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Short communication

Colorimetric determination of β-blockers in pharmaceutical formulations

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Abstract

A simple, accurate, precise and sensitive colorimetric method for the determination of some β-blockers as atenolol (Ateno), metoprolol (Metop), sotalol (Sot) and nadolol (Nad) is described. This method is based on the formation of charge transfer complex with 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD–Cl) in methanolic–aqueous (for Ateno and Metop) or acetone–aqueous (for Sot and Nad) medium [30% (v/v)]. The orange color products are measured at 485, 470, 465 and 462 nm for Ateno, Metop, Sot and Nad, respectively. The optimization of various experimental conditions is described. Beer's law is obeyed in the range 0.4–60 μg ml^{-1} while that obtained applying Ringbom is 0.8–56 μg ml^{-1}. The molar absorptivity, Sandell sensitivity, detection and quantification limits are calculated. The results obtained showed good recoveries of 99.5 ± 1.1, 100.3 ± 1.2, 100.5 ± 1.0 and 99.3 ± 1.1% with relative standard deviations of 0.74, 0.98, 1.15 and 0.87% for Ateno, Metop, Sot and Nad, respectively. Applications of the proposed method to representative pharmaceutical formulations are successfully presented.

Keywords: β-Blockers determination; 4-Chloro-7-nitro-2,1,3-benzoxadiazole; Colorimetry; Charge transfer complexes; Pharmaceutical preparations

1. Introduction

β-Blockers are a type of therapeutic drug whose optical enantiomers show significant differences in their pharmacological effects and activities and even in their toxic effects [1–4]. The stereoselective mechanisms responsible for these differences have become an interesting field in advanced investigations on β-blockers [5]. There is obviously a growing need for the development of an economical method for their determination with high sensitivity and simplicity.

Atenolol, 4-(2-hydroxy-3-isopropylaminopropoxy) phenyl acetamide [29122-68-7], metoprolol, 2-propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl) amino]-,(±)-,(*E*)-2-butanedioate] (2:1) salt [119637-66-0], nadolol, 2,3-naphthalenediol, 5-[3-[(1,1)-dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-,*cis*-1-(*tert*-butyl)amino)-3-[(5,6,7,8-tetrahydro-*cis*-6,7-dihydroxy-1-

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naphthyl)oxy]-2-propanol, [42200-33-9], and sotalol HCl, (*RS*)-4′-(1-hydroxy-2-isopropylaminoethyl) methanesulphonanilide hydrochloride [959-24-0] are examples of the management of hypotension, angina pectoris, cardiac dysrhythmias and myocardial infraction, where it acts preferentially upon the β-adrenergic receptors in the heart.

Many methods have been described for the quantitative determination of β-blockers including non-aqueous titrimetric [6], colorimetric [7–9], spectrophotometric [10–12], fluorimetric [13,14], gas chromatographic [15], liquid chromatographic [16,17] and high performance liquid chromatographic [18–24]. Nevertheless, methods with higher sensitivity are still needed when the amount of pure sample available in dosage forms is limited to a very small concentration.

The molecular interaction between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region [25]. The colorimetric methods based on these interactions are simple and convenient because of the rapid formation of the complexes. 4-Chloro-7-nitro-2,1,3-benzoxadiazole has been the subject of many investigations in analytical methods as a chromogenic reagent [26]. Yet, no work has been performed to use NBD–Cl as a π-acceptor. The goal of the present work is to apply NBD–Cl to react with atenolol (Ateno), metoprolol (Metop), sotalol (Sot) or nadolol (Nad) yielding a colored CT complex and presenting a simple and rapid assay procedure for the studied drugs in pure and in pharmaceutical formulations. This work describes a colorimetric method that can be used in laboratories where modern and expensive apparatus such as that required for GLC or HPLC is not available.

2. Experimental

2.1. Reagents

Analytical reagent grade chemicals were used whenever possible, unless otherwise stated.

Stock solution 1.5×10^{-2} M of NBD–Cl (Aldrich product) was prepared by dissolving 0.2994 g of pure reagent in 10 ml methanol or acetone in a 100 ml calibrated flask and completed to the mark with the same solvent.

