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ANALYSIS

APPLICATION OF DERIVATIVE SPECTROPHOTOMETRY FOR DETERMINATION OF ENALAPRIL, HYDROCHLOROTHIAZIDE AND WALSARTAN IN COMPLEX PHARMACEUTICAL PREPARATIONS

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Abstract: A derivative spectrophotometry method was developed to determine enalapril, hydrochlorothiazide, candesartan and walsartan in complex antihypertensive drugs. The pharmaceutical preparations containing hydrochlorothiazide and one of the angiotensin convertase inhibitors were investigated.
It was found that the developed method enables the constituents of the investigated drugs to be determined directly despite evident interference of the zero order absorption spectra. For determination of enalapril and hydrochlorothiazide as well as candesartan and hydrochlorothiazide the first derivative was used, while for walsartan and hydrochlorothiazide the second derivative was employed. The method was of high sensitivity; the LOD accuracy for enalapril was 2.81 $\mu g \times mL^{-1}$, 0.56 $\mu g \times mL^{-1}$ for candesartan, 4.02 $\mu g \times mL^{-1}$ for walsartan and ranged from 0.31 $\mu g \times mL^{-1}$ to 1.78 $\mu g \times mL^{-1}$ for hydrochlorothiazide, depending on preparation under investigation . The recovery of individual constituents was within the limit of 100% ± 5%, RSD varied from 1.11% to 2.94%, and the linearity range was from 4.1 $\mu g \times mL^{-1}$ to 20.5 $\mu g \times mL^{-1}$ for enalapril, from 6.45 $\mu g \times mL^{-1}$ to 32.25 $\mu g \times mL^{-1}$ for walsartan, from 2.36 $\mu g \times mL^{-1}$ to 11.80 $\mu g \times mL^{-1}$ for candesartan, and from 0.96 $\mu g \times mL^{-1}$ to 26.00 $\mu g \times mL^{-1}$ for hydrochlorothiazide.

Keywords: enalapril, walsartan, candesartan, hydrochlorothiazide, derivative spectrophotometry

The use of complex drug preparations is intended to enhance efficiency in chronic disease management due to synergic effects. The drugs belonging to angiotensin convertase inhibitors are often administered in combination with diuretics, thus increasing their hypotensive activity.

There are many analytical study literature reports related to the determination of enalapril, walsartan, candesartan and hydrochlorothiazide in pharmaceutical products and body fluids (1).

Hydrochlorothiazide and photodegradation products were determined spectrophotometrically using the first derivative spectra and HPLC, often in combination with mass spectrometry (2-5). Good results were obtained in the determination of hydrochlorothiazide using voltamperometry (6). Bioavailabilities of hydrochlorothiazide were determined by the use of HPLC with the Diode Array Detector (7).

To determine enalapril, liquid chromatography in combination with mass spectrometry (8, 9) and spectrofluorometry were used (10). The GC-MS method was employed for determining enalapril and its active metabolite in blood plasma and body fluids (11). Good results were obtained when determining enalapril in pharmaceutical preparations using proton magnetic resonance spectroscopy (^{1}H NMR) and capillary electrophoresis (12, 13).

Walsartan and candesartan and their metabolites were determined by gas chromatography in combination with mass spectrometry and spectrofluorometry (14, 15). Walsartan was determined in pharmaceutical products by employing derivative spectrophotometry and the second derivative spectra (16). Simultaneous determination of hydrochlorothiazide and walsartan and candesartan was carried out using micellar electrokinetic capillary chromatography and capillary electrophoresis (17).

The constituents of complex drugs often have similar physicochemical properties, thus posing problems in selection of appropriate determination methods. The methods recommended for analysis of single-component drugs usually prove unsuccessful and this is why it is still necessary to seek for new methods. We have therefore attempted to develop a derivative spectrophotometry method for

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determining hydrochlorothiazide in pairs with enalapril, walsartan and candesartan appearing in complex drugs.

