak 2000).

What is new and important about wavelet decomposition methodology is that the wavelet basis functions have what is called compact support. This means that the basis functions are non-zero only on a finite interval. The compact support of the wavelet basis functions allows the wavelet transformation to efficiently represent functions or signals which have localized features. The efficiency of the representation is important in applications such as compression, signal detection, denoising, and interference excision. The common thread throughout all these applications is that the structured component of a signal is well represented by relatively few of the wavelet basis functions, whereas the unstructured component of the signal projects almost equally onto the entire basis functions. The structured and unstructured parts of the signal are then easily separated in the wavelet transform domain.

The wavelet approach with chemometric calibration techniques and zero-crossing point (Dinc and Baleanu 2004a, 2004b; Dinc et al. 2003, 2004a, 2004b, 2005) has been used for the multicomponent analysis of mixtures containing two

or more active compounds in the presence of their strongly overlapping spectra without using any separation procedure. This study reports a powerful tool, giving a fast and very cheap approach to the simultaneous quantitation of DIMA and PHE in veterinary granules for injection by two different wavelet approaches, db4 (a = 180) and rbior3.7 (a = 125), providing accurate and precise results. In our study, db4 (a = 180) was applied to the original absorption spectra of both drugs. At the same time, ratio spectra of the above mentioned drugs were subjected to rbior3.7 (a = 125). The two spectral wavelet approaches described were validated and compared successfully with each other.

2. Investigations, results and discussion

2.1. Wavelet transform

The wavelets are a new type of functions, which provide an excellent orthonormal basis for functions of one or more variables. A set of functions $\Psi_{a,b}(x)$ is obtained from a mother wavelet $\Psi(x)$ by scaling (or dilatation) and shifting (or translation) as follows:

$$\Psi_{a,b}(x) = \frac{1}{\sqrt{|a|}}\,\Psi\left(\frac{x-b}{a}\right) \quad a \neq 0, \quad a, b \in R \qquad (1)$$

where a represents the scale parameter, which is a variable, used to control the scaling and b represents the translation parameter controlling the translation and R is the domain of real numbers.

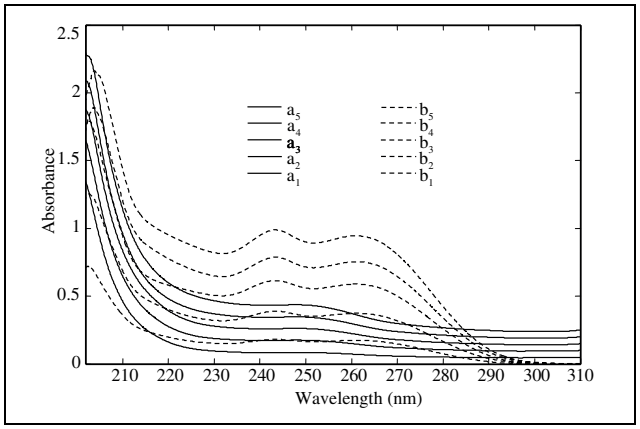

Fig. 1: Absorption spectra of DIMA (——) a1) 4 µg/ml, a_2) 8 µg/ml, a_3) 12 µg/ml, a_4) 16 µg/ml, a_5) 20 µg/ml and PHE (- - - -) b_1) 4 µg/ml, b_2) 8 µg/ml, b_3) 12 µg/ml, b_4) 16 µg/ml, b_5) 20 µg/ml in 0.01 M NaOH and methanol (50 : 50, V/V)