Atenolol was of pharmaceutical grade, obtained from the Egyptian International Pharmaceutical Industries Company [EIPICO]. Metoprolol was supplied from Bristol-Myers-Squibb Company, sotalol from Amoun Pharmaceutical Company and nadolol from Chemical Industries Development, Egypt, and used as received. 100 μg ml^{-1} of drug was prepared by dissolving 0.010 g in 10 ml of bidistilled water and then diluted to the mark in a 100 ml calibrated flask. Working solutions of lower concentration were freshly prepared by appropriate dilution of the standard solution.

2.2. Apparatus

A Perkin–Elmer Lambda 3B and Shimadzu 260 spectrophotometers with matched 10 mm quartz cells were used for all absorbance measurements during the development of the procedure.

2.3. General procedure

Aliquot containing 4.0–600 μg of the standard drug solution was transferred into a 10 ml calibrated flask. 1.0 ml of 1.5×10^{-2} M solution of NBD–Cl was added and heated on a water bath at 70 °C for 10 min. After cooling, the mixture was diluted with methanol (for Ateno and Metop) or acetone (for Sot and Nad) and water to achieve 30% (v/v). The absorbance was measured at 485, 470, 465 and 462 nm for Ateno, Metop, Sot and Nad, respectively, against a reagent blank prepared in the same manner.

2.4. Stoichiometric ratio

The molar ratio and continuous variation methods were applied to study the stoichiometric ratio of the charge transfer formed. A 5×10^{-3} M standard solution of each drug and reagent were used. In the former method a constant volume of 5×10^{-3} M drug solution was employed and the reagent concentration was changed to obtain

different ratios for the CT complex, while in the latter method, a series of solutions was kept at 2.0 ml. The reagent was mixed in various proportions and heated in a water bath of 70 °C for 10 min, cooled and then diluted to volume in a 10 ml calibrated flask with methanol or acetone and water to achieve 30% (v/v) solvent ratio as mentioned in the general procedure.

2.5. Procedure for pharmaceutical formulations

Ten tablets were weighed and powdered. An accurately weighed portion of powder equivalent to 10 mg of drug was dissolved in 10 ml water, filtrated and placed in a 100 ml calibrated flask and then diluted to the mark with water. An accurately measured volume of this solution was assayed as described above under general procedure.

3. Results and discussion

Atenolol, metoprolol, sotalol and/or nadolol, which does not have a chromophore that absorbs above 330 nm, can be determined colorimetrically by the formation of methanolic or acetone–aqueous soluble complex with NBD–Cl. The formation of orange color species of charge transfer complex is based on π–π^* interaction between benzene ring present in the donating drug to that present in the NBD–Cl moiety and produces a bathochromic shift of 115, 100, 95 and 92 nm for Ateno, Metop, Sot and Nad, respectively, since NBD–Cl absorbed at 370 nm. The absorbance of the complex is then measured at its maximum wavelength (485, 470, 465 and 462 nm for Ateno, Metop, Sot and Nad, respectively). Investigations were carried out to establish the most favorable conditions for the charge transfer formation. The influence of some variables on the reaction has been tested as follows.

3.1. Effect of reagent concentration

The amount of NBD–Cl necessary to obtain a linear graph for a drug concentration was studied. When various concentrations of the reagent were added to 30 μg ml^{-1} of the drug, 1.0 ml of 1.5×10^{-2} M NBD–Cl solution was found to be sufficient for the production of maximum and reproducible color intensity. Higher concentrations of NBD–Cl did not affect the color intensity (Fig. 1).

3.2. Effect of time and temperature

The optimum reaction time was determined by following the color development at ambient temperature (25 ± 2 °C). Complete color development was attained after 2 h, whereas this time was reduced to 10 min on raising the temperature to 70 °C on a water bath to obtain complete color development. The color remained stable for 18, 15, 18 and 24 h for Ateno, Metop, Sot and Nad charge transfer complexes, respectively (Fig. 2). Therefore the proposed method can be used as a stability-indicating method.

