

# Simultaneous Quantitative Resolution of Irbesartan and Hydrochlorothiazide in a Pharmaceutical Dosage Form By Signal Processing Techniques

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*Simultaneous Quantitative Resolution of Irbesartan and Hydrochlorothiazide in a Pharmaceutical Dosage Form By Signal Processing Techniques*

*Bir Farmasötik Dozaj Formundaki İrbesartan ve Hidroklorotiazid'in Sinyal İşleme Teknikleri ile Aynı Anda Miktar Tayini*

## SUMMARY

In this study, the continuous wavelet transform (CWT) was applied to the original absorption spectra and their ratio spectra for the determination of irbesartan (IRB) and hydrochlorothiazide (HCT) in a tablet dosage form. The proposed signal processing methods do not require a preliminary separation procedure. Calibration graphs were obtained by measuring CWT signals of the original spectra and ratio spectra at 243.0 nm and 235.9 nm for IRB and 273.8 nm and 262.8 nm for HCT, respectively. The validation of the proposed CWT methods was performed by using recovery studies, standard addition experiments and inter-day and intra-day analyses. The proposed CWT signal processing methods were successfully applied to the simultaneous quantitative estimation of IRB and HCT in tablets.

**Key Words:** Continuous wavelet transform, irbesartan, hydrochlorothiazide, signal processing techniques, quantitative analysis

## ÖZET

Bu çalışmada, tablet dozaj formunda bulunan irbesartan (IRB) ve hidroklorotiazid (HCT) etken maddelerinin tayini için bu maddelerin orijinal ve spektrum oranlarına sürekli dalgacık dönüşüm (SDD) yöntemi uygulandı. Önerilen sinyal işleme yöntemleri herhangi bir ön ayırma işlemi gerektirmemektedir. Kalibrasyon grafikleri, orijinal ve spektrum oranlarına sırasıyla, IRB için 243.0 nm ve 235.9 nm, HCT için 273.8 nm ve 262.8 nm'de SDD sinyallerinin ölçülmesi ile elde edildi. Önerilen SDD yöntemlerinin validasyonu geri kazanım çalışması, standart ekleme deneyleri ve gün içi – günler arası analizleri ile gerçekleştirildi. Önerilen SDD sinyal işleme yöntemleri, tabletlerdeki IRB ve HCT'in aynı anda miktar tayinlerine başarıyla uygulandı.

**Anahtar kelimeler:** Sürekli dalgacık dönüşüm, irbesartan, hidroklorotiazid, sinyal işleme teknikleri, miktar tayini

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## INTRODUCTION

Nowadays, the use of the combined pharmaceutical dosage forms containing two or more drug substances has been increased to get more effective treatments. The simultaneous quantitative analysis of drug compounds in pharmaceutical samples with the presence of excipients is one of the main problems of analytical chemistry. This requires very powerful analytical methods giving reliable, precise and accurate assay results.

For this aim, the analytical methods e.g. spectrophotometry, mass spectrometry, chromatography and electrophoresis, electrochemistry and their combined devices have been used. For example, the separation techniques, LC and CE combined with various spectroscopic systems (hyphenated techniques namely LC-MS and CE-MS) have been applied to obtain additional chemical information and to decrease the complexity of multicomponent mixtures in chemical and pharmaceutical analysis. Particularly, LC method of them has been proposed as main or comparison method for the analysis of active compounds in pharmaceutical and other samples. In applications, these chromatographic analysis approaches require a preliminary separation and other tedious analytical processes during analysis for searching optimal separation and other experimental conditions. In addition, these methods using the combined devices bring high cost and time-consuming for analysis. In some cases, the separation methods based on high technology may not provide desirable analytical results for the combined pharmaceutical preparations.

Due to the disadvantages of the separation techniques, the use of spectroscopic methods instead of the mentioned chromatographic methods are preferable to get rapid analysis with low cost for the complex pharmaceutical dosage forms.

