To improve therapy of cardiovascular system diseases, a number of medicinal substances are used in the form of complex drugs, as in the case of hydrochlorothiazide and metoprolol (1). Both constituents of this drug have similar physicochemical properties, thus arising difficulty in their identification and quantitative determination. This is why separating methods with the use of chromatography and electrophoresis predominate in analytical reports.

To determine hydrochlorothiazide in medicinal products (beside lisinopril, amilorid, methyldopa and losartan) chromatographic methods were used (2–4). Angiotensin convertase inhibitors were determined along with hydrochlorothiazide by capillary electrophoresis method (5). Good results of quantitative analysis for this substance in complex drugs beside enalapril, amilorid, atenolol, propranolol and triamteren were obtained with UV spectrophotometry (6–10).

As no similar analyses were found in available literature it seems justifiable to develop a simple, quick and easily available spectrophotometric method for drug quality control purposes.

EXPERIMENTAL

Materials

- **MET – metoprolol tartrate** – (Astra Hässle, Germany)
- **HYD – hydrochlorothiazide** – (Merck, Germany)
- **Metoprolol-Ratiopharm** – (Ratiopharm, Germany)
- **Methanol** – (Merck, Germany)

Apparatus

(a) **Spectrophotometer UV–Vis Cary 100** (Varian), 10 mm quartz cells
(b) **Computer** – PC Pentium MMX, 16 MB RAM, Hewlett–Packard LaserJet 6L printer and software (Microsoft Office 97, Statistica 5.1 edition 97).
Metoprolol and hydrochlorothiazide standard solutions

Standard solutions were prepared in methanol: metoprolol at concentrations from 100.0 µg·mL⁻¹ to 300.0 µg·mL⁻¹ by dilution of basic solution of 2.0 mg·mL⁻¹, hydrochlorothiazide at concentrations from 12.5 µg·mL⁻¹ to 37.5 µg·mL⁻¹ by dilution of basic solution of 0.25 mg·mL⁻¹.

Sample solutions

From powdered mass of 20 drug tablets 0.35 g was weighed and 5.0 mL of methanol was added. The mixture was shaken for 15 minutes. The obtained suspension was filtered and 1.0 mL of clear solution was taken and filled up to 100 mL with methanol.

RESULTS AND DISCUSSION

Establishing the measurement conditions

There were well developed zero-order absorption spectra recorded for standard solutions (Fig. 1). There are two absorbance maxima for hydrochlorothiazide, higher one at λ~271 nm and lower at λ~317 nm. For metoprolol there is a maximum at λ~276 nm and characteristic inflexion at λ~282 nm (Fig. 1).

The solution absorption spectrum recorded for a mixture in which the concentrations of both constituents are comparable to those of the preparation under investigation, shows spectral interferences originated from individual constituents, thus making simultaneous determination impossible. By using the characteristic inflexion at λ~282 nm favourable conditions were established for derivative spectrophotometry (14) (Fig. 2).

Figure 1. Zero order uv spectra for preparation (A) hydrochlorothiazide (B) and metoprolol (C).

Figure 2. Third order uv derivative spectra for hydrochlorothiazide (A) and metoprolol (B) (c₁= 100.0 µg·mL⁻¹, c₂=150.0 µg·mL⁻¹, c₃= 200.0 µg·mL⁻¹, c₄= 250.0 µg·mL⁻¹, c₅= 300.0 µg·mL⁻¹).
There are well developed third order derivative absorption spectra (Figure 2) showing clearly indicated extremes. At wavelength $\lambda\sim281$ nm chosen for determining metoprolol, the value of third derivative absorption spectrum originated from hydrochlorothiazide is zero. No hydrochlorothiazide interferences are observed even at different concentrations (Fig. 3). To determine hydrochlorothiazide the first order derivative spectra were used by making measurements at $\lambda\sim282$ nm (Fig. 4), at which $D_1=0$ for metoprolol. Any change in metoprolol concentration has no effect on the measurements of derivative $D_1$, chosen for quantitative determination of hydrochlorothiazide (Fig. 5).

In the next step of this study the conditions of method were validated by determining specificity, linearity range, detection limit and quantitation limit as well as accuracy based on the results of analysis obtained for the drug under investigation (15).

**Specificity**

To find an effect of matrix constituents on the results of determination, comparative analysis was carried out for standard solution containing active components at concentrations comparable to those of the analyzed preparation (Fig. 6). The values of derivatives at selected wavelengths for the sample and standard solution were within admissible errors.
of spectrophotometric method, thus one can conclude that the results of determination remain unaffected by auxiliary constituents of the drug.

**Linearity**

To check the range of linearity 5 measurements were made for each solution at concentrations from 100.0 µg·mL⁻¹ to 300.0 µg·mL⁻¹ and from 12.50 µg·mL⁻¹ to 37.50 µg·mL⁻¹ for metoprolol and hydrochlorothiazide, respectively. The following results were obtained by using equations of linear regression:

for metoprolol \[ (D_3) = -0.0010 + 0.0002 \cdot c, \quad r = 0.9995 \]

for hydrochlorothiazide \[ (D_1) = -0.0002 - 0.0041 \cdot c, \quad r = 0.9983 \]

**Detection limit and quantitation limit**

The detection limit and quantitation limit were established from analysis of solutions of decreasing concentrations of analyzed substances. It was found that the detection limit is 5.0 µg·mL⁻¹ for metoprolol, while its quantitation limit is 15.0 µg·mL⁻¹. The values for hydrochlorothiazide are 1.5 µg·mL⁻¹ and 4.5 µg·mL⁻¹, correspondingly.

**Accuracy**

The accuracy of the method was determined from percentage recovery by analyzing concentrations of metoprolol and hydrochlorothiazide added to sample solution at amounts from 80% to 120% of the declared values. The obtained results along with statistical evaluation, including mean ($\bar{X}$), standard
deviation (S_x), relative standard deviation ([%]RSD) and confidence interval (t_{0.95}) are listed below: metoprolol [%]: 98.76, 100.41, 97.63, 98.46, 98.69, \bar{X} = 98.79, S_x = 1.0112, t_{0.95} = \pm 1.2558, [%]RSD = 1.02; hydrochlorothiazide [%]: 103.28, 98.39, 100.0, 98.41, 96.88, \bar{X} = 99.39, S_x = 2.4375, t_{0.95} = \pm 3.0263, [%]RSD = 2.45.

Suitability of the developed method for determining metoprolol and hydrochlorothiazide was successfully checked for the complex drug Metoprolol–Ratiopharm comp, containing both analyzed substances (Table 1).

CONCLUSIONS

A quick and accurate method for determining metoprolol and hydrochlorothiazide was developed by using derivative spectrophotometry.

The advantage of this method is that both constituents can be determined directly in a single sample without the need to be separated.

It was also found that auxiliary drug components had no effect on the results of determination obtained under established conditions.

The method gives results of high accuracy and high recovery of 98.79% and 99.39% for metoprolol and hydrochlorothiazide, respectively at good precision; [%]RSD does not exceed 2.5%.

Satisfactory results were obtained also for the drug under investigation and the obtained values do not differ from those declared by the manufacturer.

REFERENCES


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