Validation of a New Spectrophotometric Method for the Assay of Bisoprolol Fumarate using Tropaeolin 00

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A spectrophotometric method for the assay of bisoprolol fumarate was established based on an ion pair complex formed between bisoprolol and tropaeolin 00, in acidic medium, which could be quantified after being extracted in dichloromethane, by measuring its maximum absorbance at 412 nm. The working procedure was established and the analysis method was validated. Thus, 1 mL 0.05M hydrochloric acid and 1 mL of 0.01% tropaeolin 00 aqueous solutions were added to 1.0 mL bisoprolol fumarate solution. Fifteen minutes later the absorbance was measured at 412 nm, using as reference a blank sample prepared in the same conditions. The method presented a good linearity in the range 5-30 μg·mL^{-1} and the correlation coefficient was r = 0.9995. The limit of detection (LOD) was 0.67 μg·mL^{-1} and the limit of quantification (LOQ) was 2.23 μg·mL^{-1}. The relative standard deviation for the precision of the method was 0.78. While studying the accuracy of the method a mean recovery of 100.3 % was established. The experimental data obtained showed a good sensitivity of the method and the obtained value of the specific absorbance for this method was much higher than that of the corresponding bisoprolol solution in the UV.

Key words: bisoprolol, Vis spectrophotometric method, assay, tropaeolin 00

Beta-blockers belong to amine derivatives and have quite important biological function [1,2].

Bisoprolol fumarate is a highly selective β_1-adrenoreceptor antagonist used for the treatment of coronary disease and hypertension [3,4]. Chemically, bisoprolol fumarate is (±)-1-[4-[[2-(1-Methylethoxy) ethoxy]methyl] phenoxy]-3[(1-methylethyl)amino]-2-propanol(E)-2-butenedioate (2:1) (salt) [5]. It possesses an asymmetric carbon atom in its structure and it is provided as a racemic mixture. The S(-) enantiomer of bisoprolol is responsible for the therapeutic effect of reducing blood pressure and most of its beta-blocking activity. Bisoprolol has also shown beneficial cardiac effects in the treatment of hypertension [6-8].

Bisoprolol fumarate can be quantitatively determined in biological fluids and in pharmaceutical formulations by various methods such as UV spectrophotometry, HPLC, HPTLC, densitometry [9-15]. One method has been reported for the quantitative determination of bisoprolol by visible region spectrophotometry [16].

This paper presents a new spectrophotometric method for the assay of bisoprolol using tropaeolin 00 as reagent. The developed method was validated using pure substance [17-22].

Experimental part

Materials and method

Apparatus

Absorbance was measured in quartz cuvettes using a Hewlett Packard 8453 UV–Vis spectrophotometer while maintaining the temperature at 25^{0}C.

Reagents

Only analytical grade chemicals were used as reagents, such as: bisoprolol fumarate (100.07% pure substance provided by Unichem Laboratories LTD, India), hydrochloric acid (Tunic Prod, Romania), dichloromethane (Fluka, Germany); tropaeolin 00 (Tunic Prod, Romania).

A 100 μg·mL^{-1} stock bisoprolol solution was then diluted to obtain standard solutions of various concentrations.

Assay procedure: 1 mL 0.05 M hydrochloric acid and 1 mL 0.01% (w/v) tropaeolin 00 aqueous solution were added to each 1.0 mL of bisoprolol fumarate solution with a concentration in between 5-30 μg·mL^{-1}. The complex was then extracted using dichloromethane. Fifteen minutes later the absorbance was measured at 412 nm, using as reference a blank sample prepared in the same conditions.

Assay validation

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following formulae:

$LOD = 3 \cdot SD \cdot Slope^{-1}$

$LOQ = 10 \cdot SD \cdot Slope^{-1}$

where:

SD = standard deviation of the intercept;

slope = the slope of the calibration curve equation.

Method precision was evaluated through repeatability and reproducibility. Using the experimental data the sample concentration was calculated using the calibration curve equation.

Standard addition method was used to evaluate the accuracy of the method.