Particularly derivative spectrophotometry and its modified versions have been intensively utilized in fast quantitative resolution of multi-component mixtures without separation step. However, in all cases, these spectral methods may not give expected analytical results due to strongly overlapping spectra of the analyzed compounds, interference of main peaks with noise, baseline problems, decreasing signal intensity and worsening signal-to-noise ratio (S/N) for higher derivative orders. Recent developments in signal processing methods provided us more opportunity for the better quantitative resolution of the complex analytical problems as well as other areas of science. One of the newest signal processing methods is wavelets for the spectral estimation of compounds in mixtures. Wavelet transform (WT) has gained wide acceptance as a powerful tool for signal analysis, due to their wide range of applications. WT is classified into two categories; discrete wavelets transform (DWT) and continuous wavelets transform (CWT) (1).

In analytical chemistry and related branches, the CWT approach has been used for data reduction, de-noising,

baseline correction and resolution of overlapping spectra (2, 3). The applications of spectroscopic techniques, especially UV-Vis spectrophotometry combined with CWT methods have been increased the potential power of spectroscopic analysis of multicomponent mixtures. In previous studies, the combined use of the CWT methods with zero-crossing technique and ratio signals gives new opportunities for the spectrophotometric simultaneous resolution of mixtures without using preliminary separation step (4-9). Several analytical methods, including derivative spectrophotometric methods (10-12) and high performance liquid chromatographic methods (13-15) were reported for the analysis of IRB and HCT in pharmaceutical and biological studies.

In this study, new signal processing methods Symlet5 continuous wavelet transform – zero crossing technique (SYM5-CWT) and ratio spectra-bior1.3 continuous wavelet transform (RS-bior1.3-CWT) were applied to the simultaneous quantification of IRB and HCT in tablets. The CWT signal processing methods were validated and applied to the real samples containing IRB and HCT tablets. A good agreement was reported for the assay results.

## EXPERIMENTAL SECTION

### Instruments

In the UV data collection, the absorption spectra of the compounds and their samples in the spectral region of 200-305 nm were recorded by using a Shimadzu UV-1601 double beam UV-VIS spectrophotometer having a fixed slit width (2 nm) connected with a computer loaded with Shimadzu UVPC software and a LEX-MARK E-320 printer. In the application of the approaches, the Microsoft EXCEL and Wavelet Toolbox in Matlab 7.0 software were used for data treatments, regressions and statistical analysis.

### Commercial tablet product

A commercial tablet formulation (KARVEZIDE® Tablet, Sanofi-Aventis. Ind., Istanbul, Turkey), containing 150 mg of IRB and 12.5 mg of HCT per tablet was collected from local Turkish market. IRB and HCT reference substances were kindly donated from National Pharm. Ind. Companies, Turkey).

### Standard solutions

A stock standard solution of IRB and HCT was separately prepared by dissolving 10 mg of each drug in 100 mL methanol. For the spectral analysis, a calibration series for each drug between 4.0-32.0  $\mu\text{g mL}^{-1}$  for IRB and 2.0-9.0  $\mu\text{g mL}^{-1}$  for HCT in the above solvent was prepared from the standard stock solutions. An independent validation set consisting of 16 synthetic mixture solutions of IRB and HCT in the above working concentration range was prepared. For the standard addition technique, the sample solutions were prepared by adding the stock solution of each compound to tablets at three different concentration levels for six replicates to evaluate the interference of excipients on the analysis.

