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SIMULTANEOUS DETERMINATION OF TRIAMTERENE AND HYDROCHLOROTHIAZIDE IN TABLETS USING DERIVATIVE SPECTROPHOTOMETRY

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Abstract: A quick and accurate method for determining triamterene and hydrochlorothiazide in complex drugs of diuretic activity by using first-derivative (D1) and second-derivative (D2) spectrophotometry was developed. The zero-crossing technique was employed in measurements, using D1 at λ = 240.9 nm and D2 at λ = 278.2 nm for determining triamterene and D1 at λ = 255.7 nm and D2 at λ = 283.2 nm for hydrochlorothiazide. The linear relationship between the values of derivatives and analyte concentrations are maintained for concentrations from 2.40 $\mu g \times mL^{-1}$ to 12.00 $\mu g \times mL^{-1}$ for triamterene and from 1.25 $\mu g \times mL^{-1}$ to 6.25 $\mu g \times mL^{-1}$ for hydrochlorothiazide. LOD for triamterene was 0.90 $\mu g \times mL^{-1}$ or 1.02 $\mu g \times mL^{-1}$, while LOQ was 2.73 $\mu g \times mL^{-1}$ or 3.08 $\mu g \times mL^{-1}$. The corresponding values for hydrochlorothiazide were: LOD 0.25 $\mu g \times mL^{-1}$ or 0.17 $\mu g \times mL^{-1}$ and LOQ 0.77 $\mu g \times mL^{-1}$ or 0.51 $\mu g \times mL^{-1}$ depending on the derivative used. The determination results of drug constituents are of high accuracy, percentage recovery ranging from 97.17% to 99.74% for triamterene and from 102.44% to 102.64% for hydrochlorothiazide, and good precision. The computed values of RSD are smaller than 2.73% for triamterene and below 1.63% for hydrochlorothiazide. Selectivity and sensitivity of the developed method are satisfactory.

Keywords: triamterene, hydrochlorothiazide, derivative spectrophotometry, drug analysis

The use of mixtures of diuretics is targeted at intensification of its efficiency by achieving synergic effects. In complex mixtures the combinations of thiazides and diuretics are often used. The advantages of complex drugs include reduced dosages, while intensifying hypertension activity and reducing the hypokalemia symptoms. Such activity can be assigned to the medicine containing 50 mg of triamterene and 25 mg of hydrochlorothiazide in a Diureticum-Verla tablet.

There are numerous papers devoted to analytical control of particular constituents where conditions for determining diuretics in pharmaceutical products and body fluids are described.

Hydrochlorothiazide and the products of its photodegradation were determined spectrophotometrically using first-derivative spectroscopy and HPLC combined often with mass spectrometry (1-4). Good results were obtained by employing voltammetry for hydrochlorotiazide (5). Bioavailabilty of hydrochlorothiazide was determined by HPLC with the application of the Diode Array Detector (6).

To determine triamterene and its metabolite in blood and urine HPLC (7) or LC (8) were used. The mixtures of triamterene with other diuretics such as spironolactone, amiloride or chlorthalidone in pharmaceutical preparations were determined quantitatively using micellar liquid chromatography (9), while both chromatographic and spectrophotometric methods (9-12) were used for analysis of mixtures of diuretics with β-adrenolytics or angiotensin convertase blockers.

The constituents of complex drugs are often of similar physicochemical properties, thus causing a problem connected with selection of suitable methods for their determination. The methods recommended for analyzing one-component drugs often fail thus it is still necessary to seek for new methods. In this paper the research studies were performed to develop a method for determination of mixed triamterene and hydrochlorothiazide using derivative spectrophotometry.

EXPERIMENTAL

Apparatus

Spectrophotometer UV-VIS Cary 100 (Varian), quartz cuvettes (l = 1 cm). Computer: PC Pentium MMX, 16 MB RAM, Brother HL – 1430 printer and

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software (Microsoft Office 2003, Statistica 7.1 edition 2007).

