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Continuous wavelet transformation applied to the simultaneous quantitative analysis of two-component mixtures

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In this paper we developed a graphical method based on Haar (HA) and Mexican (MEX) one-dimensional continuous wavelet transforms and we applied it to a mixture of hydrochlorothiazide (HCT) and spironolactone (SP) in the presence of strongly overlapping signals. Keeping in mind to obtain an appropriately transformed spectrum, we tested several values of the scaling parameter a and the point number of the analysed spectrum in the concentration range of 2–22 μg/ml for both active compounds. The optimal values of the scale parameters and the corresponding frequencies were found to be a = 32 and 0.031 for HA and a = 30 and 0.008 for MEX corresponding to 400 points. HA and MEX methods based on a zero crossing technique were applied to the analysed signal and their regression lines at the selected points were obtained. The validation of the above methods was carried out by analysing different synthetic mixtures containing HCT and SP. MATLAB 6.5. Software was used for one-dimensional wavelet analysis and the basic concepts about wavelet method were briefly explained. The method developed in this paper is rapid, easy to apply, inexpensive and is suitable for analysing the overlapping signals of compounds in their mixtures without any chemical pre-treatment.

1. Introduction

The fast development of new methods in analytical chemistry, e.g, graphical and numerical techniques, permits to solve many problems of the quantitative analysis of two-component and multi-component mixtures. In the past, to solve these problems various graphical (Haver 1976; Salinas et al. 1990; Berzas Nevado at el. 1992; Dinc and Onur 1998; Dinc 1999) and numerical methods (Dinc 1999; Beebe and Kowalski 1987; Haaland and Thomas 1988; Wang et al. 2000; Dinc et al. 2001; Dinc and Baleanu 2002) were developed but still the resolving of multi-mixtures has drawbacks in some cases. It may happen that the classical methods, e.g. derivative and ratio spectra derivative methods do not give the best resolution of a two-mixture system. For example, since the higher derivative procedure reduces the peak amplitude, the process of finding zero-crossing points is very difficult and the sensitivity of the method is decreasing. Particularly, the ratio spectra derivative method leads to an infinite value of ratio spectra in some cases. We observed some non-resolving determinations of analytes using the absorption spectra.

Another way to bypass the above-mentioned problems is to use the wavelet transform (WT) WT (Tang et al. 2000; Vetterli and Kovacevic 1995; Meyer 1992; Daubechies 1992; Leung et al. 1998) rapidly developed during the last decade in various branches, analysis of electrochemical noise data (Zou and Mo 1997; Shao et al. 2000), or resolving simulated overlapped spectra (Zang et al. 2001).

The basis idea of continuous one-dimensional wavelet transformation (CWT) is to represent any arbitrary function as a superposition of wavelets. A fast implementation method for discrete wavelet method (DWT) was discovered by Mallat and Hwang (1992) and is an effective tool for processing chemical data. It was recently proved that CWT based on the use of the zero-crossing technique could give better results for the resolution of the mixtures (Dinc and Baleanu 2003a; Dinc and Baleanu 2003b).

DWT and CWT have been applied only to the signal analysis such as compression and de-nosing of spectra. Our proposed approach contains a direct application of DWT and CWT to the two-component mixture analysis on the contrary of the studies of signal compression and de-nosing given in the literature (Zou and Mo 1997; Shao et al. 2000; Zang et al. 2001)

For these reasons, MEX-CWT and HA-CWT were subjected to the simultaneous determination of HCT and SP in tablets. The obtained results were successfully compared among each other as well as with those obtained by other literature methods.

2. Investigations, results and discussion

2.1. Wavelet transform

A wavelet transform involves the decomposition of a signal function or vector into simpler, fixed building blocks at different scales and positions.