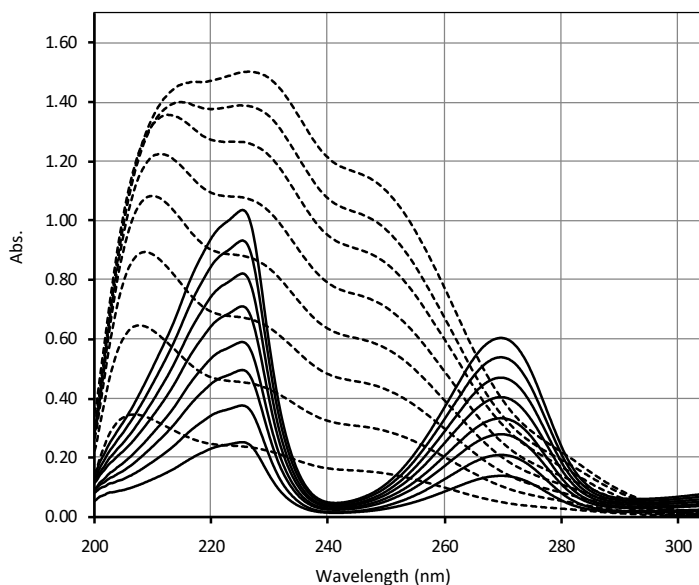
In addition, the synthetic sample solutions at three different concentration levels for six times were prepared for testing intra-day and inter-day analysis.

#### Sample solutions preparation

For analysis of commercial tablets; twenty tablets containing IRB and HCT were weighed and crushed into fine powder. A quantity of powder equivalent to one tablet was transferred to in 100 ml volumetric flask and then volume was made up to mark with methanol. The content of the flask was mechanically shaken for 30 min. After filtration, the supernatant was diluted with methanol to obtain final concentrations. This sample preparation was repeated ten times.

## RESULTS and DISCUSSION

This study aims to apply new powerful signal processing methods to the simultaneous quantification of IRB and HCT in their synthetic mixtures and tablets. The UV spectra of IRB and HCT standard series and tablet solution were recorded between 200-305 nm as shown in Figure 1. As can be seen, the UV spectra of two drugs overlapped strongly in same spectral region. In this situation, the simultaneous quantitative spectral analysis of IRB and HCT is not possible by direct conventional absorbance measurement due to the overlapping spectra of the analyzed drugs. However, we focused mainly on the application of the SYM5-CWT and RS-bior1.3-CWT spectra treatment to the quantitative resolution of IRB-HCT mixtures without using a separation procedure.



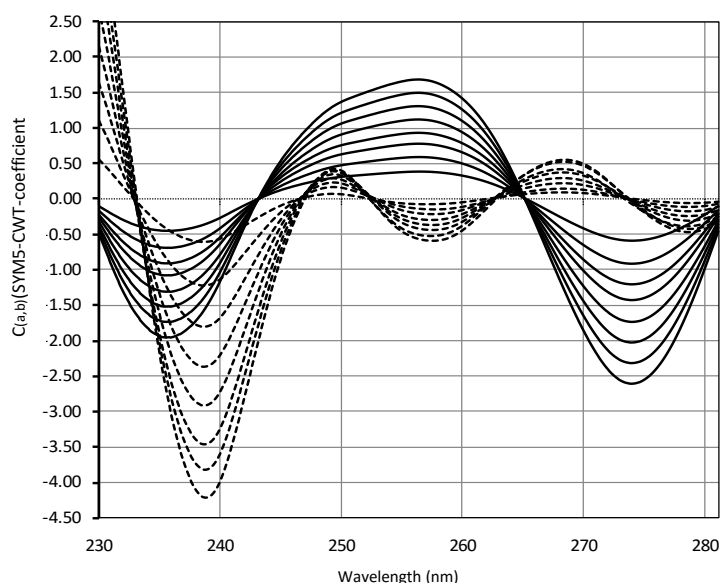
**Figure 1.** The UV absorption spectra of 4.0-36  $\mu\text{g mL}^{-1}$  IRB (---) and 2.0-9.0  $\mu\text{g mL}^{-1}$  HCT (—) in methanol