Standards and standard solutions

a) Standard solutions were prepared in methanol at the following concentrations:

triamterene (Sidaco/The Netherlands) 0.48 mg×mL^{-1} (solution 1)

hydrochlorothiazide (Merck, USA) 0.25 mg×mL^{-1} (solution 2)

For direct determination purposes the solutions were diluted with methanol up to concentrations ranging from 2.40 µg×mL^{-1} to 12.0 µg×mL^{-1} for triamterene and from 1.25 µg×mL^{-1} to 6.25 µg×mL^{-1} for hydrochlorothiazide.

b) Standard mixture

In 10.0 mL volumetric flasks 50, 100, 150, 200 and 250 µL of solution 1 and 2 were pipetted and filled up with methanol to the specified volume, thus obtaining a mixture of standards of compositions comparable to those of the complex preparation Diureticum Verla (Di-V).

Sample solutions

From powdered mass of 10 tablets of the preparation Diureticum Verla an amount of 26.00 mg to 38.50 mg were weighed up to 0.1 mg. The weighed amounts were poured with 10 mL of methanol and shaken for 15 minutes, and then centrifuged at 1500 rpm. For direct determination 100 µL of solution was diluted up to the final volume of 10 mL.

Solvent

Methanol of analytical purity (Merck).

RESULTS AND DISCUSSION

Determination conditions

First, the zero-order absorption spectra were recorded within 200 nm – 400 nm for appropriate standard solutions containing constituents at concentrations comparable to those of composition of the drug existing individually and in appropriate mixtures. Spectrophotometric measurements for methanol were made as a blind test. The obtained results are shown in Figure 1.

The absorption spectra of individual constituents show absorbance maxima at λ = 233 nm and 270 nm and at λ = 225 nm and λ = 269 nm for the mixture. Both absorption spectra and absorbance maxima recorded for the mixtures indicate an interference of constituents that is confirmed by the value of absorbance for the mixture being a sum of individual constituent absorbance at appropriate wavelengths (Figure 1). It is noticeable that the value of absorbance for triamterene is almost five times higher than that for hydrochlorothiazide, when keeping the proportion of both mixture constituents. After converting the zero-order spectra into D1 and D2 derivatives, the spectra become highly differentiated in well developed absorption maxima at different wavelengths. By employing the zero-crossing technique the wavelengths were found at which no interference of measured quantities is observed in model solutions. For the first derivative (D1) the concentration of triamterene (T) was determined at $\lambda_{240.9nm}$, whereas for hydrochlorothiazide (H) $\lambda_{255.7nm}$ was chosen. In the case of second derivative (D2), the wavelengths were $\lambda_{278.2nm}$ and $\lambda_{283.2nm}$ for (T) and (H), respectively (Figures 2, 3).

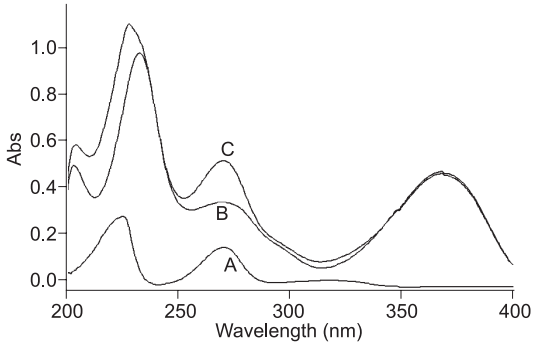

Figure 1. The zero order absorption spectra for hydrochlorothiazide (A), triamterene (B) and preparation Diureticum Verla (C).

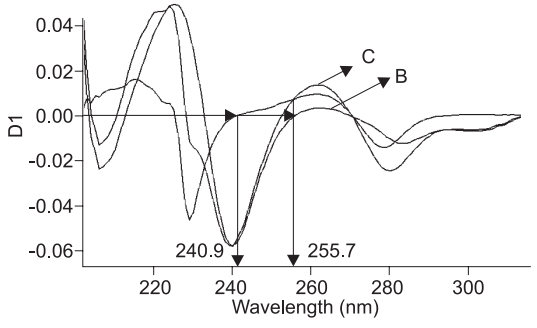

Figure 2. The first derivative values for hydrochlorothiazide (A), triamterene (B) and preparation Diureticum Verla (C).

