Acta Poloniae Pharmaceutica – Drug Research, Vol. 63 No. 3 pp. 169-173, 2006 ISSN 0001-6837
Polish Pharmaceutical Society

DETERMINATION OF METOPROLOL AND HYDROCHLOROTHIAZIDE BY DERIVATIVE SPECTROPHOTOMETRIC METHOD IN PHARMACEUTICAL PREPARATIONS.

MARIUSZ STOLARCZYK, RADOSŁAW EKIERT, JAN KRZEK and WŁODZIMIERZ RZESZUTKO

Department of Inorganic and Analytical Chemistry,
Collegium Medium, Jagiellonian University,
9 Medyczna St,.30-688 Cracow, Poland

Abstract:A procedure for simultaneous determination of metoprolol and hydrochlorothiazide in tablets by employing derivative spectrophotometry, "zero-crossing" method was developed. The third order derivative absorption spectra at λ~281 nm were used for metoprolol and the first order derivative spectra at λ~282 nm were used for hydrochlorothiazide. No interferences were found between both determined constituents and those of matrix. A good accuracy and precision of simultaneous determination of metoprolol and hydrochlorothiazide were confirmed by statistical analysis. The recovery of individual constituents under established conditions is very high and ranges from 98.79% to 99.39%. Linearity is maintained within a wide concentration range from 100.0 $\mu g \cdot mL^{-1}$ to 300.0 $\mu g \cdot mL^{-1}$ and from 12.5 $\mu g \cdot mL^{-1}$ to 37.5 $\mu g \cdot mL^{-1}$ for metoprolol and hydrochlorothiazide, respectively. The detection limit is 5.0 $\mu g \cdot mL^{-1}$ for metoprolol and 1.5 $\mu g \cdot mL^{-1}$ for hydrochlorothiazide. The corresponding quantitation limits are 15.0 $\mu g \cdot mL^{-1}$ for metoprolol and 4.5 $\mu g \cdot mL^{-1}$ for hydrochlorothiazide.

Keywords: metoprolol, hydrochlorothiazide, drug analysis, spectrophotometry

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 hare 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).

Metoprolol as well as hydrochlorothiazide in complex drugs were determined with chromatographic methods (11–13).

In this paper a new spectrophotometric method for simultaneous determination of hydrochlorothiazide and metoprolol is presented. Due to interferences in zero–order spectra and significant differences in concentration of both constituents in the preparation, derivative spectrophotometry was used for quantitative analysis by using a slight inflexion at λ~282 nm in the zero–order spectrum. An attempt was made to find suitable derivatives and wavelength for quantitative analysis at which both constituents show no interference.

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 comp tablets – (Rathiopharm, 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},
hydrochlorotiazide 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).

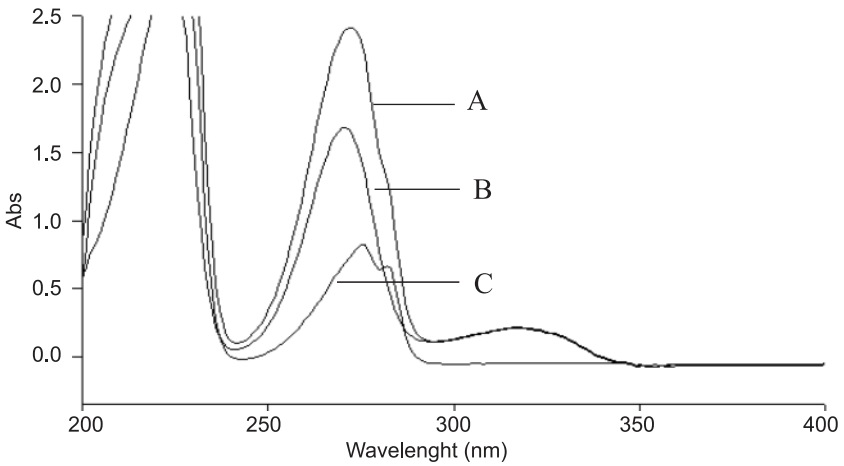

Figure 1. Zero order uv spectra for preparation (A) hydrochlorothiazide (B) and metoprolol (C).

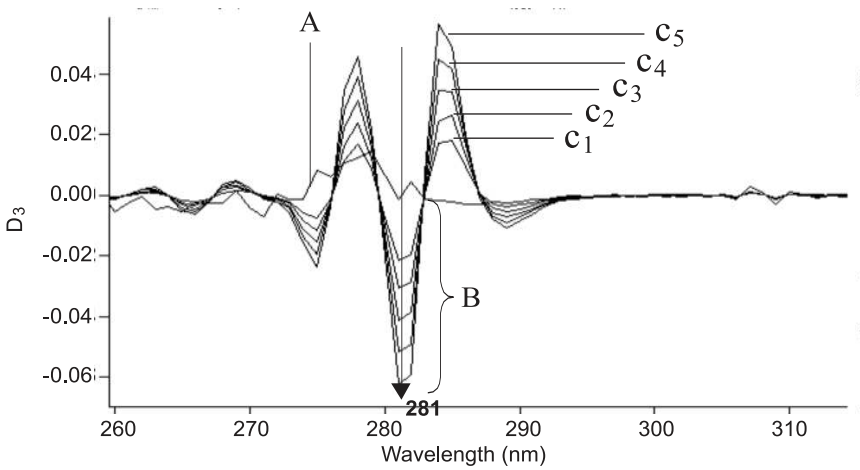

Figure 2. Third order uv derivative spectra for hydrochlorothiazide (A) and metoprolol (B) (c_1= 100.0 μg·mL^{-1}, c_2=150.0 μg·mL^{-1}, c_3= 200.0 μg·mL^{-1}, c_4= 250.0 μg·mL^{-1}, c_5= 300.0 μg·mL^{-1}).

