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Spectrophotometric determination of β-adrenergic blocking agents in pharmaceutical formulations

Hesham Salem *

Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia, Egypt

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Abstract

Simple, rapid and sensitive spectrophotometric procedures were developed for the analysis of atenolol, timolol maleate, propranolol hydrochloride, metoprolol tartarate, betaxolol hydrochloride, levobunolol hydrochloride and bisprolol fumarate in pure form as well as in their pharmaceutical formulations. The methods are based on the reaction of these drugs as n-electron donors with the sigma-acceptor iodine, and the pi-acceptors: 7,7,8,8-tetracyanoquinodimethane, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tetracyanoethylene, 2,3,5,6-tetrabromo-1,4-benzoquinone (bromanil) and 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil). The obtained charge-transfer complexes were measured at 365 nm for iodine (in 1,2-dichloroethane), at 840, 420, and 470 nm for 7,7,8,8-tetracyanoquinodimethane, tetracyanoethylene and 2,3-di-chloro-5,6-dicyano-1,4-benzoquinone (in acetonitrile), respectively, and at 450 and 440 nm for bromanil and chloranil (in ethanol), respectively. Due to the rapid development of colors at ambient temperature, the obtained results were used on thin-layer chromatograms for the detection of the investigated drugs. Beer's plots were obeyed in a general concentration range of 4–120 μg ml^{-1} with correlation coefficients not less than 0.9991. The proposed procedures could be applied successfully to the determination of the investigated drugs in tablets and ophthalmic solutions with good recovery; percent ranged from 98.03 ± 0.98 to 100.30 ± 0.90. The association constants and standard free energy changes using Benesi–Hildebrand plots were studied.

Keywords: Spectophotometry; Charge-transfer complexes; Beta-adrenergic blocking drugs; Pharmaceutical analysis

1. Introduction

β-Adrenergic blocking drugs; atenolol (1), timolol maleate (2), propranolol hydrochloride (3), metoprolol tartarate (4), betaxolol hydrochloride (5), levobunolol hydrochloride (6) and bisprolol fumarate (7) are chemical agents that exert their principle pharmacological and therapeutic effects by acting at peripheral sites to either enhance or reduce the activity of components of the sympathetic division on autonomic nervous system [1]. β-Adrenergic blocking drugs (1–7) are used mainly in angina pectorals, certain arrhythmia, systematic hypertension and other cardiovascular disorders, such as atrial fibrillation, flutter, myocardial infarction and sinus tachycardia [2]. Several methods have been described for the

* Tel.: +20-10-142-8104; fax: +20-86-369-075.
E-mail address: h_salem_eg@yahoo.com (H. Salem).

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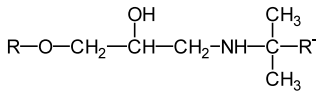
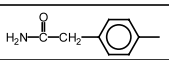
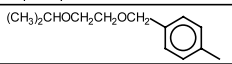
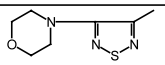
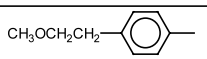
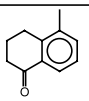
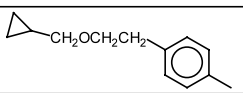
Table 1
The chemical structure of the investigated β-adrenergic blocking agents.



Drug	R	R'
Atenolol		H
Propranolol		H
Bisprolol		H
Timolol		CH_3
Metoprolol		H
Levobunolol		CH_3
Betaxolol		H

quantitative determination of the β-adrenergic blocking agents including; spectrophotometric [3–15], fluorometric [16–19], chromatographic [20–26], titrimetric [27,28] and electrochemical [29–31] methods.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensity colored charge-transfer complexes, which absorb radiation in the visible region [32]. The photometric methods based on these interactions are usually simple and convenient because of the rapid formation of the complexes. β-Adrenergic blocking drugs (1–7) are

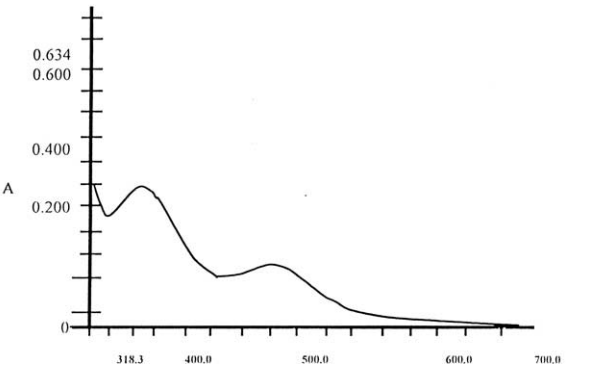
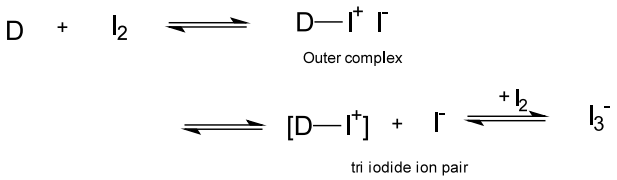


Fig. 1. Absorption spectrum of propranolol hydrochloride (18 μg ml^{-1}) with iodine in 1,2-dichloroethane. Blank: 1,2-dichloroethane.



