Fractional Wavelet Transform for the Quantitative Spectral Resolution of the Commercial Veterinary Preparations

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Fractional wavelet transform was applied to the quantitative spectral analysis of oxytetracycline and flunixin megluminein a veterinary combination. A concentration set of 15 binary mixtures containing oxytetracycline and flunixin megluminein in the range of 4-36 μg/mL oxytetracycline and 2-34 μg/mL flunixin megluminein were processed by fractional wavelet transform (FWT) method. Partial least squares (PLS) and principal component regression (PCR) were applied to the FWT-coefficients to obtain FWT-PCR and FWT-PLS. The validity of the proposed FWT-PLS and FWT-PCR approaches were carried out by analyzing the synthetic binary mixtures of the related veterinary compounds. The proposed combined approaches were used for the quality control of the commercial veterinary formulation of compounds.

Keywords: Fractional wavelets transform, partial least squares, principal component regression, commercial veterinary preparation

Flunixin meglumine (FLU) is a nicotinic acid derivate nonsteroidal antiinflaamatory drug. It produces analgesic and antiinflammatory effects by inhibiting the synthesis of prodtoglandins. Flunixin is used primarily for short-term treatment of moderate pain and inflammation. It has been particularly used for visceral pain and septic shock in horses, and also coliform mastitis in cattle (1, 2, 3). By far the most commonly used tetracycline in veterinary practice today is oxytetracycline (OXY). Oxitetracycline products are approved for use in dogs and cats, calves, non-lactating dairy cattle, beef cattle swine, fish, and poultry. It has a broad spectrum of activity including gram-positive and gra-negative bacteria, some protozoa, Ricketsiae, Chlamydia, Spirochetes, Mycoplasma and Ehrlichiae. Oxitetracycline has been available in a variety of formulations to control the release rate from an injection [1, 2, 3]. In the quantitative analyses, the spectrophotometric methods have been widely used for the determination of compounds in different samples. The most use of these spectral methods is the ultraviolet visible spectrophotometric techniques due to the resulting experimental rapidly, simplicity and the wide application. However, the simultaneous determination of active compounds by the use of the traditional spectrophotometry is difficult because, generally, the absorption spectra overlap in the same wavelength range. Therefore, the superimposed signals are not suitable for quantitative analysis of the complex mixture containing two or more compounds with sample matrix.

The fundamental problems of the signal analysis are the extraction of the largest analytical information from the complex spectral bands and the resolution of the overlapping spectra. In recent years, classical wavelet and fractional wavelet analysis methods are new and powerful tools with multipurpose use and great potential for application in the analytical chemistry as well as many branches of science.

In this context, the simultaneous use of continuous wavelet transform (CWT) and fractional wavelet transform (FWT) with multivariate statistical calibrations in analysis studies provides a new spectral quantitative resolution of complex mixture samples [4-13].

Quantitative spectrophotometry has been greatly improved by the combined use of wavelet analysis and multivariate calibration methods, particularly principal component regression and partial least square regression.

The aim of this study is to improve a new application of PCR and PLS multivariate calibrations in the fractional wavelet domain for the simultaneous quantitative resolution of a given complex mixture containing OXY and FLU antibiotics without requiring any chemical pretreatment. These FWT chemometric calibrations were denoted as FWT-PCR and FWT-PLS. The experimental results provided by applying the FWT multivariate calibration approaches were compared with those obtained by Raw-PCR and Raw-PLS.

Experimental part

Instruments and softwares

The absorption spectra of the analyzed compounds and their samples was recorded by using a Shimadzu UV-160 double beam UV–visible spectrophotometer connected to a computer loaded with Shimadzu UVPC software. The mathematical treatments of the absorption data was performed in a computer by using the Microsoft EXCEL and FFT Wavelet analysis in MATLAB 7.0 software.

Standard solutions

Stock solution for each of the pure compounds (OXY and FLU) was separately prepared dissolving 25 mg of OXY and FLU in 100 mL-calibrated flask within methanol. A concentration set corresponding to 15 binary mixtures of OXY and FLU in concentration range between 4.0-36.0 μg/

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mL and 2.0-34.0 μg/mL, respectively. For the validation of the proposed FWT chemometric calibrations, an independent set containing the different mixtures of OXY and FLU in the above ranges was prepared by using the prepared stock solutions.

