Acta Poloniae Pharmaceutica – Drug Research, Vol. 70 No. 6 pp. 967–976, 2013 ISSN 0001-6837
Polish Pharmaceutical Society

DETERMINATION OF LOSARTAN POTASSIUM, QUINAPRIL HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL PREPARATIONS USING DERIVATIVE SPECTROPHOTOMETRY AND CHROMATOGRAPHIC-DENSITOMETRIC METHOD

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Abstract: Two methods, spectrophotometric and chromatographic-densitometric ones, were developed for determination of losartan potassium, quinapril hydrochloride and hydrochlorothiazide in pharmaceutical preparations. Spectrophotometric method involved derivative spectrophotometry and zero order spectrophotometry. The measurements were carried out at λ = 224.0 nm for quinapril, λ = 261.0 nm for hydrochlorothiazide and λ = 270.0 nm for losartan when the derivative spectrophotometry was applied and λ = 317.0 nm when zero order spectrophotometry was applied for the determination of hydrochlorothiazide. In chromatographic-densitometric studies high performance thin layer chromatography (HPTLC) plates were used as stationary phase and a mixture of solvents n-butanol : acetic acid : water (15 : 5 : 1, v/v/v) as mobile phase. Under the established conditions good resolution of examined constituents was obtained. Retardation factor for quinapril hydrochloride was $R_f \sim 0.70$, for losartan potassium $R_f \sim 0.85$ and for hydrochlorothiazide $R_f \sim 0.78$. The developed methods are characterized by high sensitivity and accuracy. For quantitative analysis, densitometric measurements were carried out at λ = 218.0 nm for quinapril, λ = 275.0 nm for hydrochlorothiazide and λ = 232.0 nm for losartan.

Keywords: quinapril, losartan, hydrochlorothiazide, derivative spectrophotometry, HPTLC

Losartan (Losartanum) belongs to sartans, i.e., angiotensin receptor blockers recommended in hypertension therapy.

Quinapril belongs to the group of angiotensin-converting enzyme inhibitors. It is used in a form of prodrug. Its active form is quinaprilat which is formed as a result of biotransformation in the liver. It is recommended in therapy of hypertension and heart failure. In hypertension treatment it is used both in monotherapy and in combined therapy.

Hydrochlorothiazide is qualified to the group of saluretics, i.e., thiazide diuretics. In terms of chemical structure it is sulfonamide derivative. This drug exhibits diuretic and blood pressure lowering activity.

Main indication for this drug administration is hypertension (primary and renovascular). In hypertension therapy, this drug is used both in monotherapy and in therapy combined with other antihypertensive drugs of various targets. An application of antihypertensive drugs of separate activity mechanisms allows to obtain synergistic effect and in a consequence to increase their antihypertensive activity.

Numerous analytical methods were used in the studies on losartan, quinapril and hydrochlorothiazide analysis in pharmaceutical preparations and body fluids (1).

Hydrochlorothiazide and photodegradation products were determined spectrophotometrically using first derivative, and high performance liquid chromatography (HPLC) method often combined with mass spectrometry (2-5). Thin layer chromatography (TLC) method was used in an analysis of hydrochlorothiazide and other substances from diuretics group (6). Good results in hydrochlorothiazide determinations in pharmaceutical preparations were obtained using voltammetry method (7), HPTLC (8), reversed phase high performance liquid chromatography (RP-HPLC) (9) or capillary electrophoresis (10) methods

Liquid chromatography method with fluorimetric (11) or mass detection (12, 13) were used in losartan potassium determination in biological material.

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Quinapril, and other angiotensin convertase inhibitors, was determined using voltammetry method (14). Spectrophotometric (15, 16) and HPTLC methods (17) were used for studies in complex drugs containing hydrochlorothiazide. Pharmacologically active quinapril metabolite in blood serum was determined using ultra high performance liquid chromatography-electrospray ionization mass spectrometry (UHPLC-MS) (18).

This research aimed at a development of the method of hydrochlorothiazide, as well as quinapril and losartan co-present in complex drugs, determination with an application of derivative spectrophotometry and high performance thin layer chromatography.