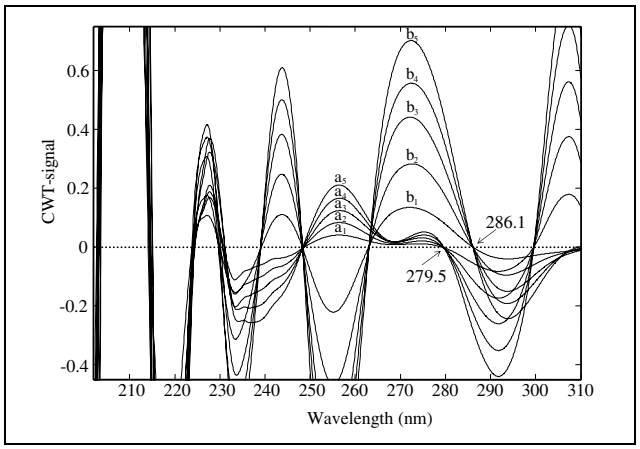

Fig. 2: CWT spectra of DIMA a_1) 4 µg/ml, a_2) 8 µg/ml, a_3) 12 µg/ml, a_4) 16 µg/ml, a_5) 20 µg/ml and PHE b_1) 4 µg/ml, b_2) 8 µg/ml, b_3) 12 µg/ml, b_4) 16 µg/ml, b_5) 20 µg/ml in 0.01 m NaOH and methanol (50 : 50, V/V)

For a given signal f, the wavelet transform consists of computation of coefficients obtained from the inner products of the signal and a family of wavelets.

CWT of f is defined as:

$$\mathrm{CWT}\,\{f; a, b\} = \int_{\infty}^{\infty} f(x)\psi^{*}_{a,b}(x)\,dx = \langle f(x), \psi_{a,b}\rangle \qquad (2)$$

where the superscript * represents the complex conjugate and $\langle f, \psi_{a,b}\rangle$ denotes the inner product of function f onto the wavelet function $\Psi_{a,b}(x)$. If the wavelet Ψ is invertible if it satisfies the admissibility condition, then the initial signal is re-obtained from $\Psi_{a,b}$:

$$f = \frac{1}{C}\int_{\infty}^{\infty}\int_{\infty}^{\infty} \mathrm{CWT}\,(a, b)\Psi_{a,b}\,\frac{da\,db}{a^2} \qquad (3)$$

Here C is given by

$$C = \int_{0}^{\infty} \frac{\hat{\Psi}^{*}(\omega)\,\hat{\Psi}(\omega)}{\omega}\,d\omega \qquad (4)$$

and $\hat{\Psi}$ is the Fourier transform of Ψ.

The Daubechies family of wavelets is one of the most important of wavelet families. Remarkably, the mother wavelet is orthogonal to all functions which are obtained by shifting the mother right or left by an integer amount. Furthermore, the mother wavelet is orthogonal to all functions which are obtained by dilating the mother by a factor of 2^j (2 to the j^{th} power) and shifting by multiples of 2^j units. The orthogonality property means that the inner product of the mother wavelet with itself is unity, and the inner products between the mother wavelet and the aforementioned shifts and dilates of the mother are zero. The collection of shifted and dilated wavelet functions is called a wavelet basis. The orthonormality of the Daubechies wavelets has a very important consequence: any continuous function may be uniquely projected on to the wavelet basis functions and expressed as a linear combination of the basis functions. The collection of coefficients which weight the wavelet basis functions when representing an arbitrary continuous function are referred to as the wavelet transform of the given function.

Some of the families are characterized by orthornormal basis functions as described above. Other wavelet families, for example the biorthogonal wavelets, are orthogonal in a more general sense than has been described. Still other families of wavelet basis functions are not orthogonal in any sense. The large number of known wavelet families and functions provides a rich space in which to search for a wavelet which will very efficiently represent a signal of interest in a large variety of applications.

For these reasons we selected the families db4 (a = 180) and rbior3.7 (a = 125) respectively for our study.

2.2. Spectral wavelet method elaboration

The present study was aimed at elaborating the new spectral wavelet approach for the resolution of binary mixtures and a commercial veterinary formulation for injection in order to (1) evaluate each method in terms of being easily applied and meaningful, (2) identy the advantages and disadvantages of the elaborated approaches, and (3) test the performances of two spectral wavelet approaches.

The outline of two spectral wavelet approaches was based on the use of the original absorption spectra and their ratio spectra. Several of the wavelet families were tested with

the original absorption and ratio spectra to provide linearity, precision and accuracy of the calibration graphs. Two spectral wavelet approaches, db4 (a = 180), and rbior3.7 (a = 125) at 0.004 frequencies were found to be optimal.

