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The derivative spectrophotometric and IP-RP-HPLC determination of the Lometazid® tablets

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Abstract

In this paper the second-derivative spectrophotometric and ion-pair reversed-phase high performance liquid chromatographic methods for the simultaneous determination of some diuretic ingredients are described. Optimal conditions of both techniques for the quantitative analysis of the two-component diuretic mixture of the Lometazid® tablets were settled. The second-derivative order of the spectra in sodium hydroxide with the wavelength modulation was used. For the determination of amiloride hydrochloride the 'zero-crossing' technique was applied, but for methyclothiazide the peak amplitude had to be corrected. In the ion-pair RP-HPLC technique tetrabutylammonium hydroxide was applied as an ion-pairing agent using acetonitrile-phosphate buffer (45:55 v/v) as a mobile phase. The validation was done of both proposed methods.

Keywords: Second-derivative spectrophotometry; Zero-crossing method; Method with correction; Ion-pair RP-HPLC; Amiloride hydrochloride; Methyclothiazide

1. Introduction

The Lometazid® tablets are a unique combination of two diuretic agents. Amiloride hydrochloride is a weak diuretic with potassium-sparing properties. Methyclothiazide is a thiazide sulfonamidic diuretic used for oedema and for hypertension.

The aim of these investigations was to develop spectrophotometric and ion-pair reversed-phase high-performance liquid chromatographic methods for a simultaneous determination of pharmaceutically active ingredients in the Lometazid® tablets. RP-HPLC with an ion-pairing agent tetrabutylammonium hydroxide was developed for the simultaneous separation, identification and determination of diuretic ingredients in the Lometazid® tablets.

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Amiloride hydrochloride is official in the USP 23 [1], British [2] and European Pharmacopea [3], but methyclothiazide only in the USP 23. Titration in non-aqueous media with perchloric acid with the potentiometric determination at the end point is prescribed for amiloride hydrochloride but for methyclothiazide it is an argentometric titration.

The derivative spectrophotometric method is a modern method which permits to resolve the problem of the overlapping spectra. It is a problem in a classical spectrophotometric method because it is impossible to determine simultaneously the components in the mixture without prior extraction. The combination of the derivative spectrophotometric method and HPLC is usualy used in laboratories. Modern scientific laboratories tend to utilize new techniques that later could be used in routine analysis with great precision, exactness, accuracy and reproductivity. The reason behind our goal was to establish conditions for two of such modern methods which could in future be implemented in both qualitative and quantitative analysis of pharmaceutical preparations and which will replace existing less accurate methods. The mentioned methods enable the determination of diuretics individually in the mixture. HPLC is a new and modern one which replaces old ones ever more frequently in analysis of drugs through monographies in international pharmacopeia.

This combination of diuretics is unique, so there are not numerous references. Amiloride hydrochloride was determined in simple pharmaceutical dosage forms or in the combinations with some other drugs. In the presence of hydrochlorothiazide it was determined spectrophotometrically [4], while in the presence of furosemide it was analysed using TLC [5] and HPLC [6] techniques. In the formulations with guanidine derivatives spectrophotometric [7] or spectrofluorimetric methods [8] were used. Methyclothiazide was analysed in human liquids mostly, but in pharmaceutical formulations polarographic [9,10] and spectrophotometric [11] methods were used.

2. Experimental

2.1. Apparatus

A UV-VIS spectrophotometer Cary 3E Varian, Australia, with 1 cm quartz cells was used. Suitable settings were: mode $^2D = d^2A/d\lambda^2$, spectrophotometric wavelength range from 200 to 400 nm, scan speed 120 nm/min, wavelength modulation $\Delta\lambda = 1$ nm and smoothing index 7.

HPLC Varian Star Chromatography Workstation, Varian, Australia, was used for the ion-pair RP-HPLC chromatographic investigations. Separations were performed on a Supelcosil LC-18 250×4.6 mm, 5 μm particles column at room temperature. The samples were introduced through a Rheodyne 7125 injector valve with a 50 μl sample loop. UV detection was performed at 268 nm.

2.2. Materials and reagents

All materials and reagents used were of analytical grade. Methanol (Zorka Pharma d.d., Šabac) was used as a solvent for the spectrophotometric assay. Acetonitrile (HPLC grade, Fluka Chemie AG, Buchs, Schweiz), potassium dihydrogen phosphate p.a. (Merck, Germany), tetrabutylammonium hydroxide (TBAH 40% puriss solution, Fluka Chemie AG, Buchs, Schweiz) and ortophosphoric acid conc. p.a. (Merck, Germany) were used for preparing the mobile phase for HPLC.