#### Continuous Wavelet Transform-Zero Crossing Method (SYM5-CWT)

The UV absorption spectra of standard calibration solutions of IRB and HCT in the linear range 4.0-36.0  $\mu\text{g mL}^{-1}$  for IRB and 2.0-9.0  $\mu\text{g mL}^{-1}$  for HCT were recorded with intervals of  $\Delta\lambda=0.1$  nm between 200.0-305.0 nm as shown in Figure 1. The UV spectra of the synthetic mixture and commercial samples were plotted as well as calibration samples. The UV absorbance vectors of standard calibration and samples of the related drugs were processed in the wavelet domain. Various wavelet

families at different scale parameters (a) were tested for the transformation of the UV absorbance vectors to obtain the best recovery results. After that, SYM5-CWT was found to be suitable for the analysis of IRB and HCT in their samples using original UV spectra. The spectra of SYM5-CWT was obtained and indicated in Figure 2.

Calibration curves for IRB and HCT were computed by measuring CWT amplitude at 243.0 nm and 273.8 nm, respectively.



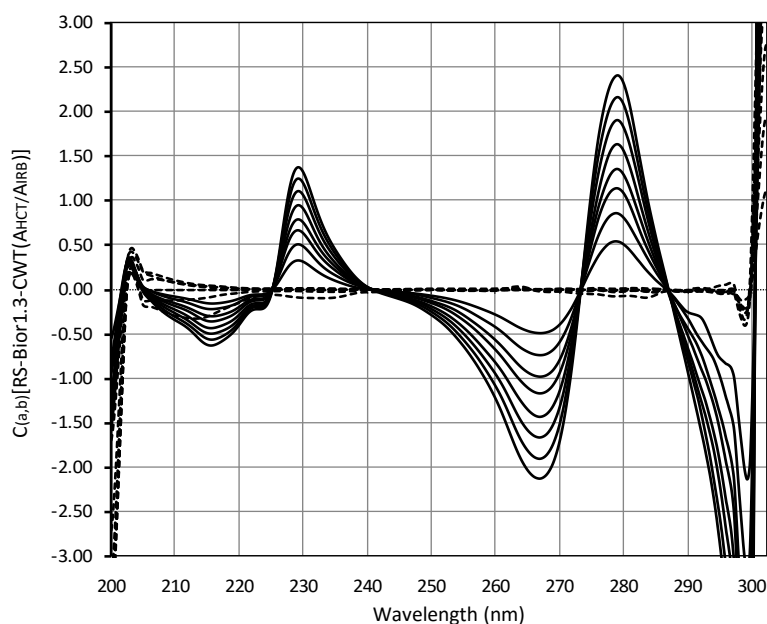
**Figure 2.** SYM5-CWT spectra obtained by transforming the UV absorption spectra of  $4.0\text{--}36\ \mu\text{g mL}^{-1}$  IRB (---) and  $2.0\text{--}9.0\ \mu\text{g mL}^{-1}$  HCT (—).

#### Ratio Spectra-Continuous Wavelets Transform Method (RS-bior1.3-CWT)

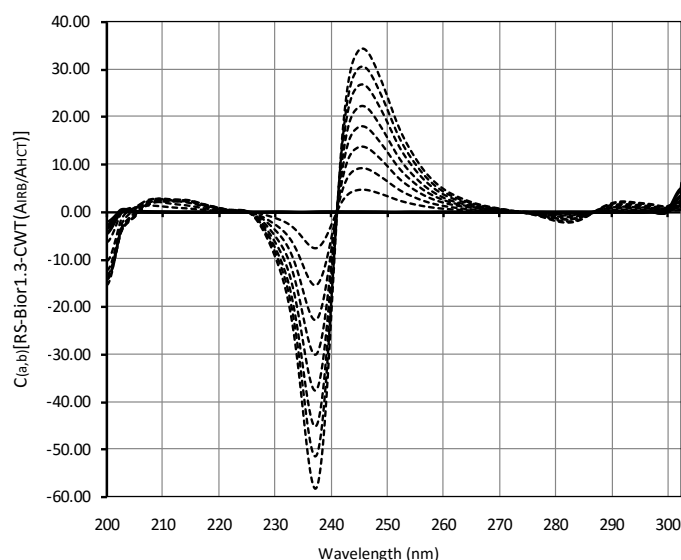
This combined approach for the determination of IRB and HCT in their mixtures is based on the application of the continuous wavelet analysis to the ratio spectra consisting of HCT/IRB and IRB/HCT, respectively. After testing many wavelet functions, bior1.3 family was found to be adequate for the quantitative analysis of the mentioned drugs. The application of bior1.3 continuous wavelet transform to the ratio spectra of the ana-

lyzed drugs denotes RS-bior1.3-CWT.