EXPERIMENTAL

Apparatus

Spectrophotometer UV-VIS Cary 100 (Varian), quartz cuvettes of layer thickness l = 1 cm. Computer – PC Pentium MMX, 16 MB RAM, Brother HL – 1430 printer and software (Microsoft Office 2003, Statistica 7.1 edition 2007).

Standard and reference solutions

The standard solutions were prepared in methanol at concentrations equal to those of the standards specified for complex drugs.

a) for preparation of Enap HL

Enalapril maleate (LGC Promochem, USA) solution 4.107 $mg \times mL^{-1}$,

Hydrochlorothiazide (Merck, USA) solution 1.04 $mg \times mL^{-1}$.

Reference solution (EHL) was prepared by mixing appropriate solutions of individual constituents.

For direct determination purposes, solutions were diluted with methanol until the following concentrations were reached: enalapril from 4.1 $\mu g \times mL^{-1}$ to 20.5 $\mu g \times mL^{-1}$ and hydrochlorothiazide from 5.2 $\mu g \times mL^{-1}$ to 26.00 $\mu g \times mL^{-1}$.

b) for preparation Co-Diovan

Walsartan (LGC Promochem, USA) solution 1.29 $mg \times mL^{-1}$,

Hydrochlorothiazide (Merck, USA) solution 3.84 $mg \times mL^{-1}$

Reference solution (COD) was prepared by mixing appropriate solutions of individual constituents.

For direct determination purposes, solutions were diluted with methanol until the following concentrations were reached: walsartan from 6.45 $\mu g \times mL^{-1}$ to 32.25 $\mu g \times mL^{-1}$ and hydrochlorothiazide from 0.96 $\mu g \times mL^{-1}$ to 4.80 $\mu g \times mL^{-1}$.

c) for preparation Blopress Plus

Candesartan cilexetil (Tianyu Pharmaceutical Co., Ltd., China) solution 0.472 $mg \times mL^{-1}$,

Hydrochlorothiazide (Merck, USA) solution 3.84 $mg \times mL^{-1}$

Reference solution (BLP) was prepared by mixing appropriate solutions of individual constituents.

For direct determination purposes, solutions were diluted with methanol until the following concentrations were reached: for candesartan from 2.36 $\mu g \times mL^{-1}$ to 11.80 $\mu g \times mL^{-1}$ and for hydrochlorothiazide from 1.92 $\mu g \times mL^{-1}$ to 9.60 $\mu g \times mL^{-1}$.

Sample solutions

10 tablets of each tested preparation were powdered and weighed to nearest 0.1 mg as follows:

a) Preparation Enap HL

10.0 mL of methanol was added to 167.00 mg of powdered tablets, shaken for 15 minutes, centrifuged at 1500 rpm. For determination purposes 150 µL of solution was taken into 10.0 mL flasks and diluted to volume with methanol.

b) Preparation Co-Diovan

10.0 mL of methanol was added to 62.70 mg of powdered tablets, shaken for 15 minutes, centrifuged at 1500 rpm. For determination purposes 50 µL of solution was taken into 10.0 mL flasks and diluted to volume with methanol.

c) Preparation Blopress Plus

10.0 mL of methanol was added to 63.00 mg of powdered tablets, shaken for 15 minutes, centrifuged at 1500 rpm. For determination purposes 100 µL of solution were taken into 10.0 mL flasks and diluted to volume with methanol.

Reagents

Methanol of analytical purity (Merck).

RESULTS AND DISCUSSION

Determination conditions

In the first stage of these research studies the absorption spectra were recorded for appropriate solutions containing the standards at concentrations close to label claims for a given constituent, individually or in relevant mixtures. The spectrophotometric measurements were made in UV at wavelength range from 200 nm to 400 nm in methanol.

The absorption spectra of individual constituents show absorbance maxima and minima at similar wavelengths, thus causing interferences when analyzed components are present together. It should be noted that there are significant differences in absorbance of enalapril, walsartan and candesartan with respect to hydrochlorothiazide, which is present together with these constituents.

The transformation of the zero order spectra into first derivatives led to significant diversification. For hydrochlorothiazide and enalapril the values of the first derivative for the former component at λ_{278nm} led to a well developed peak with no interference with the same derivative at λ_{225nm} for enalapril.