The study included pharmaceutical preparations in which one of the components is hydrochlorothiazide and the second is one of the substances of antihypertensive activity, i.e., losartan potassium or quinapril hydrochloride. Accuzide 20 containing 20.0 mg of quinapril and 12.50 mg of hydrochlorothiazide, as well as Lorista® H preparation containing 50 mg of potassium losartan and 12.50 mg of hydrochlorothiazide were used in the studies.

EXPERIMENTAL

Apparatus

Spectrophotometer UV-VIS Cary 100 Varian (Australia), quartz cuvettes of a layer thickness 1 cm. Computer – Dell Optiplex 755; Intel(R) Core(TM)2 Duo CPU; E4500 @ 2.20 GHz; 1.18 GHz, 1.95 GB Ram (Microsoft Office 2006, Statistica 7.1 edition 2007). Applicator for samples application LINOMAT 4; Camag (Muttenz, Switzerland). Densitometer TLC- Scanner 3 with WinCats software version 1.3.4; Camag (Muttenz, Switzerland). Microsyringe of a volume of 100 μL (Hamilton Comp. Reno, USA). Chromatographic chamber 23 × 9 × 11 cm Nano-Desaga (Germany).

Materials

Reference standards: losartan potassium, quinaprilum, hydrochlorothiazide. Tablets: Lorista® H (KRKA), Accuzide® 20 (Pfizer). Reagents of analytical grade quality: methanol, n-butanol, acetic acid. HPTLC plates $60F_{254}$ No 1.05548.0001 (Merck-Darmstadt, Germany).

Standard solutions

For spectrophotometric method: Losartan potassium (19.3 mg of losartan potassium was weighed and completed with methanol up to the volume of 10.0 mL). Quinapril hydrochloride (19.6 mg of quinapril hydrochloride was weighed and completed with methanol up to the volume of 10 mL). Hydrochlorothiazide (12.4 mg of hydrochlorothiazide was weighed and completed with methanol up to the volume of 10 mL).

For chromatographic-densitometric analysis: Methanol standard solutions for chromatographic-densitometric analysis had the following concentrations: losartan 0.44 mg/mL, quinapril 0.38 mg/mL and hydrochlorothiazide 0.52 mg/mL.

Sample solutions

Ten tablets of the studied preparations were powdered in a porcelain mortar. Weighed amounts of samples in a range of 129.8 to 240.2 mg (Accuzide 20), 99.5 to 271.3 mg (Lorista® H) were extracted using 10 mL of methanol, shaking for 15 min and centrifuged (1500 rpm). Solutions obtained directly after extraction and centrifugation were used in chromatographic-densitometric analysis, while in order to perform spectrophotometric analysis, the solutions obtained were diluted in a ratio of 1 : 100.

Spectrophotometric analysis

The absorption spectra in the range from 200 to 400 nm were registered for standard solutions and studied samples solutions in the presence of methanol. Spectra of zero order were transformed in the first order derivative (D1). The concentration of studied substances in pharmaceutical preparations was calculated using straight line equation based on the value of derivative and an absorbance read out at suitable wavelengths at $\lambda = 224.0$ nm and $\lambda = 261.0$ nm for quinapril and hydrochlorothiazide (in the case of active substances content determination in Accuzide 20 preparation), respectively, and $\lambda = 270.0$ nm for losartan potassium and $\lambda = 317.0$ nm for hydrochlorothiazide (determined in Lorista ®H preparation).

Chromatographic-densitometric analysis

Suitable standard solutions and studied samples were spread in amount of 3 mL in a form of 10 mm band using an applicator on HPTLC chromatographic plates (10 × 10 cm). Chromatograms were developed in a chromatographic chamber saturated with mobile phase composed of n-butanol : acetic acid : water – 15 : 5 : 1 (v/v/v), they were developed to a height of 9.5 cm and dried at room temperature. Densitometric registration was performed after plates drying, with the following wavelengths: $\lambda =$ 218 nm for quinapril, $\lambda = 275$ nm for hydrochlorothi-