2.3. Spectral wavelet application

The absorption spectra of two standard series of DIMA and PHE in the concentration range 4–20 μg/mL were plotted and stored over the range of 200–310 nm as shown in Fig. 1. The same procedure was repeated for the synthetic mixtures, standard addition assay and veterinary formulation for injection. The spectral wavelet approaches elaborated were applied to the recorded absorption spectra and their ratio-spectra.

2.4. Spectral db4 (a = 180) wavelet approach

The original spectral data vectors consisting of 1082 points in the wavelength range of 200–310 nm were processed by db4 (a = 180) wavelet transform. A linear regression function for PHE was obtained by measuring the transformed signal amplitude at 279.5 nm which corresponds to the zero-crossing point for DIMA. In a similar manner the calibration function for DIMA was constructed by measuring the transformed signal amplitude at 286.1 nm corresponding to the zero-crossing point for PHE (see Fig. 2). Under the optimal experimental conditions described above, the linear regression functions and their statistical parameters for both analytical characteristics are given in Table 1. The statistical parameters, limit of detection (LOD), limit of quantitation (LOQ), correlation coefficients, standard errors of calibration functions and range of linearity for DIMA and PHE were found suitable for the determination of the investigated drugs.

2.5. Spectral rbior3.7 (a = 125) wavelet approach

This approach contains a division procedure in the first step for each drug. In optimizing this elaborated approach, some divisors concentration was tested for the spectral division process. The standard spectrum corresponding to 12 μg/mL DIMA and PHE was found to be an optimal value for the division of the original spectra in the working calibration range.

The absorption spectra of the drug DIMA in the spectral range 200–310 nm were recorded and divided by the spectrum of the standard solution of 12 μg/mL PHE. The wavelength range 200–280 nm was selected for the ratio spectra procedure. Fig. 3A shows the ratio spectra of DIMA in the spectral range of 200–280 nm. Spectral rbior3.7 (a = 125) transform of the ratio spectra was obtained and shown in Fig. 3B. In the maximum amplitude at 246.6 nm for DIMA, a graph was plotted of concentration versus rbior3.7 (a = 125) amplitude values and the linear regression function obtained was used for the calculation of the DIMA concentration in samples.

Among several local maxima and minima points illustrated in Fig. 3 we retained only the point corresponding to 246.6 nm for DIMA. The other wavelength points do not have a correlation between CWT amplitude and concentration.

In the same way, the absorption spectra of the solutions of PHE were divided by the spectrum of the standard solution of 12 μg/ml DIMA and their ratio spectra were obtained in the spectral region 200–310 nm (Fig. 4A). Figure 4B shows the spectral rbior3.7 (a = 125) transform of