Similar results were obtained for the walsartan mixture at λ_{261nm} and λ_{284nm} for hydrochlorothiazide in the case of second derivative and for candesartan at $\lambda_{270.4nm}$ and hydrochlorothiazide at λ_{284nm} for the first derivative spectra (Figures. 1-3).

An analysis of derivative curves was carried out using the zero crossing technique for reference solutions. At selected wavelength the variations of a derivative versus concentration were only observed when no interferences occurred.

The obtained results enabled us to proceed further and develop a method for simultaneous determination of these constituents in pharmaceutical products.

Therefore, the specifity, linearity, limit of detection, limits of determination and accuracy were determined.

Specificity

Due to lack of information about matrix composition, solutions of appropriate constituents under consideration in reference solutions and medicines under investigation were compared. For this purpose solutions of similar compositions were prepared containing appropriate constituents at amounts equal to 75%, 100% and 125% with respect to its declared contents in pharmaceutical products. The results obtained for appropriate reference mixtures (M_m) and drugs are presented Table 1.

Analyzing the values of individual derivatives one can see that they are comparable and the differences are within the method admissible error. The results of this study indicate also no effect of matrix components on the measurements made at the selected wavelengths, thus the method seems to be specific to the analyte under investigation.

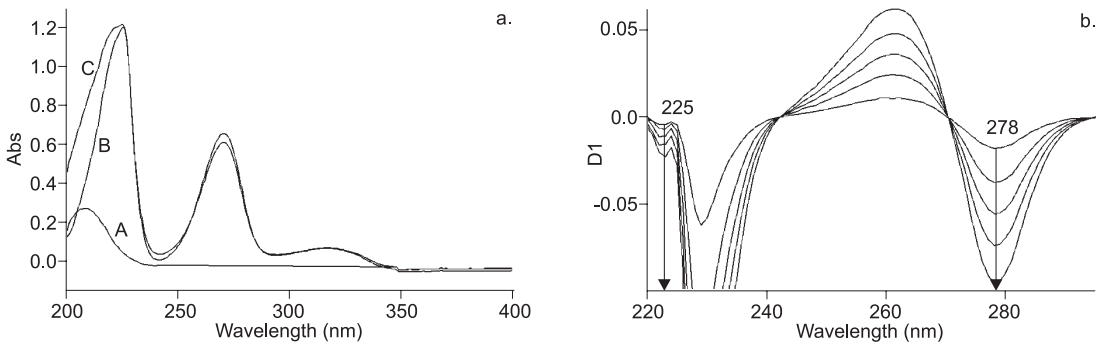

Figure 1. a) The zero order absorption spectra for enalapril (A), hydrochlorothiazide (B) and preparation Enap HL (C). b). The first derivative values vs. enalapril concentration (c_1 = 4.1 μg×mL^{-1}; c_2 = 8.2 μg×mL^{-1}; c_3 = 12.3 μg×mL^{-1}; c_4 = 16.4 μg×mL^{-1}; c_5 = 20.5 μg×mL^{-1}, λ = 225 nm) and hydrochlorothiazide (c_1 = 5.2 μg×mL^{-1}; c_2 = 10.4 μg×mL^{-1}; c_3 = 15.6 μg×mL^{-1}; c_4 = 20.8 μg×mL^{-1}; c_5 = 26.0 μg×mL^{-1}, λ = 278 nm) in reference mixtures.