Commercial veterinary formulation

A commercial veterinary formulation (Flunitet® Injection Solution, Bayer Pharm. Ind., Istanbul, Turkey) containing 300 mg OXY and 200 mg FLU per mL was analyzed by applying FWT-PCR, FWT-PLS, Raw-PCR and Raw-PLS.

Signal analysis method

Fractional wavelet transform

A new transform wavelet transform based on the fractional B-splines was initiated [1-2]. The mathematical idea of fractional derivatives has represented the subject of interest for various branches of science [3-4]. As it is already known the splines play a significant role on the early development of the theory of the wavelet transform [5-8]. The new fractional splines have all properties of the polynomial splines with the exception of compact support when the order α is non-integer. The main advantage of this construction is that we can build the wavelet bases parameterized by the continuously-varying regularity parameter α.

Results and discussions

In this study, the simultaneous determination of OXY and FLU in their mixtures is impossible by traditional spectrophotometric approaches due to the overlapping absorption bands of two compounds in the spectral region of 212.0-416.7 nm (fig. 1). Therefore, the new and powerful methods consisting of the collection of FWT with multivariate calibrations were improved to obtain more accurate and precise results for the analysis.

The proposed mathematical tools are based on the application of the FWT technique combined with PCR and PLS to the quantitative analysis of the complex veterinary mixture containing OXY and FLU drugs.

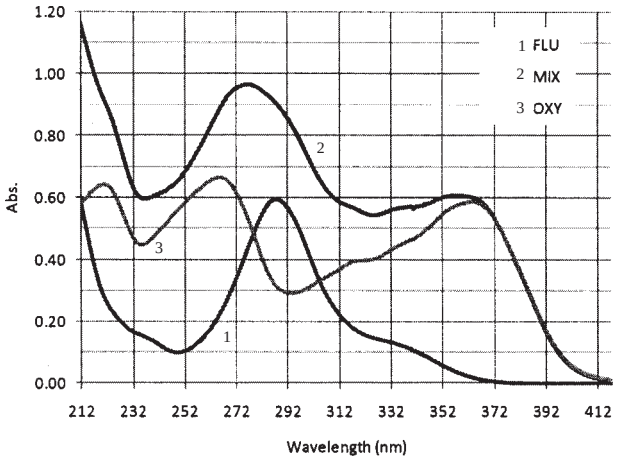

Fig. 1. UV spectra of 20 μg/mL OXY (1), their mixture in methanol (2) and 14 μg/mL FLU (3)

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FWT application

The UV spectra of the OXY and FLU compounds and their mixture were obtained between 212.0 nm and 416.7 nm as shown in figure 1. Similarly, the spectra of the training set and samples were recorded between 212.0 nm and 416.7 nm by using a UV-VIS spectrophotometer.

The absorbance data vectors having 2048 points in the above wavelength range 212.0 nm and 416.7 nm were processed by the FWT using the signal processing parameters, α = 3.50, J = 3 (depth of the decomposition =J) and type = +O. In the following step, the absorption spectra of the training set and samples were treated according to the same FWT approach. After FWT, the signals were described FWT-coefficients in the fractional wavelet domain.

In this study, FWT was used as a tool for reducing data and for removing irrelevant information from original absorbance data. FWT spectra were presented in figure 2.

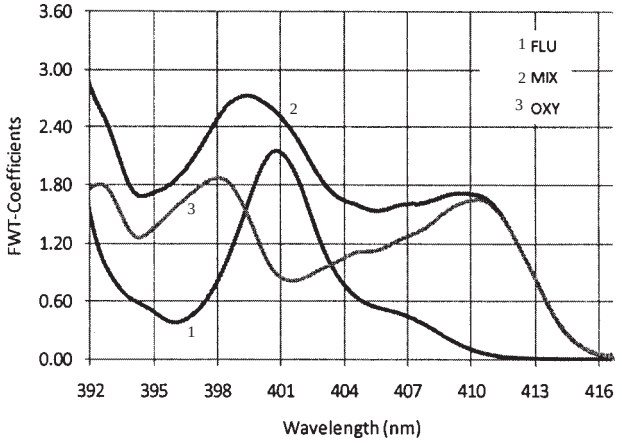

Fig. 2. FWT spectra obtained by transformation of UV spectra of 20 μg/mL OXY (1), their mixture (2), and 14 μg/mL FLU (3)

In the next step, 201 FWT-coefficients between 392.0 and 416.7 nm nm for the multivariate calibrations (FWT-PCR and FWT-PLS) were retained and other was eliminated. As it can be seen from figure 2, FWT spectra having high-amplitudes in the narrower wavelength region were obtained.