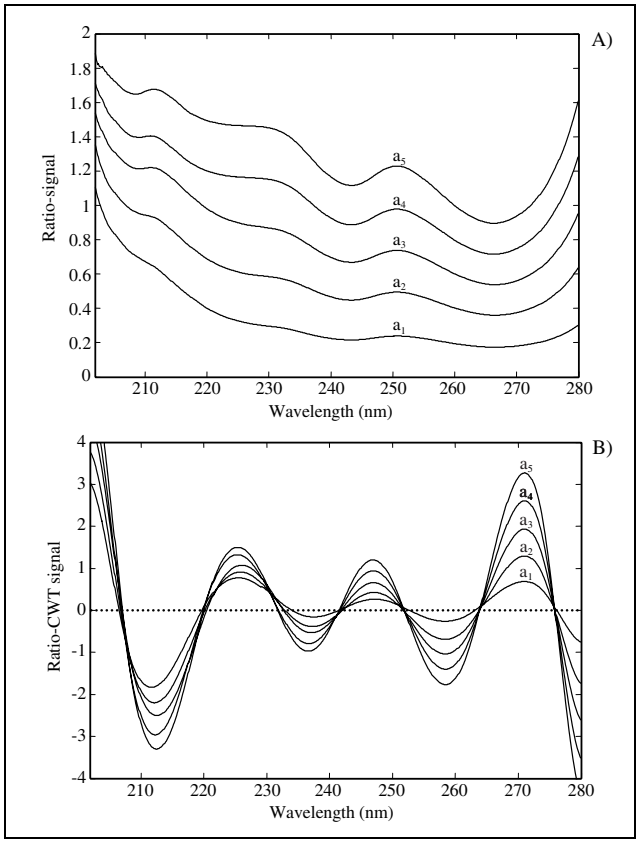

Fig. 3: Ratio spectra (A) and their CWT spectra (B) of DIMA a_1) 4 μg/ml, a_2) 8 μg/ml, a_3) 12 μg/ml, a_4) 16 μg/ml, a_5) 20 μg/ml (when 12 μg/mL PHE was used as a divisor)

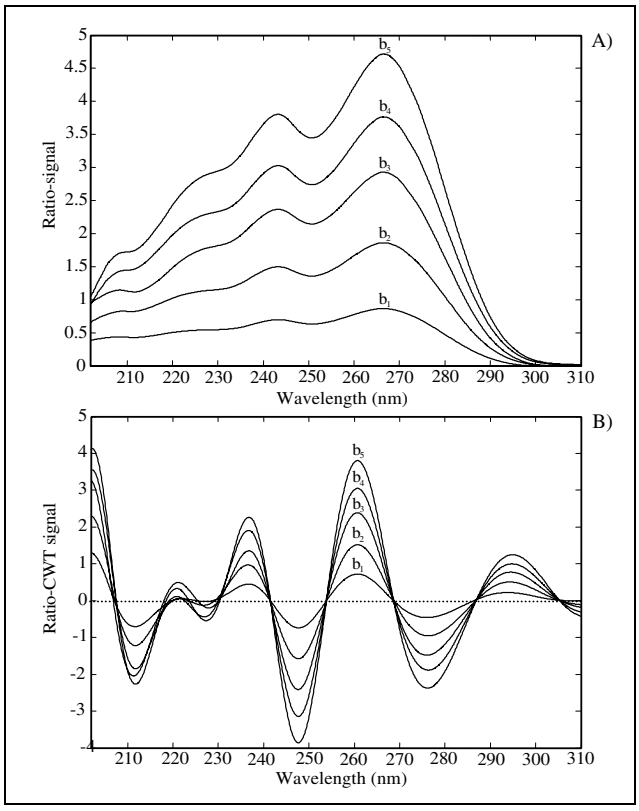

Fig. 4: Ratio spectra and their CWT spectra of PHE b_1) 4 μg/ml, b_2) 8 μg/ml, b_3) 12 μg/ml, b_4) 16 μg/ml, b_5) 20 μg/ml (when 12 μg/mL DIMA was used as a divisor)

the ratio spectra. The concentration of PHE was found to be proportional to the signals at 259.9 nm corresponding to a maximum point. The spectral rbior3.7 (a = 125) signals of ratio spectra were plotted as a graph, versus concentrations of PHE and a linear regression function was obtained.

Following the same procedure as above for finding the optimal wavelength point among the maxima and minima points from Fig. 4B we conclude that only the point corresponding to 246.6 nm for DIMA gives a linear correlation between CWT amplitude and concentration.

2.6. Method validation

The linearity of the application of two wavelet approaches to the analysis of DIMA and PHE using the original absorption spectra and their ratio spectra was tested by analyzing a series of different concentrations of each drug. In accordance with the International Conference on Harmonization (ICH) (European Agency 1996), at least five concentrations must be used. In our case five concentrations between 4 and 20 μg/ml were used for linearity. The analysis of each concentration was repeated three times. Information about the variation of the peak amplitude for the two wavelet approaches was obtained for the linear regression analysis. The linearity of individual calibration graphs for the drugs with the wavelet approaches used was verified by the high value of the correlation coefficients (see Table 1).

Ten replicate determinations at different levels of concentration were performed to test the precision of db4 (a = 180) and rbior3.7 (a = 125). The relative standard deviations for the reproducibility of the methods were calculated as shown in Table 2. Satisfactory results were obtained.