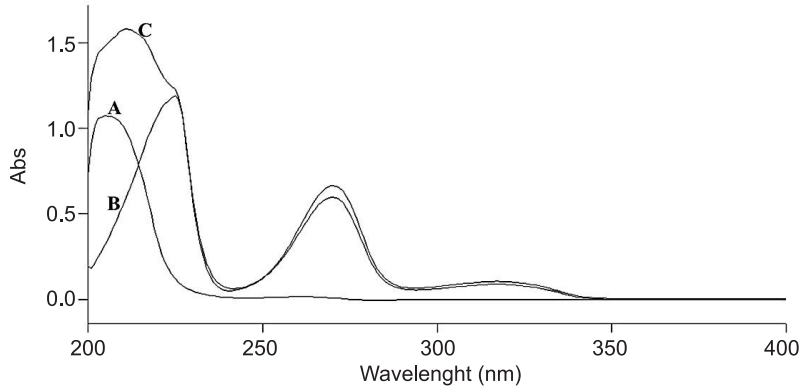

Figure 1. The zero order absorption spectra for quinapril (**A**), hydrochlorothiazide (**B**), mixture (**C**)

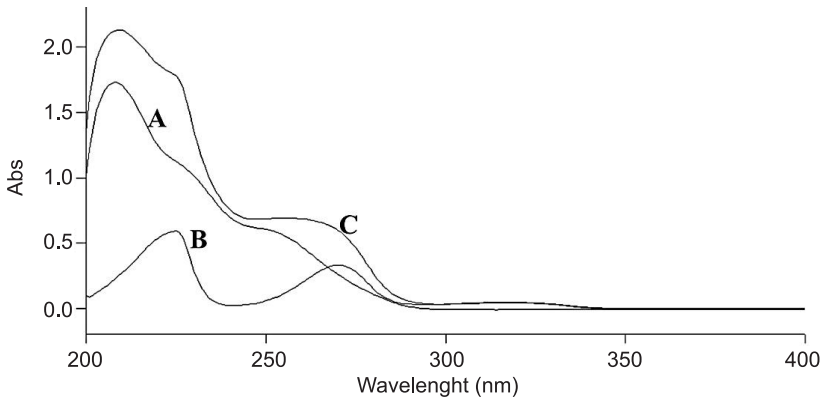

Figure 2. The zero order absorption spectra for losartan (**A**), hydrochlorothiazide (**B**), mixture (**C**)

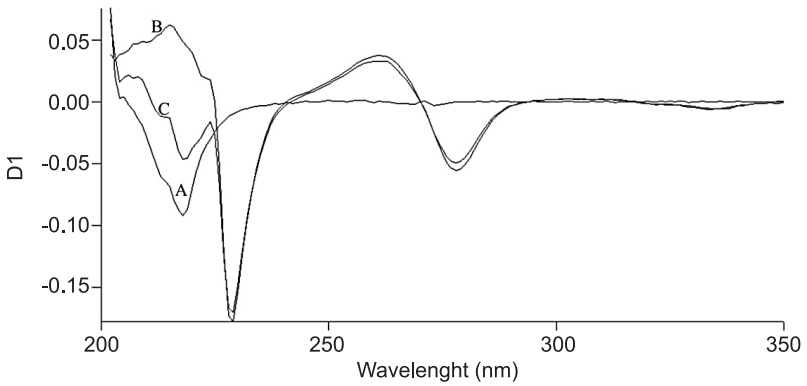

Figure 3. The first (D1) derivative spectra for quinapril (**A**), hydrochlorothiazide (**B**), mixture (**C**)

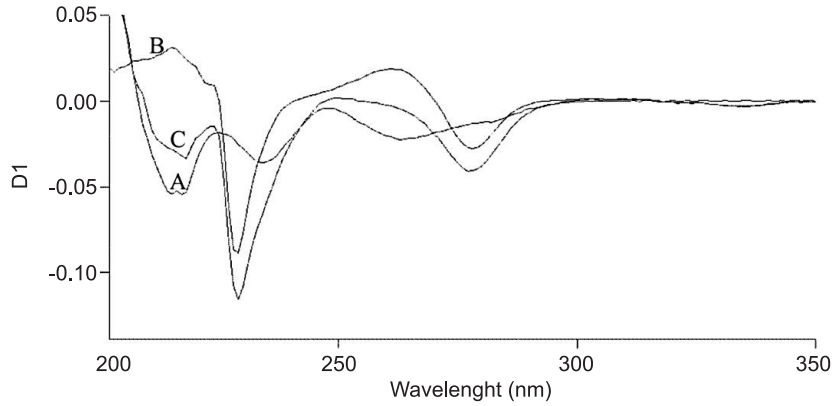

Figure 4. The first (D1) derivative spectra for losartan (**A**), hydrochlorothiazide (**B**), mixture (**C**)