Scheme 1.

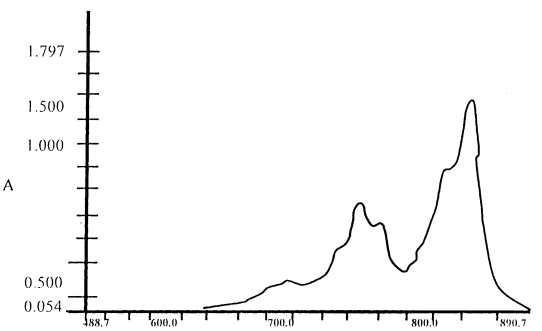
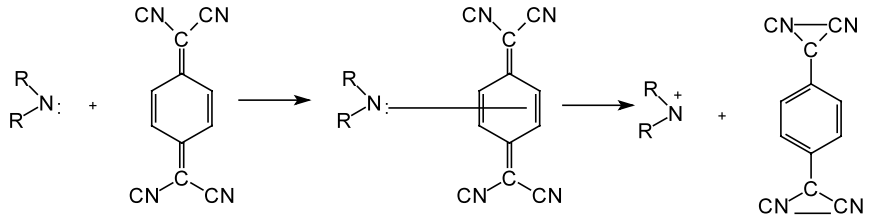


Fig. 2. Absorption spectrum of propranolol hydrochloride (20 μg ml^{-1}) with TCNQ in acetonitrile. Blank: acetonitrile.

Table 2
Optimum reaction conditions for the studied drugs with various acceptors

Parameter	Iodine	Chloranil	DDQ	Bromanil	TCNE	TCNQ
Solvent	A	B	C	B	C	C
Time (min)	5	5	20	15	20	40
Wavelength (nm)	365	440	470	450	420	840

A, 1,2-dichloroethane; B, ethanol; C, acetonitrile.



Scheme 2.

good n-electron donors and will form charge-transfer complexes with sigma or pi-acceptors.

pi-Acceptors such as 7,7,8,8-tetracyanoquinodimethane (TCNQ), tetracyanoethylene (TCNE), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 2,3,5,6-tetrabromo-1,4-benziquinone (bromanil) and 2,3,5,6-terachloro-1,4 benzoquinone (chloranil) are known to yield charge-transfer complexes and radical anions with a variety of electron donors [32–34].

The reported spectrophotometric methods are either non-specific, time consuming, indirect, or suffering from the disadvantage of low sensitivity. In addition, most of the published assay methods for β-adrenergic blocking drugs were suggested for their determination in biological fluids rather than in pharmaceutical preparations.

This study describes simple, direct, sensitive, accurate and precise spectrophotometric methods for the determination of atenolol, timolol maleate, propranolol hydrochloride, metoprolol tartarate, betaxolol hydrochloride, levobunolol hydrochloride and bisprolol fumarate via reaction with sigma and pi-acceptors in their common dosage forms and irrespective of the presence of contaminants and additives.

2. Experimental

2.1. Apparatus

Spectronic Genesys 2PC, Ultraviolet-Visible Spectrophotometer (Milton Roy Co., USA) with matched 1 cm quartz cuvettes was used. All calculations were carried out on an IBM computer using the statistical methods in analytical chemistry (SMAC) program, designed by Meier and Zund [35].

2.2. Materials and reagents

All solvents used were of analytical-reagent grade. Suppliers were as follows: atenolol and propranolol hydrochloride (Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt), bisprolol fumarate (Amoun Pharm. Co., Cairo, Egypt), timolol maleate (Egyptian Int. Pharm. Ind. Co., Tenth of Ramadan city, Egypt), metoprolol tartarate (Chem. Ind. Develop. Co., Cairo, Egypt), levobunolol hydrochloride (Allergan West Port, Co. Mayo, Ireland), and betaxolol hydrochloride (Alcon Co., Cairo, Egypt).

Iodine, resublimed (Riedel-De-Haen AG, Germany), was 25.5 mg per 50 ml (1×10^{-3} M) in 1,2-dichloroethane. The solution was found to be stable for at least 1 week at 5 °C. TCNQ (Sigma Chemical Co., USA) was 1 mg ml^{-1} in acetonitrile. The solution was found to be stable for at least 1 week at 5 °C. DDQ (Sigma Chemical Co.)