2.1.1 Continuous wavelet transform

Wavelet or "small waves" is expressed as a series of functions $\Psi_{a,b}(\lambda)$ represented by the following equation

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

Here a denotes 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. A mother wavelet $\Psi(\lambda)$ generates the set of functions $\Psi_{a,b}(\lambda)$ by scaling (or dilatation) and shifting (or translation). The wavelet continuous transform (CWT) of f (λ) is defined as:

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

where the superscript* represents the complex conjugate and $\langle f(\lambda), \Psi_{a,b}\rangle$ denotes the inner product of function f (λ) onto the wavelet function $\Psi_{a,b}(\lambda)$.
We say that the wavelet Ψ is invertible if it satisfies the admissibility condition

$$\int_{\infty}^{\infty} \frac{|\hat{\Psi}(\omega)|^2}{\omega}\,d\omega < \infty \quad (3)$$

The original signal is reobtained from $\Psi_{a,b}$ as

$$f(\lambda) = \frac{1}{C}\int_{\infty}^{\infty}\int_{\infty}^{\infty} \mathrm{CWT}\,(a, b)\,\Psi_{a,b}\,\frac{da\,db}{a^2}\,. \quad (4)$$

Here C has the following expression

$$C = \frac{\int_{\infty}^{\infty} \hat{\Psi}^*(\omega)\hat{\Psi}(\omega)}{\omega\,d\omega} \quad (5)$$

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

2.1.2. Discretization of the continuous wavelet transform

The aim of this method is to reconstruct the function f(λ) from samples taken on a discrete grid. In this case we choose the following discretization for a and b

$$a = a_0^j, b = kb_0a_0^j, \qquad j, k \in Z \quad (6)$$

where Z represent the set of integers.
The discretized family of wavelets is given by

$$\Psi_{i,j}(\lambda) = a_0^{-j/2}\Psi(a_0^{-j}t - k) \quad (7)$$

If $a_0 = 2$, $b = 1$, we obtain the dyadic case for which the orthonormal bases exist and reconstruction from transform coefficients is available.
For a given mother wavelet $\Psi$and appropriate a_0 and b_0, we have

$$f(\lambda) = \sum_j \sum_k C_{jk}\Psi_{j,k}(\lambda) \quad (8)$$

where

$$C_{j,k} = \langle f(\lambda),\ \Psi_{j,k}\rangle \quad (9)$$

MEX is defined as

$$\Psi(\lambda) = 2\pi\omega^{-1/2}[1 - 2\pi(\lambda/\omega)^2]\,e^{-\pi(\lambda/\omega)2} \quad (10)$$

where ω represents the width parameter (for example $\omega = \frac{1}{16}$).
The Haar wavelet has the property of being compactly supported which means that it vanishes outside of the finite interval.
HA is defined as

$$\Psi(t) = \begin{cases} 1 & 0 \le t < \frac{1}{2} \\ -1 & 0 \le t < \frac{1}{2} \\ 0 & \text{otherwise} \end{cases} \quad (11)$$

and the whole set of basis functions is obtained by dilation and translation as

$$\Psi_{m,n}(t) = 2^{-m/2}\Psi(2^{-2}t - n) \quad m, n \text{ are integers} \quad (12)$$

We call m the scale factor, since $\Psi_{m,n}(t)$ is of length 2^m, while n is called the shift factor, and the shift is scale dependent ($\Psi_{m,n}(t)$ is shifted by $2^m n$). The normalization factor $2^{-m/2}$ makes $\Psi_{m,n}(t)$ of unit norm.

2.2. Simultaneous determination of hydrochlorothiazide and spironolactone

Under the experimental conditions a standard solution containing HCT and SP in the concentration range of 2–22 μg/mL was prepared and its spectra were recorded in the range of 210–289 nm. We selected 400 points from the original spectra to make possible the wavelet analysis and we transferred it to MATLAB 6.5. software for the signal analysis process (see Fig. 1).
In our case the wavelength (λ) plays the role of parameter t in WT analysis, then the coefficients C_{ab} can be plotted versus wavelength number (in this case it runs from 1 to 400). The number of points plays a very important role. If the working range contains many points, the intensity of the transformed signal increased but it becomes difficult to obtain the transformed spectra zero-crossing points. For a given compound we have to establish an optimization between the number of points and the amplitude of the transformed signal. In other words we would like to find out many zero-crossing points, and the amplitude of the transformed signal to be higher than the amplitude of the analyzed signal.

