DETERMINATION OF HYDROCHLOROTHIAZIDE, TRIAMTERENE AND PROPRANOLOL HYDROCHLORIDE BY THE SPECTROPHOTOMETRIC METHOD AND HIGH–PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Abstract: Spectrophotometric and chromatographic (HPLC) methods for determination of hydrochlorothiazide, triamterene and propranolol hydrochloride were elaborated. Both methods were appropriate for the determination of three compounds in pharmaceutical preparations containing their mixtures. Both the elaborated methods for the determination of the studied compounds give comparable results and can successfully be applied to the assay in their mixtures occurring in the composition of pharmaceutical preparations.

Keywords: Hydrochlorothiazide, triamterene, propranolol hydrochloride, spectrophotometric method, reversed phase HPLC method, pharmaceutical mixtures.

Hydrochlorothiazide, triamterene and propranolol hydrochloride are applied in therapeutics as diuretic medicines, in oedema (caused by insufficiency of kidneys), hypertension and chronic insufficiency of heart muscle (1).

In the available literature neither separation nor determination of these compounds in ternary mixtures have been found.

According to the British Pharmacopoeia (BP 98), hydrochlorothiazide in drug formulations can be determined spectrophotometrically (0.01M sodium hydroxide, wavelength 273 nm, A1%cm = 520) and, for example, according to the United States Pharmacopoeia (USP 24) it can be determined in a mixture with reserpine (conditions as in BP 98).

To determine hydrochlorothiazide and triamterene in pharmaceutical preparations containing these compounds both BP 98 and USP 24 recommend the high–performance liquid chromatography method with the application of a C18 column and the following mobile phases: 0.5% ammonium chloride, 0.01M sodium perchlorate with acetonic acid; detection at 273 nm (BP 98) and a solution of phosphate buffer, propyamine (pH 5.5) with acetonitrile; detection at 280 nm (USP 24).

To determine hydrochlorothiazide and propranolol hydrochloride USP 24 is employing the following procedure: a C18 column and a solution of tetrabutylammonium hydroxide, phosphate buffer (pH 2.4) and methanol as mobile phase; detection at 220 nm.

For mixtures of hydrochlorothiazide with other compounds such as amiloride, captopril, enalapril, methyldopa, spironolactone, timolol, in various forms of medicines the HPLC method is used, (USP 24), applying most often a C18 column and mixtures of phosphate buffer solutions of different ionic strength and of various pH values (2.5–4) with acetonitrile or methanol as mobile phases. Also a phenyl column and acidified dilute methanol as mobile phase was applied.

To determine hydrochlorothiazide in a mixture with other compounds, Quang et al. (2,3) have used chemiluminescence after the post-column reaction with Ce(IV) and rhodamine on a C18 column and a mobile phase containing sodium salt of octanolphonic acid, acetic buffer and methanol (pH 3.9).

Khader et al. (4) and Shaikh et al. (5) have determined hydrochlorothiazide in biological material employing, in turn, a C8 column and acetonic buffer (pH 6.5), methanol and acetonitrile as mobile phase; detection at 254 nm and a PRP–1 column, phosphate buffer, acetonitrile, tetrahydrofuran as mobile phase; detection at 225 nm.

The spectrophotometric and chromatographic methods for the determination of hydrochlorothiazide in pharmaceutical preparations were applied by Bathia et al. (6) using a 0.01M hydrochloric acid medium and a cyanate column with a buffer (pH 6.8) and acetonitrile as mobile phase.

To determine hydrochlorothiazide beside other compounds, the spectrophotometric method was used by Panzade et al. (7) (direct method in acidic medium) and through derivatives as proposed by Jain et al. (8) and Ulvi (9,10). The polarographic method for the determination of hydrochlorothiazide in the presence of other compounds has been presented by Martin et al. (11).

To determine triamterene in capsule formula-
tions BP 98 recommends the spectrophotometric method, the absorbance being measured at 360 nm in acidic medium. For this kind of medicine USP 24 recommends the HPLC method, the same as that already described for the mixture of hydrochlorothiazide and triamterene.

El-Rayegy et al. (12) have determined triamterene in capsules employing the colorimetric method after causing a reaction with chloroanilic acid or with DDQ.