The Lometazid® tablets (10 mg amiloride hydrochloride and 5 mg methyclothiazide) is the official formulation in the tablet dosage form (Galenika a.d., Beograd, Yugoslavia).

2.2.1. Mobile phase

Solution A: Weigh 0.816 g potassium dihydrogen phosphate, dissolve in water and dilute to volume in a 1000 ml volumetric flask. Measure 550 ml of this solution, add 13 ml 40% TBAH solution and stir with a magnetic stirrer. Adjust to pH 9.0 ± 0.1 with ortophosphoric acid.

Mobile phase contains solution A–acetonitrile (55:45 v/v).

2.3. Procedure

2.3.1. Derivative spectrophotometric determination

The stock solutions were prepared by dissolving the respective standard substance in methanol to obtain the concentration of 0.2 mg/ml for amiloride hydrochloride, and 0.1 mg/ml for methyclothiazide. For the calibration curve a series of ten solutions was prepared in the concentration range from 0.006 to 0.05 mg/ml for amiloride hydrochloride and from 0.003 to 0.05 mg/ml for methyclothiazide.

To prove the validity and applicability of the proposed derivative spectrophotometric method, the laboratory mixture of standard substances of amiloride hydrochloride and methyclothiazide was made in the ratio which corresponded to the Lometazid® tablets and then measured second-derivative spectra. The stock solution of the laboratory mixture was made by dissolving 20.0 mg amiloride hydrochloride and 10.0 mg methyclothiazide with methanol in a volumetric flask of 200 ml. The concentration of that solution was 0.1 mg/ml for amiloride hydrochloride and 0.05 mg/ml for methyclothiazide.

To analyse the Lometazid® tablets, 12 tablets were accurately weighed and finally powdered. The quantity of the powdered tablets, equivalent to the average mass of two tablets was transferred into a volumetric flask of 200 ml. One hundred and eighty milliliters of methanol was added and dissolved in an ultrasonic bath for 15 min. A volumetric flask was added with methanol to the mark and after that filtered.

The second-derivative spectrum was recorded for the laboratory mixture and for the Lometazid® tablets in the range from 200 to 400 nm against the methanol.

For the accuracy of the investigation three series of laboratory mixtures ($c = 0.016$ mg/ml, $c = 0.020$ mg/ml and $c = 0.024$ mg/ml calculated to amiloride hydrochloride) were prepared with ten solutions for each concentration.

2.3.2. Ion-pair RP-HPLC determination

The stock solutions were prepared by dissolving the respective standard substance in mobile phase to obtain the concentration of 0.1 mg/ml for amiloride hydrochloride and 0.05 mg/ml for methyclothiazide. For the calibration curve a series of seven solutions was prepared in the concentration range from 0.02 to 0.15 mg/ml for amiloride hydrochloride and from 0.01 to 0.075 mg/ml for methyclothiazide.

The laboratory mixture of standard substances of amiloride hydrochloride and methyclothiazide was made in the ratio which corresponded to the Lometazid® tablets. The stock solution of the laboratory mixture was made by dissolving 25.0 mg amiloride hydrochloride and 12.5 mg methyclothiazide with mobile phase in a volumetric flask of 25 ml. After dilution, the concentration of the analysed solution was 0.1 mg/ml for amiloride hydrochloride and 0.05 mg/ml for methyclothiazide.

For analysing the Lometazid® tablets twelve tablets were accurately weighed and finally powdered. The quantity of the powdered tablets, equivalent to the average mass of one tablet, was transferred into a 10 ml volumetric flask. Five milliliters of mobile phase was added and extracted in an ultrasonic bath for 15 min. A volumetric flask was added with mobile phase to the mark and after that filtered through a Sartorius 45 μm filter. After the dilution with mobile phase, the final concentrations of amiloride hydrochloride and methyclothiazide in the sample solution were 0.1 and 0.05 mg/ml, respectively.

In the chromatographic system was injected 50 μl of the laboratory mixture or the sample solution in an isocratic mode at the flow rate of 1 ml/min at room temperature. The measured peak areas of amiloride hydrochloride and methyclothiazide were detected at 268 nm.