Results and discussions

The basic spectrophotometric conditions were designed to be simple and easy to use and reproduce and were selected after testing the different conditions that affect spectrophotometric analysis like chemical and instrumental factors.

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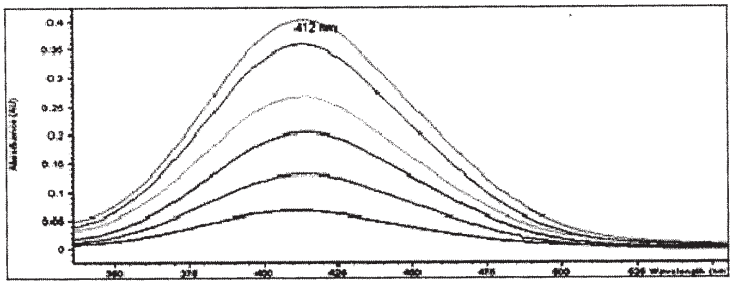

Fig. 1. Absorption spectra of reaction product against reagent blank

Hydrochloric acid ($mol \cdot L^{-1}$)	Bisoprolol concentration		Tropaeolin 00 (%)	Bisoprolol concentration	
	$5 \mu g \cdot mL^{-1}$	$30 \mu g \cdot mL^{-1}$		$5 \mu g \cdot mL^{-1}$	$30 \mu g \cdot mL^{-1}$
	Absorbance	Absorbance		Absorbance	Absorbance
0.001	0.0568	0.4025	0.005	0.0698	0.4103
0.005	0.0572	0.4105	0.01	0.0702	0.4121
0.01	0.0627	0.4112	0.05	0.0712	0.4131
0.05	0.0712	0.4129	0.1	0.0710	0.4125
0.1	0.0702	0.4105	0.5	0.0698	0.0411
0.5	0.0697	0.4102	1	0.0692	0.4023

Table 1
REAGENTS CONCENTRATION

Bisoprolol concentration ($\mu g \cdot mL^{-1}$)		Time (minutes)						
		10	15	20	25	30	40	60
5	Absorbance	0.0705	0.0716	0.0712	0.0706	0.0685	0.0682	0.0682
30		0.4102	0.4125	0.4123	0.4112	0.4053	0.4026	0.3925

Table 2
STUDY OF THE STABILITY OF THE COMPLEX

Bisoprolol concentration ($\mu g \cdot mL^{-1}$)	Absorbance						Mean
	I	II	III	IV	V	VI	
5	0.0703	0.0698	0.0705	0.0697	0.0680	0.0620	0.0684
10	0.1361	0.1354	0.1405	0.1357	0.1372	0.1380	0.1371
15	0.1998	0.2096	0.2013	0.2087	0.2008	0.1979	0.2014
20	0.2698	0.2711	0.2654	0.2685	0.2701	0.2685	0.2689
25	0.3430	0.3461	0.3397	0.3413	0.3453	0.3431	0.3431
30	0.4119	0.4099	0.4120	0.4099	0.4135	0.4198	0.4128

Table 3
LINEARITY DETERMINATION

Analyzing the absorption spectra shown in figure 1, the wavelength of maximum absorption was established at 412 nm. It was used for all the measurements.

The influence of the concentration of reagents upon maximum absorption was investigated. The optimal concentration of the tropaeolin 00 aqueous solution was found to be 0.01% (w/v) and 0.05 M hydrochloric acid was used with best results according to the data shown in table 1.

The complex formation reaction of bisoprolol and tropaeolin 00 was found to be finished after 5 min.

Stability was evaluated at ambient temperature without protection of light. The absorbance was measured after 15 min have passed since dichloromethane extraction according to the experimental data from table 2.

Validation of the method

Linearity was assessed by analyzing the obtained data shown in table 3 by linear regression and the calibration curve from figure 2 was obtained.

The parameters obtained for the validation of the method are summarized in table 4.

While studying the precision of the method, for all sets of data shown in table 5, the relative standard deviation was lower than 2% (RSD = 0.78), which proved that the proposed method was precise.