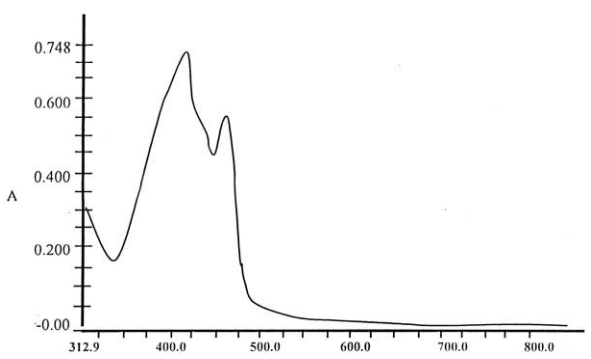
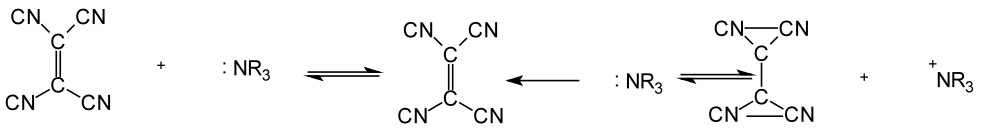


Fig. 3. Absorption spectrum of propranolol hydrochloride (24 μg ml^{-1}) with TCNE in acetonitrile. Blank: acetonitrile.



Scheme 3.

was 1 mg ml^{-1} in acetonitrile. Bromanil from Hopkin & Williams Ltd (UK) was 2 mg ml^{-1} in ethanol, chloranil from Aldrich Co., USA, was 3 mg ml^{-1} in ethanol and TCNE (Naacalai Tesque, Kyoto, Japan) was 1 mg ml^{-1} in acetonitrile, prepared fresh daily.

The adsorbent was silica gel G254-precoated plates, and the solvent system was; *n*-propanol:water:ethylacetate (80:25:10).

2.3. Pharmaceutical formulations

The following commercial dosage forms were subjected to the analytical procedure. Betaloc® tablets, Batch No. 900105 (Chem. Ind. Develop. Co.), labeled to contain 100 mg metoprolol tartarate per tablet. Concor® tablets, Batch No. 176 (Amoun Pharm. Co.), labeled to contain 10 mg bisprolol fumarate per tablet. Timolol® ophthalmic solution, Batch No. 013162 (Egyptian Int. Pharm. Ind. Co.), labeled to contain 5 mg timolol maleate per each ml ophthalmic solution. Betagan® ophthalmic solution, Batch No. E 15636 (Allergan WestPort, Co.), labeled to contain 5 mg levobunolol hydrochloride per 100 ml of the ophthalmic solution. Inderal® tablets, Batch No. 0010852, and Tenormin® tablets, Batch No. 0110275 (Kahira Pharm. & Chem. Ind. Co.), labeled to contain 40 and 50 mg propranolol hydrochloride and atenolol per tablet, respectively. Betoptic® ophthalmic solution, Batch No. 230600, (Alcon Co.), labeled to contain 0.56% betaxolol hydrochloride per each ml.

2.4. Procedures

2.4.1. Preparation of standard stock solutions

Into a 50-ml calibrated flask, 20–200 mg drug was weighed accurately and dissolved in 2 ml ethanol, completed to volume with the same solvent (for bromanil and chloranil), with 1,2-dichloroethane (for iodine) and with acetonitrile (for DDQ, TCNE and TCNQ), and diluted quantitatively to obtain the suitable concentrations.

2.4.2. General analytical procedure

In 10 ml calibrated flasks, place aliquot volumes containing 30–2000 μg drug. Add 1 ml of the reagent and dilute to the mark with the corresponding solvent (Tables 1 and 2). Measure the absorbance of the solution at the wavelength of maximum charge-transfer bands after the appropriate time at 25 °C ± 5 against reagent blank treated similarly.

2.4.3. Stoichiometric study

Job's method of continuous variation [36] was employed. Master equimolar solutions of each drug with iodine ($(2.0–3.8) \times 10^{-4}$ M), DDQ (1.0×10^{-3} M), TCNQ ($(5.1–5.7) \times 10^{-4}$ M), TCNE (2.0×10^{-3} M), bromanil (1.0×10^{-3} M) and chloranil (1.5×10^{-3} M) were prepared in 2.0 ml ethanol, and completed to volume with the same solvent (for chloranil and bromanil), with 1,2-dichloroethane (for iodine) and with acetonitrile (for DDQ, TCNE and TCNQ). A series of 10-ml portions of master solutions of each drug with the respective acceptor was made up comprising different complementary proportions (0:10, 1:9, 2:8……9:1) in 10-ml calibrated flasks.