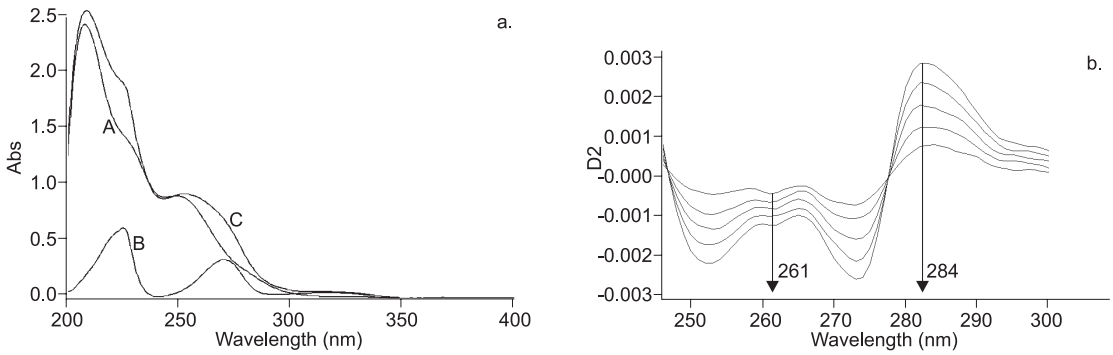

Figure 2. a) The zero order absorption spectra for walsartan (A), hydrochlorothiazide (B) and preparation Co-Diovan (C). b) The second derivative values vs. walsartan concentration (c_1=6.45 μg×mL^{-1}; c_2 = 12.90 μg×mL^{-1}; c_3 = 19.35 μg×mL^{-1}; c_4 = 25.80 μg×mL^{-1}; c_5 = 32.25 μg×mL^{-1}, λ = 261 nm) and hydrochlorothiazide (c_1 = 0.96 μg×mL^{-1}; c_2 = 1.92 μg×mL^{-1}; c_3 = 2.88 μg×mL^{-1}; c_4 = 3.84 μg×mL^{-1}; c_5 = 4.80 μg×mL^{-1}, λ = 284 nm) in reference mixtures.

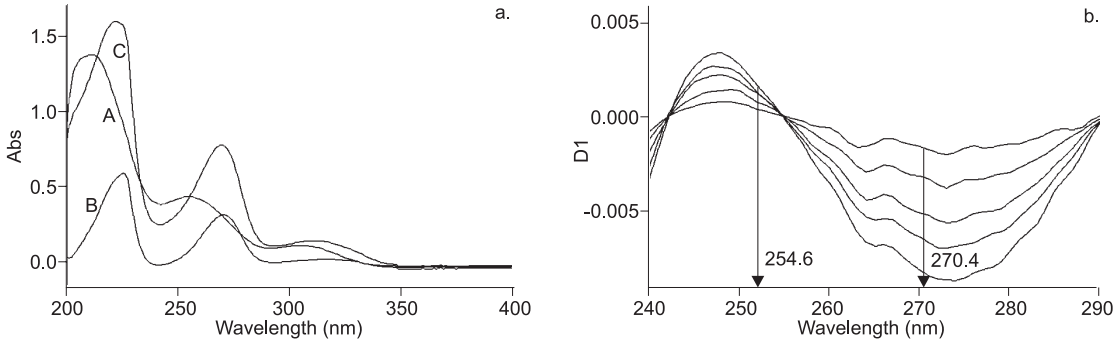

Figure 3. a) The zero order absorption spectra for candesartan (A), hydrochlorothiazide (B) and preparation Blopress Plus (C). b) The first derivative values vs. candesartan concentration (c_1 = 2.36 μg×mL^{-1}; c_2 = 4.72 μg×mL^{-1}; c_3 = 7.08 μg×mL^{-1}; c_4 = 9.44 μg×mL^{-1}; c_5 = 11.8 μg×mL^{-1}, λ = 270.4 nm) and hydrochlorothiazide (c_1 = 1.92 μg×mL^{-1}; c_2 = 3.84 μg×mL^{-1}; c_3 = 5.76 μg×mL^{-1}; c_4 = 7.68 μg×mL^{-1}; c_5 = 9.60 μg×mL^{-1}, λ = 254.6 nm) in reference mixtures.

Linearity

To check the linearity, five measurements were made for reference solutions at the following concentrations:
for enalapril
from 4.1 μg×mL^{-1} to 20.5 μg×mL^{-1}
for walsartan
from 6.45 μg×mL^{-1} to 32.25 μg×mL^{-1}
for candesartan
from 2.36 μg×mL^{-1} to 11.80 μg×mL^{-1}
for hydrochlorothiazide
from 0.96 μg×mL^{-1} to 26.00 μg×mL^{-1}

It was found that linearity was maintained within the concentration ranges under examination. The results were evaluated by using linear regression equation that specified both intersection points and correlation coefficient. The results obtained are presented in Table 2.