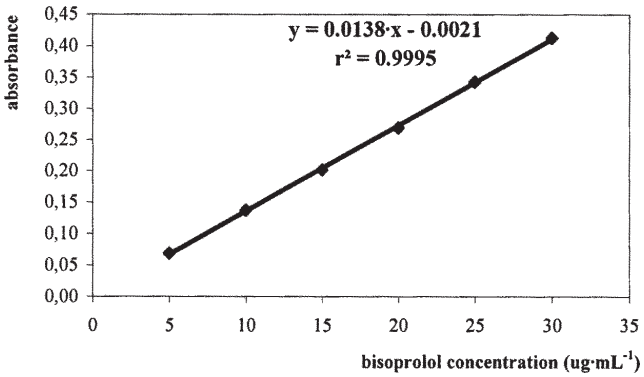

Fig. 2.Calibration curve

Parameter	Values
Wavelength (nm)	402
Linearity domain (μg·mL^{-1})	5-30
Limit of detection (μg·mL^{-1})	0.67
Limit of quantification (μg·mL^{-1})	2.23
Regression equation	Y= 0.0138 · X – 0.0021 where: Y= absorbance, X= concentration
Intercept (a)	0.0138
Slope (b)	0.0021
Correlation coefficient (r)	0.9997
Standard deviation	0.002866117

Table 4
METHOD VALIDATION SUMMARY

Table 5
PRECISION

Bisoprolol concentration (μg·mL^{-1})	Repeatability		Reproducibility	
	Absorbance	Recovery %	Absorbance	Recovery %
10	0.1381	100.39	0.1373	101.01
10	0.1375	101.47	0.1380	101.52
10	0.1376	101.54	0.1369	100.72
15	0.2078	101.71	0.2081	101.54
15	0.2068	101.23	0.2105	102.27
15	0.2081	101.86	0.2068	100,91
20	0.2749	100.67	0.2753	100.51
20	0.2721	99.66	0.2723	99.42
20	0.2758	101.00	0.2746	100.25
Statistical data	Mean = 101.05 SD = 0.71 RSD = 0.70		Mean = 100.95 SD = 0.87 RSD = 0.86	

Table 6
ACCURACY

Theoretical concentration (μg/·mL^{-1})	Absorbance	Calculated concentration (μg·mL^{-1})	Recovery %
10	0.1374	10.14	101.41
	0.1367	10.06	100.60
	0.1353	9.98	99.87
11.25	0.1538	11.30	100.44
	0.1525	11.21	99.64
	0.1519	11.16	99.20
15	0.2081	15.28	101.88
	0.2047	15.03	100.23
	0.2026	14.88	99.23
18.75	0.2588	18.91	100.85
	0.2548	18.62	99.31
	0.2574	18.81	100.32
Statistical Data		Mean	100.30
		min	99.20
		max	101.88

It was established that the recovery for the studied concentration range was in between 99.20% and 101.88% and the mean was 100.3%. These values proved that the proposed method was accurate (table 6).

The values of specific absorbance for bisoprolol and reaction product in dichlormethane solutions are $A^{1\%}_{1cm,223nm}$=395 and $A^{1\%}_{1cm,223nm}$= 1095 respectively. So,

the sensibility of this method is three times greater than of the direct UV determination of bisoprolol.

Conclusions

This study describes the successful development of a simple spectrophotometric method for the determination of bisoprolol fumarate.. The proposed method is based on the formation of a colored complex combination with tropaeolin 00. The method was validated by determining the following parameters: linearity range 5-30 $\mu g \cdot mL^{-1}$, r = 0.9997, r^2 = 0.9997, LOD = 0.67 $\mu g \cdot mL^{-1}$, LOQ = 2.23$\mu g \cdot mL^{-1}$, precision (RSD = 0.78 %) and accuracy (100.3 % mean recovery).

All analytical reagents used are inexpensive, quite stable and widely available in analytical laboratory. Complex procedures are not required. The earlier reported methods involved costlier techniques.

The methods are suitable for routine analysis in quality control laboratories.