The UV spectra of the IRB and HCT, and their tablet solution were recorded in the range  $200.0\text{--}305.0\ \text{nm}$  and divided by the standard spectrum of  $16\ \mu\text{g mL}^{-1}$  IRB. The same procedure was repeated for the standard spectrum of  $4\ \mu\text{g mL}^{-1}$  HCT as a divisor, respectively. Thus, the ratio spectra of HCT/IRB and IRB/HCT were obtained and presented in Figure 3 and Figure 4, respectively.



**Figure 3.** RS-bior1.3-CWT spectra obtained by transforming the UV absorption spectra of  $4.0\text{--}36\ \mu\text{g mL}^{-1}$  IRB (---) and  $2.0\text{--}9.0\ \mu\text{g mL}^{-1}$  HCT (—) (Divisor:  $16\ \mu\text{g/mL}$  IRB)



**Figure 4.** RS-bior1.3-CWT spectra obtained by transforming the UV absorption spectra of  $4.0\text{--}36\ \mu\text{g mL}^{-1}$  IRB (---) and  $2.0\text{--}9.0\ \mu\text{g mL}^{-1}$  HCT (—). (Divisor:  $4\ \mu\text{g/mL}$  HCT)

The calibration graphs for IRB and HCT were calculated at the selected wavelength points ( $235.9\ \text{nm}$  for IRB and  $262.8\ \text{nm}$  for HCT). The statistical results of the calibration graphs calculated by using the linear regression analyses were summarized in Table 1.

#### Validation of the Proposed Methods

In this study, the proposed methods were validated by using the recovery studies, intra-day and inter-day assays and standard addition technique. In the application of the developed methods, a good linearity with the higher correlation coefficients was reported in the concentration ranges as indicated in Table 1.

**Table 1.** Linear regression analysis and its statistical results for the proposed methods

Method	SYM5-CWT		RS-bior1.3-CWT	
Parameter	IRB	HCT	IRB	HCT
$\lambda$ (nm)	243.0	273.8	235.9	262.8
m	$-6.55 \times 10^{-2}$	-0.2852	-1.61	$-1.86 \times 10^{-1}$
n	$-4.19 \times 10^{-2}$	-0.0381	$-9.08 \times 10^{-2}$	$-1.91 \times 10^{-2}$
r	0.9994	0.9996	0.9996	0.9994
SE (m)	$9.40 \times 10^{-4}$	$3.23 \times 10^{-3}$	$1.97 \times 10^{-2}$	$2.55 \times 10^{-3}$
SE (n)	$3.90 \times 10^{-3}$	$1.92 \times 10^{-3}$	$3.98 \times 10^{-2}$	$1.05 \times 10^{-2}$
SE (r)	$2.44 \times 10^{-2}$	$2.09 \times 10^{-2}$	$5.11 \times 10^{-1}$	$1.65 \times 10^{-2}$
LOD ( $\mu\text{g/mL}$ )	0.51	0.06	0.21	0.48
LOQ ( $\mu\text{g/mL}$ )	1.68	0.19	0.70	1.60