3.3. Effect of pH

When using acidic, neutral or basic buffer media, reagents form an orange yellow color which is absorbed at λ_{max} 373 and 450 nm. This

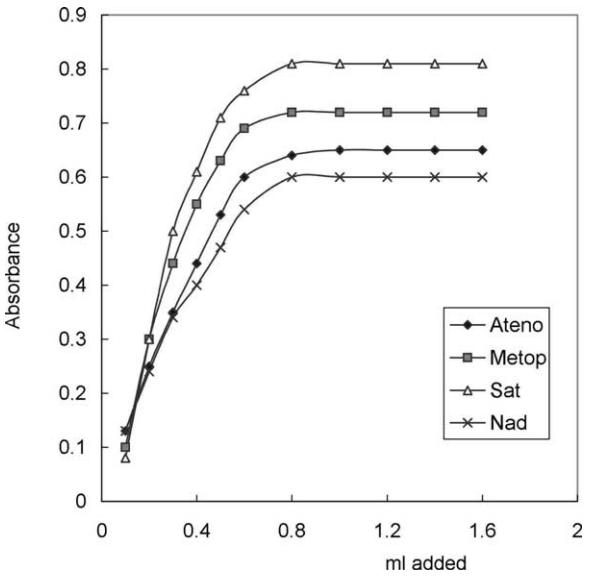

Fig. 1. Effect of reagent concentration (1.5×10^{-2} M) on the formation of the orange colored CT complexes, [drug = 30 μg ml^{-1}].

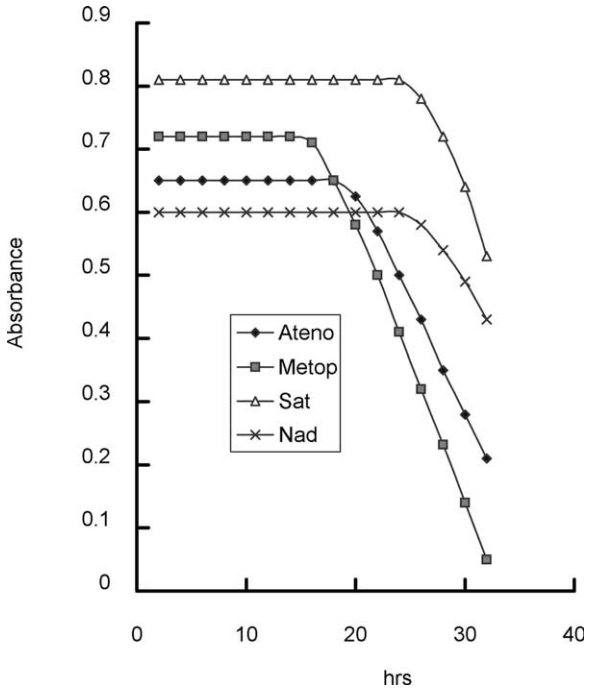

Fig. 2. Stability of the colored CT complexes, [drug] = 30 μg ml^{-1}, [NBD–Cl] = 1.5×10^{-3} M.

will decrease the absorbance of the sample solution on using it as blank, so the sensitivity of the procedure decrease in addition to increase the detection and quantification limits.

3.4. Effect of solvent

Several organic solvents i.e. methanol, ethanol, propanol, acetone, dioxane, acetonitrile and dimethylformamide (DMF) were investigated. Methanol was found to be the best solvent for Ateno and Metop–NBD–Cl charge transfer complex formation, whereas acetone is the optimum for Sot and Nad complex formation because it has a high relative permittivity which ensures the maximum yield of CT complexes. Moreover, the percentage of solvent was also investigated and it was found that 30% (v/v) gave the highest absorbance. On the other hand, ethanol and acetonitrile are possible substitutes, but it takes more time to achieve the same sensitivity using the former, whereas using the latter a yellow orange reagent blank is formed which decreases sensitivity of the procedure.

3.5. Stiochiometric ratio

The stoichiometric ratio of the drug to NBD–Cl in each of the colored complexes was determined using the molar ratio and continuous variation methods. It is apparent from the data that charge transfer complexes with drug to NBD–Cl ratio 1:1 are formed. The logarithmic stability constants of the formed complexes are calculated from the Harvey and Manning method [27] using the data of the molar ratio and continuous variation method (Table 1).

3.6. Quantification

A linear correlation was obtained between absorbance and concentration in the range given in Table 1. For more accurate analysis, the Ringbom optimum concentration range was obtained by plotting the percentage of transmittance versus the logarithmic value of concentration in μg ml^{-1}.