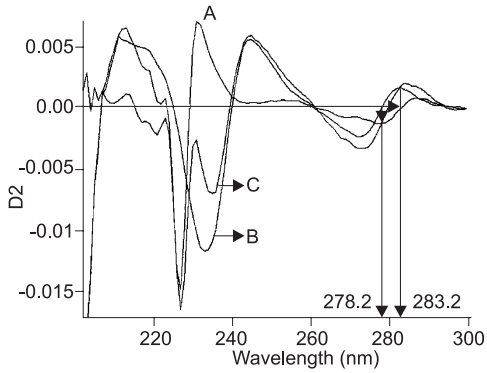

Figure 3. The second derivative values for hydrochlorothiazide (A), triamterene (B) and preparation Diureticum Verla (C).

The obtained results allow further research studies to be performed to develop a method for simultaneous determination of constituents in pharmaceutical preparations.

For this purpose the method was validated, including specificity, linearity, limit of detection, limits of quantification and accuracy.

Specificity

Since no information of placebo composition is available, the comparative study was carried out for standard solutions and the medicine under investigation. To do it, solutions of identical composition containing 75%, 100% and 125% of individual constituents were prepared and the values of derivatives were measured at selected wavelengths.

The results of D1 and D2 measurements for solutions of standard mixtures (M_w) and the medicine (Di-V) are presented below Table 1.

When comparing the values of appropriate derivatives for standard solution and the preparation one can conclude that the method is specific for the analyte used and the coexisting constituents do not affect the measurements.

Linearity

To check linearity, five measurements were made for each standard solution (Fig. 4 and 5) within the range: from 2.40 μg×mL^{-1} to 12.0 μg×mL^{-1} for triamterene and from 1.25 μg×mL^{-1} to 6.25 μg×mL^{-1} for hydrochlorothiazide.

Within the concentration ranges under investigation the linearity is maintained. The linear regression equation used for assessing the results has the parameters (intersection point, correlation coefficient) shown in Table 2.

At the wavelengths selected the method was specific to analytes composed of constituents under investigation. No interference of matrix components was observed, thus confirming selectivity of the method. Linearity was maintained in the wide concentration range, i.e. from 1.25 μg×mL^{-1} to 6.25 μg×mL^{-1} for triamterene and from 1.25 μg×mL^{-1} to 12.00 μg×mL^{-1} for hydrochlorothiazide at good correlation both for D1 r = 0.99807 (T) and r = 0.99944 (H) as well for D2, r = 0.99753 (T) and r = 0.99975 (H), respectively. The intersection point of appropriate straight lines did not differ significantly from zero.

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

The limit of detection and the limit of quantification were calculated using the statistical parameters for appropriate calibration curves from the following formulas: LOD = $3.3 \cdot S_Y/a$ and LOQ= $10.0 \cdot S_Y/a$, where S_Y – standard estimation error, a – slope of the straight line.

The method was highly sensitive; LOD and LOQ for particular constituents using appropriate derivative were as follows: D1 LOD for triamterene was 0.90 μg×mL^{-1}, while for D2 1.02 μg×mL^{-1} and LOQ was 2.73 μg×mL^{-1} and 3.08, respectively. For hydrochlorothiazide D1 LOD was 0.25 μg×mL^{-1} and for D2 0.17 μg×mL^{-1}, while LOQ was 0.77 μg×mL^{-1} and 0.51 μg×mL^{-1}, correspondingly.

Table 1.