3. Results and discussion

The classical spectrophotometric method is not suitable for analysing a multicomponent mixture because of the overlapping of the absorption spectra of the ingredients because it is often complicated by an interference from the formulation matrix. Also, energy changes in molecules are very composite, causing wide and complex absorption bands, but not too characteristic for

identification or quantification of the substances in a multicomponent mixture. The derivative spectroscopy is a simple technique for magnifying the fine structure of spectral curves. It consists of calculating the first, second, or higher order derivative of a spectrum with respect to wavelength or frequency and plotting this derivative rather that the spectrum itself. The higher order derivative spectrophotometry permits to resolve problems of the overlapping bands of the spectra. Resolution increases with the derivative order. It is most important to choose the optimal derivative order to resolve the absorption spectra. For a quantitative analysis it is necessary to measure the peak amplitude of the derivative spectra in the concentration range of the Lambert–Beer linearity.

The zero order, first, second, third and fourth-derivative spectra for all investigated ingredients of the Lometazid® tablets were recorded in the wavelength range from 200 to 400 nm. For a simultaneous determination the authors chose the second-derivative order ^2D (Fig. 1).

Amiloride hydrochloride was determined at 300 nm, while methyclothiazide was determined at 226 nm. The signal at 300 nm corresponded to amiloride hydrochloride, while the signal of methyclothiazide at the same wavelength was zero. That was the reason why the authors chose the 'zero crossing' technique for the determination of amiloride hydrochloride in the combination with methyclothiazide at 300 nm. But for determining methyclothiazide it was necessary to correct the peak amplitude. It demanded a calculation of the participation (factor of correction) for amiloride hydrochloride at the same wavelength for methyclothiazide determination. The factor showed the participation of one component in the mixture with the other when the bands overlapped. It could be calculated from the calibration spectra of amiloride hydrochloride, because the ratios of the characteristic signals at the chosen wavelength (in our case: for amiloride hydrochloride at $\lambda = 226$ nm) and the signals at the wavelength where methyclothiazide 'was zero' ($\lambda = 300$ nm) were of constant value:

$$f = \frac{D^{A}_{300}}{D^{A}_{226}}$$

where D^{A}_{300} is the peak amplitude for amiloride hydrochloride in the second-derivative spectra at 300 nm and D^{A}_{226} is the peak amplitude for amiloride hydrochloride in the second-derivative spectra at 226 nm.

It means that the participation of amiloride hydrochloride in the mixture with methyclothiazide at 226 nm is given by:

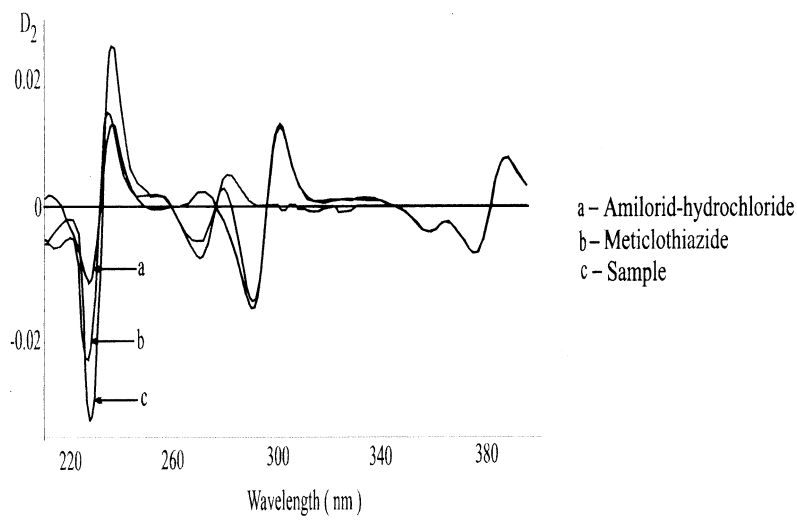

Fig. 1. Second-derivative spectra of amiloride hydrochloride (a), methyclothiazide (b) and laboratory mixture which corresponds to Lometazid® tablets (c) in methanol.