FWT-PCR and FWT-PLS

A concentration (training) set of 15 mixtures obtained by mixing *OXY* and FLU in the range of 4.0-36.0 μg/mL and 2.0-34.0 μg/mL, respectively was symmetrically designed corresponding to a 15x2-dimentional concentration data matrix as presented in table 1. Absorbance values of the spectra were measured at 201 wavelengths between 392.0 nm and 416.7 nm for the above concentration set and then a 15x512-dimensional absorbance data matrix were obtained.

FWT was applied to each of the row vectors of absorbance data matrix. After FWT, a matrix of 15x201-FWT coefficients was reconstructed in selected fractional wavelet domain between 392.0-416.7 nm. FWT-PCR and FWT-PLS calibrations were computed by applying the algorithms of PCR and PLS to the relationship between training (concentration) set and FWT- coefficients matrices. FWT-PCR and FWT-PLS were applied to the quantitative resolution of the mixture containing OXY and FLU compounds.

Raw data-PCR and Raw data-PLS calibrations

Raw data-PCR and Raw data-PLS calibrations were used for the comparison of the prediction results obtained by the FWT-PCR and FWT-PLS methods. For a comparison, Raw data-PCR and Raw data-PLS calibrations were constructed by using the mathematical relationship based on the training (concentration) set (15x2-dimentional) and absorbance data (19x2048-dimentional) matrices. The

No.	Concentration (μg/mL) OXY	FLU	No.	Concentration (μg/mL) OXY	FLU
1	4.0	2.0	11	20.0	10.0
2	4.0	10.0	12	20.0	18.0
3	4.0	18.0	13	28.0	2.0
4	4.0	26.0	14	28.0	10.0
5	4.0	34.0	15	36.0	2.0
6	12.0	2.0			
7	12.0	10.0			
8	12.0	18.0			
9	12.0	26.0			
10	20.0	2.0			