D1						
Level	75%		100%		125%	
M_w / $Di - V$	$\lambda_{255.7nm}$ 0.0078 / 0.0077	$\lambda_{240.9nm}$ 0.0429 / 0.0430	$\lambda_{255.7nm}$ 0.0108 / 0.0109	$\lambda_{240.9nm}$ 0.0575 / 0.0576	$\lambda_{255.7nm}$ 0.0138 / 0.0140	$\lambda_{240.9nm}$ 0.0739 / 0.0738
D2						
Level	75%	100%	125%			
M_w / $Di - V$	$\lambda_{283.2nm}$ 0.0017 / 0.0017	$\lambda_{278.2nm}$ 0.0009 / 0.0009	$\lambda_{283.2nm}$ 0.0022 / 0.0021	$\lambda_{278.2nm}$ 0.0011 / 0.0010	$\lambda_{283.2nm}$ 0.0030 / 0.0031	$\lambda_{278.2nm}$ 0.0015 / 0.0014

Table 2.

Derivative	λ [nm];	determined constituent	Equation of linear regression	regression coefficient (r)
D1	240.9 255.7	triamterene hydrochlorothiazide	D_1= -0.004 + 0.00785 × c; D_1= -0.001 + 0.99944 × c;	r = 0.99807 r = 0.99944
D2	278.2 283.2	triamterene hydrochlorothiazide	D_2= -0.0001 + 0.00017 × c; D_2= -0.0007 + 0.00062 × c;	r = 0.99753 r = 0.99975

Recovery

Recovery for individual constituents was expressed in percentage based on the determined concentration of analyte that was added to samples at amounts from 80% to 120% compared to those of declared concentration. The results of determination along with statistical assessment, including the mean (x_{mean}), standard deviation of the mean (S_x), relative standard deviation (RSD%) and confidence interval ($t_{0,95}$) are listed below:

D1

triamterene for n = 5 from 94.97% to 100.00%, x_{mean} = 97.17%

S_x = 2.0244, $t_{0,95}$ = ± 2.5137, [%]RSD = 2.08, $\%E_{rel}$ = 2.83;

hydrochlorothiazide for n = 5 from 93.20% to 106.21%, x_{mean} = 102.44%

S_x = 4.9007, $t_{0,95}$ = ± 6.0850, [%]RSD = 4.78, $\%E_{rel}$ = 2.44;

D2

triamterene for n = 5 from 93.23% to 102.68%, x_{mean} = 99.74%

S_x = 3.8828, $t_{0,95}$ = ± 4.8212, [%]RSD = 3.89, $\%E_{rel}$ = 0.26;

hydrochlorothiazide for n =5 from 100.83% to 105.72%, x_{mean} = 102.64%

S_x = 1.8273, $t_{0,95}$ = ± 2.2689, [%]RSD = 1.78, $\%E_{rel}$ = 2.64.

Based on the obtained results the determination procedure was established.

Quantitative analysis

Record absorption spectra at the wavelength range from 200 nm to 400 nm for the standard mixture of triamterene and hydrochlorothiazide and tested sample solutions in methanol. Convert the zero-order spectra into first-derivative (D1) and second-derivative (D2) spectra. For quantitative analysis, when using D1 read the value of first derivative at λ = 240.9 nm for triamterene and at λ = 255.7 nm for hydrochlorothiazide. Apply the same procedure for D2 spectra by recording the values of second derivative at λ = 278.2 nm and λ = 283.2 nm, respectively. The content of active components should be computed by comparing the values of relevant derivatives for the standard solution and a sample under investigation.

The results of determination for constituent concentration in the preparation under examination along with statistical analysis are listed in Table 3.