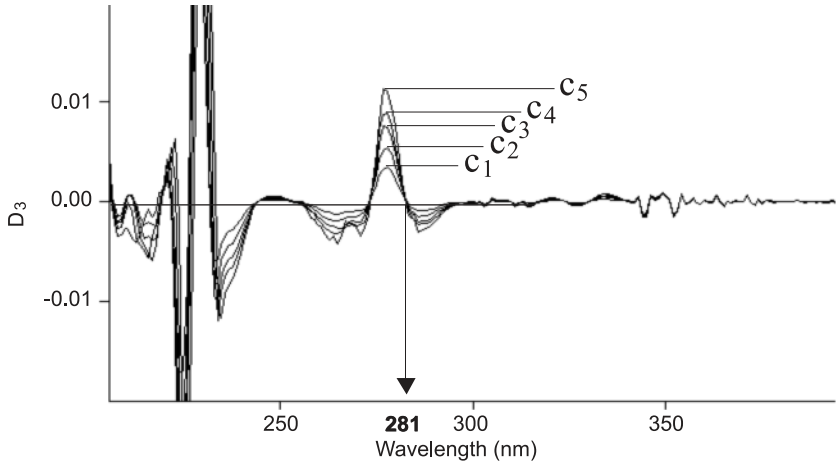

Figure 3. Third order derivative uv spectra for hydrochlorothiazide (c_1= 12.5 μg·mL^{-1}, c_2=18.8 μg·mL^{-1}, c_3= 25.0 μg·mL^{-1}, c_4= 31.3 μg·mL^{-1}, c_5= 37.5 μg·mL^{-1}). At λ~281 nm all spectra have zero value.

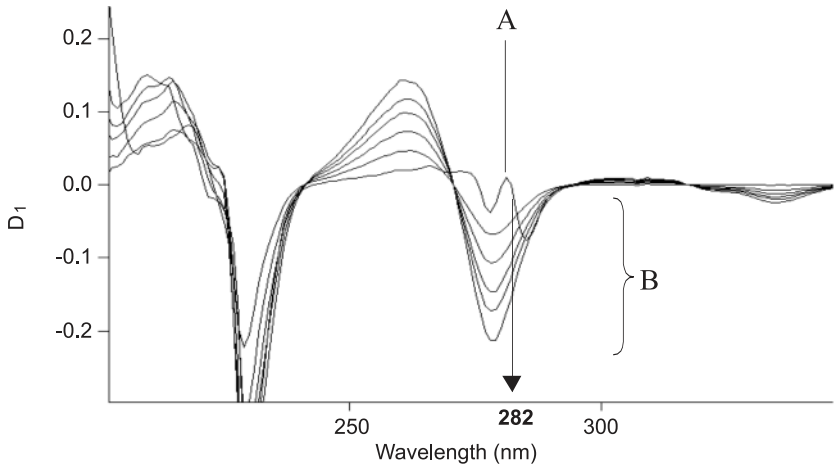

Figure 4. First order derivative uv spectra for metoprolol (A) and hydrochlorothiazide (concentrations as on Fig.3) (B).

There are well developed third order derivative absorption spectra (Figure 2) showing clearly indicated extremes. At wavelength λ~281 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 λ~282 nm (Fig. 4), at which D1= 0 for metoprolol. Any change in metoprolol concentration has no effect on the measurements of derivative D1, 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

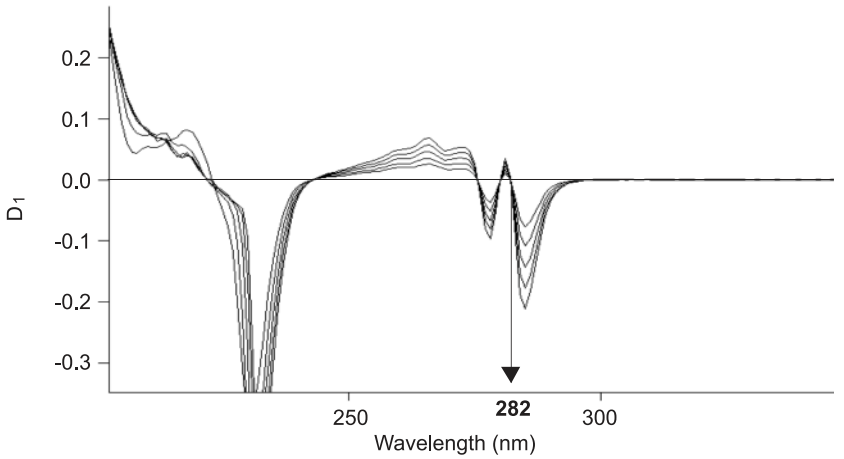

Figure 5. First order derivative uv spectra for metoprolol. At λ~282 nm all spectra have zero value.