**Table 2.** Recovery results obtained by synthetic mixtures

SYM5-CWT						RS-bior1.3-CWT			
Mixture ( $\mu\text{g mL}^{-1}$ )		Found ( $\mu\text{g mL}^{-1}$ )		Recovery (%)		Found ( $\mu\text{g mL}^{-1}$ )		Recovery (%)	
IRB	HCT	IRB	HCT	IRB	HCT	IRB	HCT	IRB	HCT
30	2.0	29.71	1.94	99.0	96.9	29.06	1.91	96.9	95.5
30	3.0	29.84	2.93	99.5	97.6	28.85	2.86	96.2	95.4
30	4.0	30.51	3.97	101.7	99.2	29.10	3.93	97.0	98.3
30	5.0	30.80	5.02	102.7	100.4	28.97	4.97	96.6	99.4
30	6.0	30.69	5.96	102.3	99.4	29.12	5.90	97.1	98.4
30	7.0	30.90	6.91	103.0	98.8	29.06	6.82	96.9	97.5
30	8.0	30.07	7.77	100.2	97.1	29.23	7.73	97.4	96.6
30	9.0	30.98	8.78	103.3	97.5	29.11	8.64	97.0	96.0
4.0	2.5	3.97	2.53	99.3	101.0	3.81	2.53	95.3	101.1
8.0	2.5	8.18	2.48	102.2	99.1	7.93	2.44	99.2	97.8
12	2.5	12.48	2.57	104.0	102.9	12.14	2.52	101.2	100.9
16	2.5	16.71	2.51	104.4	100.2	16.34	2.46	102.1	98.4
20	2.5	20.94	2.46	104.7	98.3	20.38	2.49	101.9	99.6
24	2.5	24.88	2.46	103.7	98.5	24.22	2.44	100.9	97.6
28	2.5	28.93	2.46	103.3	98.5	28.07	2.46	100.2	98.3
32	2.5	32.55	2.43	101.7	97.4	31.64	2.47	98.9	98.7
Mean				103.6		99.3		100.9	
SD				1.07		1.98		1.20	
RSD				1.03		1.99		1.19	

SD= Standard deviation  
RSD= Relative standard deviation

To test the performance of the proposed methods, the recovery studies were performed by applying the proposed signal processing methods (SYM5-CWT and RS-bior1.3-CWT) to the analysis of the independent validation set consisting of the IRB-HCT mixtures at the

different concentration levels (Table 2). Good accuracy and precision for the results obtained from the methods were reported. In addition, precision and accuracy were evaluated by the inter-day (n=6) and intra-day (n=6) tests at three different concentration levels (Table 3).

**Table 3.** Results obtained from the analysis of intra-day and inter-day samples by the proposed signal processing methods

		Intra-day (n=6)						Inter-day (n=6)					
		Added ( $\mu\text{g mL}^{-1}$ )	Found ( $\mu\text{g mL}^{-1}$ )	SD	RSD	RE	Rec. (%)	Found ( $\mu\text{g mL}^{-1}$ )	SD	RSD	RE	Rec. (%)	
SYM5-CWT	IRB	4	3.99	0.07	1.66	-0.21	99.8	4.03	0.06	1.54	0.80	100.8	
		16	16.72	0.17	1.02	4.52	104.5	16.66	0.16	0.97	4.11	104.1	
		30	31.96	0.59	1.84	6.55	100.1	32.08	0.19	0.60	6.93	106.9	
	HCT	2	1.97	0.01	0.60	-1.69	100.1	1.98	0.05	2.44	-1.12	98.9	
		5	4.84	0.04	0.90	-3.12	96.9	4.88	0.07	1.33	-2.37	97.6	
		8	7.58	0.11	1.50	-5.28	94.7	7.62	0.02	0.21	-4.76	95.2	
RS-bior1.3-CWT	IRB	4	3.89	0.07	1.88	-2.67	97.3	4.05	0.23	5.76	1.26	101.3	
		16	15.98	0.15	0.95	-0.11	99.9	15.91	0.14	0.86	-0.55	99.4	
		30	29.19	0.44	1.50	2.70	100.1	29.31	0.14	0.46	-2.29	97.7	
	HCT	2	1.99	0.01	0.65	0.71	100.1	2.00	0.05	2.50	0.20	100.2	
		5	4.79	0.04	0.92	-4.20	95.8	4.84	0.06	1.20	-3.15	96.9	
		8	7.84	0.08	1.06	-1.95	98.1	7.92	0.05	0.67	-1.06	98.9	