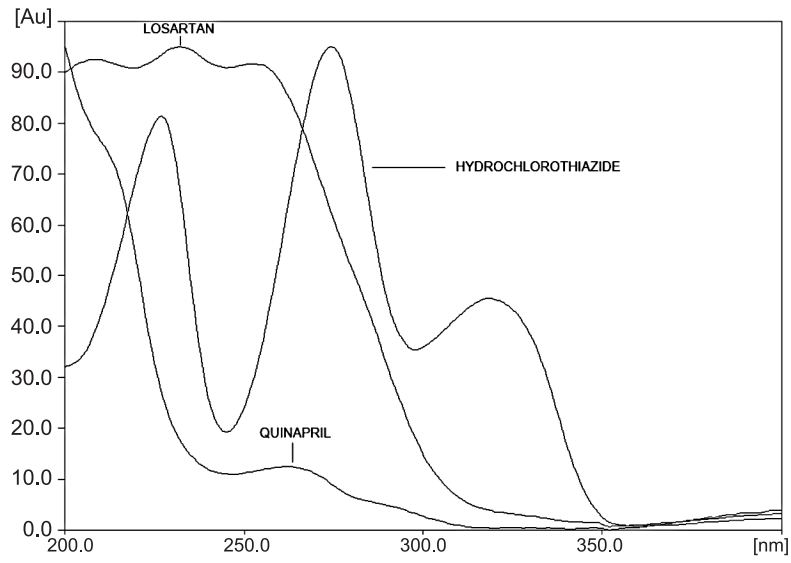

Figure 5. Absorption spectra of hydrochlorothiazide, quinapril and losartan registered directly from the chromatogram

azide and $\lambda = 232$ nm for losartan. The wavelength at which densitometric detection was performed, was selected based on spectra registered directly from chromatogram.

Content of active compounds in the preparations was calculated comparing the areas of analyzed sample peaks with an area of peaks of standard substances of known concentration.

Validation study

Validation of the methods was performed according to ICH requirements (19)

Specificity: Due to lack of data concerning placebo composition, the comparative spectrophotometric studies were performed for model solutions (R_m) and pharmaceutical forms being a subject of the study (P_f). Solutions of a comparable composi-

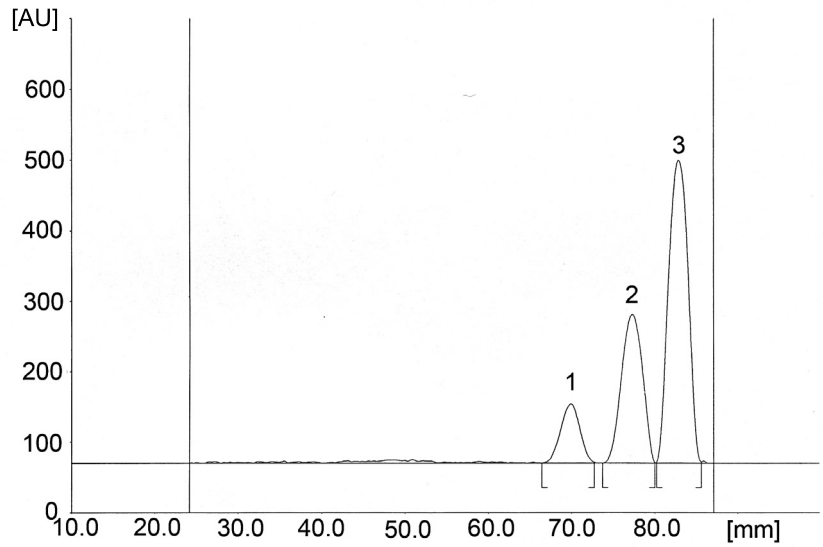

Figure 6. Densitogram of examined preparations solutions: quinapril (1) $R_f \sim 0.70$, hydrochlorothiazide (2) $R_f \sim 0.78$, losartan (3) $R_f \sim 0.85$

tion containing 75, 100 and 125% of particular compound were prepared. Measurements of derivatives values were performed at selected wavelengths. Specificity of the method for an analyte was determined comparing the values of absorbance and derivatives for standard solutions and pharmaceutical preparations.