Triamterene beside hydrochlorothiazide was determined according to BP 98 and USP 24 and Ulvi (10) as mentioned above. Oertel et al. (13) have applied the HPLC method for the determination of triamterene beside other compounds, employing a C_{18} column, a mixture of phosphate buffer (pH 4) and acetonitrile or methanol as mobile phase and spectrophotometric detection at a wavelength of 242 nm or fluorometric detection (extinction 270 nm, emission 389 nm).

To determine propranolol hydrochloride in tablets and injections, BP 98 is recommending the spectrophotometric method (methanol as measuring medium, wavelength 290 nm, A_{1%cm} = 206; USP 24 is proposing here the HPLC method with a column of C_{18} type and a mobile phase consisting of a solution of lauryl sulphate, phosphoric acid and acetonitrile; detection at 290 nm; for capsules - a C_{18} column and a mobile phase composed of a sodium phosphate solution and acetonitrile is proposed; detection at 220 nm.

The determination methods for mixtures of propranolol hydrochloride with hydrochlorothiazide were described while reviewing procedures for hydrochlorothiazide. Basci et al. (14) and Junker et al. (15) have presented HPLC methods for the determination of propranolol hydrochloride in biological material using columns of C_{18} type and a mobile phases consisting of mixtures of phosphate buffers with acetonitrile or methanol and detection at 254 and 222 nm. The method of capillary electrophoresis for the determination of propranolol hydrochloride beside other compounds in biological material have been applied by Ding et al. (16) and Pak et al. (17).

**EXPERIMENTAL**

**Apparatus**

A spectrophotometer P.U.8800 UV/VIS (Philips), a liquid chromatograph (Shimadzu) with two LC10A pumps, a detector SPD –10A, a controller SCL–10A, an integrator Chromatopac C–R4A equipped with a Rheodyne 7125 batcher with a 20 μl loop were used.

**Materials and chemicals**

The following materials and chemicals were used: hydrochlorothiazide (Pofla, Starogard), triamterene (Pliva), propranolol hydrochloride (Merck GmbH). The tablets Propa Comp.–Ratiopharm containing hydrochlorothiazide 12.5 mg, triamterene 25 mg, propranolol hydrochloride 80 mg; a mixture of 1M HCl and methanol at a ratio of 1:9 (solvent); 0.05M phosphate buffer of pH 3.5 prepared by dissolving 6.9 g of KH₂PO₄ in 800 ml of water adjusting the solution to pH 3.5 with concentrated phosphoric acid and making up the solution to 1000 ml with water; the mobile phase was filtered and degassed on a ultrasonic bath.

All the chemicals used were of analytical - reagent grade.

**Spectrophotometric method**

**Conditions for determining and establishing absorbances**

Absorption spectra of hydrochlorothiazide, triamterene and propranolol hydrochloride in various media were studied. It was found that the most suitable medium for simultaneous quantitative determination of these compounds is a mixture of 1M hydrochloric acid and methanol at a ratio of 1:9 (solvent).

The studied compounds dissolve in this solvent and can be extracted from the powdered tablet mass. Furthermore, the mutual arrangement of spectra of these compounds in this medium is suitable to perform spectrophotometric measurements – a basis for their determination (Figure 1).

The spectrum of hydrochlorothiazide shows absorption maxima at 271 and at about 320 nm, for propranolol hydrochloride at 290 nm and for triamterene at about 260 and 360 nm.

To the arrangement of spectra of the studied
Table 1. Specific absorbance of hydrochlorothiazide, triamterene and propranolol hydrochloride in a mixture of 1M HCl and methanol (129).

<table>
<thead>
<tr>
<th>Name</th>
<th>$A_{\text{f}}^m$</th>
<th>271 nm</th>
<th>290 nm</th>
<th>360 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochlorothiazide</td>
<td>670</td>
<td>72</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Triamterene</td>
<td>445</td>
<td>255</td>
<td>850</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>130</td>
<td>208</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>hydrochloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

compounds of favour is the fact that triamterene has a maximum of the bathochromic shift at 360 nm and at that wavelength, in the studied concentration range, the spectra of hydrochlorothiazide and propranolol hydrochloride solutions show no absorption thus indicating that the determination of all the three compounds without their prior separation is possible.