Limit of detection (LOD) and limit of determination (LOQ)

Both LOD and LOQ were computed using the standard estimation error (S_Y) and the slope of calibration curve (a) from the following equations: LOD = 3.3·S_Y/a and LOQ = 10.0·S_Y/a. The following results were obtained for individual consituents:

EHL
Hydrochlorothiazide:
LOD = 1.78 μg×mL^{-1} LOQ = 5.40 μg×mL^{-1}
Enalapril:
LOD = 2.81 μg×mL^{-1} LOQ = 8.51 μg×mL^{-1}
COD
Hydrochlorothiazide:
LOD = 0.31 μg×mL^{-1} LOQ = 0.95 μg×mL^{-1}
Walsartan:
LOD = 4.02 μg×mL^{-1} LOQ = 12.17 μg×mL^{-1}
BLP
Hydrochlorothiazide:
LOD = 0.69 μg×mL^{-1} LOQ = 2.10 μg×mL^{-1}
Candesartan:
LOD = 0.56 μg×mL^{-1} LOQ = 1.71 μg×mL^{-1}

Accuracy

The accuracy of the method was estimated based on the percentage recovery of the analyte that was added to weighed amounts at 80% to 120% compared to the declared amounts. The results of determinations along with statistical assessment, including the mean (x_{aver}), standard deviation (S_x), relative standard deviation (RSD%) and confidence interval ($t_{0,95}$) are presented on the next page:

Table 1.

Level	75%		100%		125%	
$\frac{M_m}{EnapHl}$	$\frac{\lambda_{225nm}\ 0.0190}{0.0192}$	$\frac{\lambda_{278nm}\ 0.0729}{0.0726}$	$\frac{\lambda_{225nm}\ 0.0272}{0.0274}$	$\frac{\lambda_{278nm}\ 0.0974}{0.1037}$	$\frac{\lambda_{225nm}\ 0.0341}{0.0342}$	$\frac{\lambda_{278nm}\ 0.1218}{0.1296}$
$\frac{M_m}{BlopressPlus}$	$\frac{\lambda_{254.6nm}\ 0.0112}{0.0107}$	$\frac{\lambda_{270.4nm}\ 0.0040}{0.0042}$	$\frac{\lambda_{254.6nm}\ 0.0161}{0.0157}$	$\frac{\lambda_{270.4nm}\ 0.0056}{0.0060}$	$\frac{\lambda_{254.6nm}\ 0.0201}{0.0198}$	$\frac{\lambda_{270.4nm}\ 0.0071}{0.0073}$
$\frac{M_m}{Co-Diovan}$	$\frac{\lambda_{261nm}\ 6.89 \cdot 10^{-4}}{7.06 \cdot 10^{-4}}$	$\frac{\lambda_{284nm}\ 0.0013}{0.0012}$	$\frac{\lambda_{261nm}\ 98.86 \cdot 10^{-4}}{10.06 \cdot 10^{-4}}$	$\frac{\lambda_{284nm}\ 0.0019}{0.0017}$	$\frac{\lambda_{261nm}\ 1.21 \cdot 10^{-4}}{1.26 \cdot 10^{-4}}$	$\frac{\lambda_{284nm}\ 0.0024}{0.0022}$

Table 2.

Mixture	λ[nm];	Determined constituent	Equation of linear regresion; correlation coefficjent (r)
EHL	225.0	enalapril	D_1= -0.01020 + 0.00260 × c; r = 0.99357
	278.0	hydrochlorothiazide	D_1= 0.00130 + 0.00544 × c; r = 0.99839
BLP	270.4	candesartan	D_1= 0.00007 + 0.00070 × c; r = 0.99921
	254.6	hydrochlorothiazide	D_1= -0.0003 + 0.00261 × c; r = 0.99821
COD	261.0	walsartan	D_2= 0.00022 + 0.00003 × c; r = 0.99589
	284.0	hydrochlorothiazide	D_2= 0.00035 + 0.00051 × c; r = 0.99855