Specificity of chromatographic-densitometric method was verified by a comparison of retardation factors (R_f) for studied substances and calculation of resolution of registered peaks (R_s – resolution factor).

Linearity: For spectrophotometric method, linearity is maintained within the studied ranges of concentrations. The equations of linear regression characterizing the points of straight line cut, correlation coefficient and statistical tests were used for the results assessment. In chromatographic-densitometric method, linearity assessment was performed by measurements for standard solutions in concentration range of 0.0078 – 1.00 mg/mL for hydrochlorothiazide, 0.0313 – 2.00 mg/mL for quinaprilum and 0.0306 – 0.49 mg/mL for losartan.

Limit of detection (LOD) and limit of quantification (LOQ): For both methods, limit of detection and limit of quantification were calculated using values of statistical parameters for suitable calibration curves according to the following formula:

$$LOD = 3.3S_y/a \text{ and } LOQ = 10.0S_y/a$$

where S_y – standard estimation error, a – gradient of a straight line.

Recovery: Determination of recovery percentage aims to determine an accuracy of the method, i.e., accordance between the real value and a value obtained as a result of analysis. It should be in the range of 95-105%. Recovery for particular components was provided as percents based on determined concentration of analyte which was added to the samples in amount from 80% to 120% with respect to the declared amounts.

Precision: Five assays were performed in order to determine the precision of the method. Amount of 3 µL of analyzed component solution was spread on chromatographic plate. In the next stage, the plates were developed in the developing mobile phase, and then densitometric analysis was performed. The areas of peaks were compared in order to determine the precision.

RESULTS AND DISCUSSION

Combination of pharmacologically active compounds in complex drugs is justified in terms of clinical practice, since it often allows to obtain improved therapeutic effects with increased undesirable activity. It is understandable that pharmacological properties and technological aspect with respect

Table 1. Linear and quadratic equation, Mandel's test, Shapiro-Wilk test of examined substances.

Substances	Methods	Linear equation	Quadratic equation	p(cx²) significance	Mandel's test p	Shapiro-Wilk test
Losartan	A	$y = 0.001x + 0.005$ $R^2 = 0.99838$	$y = 0.001x + 0.002 - 0.000001x^2$ $R^2 = 0.99747$	0.677	0.677	0.9599 (0.819)
Quinapril		$y = 0.00097x - 0.00129$ $R^2 = 0.99581$	$y = 0.001x - 0.002149 - 0.000001x^2$ $R^2 = 0.99449$	0.855	0.855	0.8932 (0.335)
Hydrochlorothiazide (D1)		$y = 0.0036x - 0.0005$ $R^2 = 0.99870$	$y = 0.0038x - 0.0013 - 0.00001x^2$ $R^2 = 0.99747$	0.551	0.551	0.8826 (0.281)
Hydrochlorothiazide (A)		$y = 0.0102x - 0.0053$ $R^2 = 0.99566$	$y = 0.0107x - 0.0086 - 0.000015x^2$ $R^2 = 0.99439$	0.780	0.780	0.8994 (0.371)
Losartan	B	$y = 17805.8x + 177.7$ $R^2 = 0.99837$	$y = 18141.5x + 158.9 - 694.1x^2$ $R^2 = 0.99799$	0.807	0.808	0.9582 (0.803)
Quinapril		$y = 2388.8x + 123.4$ $R^2 = 0.99696$	$y = 2637.7x + 66.6 - 126.1x^2$ $R^2 = 0.99724$	0.288	0.288	0.8434 (0.107)
Hydrochlorothiazide		$y = 14940.2x + 59.7$ $R^2 = 0.99812$	$y = 17278.6x - 48.1 - 4851.4x^2$ $R^2 = 0.99981$	0.0007	0.0007	0.8699 (0.150)*

* - for quadratic equation; **A** – spectrophotometric method, **B** - HPTLC

to components stability and pharmaceutical availability are considered at the stage of components selection.

The components occurring in the studied drug have similar physicochemical properties, and their absorption spectra registered using zero order spectrophotometric method interfere with each other, which prevents its application in direct analysis of quinapril, losartan and hydrochlorothiazide.