The following wavelengths were selected for measurements 271, 290 and 360 nm and for these wavelengths the absorbances were established. To do this, solutions of triamterene in the solvent at the concentration of ca. 1 mg/100 ml were prepared. For hydrochlorothiazide solutions were prepared at the concentration of ca. 1 mg/100 ml to perform measurements at 271 nm and 5 mg/100 ml for measurements at 290 nm.

The obtained results are presented in Table 1.

Model mixtures

In order to verify the additivity of absorbances in the solutions containing hydrochlorothiazide, triamterene and propranolol hydrochloride five model mixtures were prepared. By using the established absorbances in calculations, accuracy of the method for a simultaneous determination of the three compounds was verified.

To five 100 ml volumetric flasks the following amounts of the compounds were transferred: hydrochlorothiazide – from 2.987 to 7.996 mg; triamterene – from 6.056 to 16.099 mg; propranolol hydrochloride from 17.818 to 48.023 mg.

To each flask about 70 ml of the solvent was added, the flask being warmed on a ultrasonic bath (40°) for 30 min and then shaken mechanically for 60 min. The contents of the flask was made up to the mark with the solvent and then diluted to obtain solutions suitable for measuring the absorbances at 271, 290 and 360 nm.

The concentration of triamterene was calculated from the absorbance measured at a maximum at 360 nm. Then, after calculating the triamterene concentration in the investigated sample, the absorbance was established in which triamterene is participating at the measured wavelengths for hydrochlorothiazide and propranolol hydrochloride, i.e. at 271 and 290 nm. The concentration of hydrochlorothiazide (H) and propranolol hydrochloride (PC) in the mixtures was calculated according to the formula given below (from two equations, after simplification, results in grammes):

$$PC = \frac{670 \cdot A_{290} - 72 \cdot A_{271}}{130,000} \cdot \text{dilution}$$

$$H = \frac{208 \cdot A_{271} - 130 \cdot A_{290}}{130,000} \cdot \text{dilution}$$

The obtained results are presented in Table 2.

Determination of components in the preparation Propra.Comp.–Ratiopharm

To five 100 ml volumetric flasks a suitable amount of powdered tablet mass (its weight corresponding to one half of the average tablet weight i.e. about 100 mg), was transferred and further treatment was the same as for the model mixtures. The contents of the flasks, after complementation to the mark with the solvent, was filtered and diluted 25 times. The concentration of hydrochlorothiazide, triamterene and propranolol hydrochloride were calculated as already mentioned at the determination of the components in the model mixtures, considering the weighed sample of the preparation and the average mass of the tablet.

The following results were obtained: for hydrochlorothiazide $\bar{x} = 12.34 \pm 0.10$ mg (RSD = 0.68%), for triamterene $\bar{x} = 24.94 \pm 0.26$ (RSD = 0.84%), for propranolol hydrochloride $\bar{x} = 81.56 \pm 0.21$ mg (RSD = 0.21%); p = 95%, n = 5.

Chromatographic method

Conditions

The separation of the compounds studied was verified on a number of columns (Lichrosorb RP-18, Ultrasphere ODS, Nucleosil C18, Hypersil ODS, Hypersil BDS ODS, Zorbax SB phenyl, Lichrospher-CN) applying various mobile phases, e.g. a mixture of methanol and/or acetonitrile with water, buffers of various pH values and ionic strength, using also other modifiers. Finally, a Nucleosil 100 C18 150 X 4.6 mm (Merck) column was selected; the mobile phase was: acetonitrile, 0.05M phosphate buffer of pH 3.5, 17:83; flow rate = 1.5 ml/min; detection at 270 nm.

Standard solutions to construct a calibration curve

The solutions of hydrochlorothiazide, triamterene and propranolol hydrochloride were prepared in the following way. The weighed quantity of each compound was transferred to a volumetric
Table 2. Verification of the accuracy of simultaneous determination of hydrochlorothiazide, triamterene and propranolol hydrochloride; model mixtures (UV method).