The accuracy of the methods used was tested using synthetic mixtures in different possible concentrations. The average recovery data for db4 (a = 180) and rbior3.7 (a = 125) approaches were found to be 101.0% for DIMA and 100.3% for PHE, and 98.4% for DIMA and 102.3% for PHE, respectively (see Table 2). A good agreement between the numerical values provided validation of the methods investigated. Interferences and systematic errors were not reported during the analysis procedure.

According to the ICH (European Agency 1996) the limits of detection and quantitation (LOD and LOQ, respectively) can be calculated from the standard deviation of the residual signal and the slope of the calibration function. The working calibration range used for each drug depended on its amount in the commercial product and was selected to give an accurate, precise and linear response.

For validation of the procedure by another method the standard addition technique with five replicate analyses was used. The standards of two pure drugs at levels equal to the content of the veterinary granule formulation were added to the commercial sample solutions in the working concentration range. The results and their standard deviations were calculated and presented in Table 3. Results from the standard addition assay show that the matrix effect did not lead to any error in the determination.

Table 2: Recovery results obtained by applying the proposed approaches to different binary mixtures

Binary mixture		Recoveries (%)			
Added (μg/mL)		CWT-zero crossing (db4 (a = 180))		Ratio spectra-CWT (rbio3.7 (a = 125))	
DIMA	PHE	DIMA	PHE	DIMA	PHE
12	4	101.4	95.2	100.7	102.7
12	8	100.3	101.4	96.5	100.5
12	12	99.6	101.2	94.5	103.0
12	16	100.3	101.6	93.1	102.1
12	20	99.4	100.2	96.7	101.9
4	15	105.0	99.9	98.1	101.7
8	15	100.6	101.0	101.2	102.3
12	15	100.8	101.4	95.3	102.7
16	15	101.2	100.7	99.0	102.5
20	15	101.3	100.0	98.6	103.5
	Average	101.0	100.3	97.4	102.3
	RSD	1.55	1.89	2.70	0.80

Table 3: Standard addition technique and its recoveries with the statistical results

Concentration		Recoveries (%)			
		CWT-zero crossing (db4 (a = 180))		Ratio spectra-CWT (rbio3.7 (a = 125))	
DIMA (μg/mL)	PHE (μg/mL)	DIMA	PHE	DIMA	PHE
12	15	100.7	104.2	103.3	100.6
12	15	101.4	102.7	104.1	99.9
12	15	103.0	104.3	108.3	101.1
12	15	99.6	102.6	103.9	101.1
12	15	100.1	103.2	103.5	100.8
	Average	101.0	103.4	104.6	100.7
	RSD	1.16	0.70	1.77	0.44

2.7. Analysis of sample

Two wavelet approaches, db4 (a = 180) and rbior3.7 (a = 125) transforms, were applied to the quantitative analysis of DIMA and PHE in veterinary granules for injection. The assay results obtained from this veterinary formulation are presented in Table 4. These values are acceptable for routine analysis and quality control. Good agreement was observed between assay results and the label claim when the two wavelet approaches were applied to a commercial veterinary formulation.

Table 1: Linear regression functions and their statistical results

Method	λ(nm)	Regression function	r	SE(a)	SE(b)	SE(r)	LOD	LOQ
Ratio	246.6	$S = 0.0633C_{DIMA} - 0.0797$	0.9992	0.0190	0.0014	0.0181	0.80	2.67
spectra-CWT	259.9	$S = 0.1895C_{PHE} - 0.0156$	0.9990	0.0339	0.0048	0.0609	0.60	2.02
CWT-zero	286.1	$S = 0.0141C_{DIMA} + 0.0006$	0.9999	0.0012	0.0001	0.0011	0.50	1.67
crossing	279.5	$S = 0.0232C_{PHE} - 0.0028$	0.9992	0.0072	0.0005	0.0068	0.64	2.14

SE(b): Standard error of slope, SE(a): Standard error of intercept, SE(r): Standard error of regression constant, C: Concentration (μg/ml), S: Peak amplitude of derivative transform, r: Regression coefficient, LOD: Limit of detection, LOQ: Limit of quantitation

Table 4: Experimental results obtained by applying the proposed approaches to the commercial veterinary formulation