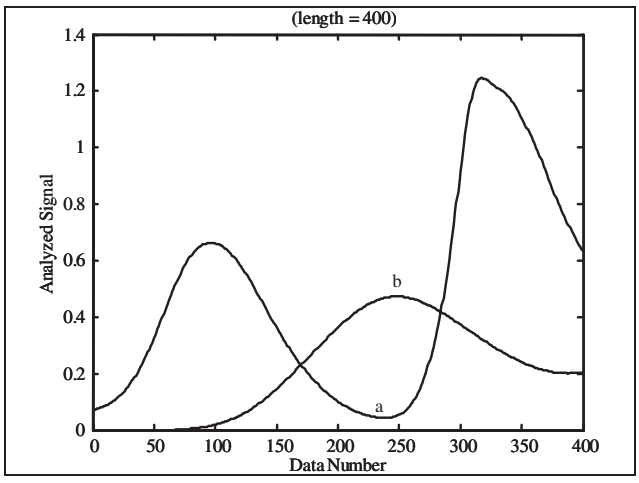

Fig. 1: Analyzed spectrum of a) 10 μg/ml HCT and b) 10 μg/ml SP

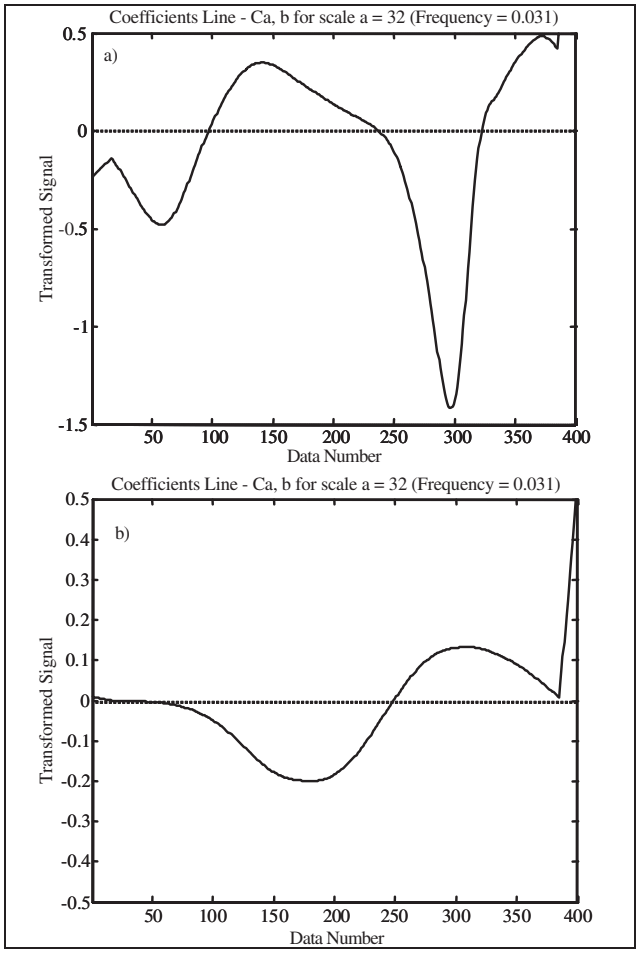

Fig. 2: Transformed spectrum of a) 10 μg/ml HCT and b) 10 μg/ml SP by using HA (a = 32)

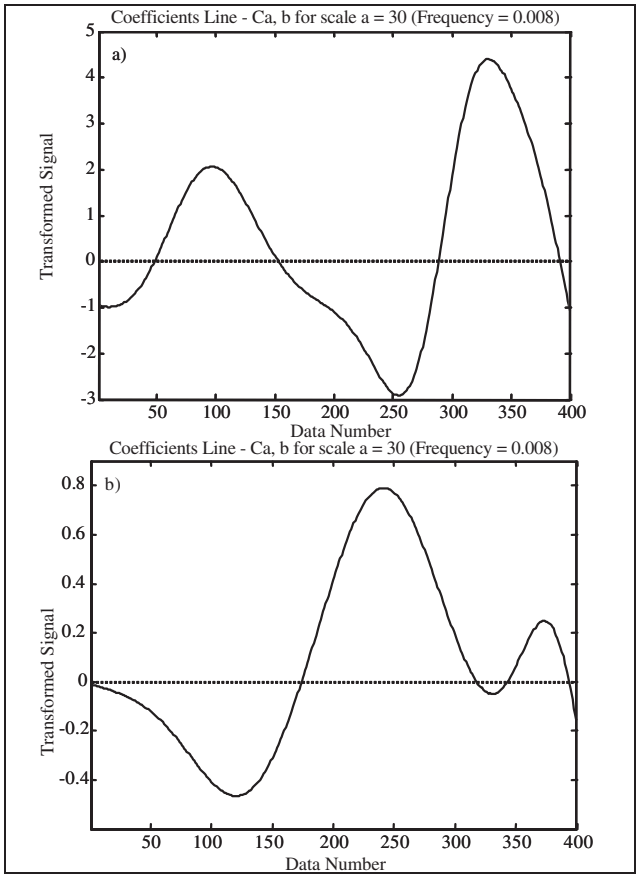

Fig. 3: Transformed spectrum of a) 10 μg/ml HCT and b) 10 μg/ml SP by using MEX (a = 30)