CONCLUSION

The constituents of the medicine under investigation are of similar physicochemical properties and their zero-order spectra interfere with each other,

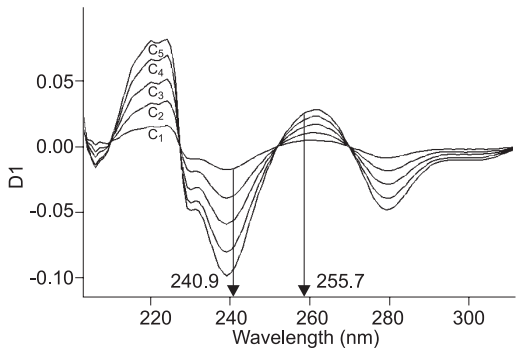

Figure 4. The first derivative values vs. hydrochlorothiazide concentration (C_1 = 1.25; C_2 = 2.50; C_3 = 3.75; C_4 = 5.00; C_5 = 6.25 μg mL^{-1}) and triamterene (C_1 = 2.40; C_2 = 4.80; C_3 = 7.20; C_4 = 9.60; C_5 = 12.00 μg/mL^{-1}) in reference mixtures.

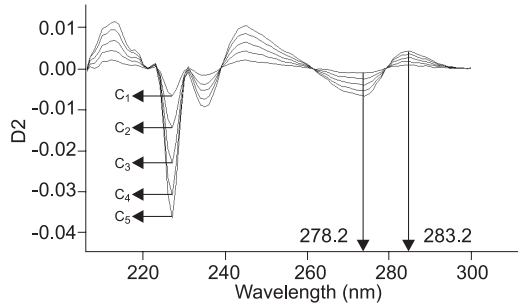

Figure 5. The second derivative values vs. hydrochlorothiazide concentration (C_1 = 1.25; C_2 = 2.50; C_3 = 3.75; C_4 = 5.00; C_5 = 6.25 μg mL^{-1}) and triamterene (C_1 = 2.40; C_2 = 4.80; C_3 = 7.20; C_4 = 9.60; C_5 = 12.00 μg/mL^{-1}) in reference mixtures.

Table 3. Results of determination of triamterene and hydrochlorothiazide in tablets.

Method	Hydrochlorothiazide	Triamterene		
	Declared content Diureticum – Verla	Determined content for n = 5 [mg/tabl.]	Declared content Diureticum – Verla	Determined content for n = 5 [mg/tabl.]
D1	25.00 [mg/tabl.]	from 24.42 to 25.02	50.00 [mg/tabl.]	from 47.27 to 48.73
First derivative		$\bar{X}$ = 24.76 S_x = 0.2699 $t_{0,95}$ = ± 0.2832 $\%E_{rel}$ = 0.96% RSD = 1.09%		$\bar{X}$ = 48.11 S_x = 0.5549 $t_{0,95}$ = ± 0.5823 $\%E_{rel}$ = 3.78% RSD = 1.15%
D2		from 24.72 to 25.67		from 49.60 to 52.95
second **derivative**		$\bar{X}$ = 25.15 S_x = 0.4100 $t_{0,95}$ = ± 0.4303 $\%E_{rel}$ = 0.60% RSD = 1.63%		$\bar{X}$ = 50.96 S_x = 1.3891 $t_{0,95}$ = ± 1.4578 $\%E_{rel}$ = 1.92% RSD = 2.73%

$\bar{X}$- mean, S_x – standard deviation of the mean, $t_{0,95}$ – confidence interval, $\%E_{rel}$ – relative error, RSD – relative standard deviation

thus making them useless for direct determination of triamterene and hydrochlorothiazide. This problem disappears when the derivative spectrophotometry method developed for direct determination of constituents under consideration is used.

The obtained results indicate that the first-derivative (D1) or second-derivative (D2) spectrophotometry allows simultaneous determination of coexisting triamterene and hydrochlorothiazide. These results can also have practical value, as the developed method can be used for a simple and quick drug quality control purposes instead of chromatographic methods.

The results of determination of individual constituents are consistent with the declared values, thus indicating good precision and accuracy, narrow confidence interval and favorable values of standard deviation of the mean (S_x), relative error ($\%E_{rel}$) and relative standard deviation (RSD) (Table 3).

The obtained results indicate also a wider application of derivative spectrophotometry in complex drug analysis, as the presented method is easily available and relatively cheap compared to such separating methods as GC, EC or HPLC.

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