Table 1
A TRAINING (CONCENTRATION) SET OF OXY AND FLU MIXTURES

Mixture (μg/mL)		Predicted concentration (μg/mL) FWT-PCR		FWT-PLS		Raw-PCR		Raw-PLS	
OXY	FLU	OXY	FLU	OXY	FLU	OXY	FLU	OXY	FLU
4.0	2.5	3.94	2.38	3.99	2.48	3.74	2.52	3.74	2.52
8.0	2.5	8.31	2.53	8.15	2.52	8.27	2.62	8.27	2.60
12.0	2.5	12.28	2.58	12.08	2.57	12.70	2.63	12.70	2.58
16.0	2.5	16.10	2.55	16.20	2.52	17.02	2.62	17.02	2.56
20.0	2.5	20.52	2.55	20.52	2.55	20.56	2.62	20.56	2.48
24.0	2.5	24.75	2.52	23.93	2.50	25.66	2.61	25.66	2.60
28.0	2.5	27.87	2.52	28.87	2.52	29.78	2.60	29.78	2.60
32.0	2.5	33.06	2.48	32.64	2.50	33.56	2.61	33.56	2.50
36.0	2.5	36.71	2.52	36.71	2.52	37.59	2.55	38.59	2.65
35.0	2.0	36.95	2.02	35.95	2.06	36.86	2.10	36.86	2.09
35.0	6.0	37.16	5.95	36.16	5.95	37.05	6.05	37.05	6.05
35.0	10.0	36.04	9.83	35.43	9.73	36.94	9.94	36.34	9.94
35.0	14.0	35.86	13.95	36.86	13.98	36.76	13.80	36.76	13.80
35.0	18.0	35.10	18.47	35.10	17.46	37.01	17.67	37.01	17.67
35.0	22.0	35.96	22.45	36.06	21.47	36.86	21.55	36.86	21.55
35.0	26.0	35.20	26.28	35.19	25.28	37.09	25.50	37.09	25.51
35.0	30.0	36.64	31.79	36.01	29.79	36.55	29.98	37.55	29.98
35.0	34.0	36.69	33.52	35.07	33.95	37.58	33.34	37.58	33.34
		Recovery (%)							
		FWT-PCR		FWT-PLS		Raw-PCR		Raw-PLS	
		OXY	FLU	OXY	FLU	OXY	FLU	OXY	FLU
		98.4	95.1	99.8	99.1	93.4	100.8	93.4	100.8
		103.8	101.1	101.9	100.7	103.4	104.8	103.4	104.0
		102.4	103.0	100.7	102.6	105.8	105.1	105.8	103.2
		100.6	102.1	101.3	100.9	106.3	104.6	106.3	102.2
		102.6	102.1	102.6	101.9	102.8	104.9	102.8	99.2
		103.1	101.0	99.7	100.2	106.9	104.4	106.9	104.0
		99.5	100.7	103.1	100.7	106.3	104.0	106.3	104.0
		103.3	99.4	102.0	100.2	104.9	104.4	104.9	100.2
		102.0	100.6	102.0	100.6	104.4	102.0	107.2	106.0
		105.6	101.1	102.7	103.1	105.3	105.0	105.3	104.5
		106.2	99.2	103.3	99.2	105.9	100.8	105.9	100.8
		103.0	98.3	101.2	97.3	105.5	99.4	103.8	99.4
		102.5	99.6	105.3	99.9	105.0	98.6	105.0	98.6
		100.3	102.6	100.3	97.0	105.7	98.2	105.7	98.2
		102.7	102.0	103.0	97.6	105.3	97.9	105.3	97.9
		100.6	101.1	100.6	97.2	106.0	98.1	106.0	98.1
		104.7	106.0	102.9	99.3	104.4	99.9	107.3	99.9
		104.8	98.6	100.2	99.9	107.4	98.1	107.4	98.1
	Mean	102.6	100.8	101.8	99.9	104.7	101.7	104.9	101.1
	SD	2.09	2.28	1.49	1.77	3.04	2.91	3.16	2.65
	RSD	2.04	2.27	1.46	1.77	2.91	2.86	3.01	2.62

Table 2
RECOVERY RESULTS FOR OXY AND FLU IN THEIR MIXTURES BY FWT-PCR, FWT-CWT, RAW-PCR AND RAW-PLS

SD = Standard deviation and RSD = Relative standard deviation

simultaneous prediction of OXY and FLU in samples was performed by using Raw data-PCR and Raw data-PLS calibrations based on the use of the original absorbance values.

Method validation

For the validity of the proposed methods, an independent set of 18 synthetic mixtures consisting of OXY and FLU in the range of 4.0-36.0 μg/mL and 2.0-34 μg/mL, respectively was illustrated in table 2.

The experimental results of the validation samples were predicted by applying FWT-PCR and FWT-PLS. Recovery results and relative standard deviations (RSD) were presented in table 2. The recoveries obtained by applying Raw data-PCR and Raw data-PLS, were shown on same table.

Conclusions

In this study a new method was developed based on the simultaneous use of fractional wavelet analysis and

chemometric multivariate calibrations (PCR and PLS). This approach was applied to the quantitative resolution of OXY and FLU compounds in their samples without using any separation procedure.

To compare the results of FWT-PCR and FWT-PLS, the PCR and PLS approaches based on the use of Raw data (original absorbance data) was subject to the analysis of binary mixture containing related compounds. By analyzing the obtained results we conclude that our new proposed FWT-PCR and FWT-PLS method gives better results than those obtained by the Raw data-PCR and Raw data-PLS.

As it is well known, various traditional spectral methods have been used for the simultaneous quantitative analysis of a complex mixture containing chemical compounds. However, these methods may not give always expected results due to overlapping spectra together with noise and irrelevant information. Taking into account the above restrictions for the spectral quantitative evaluation, we need to develop new and efficient analytical methods and approaches. In our work, we concluded that FWT-PCR and FWT-PLS gave more accurate and precise analysis results than those obtained by Raw data-PCR and Raw data-PLS.

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