In our case this analysis was possible by investigating the different values of the scale parameter a and frequency and we found the optimal values to be a = 32 and 0.031 for HA and a = 30 and 0.008 for MEX corresponding to the signal analysis of HCT and SP (see Figs. 2 and 3). A zero-crossing technique on transformed signals was used. As a remark, we observed that when the length of the signal decreased the peak intensity of the transformed signals becomes smaller and the zero crossing points were diminished.

The maximum amplitudes of the transformed signals depend on the applied wavelet (see Figs. 2 and 3).

2.2.1. CWT and Beer-Lambert law

We consider a binary mixture containing two analytes (X and Y). If the Beer-Lambert law is valid for two analytes at the whole points in the working range, the analysed signal of the binary mixture at the point i are given

$$S_{mix,i} = \alpha_{X,i} C_X + \beta_{Y,i} C_Y \qquad (13)$$

Here $S_{mix,i}$ represents the analysed signal of the binary mixture at the point i, $\alpha_{X\,i}$, β_{y,p_i} denote the constants of X and Y; CX and CY are the concentration of X and Y respectively.

We apply now CWT to Eq. (13) and taking into account its definition we obtain

$$\text{CWT}\, S_{mix,i} = \text{CWT}\, \alpha_{X,i}\, C_X + \text{CWT}\, \beta_{Y,i}\, C_Y \qquad (14)$$

Eq. (14) represents the Beer-Lambert law for the transformed signal.

If the transformed amplitude (CWT $S_{Y,i}$) of C_Y corresponds to a zero crossing point, Eq. (14) will be:

$$\text{CWT}\, S_{mix,i} = \text{CWT}\, \alpha_{X,i}\, C_X \qquad (15)$$

Eq. (15) indicated that the "transformed spectrum" of the binary mixture depends only C_X. The amplitude value (CWT $S_{X,i}$) of X is plotted versus C_X at the point corresponding to zero crossing of the transformed signal of Y in the same point (CWT $S_{Y,i}$ is equal to zero).

Linear regression line of X is obtained by plotting the transformed amplitude values (CWT $S_{X,i}$) versus the concentration of X at i corresponding to the zero crossing points of the transformed amplitude corresponding to Y. Analysis of Y is subjected to a similar procedure.

2.2.2. Calibration equations

The calibration graphs of HCT for the wavelet methods were constructed by plotting the transformed signals versus the concentration at the zero-crossing points of SP and vice versa.

The calibration graphs of MEX were obtained by measuring the transformed signals at 174 ($^{mex}S_{174}$), 318 ($^{mex}S_{318}$) and 343 ($^{mex}S_{343}$) for HCT (corresponding to zero crossing point of SP) and at 48 ($^{mex}S_{48}$), 153 ($^{mex}S_{153}$) for SP (corresponding to zero crossing point of HCT). By a similar procedure, the calibration graphs for HA were constructed by measuring the signal amplitude at 249 ($^{haar}S_{249}$) for HCT and at 98 ($^{haar}S_{98}$) and 237 ($^{haar}S_{237}$) for SP (see Table 1).

The amplitude values of the transformed signals were drawn as a graph versus concentrations of HCT and SP