Table 1
Second-derivative spectrophotometric determination of Lometazid® tablets

Parameters	Amiloride hydrochloride ($\lambda = 300$ nm)	Methyclothiazide ($\lambda = 226$ nm)
Concentration range (mg/ml)	0.006–0.050	0.003–0.05
$y = ax + b$	$y = 0.319x + 0.022$	$y = 1.216x - 0.010$
Correlation coefficient	$r = 0.9995$	$r = 0.9993$
Standard deviation of the slope	$S_a = 3.9 \times 10^{-3}$	$S_a = 1.8 \times 10^{-2}$
Standard deviation of the intercept	$S_b = 2.7 \times 10^{-5}$	$S_b = 6.2 \times 10^{-5}$
Standard deviation of regression coefficient	$S_r = 4.9 \times 10^{-5}$	$S_r = 1.1 \times 10^{-4}$
t_α ($k = 8$)	$t_\alpha = 2.361$	$t_\alpha = 1.882$
Limit of detection	LD = 0.0008 mg/ml	0.0004 mg/ml
Limit of quantification	LQ = 0.003 mg/ml	0.0015 mg/ml
Lometazid® tablets		
Taken (mg/tbl)*	10.0	5.0
Found (mg/tbl)	10.24	4.88
Standard deviation (mg/tbl)	0.16	0.10
Coefficient of variation (%)	CV = 1.55	CV = 1.98
Recovery (%)	$R = 102.4$	$R = 97.7$

* $n = 10$.

$$D^{A}_{226} = \frac{D^{A}_{300}}{f}$$

The total peak amplitude (D^{L}_{226}) of the mixture of amiloride hydrochloride and methyclothiazide at 226 nm is:

$$D^{L}_{226} = D^{A}_{226} + D^{M}_{226}$$

where D^{M}_{226} is the peak amplitude for methyclothiazide at 226 nm.

Under described experimental conditions the calibration curves, obtained by plotting ^2D values versus concentration at the mentioned characteristic wavelengths, show linear relationships in the following concentration ranges: 0.006–0.05 mg/ml for amiloride hydrochloride and 0.003–0.05 mg/ml for methyclothiazide. The calibration curves were in agreement with the Beer's law. The regression equations for the investigated substances were calculated including the standard deviation of the slope (S_a), standard deviation of the intercept (S_b), correlation coefficient (r) and standard deviation of the correlation coefficient (S_r) (Table 1).

For quantitative analyse of the Lometazid® tablets ten solutions were prepared. The amount of the ingredients was calculated by the method of standard. Table 1 presents the results of the determination of amiloride hydrochloride and methyclothiazide in the Lometazid® tablets under described experimental conditions.

The validation parameters (linearity, selectivity, accuracy, precission, limit of detection and limit of quantification) were also determined (Table 1). The derivative spectrophotometric method is selective because the excipient did not interfere during the determination of the ingredients of the Lometazid® tablets. Small amounts of the coefficient t_α (2.361 for amiloride hydrochloride and 1.882 for methyclothiazide) show good accuracy and the coefficient of variation (1.55 and 1.98%, respectively) confirms a precission of the method.

The Lometazid® tablets were also analysed using the ion-pair RP-HPLC (RP-IPC) method with tetrabutylammonium hydroxide as a pairing agent. Under described experimental conditions the retention time for amiloride hydrochloride was 2.451 min and for methyclothiazide was 5.249 min (Fig. 2). The capacity factor k', selectivity factor α and resolution factor R_s were calculated (Table 2).

The calibration curves, obtained by measuring the peak areas versus concentration at the detected wavelength, show linear relationships in the following concentration ranges: from 0.02 to 0.15 mg/ml for amiloride hydrochloride and from 0.01 to 0.075 mg/ml for methyclothiazide. The regression equations for investigated substances were calculated including the standard deviation of the slope (S_a), standard deviation of the intercept (S_b), correlation coefficient (r) and standard deviation of the correlation coefficient (S_r)

For a quantitative RP-IPC analysing of the Lometazid® tablets ten solutions were prepared in

the same way as in the spectrophotometric method. The amount of the ingredients was calculated by the method of external standard. Table 2 presents the results of the determination of amiloride hydrochloride and methyclothiazide in the Lometazid® tablets under described experimental chromatographic conditions. The quantity Q (mg/tbl) of amiloride hydrochloride (or methyclothiazide) was calculated from the following equation:

$$Q = \frac{P_s}{P_{st}} \cdot \frac{C_{st} \times B}{W}$$

P_s, peak area of amiloride hydrochloride (methyclothiazide) in the sample.