This problem is not observed in the case of an application of derivative spectrophotometry method, which was developed for the needs of direct analysis of components being a subject of the research. Equally good results may be obtained using chromatographic-densitometric method in an analysis of selected preparations. The spectra of zero order in the range of 200 – 400 nm were registered in the first stage of the research for suitable standard solutions containing active substances studied. Spectrophotometric measurements were performed in the presence of methanol as a reference substance, and their results are presented in Figures 1 and 2.

Analyzing the course of absorption spectra of quinapril and hydrochlorothiazide mixture (Fig. 1) as well as losartan and hydrochlorothiazide (Fig. 2), a clear interference of absorption maxima in the range of 200-300 nm was noted, which prevents direct analysis of the studied substances. Transformation of the obtained spectra into the first order derivative curves (Figs. 3, 4) and an application of zero-crossing technique allowed to determine the wavelengths at which interference phenomena were not observed.

For quantitative analysis purposes, suitable wavelengths were selected: λ = 224 nm for quinapril and λ = 261 nm for hydrochlorothiazide in their mixture. The analysis of losartan and hydrochlorothiazide mixture was possible with an application of relationship D1 = f(c) at λ = 270 nm for determination of losartan, and relationship A = f(c) λ = 317 nm for hydrochlorothiazide determination. The results obtained allow to conclude that the method of the first order derivative spectrophotometry (D1) or combination of derivative spectrophotometry method and zero order spectrophotometry in case of concurrent determination of losartan potassium and hydrochlorothiazide, allows to perform an analysis of active substances in the studied

Table 2.Validation of the developed methods with statistical evaluation.

	SPECTROPHOTOMETRY				**HPTLC**		
	Quinapril λ = 224.0 nm	Hydrochlorothiazide λ = 261.0nm	Losartan λ = 270.0nm	Hydrochlorothiazide λ = 317.0nm	Quinapril R_F ~ 0.70	Hydrochlorothiazide R_F ~ 0.78	Losartan R_F ~ 0.85
LOD	1.95 [µg/mL]	0.68 [µg/mL]	1.48 [µg/mL]	2.01 [µg/mL]	0.631 [µg/spot]	0.295 [µg/spot]	0.200 [µg/spot]
LOQ	5.91 [µg/mL]	2.06 [µg/mL]	4.48 [µg/mL]	6.08 [µg/mL]	1.912 [µg/spot]	0.893 [µg/spot]	0.605 [µg/spot]
Recovery 80% [%]	$\bar{x}$ = 103.31 S_x = 1.2236 $t_{95\%}$ = ±1.3914 RSD = 1.18%	$\bar{x}$ = 100.27 S_x = 0.5750 $t_{95\%}$ = ±0.6538 RSD = 0.57	$\bar{x}$ = 99.06 S_x = 2.576 $t_{95\%}$ = ±2.9321 RSD = 2.60	$\bar{x}$ = 99.66 S_x = 2.622 $t_{95\%}$ = ±2.9811 RSD = 2.63	$\bar{x}$ = 101.04 S_x = 1.2542 $t_{0.95}$ = ±1.5573 RSD = 1.24%	$\bar{x}$ = 101.38 S_x = 1.4360 $t_{0.95}$ = ±1.783 RSD = 1.42%	$\bar{x}$ = 99.44 S_x = 1.6861 $t_{0.95}$ = ±2.0936 RSD = 1.70%
Recovery 100% [%]	$\bar{x}$ = 98.28 S_x = 0.7245 $t_{95\%}$ = ±0.8238 RSD = 0.74	$\bar{x}$ = 104.93 S_x = 0.4679 $t_{95\%}$ = ±0.5321 RSD = 0.45%	$\bar{x}$ = 100.90 S_x = 3.986 $t_{95\%}$ = ±4.5300 RSD = 3.95	$\bar{x}$ = 100.48 S_x = 2.933 $t_{95\%}$ = ±3.2120 RSD = 2.57	$\bar{x}$ = 100.74 S_x = 0.8961 $t_{0.95}$ = ±1.1127 RSD = 0.89%	$\bar{x}$ = 102.74 S_x = 2.1478 $t_{0.95}$ = ±2.6668 RSD = 2.09%	$\bar{x}$ = 101.86 S_x = 1.6876 $t_{0.95}$ = ±2.0954 RSD = 1.66%
Recovery 120% [%]	$\bar{x}$ = 97.18 S_x = 2.1732 $t_{95\%}$ = ±2.4713 RSD=2.24	$\bar{x}$ = 102.43 S_x = 2.0369 $t_{95\%}$ = ±2.3164 RSD = 1.99	$\bar{x}$ = 97.26 S_x = 1.683 $t_{95\%}$ = ±1.9112 RSD = 1.73	$\bar{x}$ = 100.26 S_x = 2.177 $t_{95\%}$ = ±2.4700 RSD = 2.17	$\bar{x}$ = 100.68 S_x = 1.4342 $t_{0.95}$ = ±1.7808 RSD = 1.42%	$\bar{x}$ = 102.50 S_x = 1.2207 $t_{0.95}$ = ±1.5156 RSD = 1.19%	$\bar{x}$ = 103.44 S_x = 0.9813 $t_{0.95}$ = ±1.2185 RSD = 0.95%
Precision $\bar{x}$ S_x $t_{95\%}$ RSD	 20.06 [mg/tab] 0.2873 ±0.6011 0.30%	 12.43 [mg/tab] 0.3211 ±0.6011 0.56%	 49.85 [mg/tab] 0.4223 ±0.7422 0.30%	 12.68 [mg/tab] 0.4723 ±0.8214 1.44%	 2609.82 [mm^2] 51.404 ±63.827 1.97%	 2874.48 [mm^2] 48.1515 ±59.788 1.68%	 5313.66 [mm^2] 21.8399 ±27.118 0.41%