Table 1: Linear regression analysis and its results

Methods	Range µg/ml	Equation	r	S_r	S_m	S_b	LOD µg/ml	LOQ µg/ml
MEX	2–22	$^{mex}S_{174} = -0.0050 - 0.0611\ C_{HCT}$	−0.9999	0.1647	0.0655	0.0672	0.50	1.68
		$^{mex}S_{318} = 0.0590 + 0.3827\ C_{HCT}$	0.9995	0.3481	0.1384	0.1421	0.51	1.69
		$^{mex}S_{343} = 0.2037 + 0.3783\ C_{HCT}$	0.9990	0.3472	0.1381	0.1417	0.39	1.29
		$^{mex}S_{48} = -0.0068 - 0.0104\ C_{SP}$	0.9994	0.0696	0.0277	0.0284	0.14	0.46
		$^{mex}S_{153} = 0.0061 - 0.0292\ C_{SP}$	−0.9987	0.1155	0.0459	0.0472	0.44	1.46
HA	2–22	$^{haar}S_{249} = 0.0040 - 0.0090\ C_{HCT}$	−0.9999	0.0646	0.0257	0.0264	0.56	1.86
		$^{haar}S_{98} = 0.0046 + 0.0004\ C_{SP}$	−0.9999	0.0465	0.0185	0.0190	0.26	0.88
		$^{haar}S_{237} = 0.0054 + 0.0005\ C_{SP}$	−0.9971	0.0501	0.0199	0.0205	0.51	1.70

C_{HCT} = HCT concentration (µg/mL)
C_{SP} = SP concentration (µg/mL)
^{mex}S and ^{haar}S = transformed signal of MEX and HA

r = Regression coefficient
Sr = Standard deviation of regression
Sm = Standard deviation of slope
Sb = Standard deviation of intercept
LOD = Limit of detection
LOQ = Limit of quantification

Table 2: Recoveries of HCT and SP in their mixtures by the applied methods

Added µg/mL		Recovery (%)							
		MEX (a = 30)					HA (a = 32)		
HCT	SP	HCT			SP		HTC	SP	
		$^{mex}S_{174}$	$^{mex}S_{318}$	$^{mex}S_{343}$	$^{mex}S_{48}$	$^{mex}S_{153}$	$^{haar}S_{249}$	$^{haar}S_{98}$	$^{haar}S_{237}$
2	10	105.9	103.8	101.3	95.9	103.8	100.8	103.0	99.2
6	10	105.2	113.1	95.0	95.3	103.9	96.4	101.7	98.9
10	10	100.8	103.0	97.9	103.4	99.4	97.9	101.4	96.6
14	10	100.1	106.0	98.4	108.4	96.1	97.1	96.1	97.0
18	10	101.3	104.8	98.2	108.4	96.2	101.1	94.9	97.8
22	10	97.4	97.9	92.3	103.4	100.8	98.5	97.8	97.2
10	2	99.8	108.2	100.3	105.8	95.9	99.8	92.8	96.9
10	6	99.7	103.0	97.8	102.4	95.3	95.6	98.3	98.0
10	10	99.8	103.0	96.8	103.1	97.5	96.8	100.2	98.7
10	14	108.8	105.6	102.3	104.8	105.0	97.9	104.4	106.6
10	18	104.5	103.2	95.3	101.6	104.1	99.0	101.5	102.6
10	22	107.4	104.3	96.3	101.5	102.4	96.8	99.4	101.4
	Mean =	102.5	104.6	97.7	102.8	100.0	98.1	99.3	99.2
	RSD =	1.06	0.33	3.57	3.83	1.04	2.91	2.60	1.54

RSD = Relative standard deviation

and a straight line was obtained for both active compounds in the above-indicated points. The quantitative analysis of HCT and SP in the samples was achieved by using the calibration equations (see Table 1). In all calibration equations the linear regression coefficients were higher than 0.9989. Detection limit (LOD) (signal to noise ratio 3 : 1) and quantitation limit (LOQ) (signal to noise ratio 10 : 1) were computed using the data obtained from ten replicate for standard solution of 10 µg/mL HCT and 10 µg/mL SP. All parameters of the linear regression analysis are indicated in Table 1. The linear regression lines obtained at the selected zero-crossing points indicated us that they are suitable for the determination of the compounds in this study.