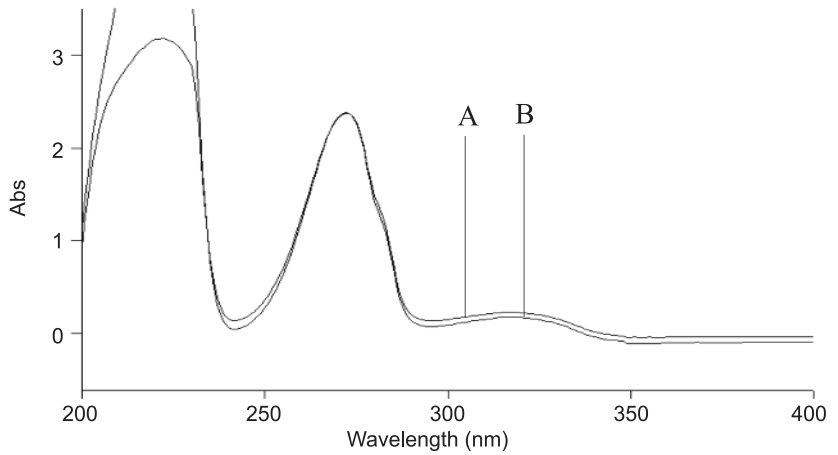

Figure 6. Uv spectra for analyzed preparation (A) and standard solution (B).

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 $(D_3) = -0.0010 + 0.0002 \cdot c$, $r = 0.9995$

for hydrochlorotiazide $(D_1) = -0.0002 - 0.0041 \cdot c$, $r = 0.9983$

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

Table 1. Results of determination of metoprolol and hydrochlorothiazide in tablets.

Determined constituent	Determined quantity [mg/ tablet]	Statistical assessment	
metoprolol [100 mg/ tablet]	103.4 98.5 102.9 100.7 100.7 103.2	$\bar{X}$ S_X $t_{0,95}$ [%]RSD	101.57 1.9346 ± 2.030 1.90
hydrochlorothiazide [12,5 mg/ tablet]	12.4 13.5 12.6 13.0 13.1 13.4	$\bar{X}$ S_X $t_{0,95}$ [%]RSD	13.0 0.4336 ±0.455 3.34

$\bar{X}$ – mean, S_x – standard deviation, [%]RSD – relative standard deviation, $t_{0,95}$ – confidence interval

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, $t_{0,95}$ = ±1,2558, [%]RSD = 1,02;
hydrochlorothiazide [%]: 103.28, 98.39, 100.0, 98.41, 96.88,
$\bar{X}$= 99.39, S_x = 2.4375, $t_{0,95}$ = ±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

1. Podlewski J, Chwalibogowska–Podlewska A.: Leki współczesnej terapii, Split Trading Sp. z o.o., Warszawa (1999).
2. El–Gindy A.,Ashour A., Abdel–Fattah L., Shabana M.M.: J. Pharm. Biomed. Anal. 24, 923 (2001).
3. Zecevic M., Zivanovic L., Agatonovic–Kustrin S.,Minic D.: J. Pharm. Biomed. Anal. 24, 1019 (2001).
4. Carlucci G., Palumbo G.,Mazzeo P., Quaglia M.G., J. Pharm. Biomed.Anal. 23, 185 (2000).
5. Hillaret S., De Grauwe K., Van den Bossche W.: J. Chromatogr. 924, 439 (2001).
6. el Walily A.F., Belal S.F., Heaba E.A., el Kersh A.: J. Pharm. Biomed. Anal. 13 851 (1995).
7. Parasad C.V., Parihar C., Sunil K., Parimoo P.: J. Pharm. Biomed. Anal. 17, 877 (1998).
8. Dinc E., Baleanu D.: J. Pharm. Biomed. Anal. 30, 715 (2002).
9. Jonczyk A. Nowakowska Z.: Acta Pol. Pharm., 58, 339 (2001).
10. Erk N.: J. Pharm. Biomed. Anal. 27, 901 (2002).
11. Vujic Z., Radulovic D., Agbaba D.: J. Pharm. Biomed. Anal. 15, 581 (1997).
12. Yang X., Fukushima T., Santa T., Homma H., Imai K.: Analyst 122, 365 (1997).
13. Fente C.A., Vazquez B.I., Franco C., Cepeda A., Gigosos P.G.: J. Chromatogr. B. 726, 133 (1999).
14. Kuś S., Marczenko Z., Obarski N.: Chem. Anal. (Warsaw), 41, 899, (1996)
15. Zając M., Jelińska A., Muszalska I.: Nogowska, Ocena jakości substancji leczniczych i preparatów farmaceutycznych według wymagań farmakopealnych i ICH, Wydawnictwo Kontekst, Poznań (2000).

Received: 31.03.2006