Enap HL
Enalapril (%): 103.39, 97.52, 98.72, 103.44, 103.72, x_{aver} = 101.36
S_x = 2.9888, $t_{0,95}$ = ± 3.7111, (%)RSD = 2.94, $\%E_{rel}$ = 1.36;
Hydrochlorothiazide (%): 100.53, 102.70, 98.70, 101.34, 102.40, x_{aver} = 101.13
S_x = 1.6114, $t_{0,95}$ = ± 2.0008, (%)RSD = 1.59, $\%E_{rel}$ = 1.13;
Blopress Plus
Candesartan (%): 92.72, 94.80, 97.50, 92.30, 94.28, x_{aver} = 94.32
S_x = 2.0606, $t_{0,95}$ = ± 2.5586, (%)RSD = 2.18, $\%E_{rel}$ = 5.68;
Hydrochlorothiazide (%): 106.30, 100.80, 105.50, 101.40, 102.35, x_{aver} = 103.27
S_x = 2.4798, $t_{0,95}$ = ± 3.0791, (%)RSD = 2.40, $\%E_{rel}$ = 3.27;
Co-Diovan
Walsartan (%): 93.48, 91.68, 97.24, 95.30, 97.49, x_{aver} = 95.04
S_x = 2.4816, $t_{0,95}$ = ± 3.0813, (%)RSD = 2.61, $\%E_{rel}$ = 4.96;
Hydrochlorothiazide (%): 95.30, 97.01, 94.23, 95.80, 96.42, x_{aver} = 95.75
S_x = 1.0668, $t_{0,95}$ = ± 1.3246, (%)RSD = 1.11, $\%E_{rel}$ = 4.25;

Based on the obtained results of validation, the quantitative analysis procedure was established.

Quantitative analysis

Record spectra at the wavelength range from 200 nm to 400 nm, using methanol as reference material for standard mixture solutions of hydrochlorothiazide and appropriate constituents under investigation and for sample solutions. Transform the obtained spectra into the first or second derivative spectra depending on the pharmaceutical product under investigation. Compute the contents of active substances by comparing the derivative values at wavelength λ_{225nm} for enalapril and λ_{278nm} for hydrochlorothiazide in preparation Enap HL, $\lambda_{270.4nm}$ for candesartan and $\lambda_{254.6nm}$ for hydrochlorothiazide in preparation Blopress Plus, and λ_{261nm} for walsartan and λ_{284nm} for hydrochlorothiazide in preparation Co-Diovan.

The results of constituent concentration determination in preparations under consideration and statistical evaluation are presented in Table 3.

DISCUSSION AND CONCLUSIONS

As mentioned above, combination of pharmacologically active constituents in complex drugs is justified in clinical practice, as this enables often better therapeutic effects to be achieved with lesser adverse effects. Thus, it is quite obvious that when selecting components their pharmacological properties and technological aspects related to their durability and pharmaceutical availability are taken into account.

The fact that constituents of analyzed complex drugs often have similar physicochemical properties and their zero order spectra interfere with each other, makes any direct analysis impossible. It seems that the difficulties mentioned above cause that spectrophotometric methods are replaced by resolving ones.

However, when considering the advantages of spectrophotometric methods: easiness to make measurements, accuracy and availability as well as great advances in the development of new instruments, it was possible to prove that such methods can be widely used for fast and accurate quantitative analysis. Wider application of spectrophotometry was possible thanks to new numerical techniques, that by differentiating the zero order spectra increase the analytical selectivity and sensitivity compared to traditional zero order spectrometry.

The assay results presented above allow to conclude that the use of derivative spectrophotometry and spectra transformation into the first derivative spectra in the case of the simultaneous determination of hydrochlorothiazide, enalapril and can-

Table 3. Results of determination of hydrochlorothiazide, enelapril, candesartan and walsartan in tablets.