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References

1.MAMEDOV, M.N., Cardiol., 2012, **52**, p. 74.
2.MAINGUY, V., GIRARD, D., MALTAIS, F., SAEY, D., MILOT, J., SÉNÉCHAL, M., POIRIER, P., PROVENCHER, S., Am. J. Cardiol., **110**, 2012, p. 258.
3.BUDAVARI, S., O'NEIL, M., SMITH, A., HECKELMAN, P., OBENCHAIN, J., The Merck Index, 12, White House Station, New Jersey, 1996, p. 1328.
4.BRUNTON, L., CHABNER, B., KNOLLMANN, B.C., The Pharmacological Basis of Therapeutics, **11**, McGraw Hill Medical publishing Division, New York, 2006, p. 285.
5.MOFFAT, A., OSSELTON D., WIDDOP, B., WATTS, J., Clarkes's Analysis of Drugs and Poisons, 4, **1**, Pharmaceutical Press, London, 2005, p.703.
6.COPIE, X., HEUZEY, X., LECHAT, P., BOISSEL, J.P., POUSSET, F., FILLETTE, F., REMME, W., HETZEL, M., GIUZE, L., J. Am. Coll. Cardiol., **25**, 1995, p. 417
7.BUTNARIU, R., CAPROSU, M., BEJAN, V., UNGUREANU, M., POIATA, A., TUCHILUS, C., FLORESCU, M., MANGALAGIU, I.I., J. Heterocyclic Chem., **44**, 2007, p. 1149.
8. DROCHIOIU, G., MANGALAGIU, I.I., AVRAM, E., POPA, K., DIRTU, A.C., DRUTA, I., Anal. Sci., **20**, 2004, p. 1443.
9.DROCHIOIU, G., MANGALAGIU, I.I., TATARU, V., Analyst, **125**, 2000, p. 939.
10.EASTWOOD, R.J., JERMAN, J.C., BHAMRA, R.K., HOLT, D.W., Biomed. Chromatog., **4**, 2005, p. 17**8.**
11.ROLIM, C.B., BRUM- FRONZA, J.R.L., MALESUIK, M.D., LISIANE BAJERSKI, L., DALMORA, S. L., J. Liq. Chromatogr. & Rel. Tech. **28**, 2005; p. 477.
12. ULU S. T., KEL, E., Opt. Spectrosc., **112**, 2012, p. 864.
13. SHARMA, S., SHARMA, M. C., J. Optoelectron. B.M., **2**, 2010, p. 223.
14.KRZEK, J., KWIECIEN, A., J. Planar. Chromatogr., **18**, 2005, p. 308.
15.WITEK, A., HOPKALA, H., MATYSIK, G., Chromatogr. **50**, 1999, p. 41.
16.GUDRUMAN, A ., ANDREEA MURĂRAŞU, A., DORNEANU, V., Farm., **60**, 2012, p. 634.
17.BALAN, A.M., ZBANCIOC, GH., BENTEA, E., CARJA, I., UNGUREANU, M., TUCHILUS, C., MANGALAGIU, I.I., Rev. Chim. (Bucharest), **60**, no. 10, 2009, p. 1021.
18.SANDU, I., MIRCEA, O., SANDU, A.V., SARGHIE, I., SANDU, I.G., VASILACHE, V., Rev. Chim. (Bucharest), **61**, no. 11, 2010, p. 1054.
19.OPREAN, R., ROZET, E., DEWÉ, W., BOULANGER, B., HUBERT, P., Ghid de validare a procedurilor analitice cantitative, Ed. Medicală Universitară "Iuliu Haţieganu", Cluj Napoca, 2007, p. 57.
20. ROMAN, L., BOJIŢĂ, M., SĂNDULESCU, R., Validarea metodelor de analiză şi control, Ed. Medicală, Bucureşti, 1998, p.520.
21.TANTARU, G., APOSTU, M., Rev. Chim. (Bucharest), **61,** no.7, 2010, p. 632.
22.ÖZDEMIR, D., DINÇ, E., BALEANU, D., Rev. Chim. (Bucharest), **61,** no. 2, 2010, p.146.

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