2.2.3. Validation of the calibration graphs

In order to validate the above calibration graphs different composition mixtures were prepared. We applied the above signal analysing procedure on the synthetic mixture

Table 3: Analysis of the pharmaceutical formulation by the applied methods

Compound	HCT mg/tablet				SP mg/tablet			
Methods	MEX (a = 30)			HA (a = 32)	MEX (a = 30)		HA (a = 32)	
Point	$^{mex}S_{174}$	$^{mex}S_{318}$	$^{mex}S_{343}$	$^{haar}S_{249}$	$^{mex}S_{48}$	$^{mex}S_{153}$	$^{haar}S_{98}$	$^{haar}S_{237}$
Mean	24.59	25.64	24.20	24.54	26.03	24.27	24.73	24.91
SD	0.78	0.72	0.75	0.26	1.50	0.76	0.42	0.60
RSD	3.16	2.81	3.08	1.07	5.76	3.13	1.70	2.39
SE	0.45	0.42	0.43	0.15	0.87	0.44	0.24	0.34
CL (P = 0.005)	0.91	0.84	0.87	0.31	1.74	0.88	0.49	0.69

SD = Standard deviation. RSD= Relative standard deviation
SE = Standard error. CL= Confidence limit
Obtained results are the average of ten experiments for each method

for determination of both compounds and we observed that MEX and HA gave us satisfactory results (see Table 2). These results show that both methods are effective for the analysis of the two active compounds in synthetic mixtures.

2.2.4. Analysis of tablet content

The quantitative determination of HCT and SP was carried out using MEX and HA methods. The experimental results of the tablet formulation are summarised in Table 3. Good coincidence was observed from the assay results of the pharmaceutical formulations by application of the two methods described in this paper.

The excipients in tablets or matrix effect give a constant signal, which is eliminated in the working range of the transformed spectrum. This is a consequence of the wavelet transforms as well as it is a matter of choosing the zero-crossing points. In other words our method can be realized in presence of the interference of transformed spectra of both active compounds and in presence of the tablet excipients.

In the present study, CWT was successfully applied to the signal analysis of HCT and SP and their mixtures. The quantitative analysis of HCT and SP in tablets was carried out by the simultaneous use of the transformed spectra and the zero-crossing techniques.

A continuous one-dimensional MEX and HA followed by a calibration method similar to the zero-crossing method on the transformed signals have been applied to analyse a combination of HCT and SP without any chemical separation procedure. The transformed signal and the concentration have a very good linear correlation for the measured amplitudes corresponding to the zero crossing points of the working length. Another advantage of this method is that several calibration graphs can be used in the prediction of the contents of two active compounds. On the other hand the investigated method does not require any other complex calibration technique and it is a powerful tool for the resolution of binary mixture systems. The method is easy to apply, fast and cheap.

The obtained results are reliable in comparison with those given by chemometric methods (Martin et al. 1997b) and they are comparable with those achieved by HPLC (Bachman and Stewart 1990).

Taking into account the advantages of this new method we believe that it is appropriate to quality control and the routine analysis of multi-component mixtures and the pharmaceutical formulations.

On the other hand the software of CWT can be successfully implemented on UV-VIS spectrophotometry as well as to other analytical instruments.

3. Experimental

3.1. Instruments

A Shimadzu UV-1600 double beam UV-VIS spectrophotometer connected to a computer having Shimadzu UVPC software and an HP DeskJet 600 printer were used to record the UV-VIS absorption spectra.

The analysed signal subjected to MEX and HA transformations represents the recorded absorption spectra in the range of 210.0–289.8 nm. Calculations and the signal analysis were obtained by using EXCEL and MATLAB 6.5.

3.2. Pharmaceutical formulation

In this paper a pharmaceutical tablet formulation Aldactazide tablet (produced by Ali Raif Ileac Ind., Turkey. Batch no. 1C260) containing 25 mg SP, 25 mg HCT and excipients (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil and magnesium stearate) were analysed by the MEX and HA transformation methods.

3.3. Stock solutions

Acetic acid-sodium acetate buffer solution, 0.2 M and pH = 5, was prepared using analytical-reagent grade reagents. Stock solutions of 100 mg/ml of HCT and SP were prepared in a solvent containing methanol and 0.2 M acetate buffer solution, pH = 5 (80 : 20).

3.4. Standard solutions

A standard series of solutions containing between 2–20 μg/ml for HCT and SP was made from stock solutions. A validation set of 12 synthetic mixtures containing various concentrations of two active compounds was also prepared from the same stock solutions.

3.5. Tablet content analysis

Ten tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in methanol and 0.2 M acetate buffer solution, pH = 5 (80 : 20) in a 100 ml calibrated flask by sonication. The solution was filtered into a 100 ml calibrated flask through Whatman no.42 filter paper and diluted to an appropriate volume with the same solvent. The analysis of the solutions was performed with MEX and HA transforms in the spectral range of 210–290 nm.

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