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Hydrochlorothiazide (H) (mg)</th>
<th>Recovery %</th>
<th>Triamterene (T) (mg)</th>
<th>Recovery %</th>
<th>Propranolol hydrochloride (PC) (mg)</th>
<th>Recovery %</th>
<th>Ratio of mass of components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>added</td>
<td>found</td>
<td>added</td>
<td>found</td>
<td>added</td>
<td>found</td>
<td>added</td>
</tr>
<tr>
<td>1</td>
<td>2.987</td>
<td>2.957</td>
<td>98.99</td>
<td>6.056</td>
<td>6.047</td>
<td>99.85</td>
<td>18.166</td>
</tr>
<tr>
<td>2</td>
<td>5.185</td>
<td>5.263</td>
<td>101.50</td>
<td>10.471</td>
<td>10.470</td>
<td>99.99</td>
<td>20.860</td>
</tr>
<tr>
<td>3</td>
<td>5.861</td>
<td>5.735</td>
<td>97.85</td>
<td>6.730</td>
<td>6.794</td>
<td>100.95</td>
<td>18.019</td>
</tr>
</tbody>
</table>

$\bar{x} = 99.69\% \pm 1.70\%$

$p = 95\%$

RSD = 1.37\% (n = 5)

$\bar{x} = 99.98\% \pm 0.87\%$

$p = 95\%$

RSD = 0.70\% (n = 5)

$\bar{x} = 99.24\% \pm 2.00\%$

$p = 95\%$

RSD = 1.62\% (n = 5)

Table 3. Relation between the peak area (S) and the concentration (C) for hydrochlorothiazide, triamterene and propranolol hydrochloride.

<table>
<thead>
<tr>
<th>Hydrochlorothiazide (H)</th>
<th>Triamterene (T)</th>
<th>Propranolol hydrochloride (PC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c, mg/ml</td>
<td>S</td>
<td>S/c</td>
</tr>
<tr>
<td>0.000452</td>
<td>13937</td>
<td>30834070</td>
</tr>
<tr>
<td>0.000740</td>
<td>22894</td>
<td>30937838</td>
</tr>
<tr>
<td>0.002704</td>
<td>81632</td>
<td>30189349</td>
</tr>
<tr>
<td>0.004092</td>
<td>119922</td>
<td>29306329</td>
</tr>
<tr>
<td>0.010384</td>
<td>319742</td>
<td>30791795</td>
</tr>
<tr>
<td>0.020650</td>
<td>616871</td>
<td>29872688</td>
</tr>
<tr>
<td>0.040980</td>
<td>1241502</td>
<td>30293530</td>
</tr>
<tr>
<td>0.076760</td>
<td>2290352</td>
<td>29837838</td>
</tr>
<tr>
<td>0.137350</td>
<td>4097236</td>
<td>29830622</td>
</tr>
<tr>
<td>0.201590</td>
<td>6066946</td>
<td>30095471</td>
</tr>
</tbody>
</table>

$\bar{x} = 30201861 \pm 254660$

$p = 95\%$

$\bar{x} = 21217099 \pm 197804$

$p = 95\%$

$\bar{x} = 5650568 \pm 44337$

$p = 95\%$
flask, 20% (v/v) of acetonitrile was added and the solution was put on a ultrasonic bath for 2 min. Then 4% (v/v) of concentrated acetic acid was added and the solution was put again on a ultrasonic bath for 2 min. Finally, 20% (by volume) of water was added and the solution was put on a ultrasonic bath for 20 min and then on a shaker for 30 min and made up to the mark with water.

The same procedure was adopted while preparing all the solution and it was named „dissolved”. Then the prepared solutions were diluted to proper concentrations with the mobile phase. The concentrations were as follows: for hydrochlorothiazide from 0.00045 mg/ml to 0.2 mg/ml, for triamterene from 0.0006 mg/ml to 0.2 mg/ml and for propranolol hydrochloride from 0.0012 mg/ml to 0.25 mg/ml.

Model mixtures

To six volumetric flasks a suitable amount of powdered tablet mass (its weight corresponding to one forth of the average tablet mass) was transferred. Then to each flask weighed amounts of the studied compounds were transferred, their weights being conformed with the amounts of the particular components in the weighed tablet mass, i.e. about 3.2 mg of hydrochlorothiazide, about 6.5 mg of triamterene and about 20 mg of propranolol hydrochloride. The contents of the flasks was „dissolved” in the way as mentioned above, the solution was made up to the mark with water, filtered and diluted ten times with the mobile phase. The concentration of the compounds was determined with relation to the solutions of the particular standard substances, prepared independently, at the concentrations of ca. 0.006 mg/ml for hydrochlorothiazide, 0.013 for triamterene and 0.04 mg/ml for propranolol hydrochloride. In calculations of the recovery, the amount of the particular substances originating from the preparation, determined in tablets by the HPLC method, was considered.