SD= Standard deviation  
RSD= Relative standard deviation  
SE= Standard error



The selectivity of the signal processing methods was studied by calculating the mean recovery of the subject matter compounds by adding standards known concentrations to the tablet samples. Appropriate volumes of the standard stock solutions of IRB and HCT compounds at three different concentration levels were

added to the tablet sample solutions, respectively. This procedure was repeated six times for each concentration level (Table 4). According to the recovery studies and standard addition technique, it was observed that the numerical results obtained were in a good agreement.

**Table 4.** Results obtained from the analysis of the standard addition samples by the proposed signal processing methods

		Added ( $\mu\text{g/mL}$ )	Recovery (n=6)					Mean	SD	RSD	RSE
SYM5-CWT	IRB	4.0	101.2	100.7	103.5	101.1	99.9	101.3	1.33	1.31	1.29
		8.0	100.1	97.3	97.6	101.2	100.1	99.3	1.71	1.72	-0.74
		12.0	98.1	100.7	100.2	98.6	101.2	99.7	1.34	1.34	-0.25
	HCT	2.0	103.7	104.8	104.6	105.2	105.7	104.8	0.74	0.71	4.83
		4.0	102.4	102.8	102.9	101.9	101.9	102.4	0.48	0.47	2.40
		8.0	98.9	99.8	100.1	99.7	99.7	99.6	0.46	0.47	-0.35
RS-bior1.3-CWT	IRB	4.0	101.4	101.7	98.4	97.7	101.4	100.1	1.91	1.90	0.10
		8.0	103.6	105.5	105.5	104.1	104.0	104.5	0.88	0.84	4.50
		12.0	102.6	102.5	101.2	100.4	100.6	101.5	1.07	1.05	1.46
	HCT	2.0	101.9	101.8	102.2	104.5	103.6	102.8	1.18	1.15	2.80
		4.0	100.8	101.0	100.8	100.3	99.9	100.5	0.45	0.45	0.54
		8.0	97.5	98.5	98.7	98.6	98.5	98.4	0.49	0.50	-1.65

SD= Standard deviation, RSD= Relative standard deviation, RSE= Relative standard error

## ASSAY RESULTS of COMMERCIAL TABLETS

Assay results obtained by the application of the proposed signal processing methods to the IRB-HCT tablet samples were indicated in Table 5. A good coincidence was reported between the assay tablet results obtained by

the proposed signal processing methods. In the tablet analysis, the interference of the tablet excipients on the determination of the related compounds as well as the analysis of standard addition samples, was not observed in application of proposed methods to the commercial tablets.

**Table 5.** Analysis results of tablets containing IRB and HCT by the proposed methods

mg tablet <sup>-1</sup> (n=10)				
SYM5-CWT			RS-bior1.3-CWT	
	IRB	HCT	IRB	HCT
Mean	151.75	12.38	147.98	12.37
SD	1.12	0.13	1.04	0.13
RSD	0.74	1.02	0.71	1.09
SE	0.35	0.04	0.33	0.04
CL	0.69	0.08	0.65	0.08

SD= Standard deviation, RSD= Relative standard deviation, SE= Standard error, CL= Confidence limit

## CONCLUSIONS

In this work, new hyphenated signal processing approaches, SYM5-CWT and RS-bior1.3-CWT were proposed and applied to the spectral quantification of IRB and HCT in tablets. These signal processing methods do not require any separation step for the analysis of both compounds having the strong overlapping spectra in the same spectral region (see Figure 1). The use of

the CWT method is new and promising approaches for the quantification of the related drugs. It was concluded that the proposed CWT methods were more precise, accurate and economic than conventional analytical methods.

In conclusion, the proposed methods can be used for the quantitative estimation and routine quality control of the tablets containing IRB and HCT.

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