$\bar{x}$ - mean value, S_x – standard deviation, RSD% - relative standard deviation, $t_{0.95}$ – confidence interval for 95% probability.

Table 3. Determination results with statistical evaluation.

Pharmaceutical preparation	Determined content (hydrochlorothiazide, mean n = 10) [mg/ tabl.]	Statistical assessment	Determined content (quinapril, mean n = 10) [mg/ tabl.]	Statistical assessment	Determined content (losartan, mean n = 10) [mg/ tabl.]	Statistical assessment
SPECTROPHOTOMETRY						
Accuzide 20	12.36	S_x = 0.2103 $t_{0.95}$ = ±0.6751 $\%E_{rel}$ = 1.12 RSD = 1.07%	19.82	S_x = 0.3660 $t_{0.95}$ = ±0.6751 $\%E_{rel}$ = 0.90 RSD = 1.85%	———	———
LogistaH	12.49	S_x = 0.2772 $t_{0.95}$ = ±0.7074 $\%E_{rel}$ = 0.08 RSD = 2.22%	———	———	49.91	S_x = 0.4931 $t_{0.95}$ = ±0.7074 $\%E_{rel}$ = 0.18 RSD = 0.99%
HPTLC						
Accuzide 20	12.45	S_x = 0.3192 $t_{0.95}$ = ±0.3964 $\%E_{rel}$ = 0.40 RSD = 2.56%	20.06	S_x = 0.2613 $t_{0.95}$ = ±0.3244 $\%E_{rel}$ = 0.30 RSD = 1.30%	———	———
LoristaH	12.69	S_x = 0.1704 $t_{0.95}$ = ±0.2116 $\%E_{rel}$ = 1.52 RSD = 1.34%	———	———	47.78	S_x = 1.3005 $t_{0.95}$ = ±1.6147 $\%E_{rel}$ = 4.44 RSD = 2.72%

S_x – standard deviation, RSD% - relative standard deviation, $t_{0.95}$ – confidence interval for 95% probability

forms of drugs. Due to characteristic course of derivative curves and absence of zero sites, which would have been used for determination of components in three-components mixture, especially with respect to losartan potassium, the method of derivative spectrophotometry is not recommended for such mixtures analysis. This is not a factor determining its usefulness in quantitative analysis of these active substances, since studied substances are not observed in three component combination in a form of a drug. The chromatographic-densitometric method with an application of HPTLC plates and mobile phase composed of n-butanol, acetic acid and distilled water in a ratio of 15 : 5 : 1 (v/v/v) may be successfully used for determination of substance in three-component mixture in developed conditions of analysis. Well separated peaks were obtained in such conditions, which was confirmed by R_f values.