Preparation	Determined quantity of hydrochlorothiazide [mg/ tabl.]	Statistical assessment	Determined quantity of enalapril [mg/ tabl.]	Statistical assessment	Determined quantity of candesartan [mg/ tabl.]	Statistical assessment	Determined quantity of walsartan [mg/ tabl.]	Statistical assessment
Enap HL	12.68 12.39 12.59 12.28 12.39	$\bar{X}$ = 12.47 S_x = 0.1638 $t_{0.95}$ = ± 0.2034 $\%E_{rel}$ = 0.24 RSD = 1.31%	9.66 10.15 10.07 9.38 10.60	$\bar{X}$ = 9.97 S_x = 0.4700 $t_{0.95}$ = ± 0.5835 $\%E_{rel}$ = 0.3 RSD = 4.71%				
Blopress Plus	12.61 12.68 12.24 12.56 12.37	$\bar{X}$ = 12.49 S_x = 0.1819 $t_{0.95}$ = ± 0.2258 $\%E_{rel}$ = 0.08 RSD = 1.46%			16.47 16.76 15.47 15.84 16.03	$\bar{X}$ = 16.11 S_x = 0.5101 $t_{0.95}$ = ± 0.6334 $\%E_{rel}$ = 0.69 RSD = 3.17%		
Co-Diovan	12.37 11.49 12.63 11.91 12.40	$\bar{X}$ = 12.16 S_x = 0.4566 $t_{0.95}$ = ± 0.5670 $\%E_{rel}$ = 2.72 RSD = 3.75%					73.48 71.68 77.24 75.30 77.49	$\bar{X}$ = 75.04 S_x = 2.4816 $t_{0.95}$ = ± 3.0813 $\%E_{rel}$ = 6.2 RSD = 3.31%

$\bar{X}$ - mean, S_x – standard deviation, $t_{0.95}$ – confidence interval, $\%E_{rel}$ – relative error RSD – relative standard deviation

desartan as well as the second derivative spectra in the case of hydrochlorothiazide and walsartan, enable concurrent analysis of active substances in preparations under investigation.

It was proven that the method is specific to analytes at selected wavelengths. No interference of matrix components was observed, thus indicating good selectivity of the method. The linearity was maintained in a wide concentration range, i.e. from 4.1 $\mu g \times mL^{-1}$ to 20.5 $\mu g \times mL^{-1}$ for enalapril, from 6.45 $\mu g \times mL^{-1}$ to 32.25 $\mu g \times mL^{-1}$ for walsartan, from 2.36 $\mu g \times mL^{-1}$ to 11.80 $\mu g \times mL^{-1}$ for candesartan and from 0.96 $\mu g \times mL^{-1}$ to 26.00 $\mu g \times mL^{-1}$ for hydrochlorothiazide. The intersection point of the straight line did not significantly differ from zero. The method was highly sensitive: LOD and LOQ values for particular constituents were as follows: 2.81 $\mu g \times mL^{-1}$ for enalapril and 8.51 $\mu g \times mL^{-1}$, 4.02 $\mu g \times mL^{-1}$ and 12.17 $\mu g \times mL^{-1}$ for walsartan, 0.56 $\mu g \times mL^{-1}$ and 1.71 $\mu g \times mL^{-1}$ for candesartan, and 0.31 $\mu g \times mL^{-1}$ and 5.40 $\mu g \times mL^{-1}$ for hydrochlorothiazide, depending on the preparation type.

The obtained results are valuable not only from the scientific viewpoint but can also have practical value, as the developed method can be used for easy and quick drug quality control. Thus, one may conclude that this method can serve as an alternative to commonly used but expensive chromatographic methods.

There are no discrepancies between the results of determination and the declared values for individual constituents. Thus, the method is of good precision and accuracy, narrow confidence interval and advantageous values of standard deviation of the mean (S_x), relative error ($\%E_{rel}$) and relative standard deviation (RSD) (Table 3).

Finally, one can conclude that the newly developed method meets the quantitative analysis requirements for pharmaceutical analysis purposes pertaining simultaneous determination of hydrochlorothiazide along with enalapril, walsartan and candesartan and seems to be worthy to recommend it for routine analysis of complex preparations.

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