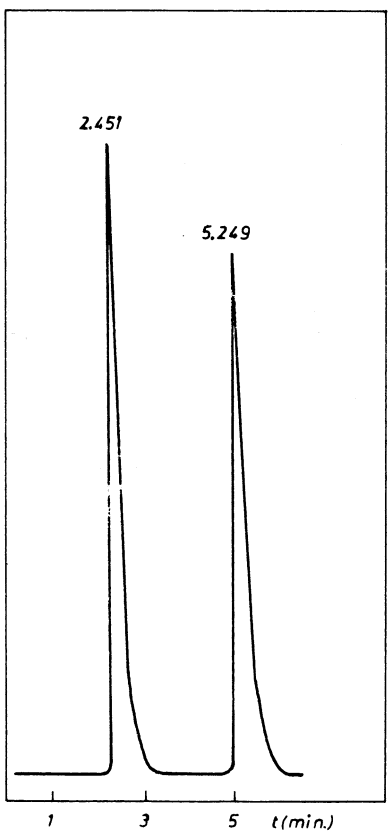

Fig. 2. Chromatogram of amiloride hydrochloride (a) and methyclothiazide (b) in Lometazid® tablets. Mobile phase, phosphate buffer–acetonitrile (55:45 v/v), flow rate 1 ml/min and detection wavelength 268 nm.

Table 2
Ion-pair reversed-phase high performance liquid chromatographic determination of Lometazid® tablets*

Parameters	Amiloride hydrochloride	Methylclothiazide
Retention time (min)	$t = 2.451$	$t = 5.249$
Capacity factor	$k' = 2.410$	$k' = 6.222$
Concentration range (mg/ml)	0.02–0.15	0.01–0.075
$y = ax + b$	$y = 6.45 \times 10^6 x + 2192$	$y = 10.85 \times 10^6 x + 8775$
Correlation coefficient	$r = 0.9999$	$r = 0.9996$
Standard deviation of the slope	$S_a = 2.8 \times 10^4$	$S_a = 1.3 \times 10^5$
Standard deviation of the intercept	$S_b = 2622$	$S_b = 6013$
Standard deviation of regression coefficient	$S_r = 3074$	$S_r = 7050$
$t_\alpha = (k = 5)$	$t_\alpha = 0.425$	$t_\alpha = 10.330$
Limit of detection	LD = 0.002 mg/ml	0.001 mg/ml
Limit of quantification	LQ = 0.006 mg/ml	0.003 mg/ml
Lometazid® tablets		
Taken (mg/tbl)*	10.0	5.0
Found (mg/tbl)	9.96	4.95
Standard deviation (mg/tbl)	0.10	0.05
Coefficient of variation (%)	CV = 1.06	CV = 1.08
Recovery (%)	R = 99.65	R = 99.10

* $n = 10$; Selectivity factor $\alpha = 2.58$; Resolution factor $R_s = 4.07$.

P_{st}, peak area of amiloride hydrochloride (methyclothiazide) in the standard solution.

C_{st}, concentration of the standard solution of amiloride hydrochloride (methyclothiazide) in mg/ml.

W, weighed mass of tablets in mg.

B, average mass of tablets in mg.

The validation parameters (linearity, selectivity, accuracy, precission, limit of detection and limit of quantification) were also determined (Table 2). The placebo tablet did not give any chromatographic signal, so the method is selective for analysing the Lometazid® tablets. Small amounts of the coefficient t_α (0.425 for amiloride hydrochloride and 0.330 for methyclothiazide) show good accuracy and the coefficient of variation

(1.06 and 1.08%, respectively) confirms precission of the method.

The robustness of a method describes the effect of minor changes in the analytical parameters, such as pH values, temperature, flow rate, etc. In our investigations, it can be concluded that RP-IPC method is robust, because slight variations in some experimental parameters have little or no effect on the results, beside eluent composition. There were no remarkable changes of the retention time except that the increase of temperature decreased the retention time and the shape of the peak was finer.

Tendency of modern laboratories is learning towards repalcement of archaic methods (including titrations) with new spectroscopic and especialy methods of separations such as HPLC and CE.

From the point of time factor, it is clear that HPLC, under previously well established conditions is allmost an ideal method pertaining to sensitivity, exactness and precission providing possibility for analysis of much more elaborate compounds, including the products of degradations, excipience, colours, additives, etc.

4. Conclusion

The second-derivative order of the spectra of the investigated substances and the ion-pair reversed-phase high-performance liquid chromatographic method are suitable for a simultaneous determination of the Lometazid® tablets. The obtained results are accurate and precise and confirmed by statistical parameters. There was no interference of the excipient in the tablets. The authors propose the second-derivative spectrophotometric and ion-pair RP-HPLC method for a simple, rapid, simultaneous, selective, accurate and precise determination of the Lometazid® tablets or a corresponding multicomponent mixture.

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