With wavelengths selected for measurements, spectrophotometric and chromatographic methods are specific for analyte represented by the studied compounds. Specificity in spectrophotometric method was determined comparing derivatives values according to the relationship (R_m – first derivative value of model solution; P_f – first derivative value of pharmaceutical formulation). It was found that they do not differ considerably from value of 1 and are within the range from 0.96 to 1.05, which proves almost identical course of derivative curves for standard solutions and pharmaceutical preparation solutions. It may be concluded on this basis that placebo used for formation of suitable drug form does not influence quantitative determination of an active substance.

Well separated peaks of retardation factor values of $R_f \sim 0.70$, $R_f \sim 0.78$ and $R_f \sim 0.85$ for quinapril, hydrochlorothiazide and losartan, respectively, are observed on registered densitograms in chromatographic-densitometric method. Satisfactory separation of the peaks is confirmed by resolution factors $R_s = 1.36$ for quinapril and hydrochlorothiazide peaks, and $R_s = 1.12$ for hydrochlorothiazide and losartan peaks (Figs. 5, 6)

Linearity is maintained in a wide range of concentrations, i.e., from 4.90 to 29.40 μg/mL for quinapril, and from 3.10 to 18.60 μg/mL for hydrochlorothiazide, as well as from 4.83 to 28.95 μg/mL for losartan potassium and from 1.24 to 7.44 μg/mL for hydrochlorothiazide, for spectrophotometric method.

Linearity range determined by the chromatographic-densitometric method was from 0.0313 to 2.00 mg/mL for quinapril, from 0.0306 to 0.49 mg/mL for losartan and from 0.0078 to 1.00 mg/mL for hydrochlorothiazide.

Linear determination coefficient does not determine the linearity of calibration method in an unequivocal manner. Therefore, Mandel's test was used for linearity assessment. The obtained results of linear and square adjustment prove linear adjustment of calibration curve in all the cases except for determination of hydrochlorothiazide using chromatographic-densitometric method. Normality of residues distribution was confirmed using Shapiro-Wilk test (20). Points of straight line crossing do not diverge considerably from zero (Tab. 1).

Sensitivity of developed method is high, for spectrophotometric method LOD was in the range from 0.68 to 2.01 μg/mL and LOQ from 2.06 to 6.08 μg/mL. For chromatographic-densitometric method LOD was in the range from 0.20 to 0.63 μg/spot and LOQ from 0.61 to 1.91 μg/spot.

Percentage of recovery of the studied components presented as the mean values for three levels of concentrations is high, and in the range of 100 ± 5%, both in spectrophotometric and chromatographic-densitometric method. The results of determination for individual constituents are of similar precision, RSD is within a narrow range from 0.30 to 1.97% (Tab. 2).

The results obtained in the study are of a practical value and may be applied in drug quality control.

The results of particular components determination do not diverge from declared values, and are thus characterized by good precision and accuracy, narrow confidence interval and beneficial values of mean standard deviation (S_x), relative error ($\%E_{rel}$) and relative standard deviation (RSD) (Tab. 3). They may also be an indicator for wider application of derivative spectrophotometry method and HPTLC for complex drugs analysis.

CONCLUSIONS

Spectrophotometric and chromatographic-densitometric methods have been proposed for the determination of losartan potassium, quinapril hydrochloride and hydrochlorothiazide present in the complex pharmaceutical formulations. Spectrophotometric method utilizes a "zero-crossing" technique and the corresponding experimentally determined wavelengths. This technique enables to eliminate interferences between determined constituents which are clearly visible in the zero order spectra. The method is characterized by a wide linearity range confirmed by statistical tests, high precision (RSD in the range 0.30 - 1.44%), low values of LOD and LOQ and specificity in relation to the matrix

components. Separation conditions developed by a chromatographic-densitometric method (stationary phase and mobile phase) allow for a satisfactory separation of components of the mixture (confirmed by R_f values). Good separation of the constituents enables qualitative and quantitative determination of the three components in the analyzed mixture.

Analytical procedures presented in this manuscript do not require complicated sample preparation, they are simple and quick to perform and can be used in the quality control of medicinal products, as an alternative to the pharmacopoeial methods.

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Received: 28. 02. 2013