Regression plots showed that there was a linear dependence of absorbance on concentration over the Beer's law ranges. The molar absorptivity, Sandell sensitivity, slope, intercept and correlation coefficients obtained by the linear least-squares treatment of the results are also given in Table 1.

The relative sensitivity for the drugs under consideration can be determined by comparing the molar absorptivities (ε) of the chromogens (Table 1). Sotalol exhibited the most intense band and was therefore selected for all further work. The most important spectral characteristics of the reaction of Ateno, Metop, Sot and Nad with NBD–Cl investigated are presented in Table 1. The precision of the method was tested by analyzing eight replicate samples of each drug (30 μg ml^{-1}). The relative standard deviation and range of recovery obtained are given in Table 1.

The standard deviation of the absorbance measurements was obtained from a series of 13 blank solutions. The limits of detection ($K = 3$) and of quantification ($K = 10$) of the methods were established according to IUPAC definition ($C_l = KS_o/s$ where C_l is the limit of detection, S_o is the

standard deviation of blank determination, s is the slope of the standard curve and K is the constant related to the confidence interval [28]). The values were calculated and recorded in Table 1.

The performance of the method was assessed by calculation of the t- and F-values compared with the official method [29,30] (based on non-aqueous potentiometric titration with 0.1 M perchloric acid using crystal violet as indicator for Ateno and Nad, whereas, for Sot, potentiometric titration with 0.1 M mercury (II) acetate was made. For Metop, measuring the absorbance at 274 nm used a spectrophotometric procedure). Mean values were obtained in Student's t- and F-tests at 95% confidence limits for five degrees of freedom [31], and the results recorded in Table 1 showed that the calculated t- and F-values did not exceed the theoretical values.

Comparison of the recovery obtained with the proposed method with the purity of the studied drug as determined according to the official methods [29,30] showed a high accuracy of the present method. The proposed method is simpler, less time consuming and more sensitive than the official method. Moreover, the proposed method could be used for the routine determination of Ateno, Metop, Sot and Nad in pure form or in pharmaceutical formulations.

Comparison of the results obtained by the proposed method with those obtained by HPLC methods [18–24] showed that the recommended procedure is more economical as regards reagent consumption and time required for the analysis without any loss of accuracy or precision in addition to a wider range of determination.

3.7. Interferences

The diluents and additives such as calcium lactate, lactic acid, zinc stearate, magnesium stearate, lactose, starch, and carboxymethyl cellulose did not interfere with the analysis of the drugs in the proposed method, even when present in high concentration. Also there is no interference from either the synthesis respective byproducts or from the degraded products resulting from thermal and

Table 1
Optical characteristics and precision data

Parameter	Ateno	Metop	Sot	Nad
λ_{max} (nm)	485	470	465	462
Logarithmic stability constants	7.8	6.5	7.1	6.3
Stability (h)	17	15	24	24
Beer's law limits (μg ml^{-1})	0.5–56	0.8–60	0.4–48	0.5–52
Ringbom conc. ranges (μg ml^{-1})	1.0–53.5	1.5–56	1.0–45	1.2–50
Detection limits (μg ml^{-1})	0.14	0.24	0.11	0.15
Quantification limits (μg ml^{-1})	0.48	0.78	0.37	0.52
Molar absorptivity (L mol^{-1} cm^{-1})	5.77×10^3	6.32×10^3	8.36×10^3	6.19×10^3
Sandell sensitivity (μg cm^{-2})	0.046	0.042	0.037	0.050
Regression equation[a]				
Slope (a)	0.022	0.024	0.027	0020
RSD% of the slope	0.57	0.49	0.64	0.53
Intercept (b)	−0.007	0.005	0.004	−0.009
RSD% of the intercept	0.44	0.36	0.50	0.47
Correlation coefficient (r)	0.9992	0.9996	0.9995	0.9990
Relative standard deviation (%)	0.74	0.98	1.15	0.87
Recovery (%)	99.5±1.1	100.3±1.2	100.5±1.0	99.3±1.1
Student t-test[b] (2.57)[c]	1.25	1.43	1.09	1.36
Variance F-value[b] (5.05)[c]	2.83	3.15	2.56	3.04

[a] $A = a + bC$, where C is the concentration in μg ml^{-1}.
[b] Comparison with the official method [29,30].
[c] Values in parenthesis are the theoretical t- and F-values for five degrees of freedom and 95% confidence limits.

hydrolytic treatment. The obtained results indicate a high selectivity of the method examined in the determination of the studied drugs.