	CWT-zero crossing db4 (a = 180)		Ratio-CWT rbio3.7 (a = 125)	
	DIMA	PHE	DIMA	PHE
Average	1007.5	1254.4	1015.3	1272.7
SD	20.33	16.95	19.83	17.88
RSD	2.02	1.35	1.95	1.40

The results are the mean of five replicates Claimed label: 1050 mg DIMA and 1310 mg PHE/bag

2.8. Conclusion

Two wavelet approaches, db4 (a = 180) and rbior3.7 (a = 125) transforms, offer the advantages of simplicity, rapidity and specificity without the need for a separation procedure. Nowadays a lot of wavelet families have a higher performance than the classical spectral transformation methods such as derivative spectrophotometry and the ratio-spectra derivative method. In our case the db4 (a = 180) continuous wavelet transform for DIMA and PHE was applied directly to the strongly overlapped absorption spectra in the wavelength range of 200–310 nm. As an alternative wavelet approach, the ratio spectra of the two drugs were subjected to rbior3.7 (a = 125).

The two wavelet approaches developed and presented in this study give a satisfactory result for the determination of DIMA and PHE with adequate reproductibility and recovery.

The powerful mathematical procedure of wavelet transform applied to signal analysis or spectral analysis gives important results in signal compressing and denoising. For these reasons it is considered to be superior to the classical spectral methods. This wavelet approach produces an alternative resolution of complex mixtures.

The theoretical properties of wavelet transform provide for the elimination or diminution of the noise from recording absorption spectra.

In our case other properties of these transform methods were used for the graphical procedure. Firstly, a greater peak amplitude corresponding to a zero-crossing point for an analyte in a binary mixture can be obtained by this wavelet procedure. Secondly, a constant signal of the ratio-spectra of an analyte is eliminated by the wavelet method. These two properties are new means for the spectral quantitative analysis of complex mixtures. The above mentioned characteristics of wavelet families are the basis of our application to the quantitative analysis of DIMA and PHE.

The proposed approaches can be applied quite easily to the routine analysis and quality control of commercial veterinary formulations containing these drugs.

3. Experimental

3.1. Apparatus and software

The absorption spectra were recorded using a Shimadzu UV-160 double beam UV-VIS spectrophotometer having a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK-E320 printer. The spectrum was recorded in the wavelength range of 200–330 nm against a blank (0.01 M NaOH and methanol (50 : 50, v/v)). The wavelength range of 200–310 nm was selected for data processing.

The data treatment was done with a Pentium 42.8 GHz (512 Mb RAM) computer using MATLAB 7.0 software (The Math Works, Natick, MA, USA). FWT calculations were performed in MATLAB 7.0. The calculations and calibrations were performed using Microsoft EXCEL.

3.2. Veterinary formulation

Commercial granules for injection, PIROVET® (1050 mg diminazene aceturate + 1310 mg phenazone) produced by Topkim Drug Industry, Istanbul, Turkey, were studied using the CWT approaches.

3.3. Standard solution

Stock solutions of 25 mg/100 mL DIMA and PHE were prepared in 0.01 M NaOH and methanol (50 : 50, v/v) for each compound. Two standard series of the solutions containing 4–20 μg/mL DIMA and PHE were obtained from the stock solutions for recording spectra. A validation set of 10 mixture solutions containing the two compounds was also prepared using the same stock solutions.

3.4. Preparation of sample solution

For the determination of DIMA and PHE in pharmaceuticals an accurately weighed portion of the mixed content of 10 bags equivalent to 445 mg DIMA and 550 mg PHE was dissolved in 0.01 M NaOH and methanol, 50 : 50 (v/v) in a 100 ml volumetric flask. The solution was filtered into a 100 ml volumetric flask through a 0.45-μm membrane filter. This solution was diluted to the working concentrations, 12 μg/ml for DIMA and 15 μg/ml for PHE, in a 25 ml volumetric flask. Two wavelet approaches, dba (a = 180) and rbior3.7 (a = 125), were applied to the original absorption spectra and ratio spectra, respectively, of the final sample solutions.

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