**Determination of active components in the preparation Propra. Comp.-Ratiopharm**

Preparation of the sample: to five 100 ml volumetric flasks a suitable amount of powdered tablet mass (its weight corresponding to one half of the average tablet mass) was transferred and „dissolved” as mentioned above, made up to the mark with water and filtered. The filtrate was diluted ten times with the mobile phase.

**RESULTS AND DISCUSSION**

**Spectrophotometric method**

The elaborated method enables to determine

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### Table 4: Results of determination of hydrochlorothiazide, triamterene and propranolol hydrochloride in model mixtures by the HPLC method.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Hydrochlorothiazide (mg)</th>
<th>Found (mg/ml)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.003724</td>
<td>0.000794</td>
<td>99.14</td>
</tr>
<tr>
<td>2</td>
<td>0.003649</td>
<td>0.000697</td>
<td>99.46</td>
</tr>
<tr>
<td>3</td>
<td>0.004966</td>
<td>0.000763</td>
<td>99.73</td>
</tr>
<tr>
<td>4</td>
<td>0.002588</td>
<td>0.000652</td>
<td>98.61</td>
</tr>
<tr>
<td>5</td>
<td>0.004127</td>
<td>0.000796</td>
<td>99.67</td>
</tr>
<tr>
<td>6</td>
<td>0.003708</td>
<td>0.000712</td>
<td>99.11</td>
</tr>
</tbody>
</table>

**X = 99.68% ± 2.38%**

**RSD = 2.14%**

**X = 99.90% ± 2.45%**

**RSD = 2.33%**
hydrochlorothiazide, triamterene and propranolol hydrochloride in a mixture with reasonable accuracy and sufficient precision without their separation.

The practical use of the method has been verified by determining the studied compounds in the preparation Prepra. Comp. – Ratiopharm, in which their proportions are as follows: hydrochlorothiazide: triamterene: propranolol hydrochloride = 1:2:6, respectively.

Results obtained for the model mixtures allow to assume that the method could be applied to mixtures in which the studied compounds appear in changed proportions (Table 2). The method is simple, fairly quick and the reagents are easy available.

**Chromatographic method**

As a result of the performed studies the best selected system for the determination of the studied compounds was the following: a Nucleosil column 100 C4 150 x 4.6 mm and a mobile phase consisting of acetonitrile and 0.05M phosphate buffer (KH2PO4) of pH 3.5, 17:83; flow rate – 1.5 ml/min. The retention times were: for hydrochlorothiazide – about 2.4 min, for triamterene – about 3.6 min and for propranolol hydrochloride – about 20 min. The resolution, R, (calculated according to Ph.E) between hydrochlorothiazide and triamterene must be equal to or greater than 3.

The chosen detection at 270 nm was based on the location of absorption maxima, absorbances and also on the quantitative relation of the particular components in tablets (Figure 1, Table 1).

The relation between the peak area and the concentration of the particular components was a constant value within a large concentration range and includes measured concentration in the solutions of the studied samples (Table 3).

The linearity range found was as follows: for hydrochlorothiazide – from 0.00045 mg/ml to 0.2 mg/ml, for triamterene – from 0.00006 mg/ml to 0.2 mg/ml and for propranolol hydrochloride – from 0.013 mg/ml to 0.25 mg/ml. The detection limit for these compounds was 0.05 µg/ml, 0.1 µg/ml and 0.01 mg/ml, respectively.

Results of the determination in model mixtures showed a good recovery and satisfactory precision for all the compounds (Table 4).

Results of the determination of active substances demonstrate good precision of the method and obtained: for hydrochlorothiazide – 13.08 ± 0.35 mg/tablet, for triamterene – 0.40 mg/tablet and for propranolol hydrochloride – 83.76 ± 0.68 mg/tablet at p = 95%. The RSD were 2.17%, 1.31% and 0.65%, respectively; n = 5.

**CONCLUSION**

Both the elaborated methods for the determination of the studied compounds give comparable results and can successfully be applied to the assay of hydrochlorothiazide, triamterene and propranolol hydrochloride in mixtures occurring in the composition of pharmaceutical preparations.

**REFERENCES**


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