3.8. Analytical applications

The proposed method was applied to some pharmaceutical formulations containing Ateno, Metop, Sot and Nad. The results in Table 2 indicate the high accuracy of the proposed method for the determination of the studied drugs. As can be seen from Table 2, The proposed method has the advantage of being virtually free from interferences by excipients such as glucose, lactose and starch or from common degradation products.

The results showed a good agreement with those of the pharmacopoeial method [29,30] (based on non-aqueous potentiometric titration with 0.1 M perchloric acid using crystal violet as indicator for Ateno and Nad, whereas, for Sot, potentiometric titration with 0.1 M mercury (II) acetate was made. For Metop, measuring the absorbance at 274 nm used a spectrophotometric procedure). The relative standard deviations of the proposed method were 0.74, 0.98, 1.15 and 0.87% for Ateno, Metop, Sot and Nad, respectively, whereas for the pharmacopoeial method they were 1.95, 2.45, 1.88 and 2.24%, respectively (six determinations). The percentage recoveries of the proposed procedure were 99.5 ± 101, 100.3 ± 1.2, 100.5 ± 1.0 and 99.3 ± 1.3% for Ateno, Metop, Sot and Nad, respectively. The results obtained were compared statistically by the Student's t-test (for accuracy) and the variance ratio F-test (for precision) with those obtained by the pharmacopoeial method [29,30] on samples of the same batch (Table 2). The values of t- and F-tests obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value, indicating no significant difference between the methods compared.

4. Conclusion

The proposed method is simple, sensitive, rapid, precise, selective and accurate compared with the

Table 2
Determination of Ateno, Metop, Sot and Nad in different pharmaceutical formulations using NBD–Cl

Sample	Manufacturer	Content (mg/tablet)	Found[a]			
			Official	Proposed	t-test	F-value
Atenolol tablets						
Ateno	EIPICO	50	48.5	49.4	0.98	2.12
		100	101.3	99.6	1.26	2.57
Atenolol	Pharco	50	51.2	50.4	1.72	3.25
		100	98.8	100.5	1.19	2.43
Tenormin	Kahira	50	48.2	49.3	1.37	2.66
		100	99.0	100.7	1.50	3.13
Blokium	MUPCO	50	51.4	50.3	1.11	2.28
		100	102.1	99.7	1.63	3.30
Metoprolol tablets						
Betaloc	CID	100	98.8	99.5	1.15	2.34
Sotalol tablets						
Betacor	Amoun	80	80.3	79.4	1.87	3.56
Nadolol tablets						
Corgard	Squibb	80	79.4	81.2	1.48	2.92

EIPICO: Egyptian International Pharmaceutical Industries Company. Pharco: Pharco-Pharmaceutical Company, Alexandria, Egypt. Kahira: Kahira-Pharmaceutical and Chemical Industrial Company, Egypt. CID: Chemical Industries Development, Egypt. MUPCO: Medical Union Pharmaceutical Company, Egypt. Amoun: Amoun Pharmaceutical Company, Egypt. Squibb: Bristol-Myers-Squibb Company, Egypt.

[a] Average of six determinations.

official method [29,30]. Although the color development of the charge transfer complex formed at room temperature (25±2°C) requires 2.0 h for complexation, this can be shortened to 10 min by raising the temperature to 70 °C on a water bath. The proposed method is suitable for the determination of Ateno, Metop, Sot and Nad in pharmaceutical formulations without interferences of excipients or degradation products, suggesting applications in bulk analysis.

Although the high performance liquid chromatographic methods have a higher selectivity, they require complicated sample pretreatment and use expensive apparatus. The proposed method has the advantages of sensitivity, selectivity, ease of performance, economy, wider range of determinations, less time consumption, high accuracy and precision compared to the official methods [29,30].

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