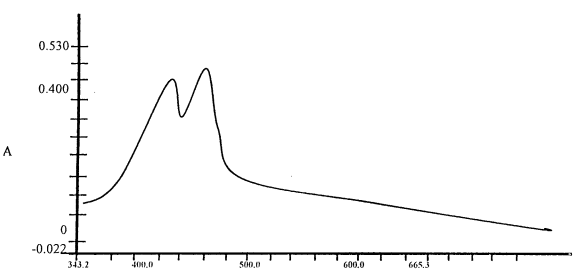
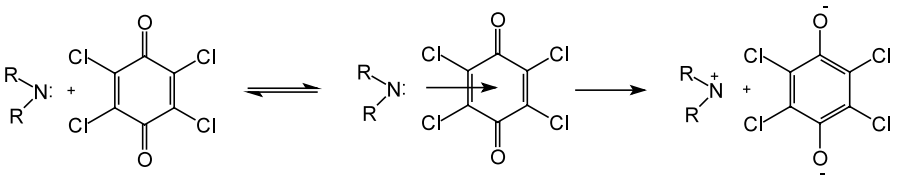


Fig. 4. Absorption spectrum of propranolol hydrochloride (16 μg ml^{-1}) with chloranil in ethanol. Blank: ethanol.



Scheme 4.

The absorbance of the resulting solutions were measured at the wavelength of maximum absorption after the appropriate time (Table 2), against reagent blanks treated similarly.

2.4.4. Analysis of tablets

Twenty tablets of the drug were weighed and powdered. A quantity of the powdered tablets equivalent to about 50 mg drug was transferred into a 50 ml calibrated flask. Then, the procedure is followed as under Section 2.4.2.

2.4.5. Analysis of drops

Dilute an appropriate volume of the solution in a volumetric flask so as to contain 1 mg ml^{-1}. Proceed as directed under tablets.

3. Results and discussion

3.1. Reaction with sigma acceptor; iodine

The immediate change of the violet color of iodine in 1,2-dichloroethane (520 nm) to a lemon yellow upon reaction with the investigated compounds was taken as suggestive of charge transfer complex formation which justified scanning in the UV range for the new bands (Fig. 1). The complex formation is distinguished from other slow oxidation or substitution reactions of the halogen with the β-adrenergic blocking drugs, by being practically instantaneous, in analogy to ionic reactions. Further conformation of the charge-transfer nature of the reaction was obtained on extracting the drugs from the complex by shaking with aqueous mineral acid, whereby the violet color of iodine in 1,2-dichloroethane was restored.

The appearance of absorption peaks at 290 and 365 nm was attributed to the formation of a charge-transfer complex between the investigated β-adrenergic blocking drugs and iodine, having an ionized structure $DI^{+}...I_3^{-}$, taking into account that the spectrum of I_3^{-} in 1,2-dichloroethane shows two absorption maxima at 290 and 365 nm.

This complex should originate from an early intermediate outer complex $D....I_2$ Scheme 1.

Measurements were carried out at 365 nm due to the interference from the native UV absorption of the studied β-adrenergic blocking drugs at 290 nm. The different variables were studied and optimized.

1,2-Dichloroethane was found to be an ideal solvent for the formation of a tri-iodide ion pair (inner complex). Methylene chloride, chloroform and carbontetrachloride produced lower absorbance readings. Polar solvents were found to be unsuitable as their blanks with iodine gave high absorbances.

The regression equations were derived using the least-squares methods [37].

3.2. Reaction with pi-acceptors

3.2.1. Reaction with TCNQ

The acetonitrile solution of β-adrenergic blocking drugs (Lewis base) when mixed with acetoni-

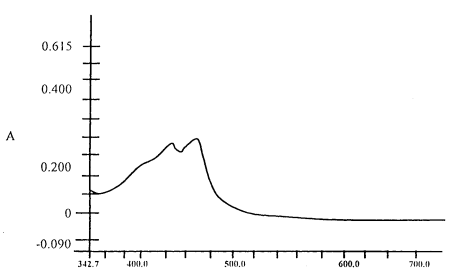
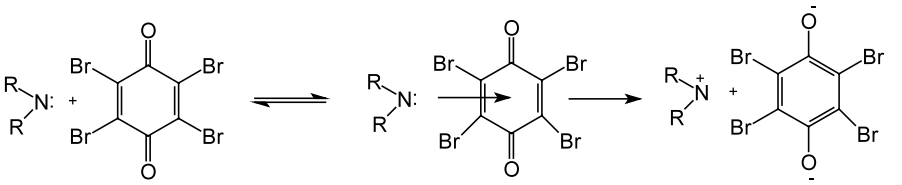


Fig. 5. Absorption spectrum of propranolol hydrochloride (60 μg ml^{-1}) with bromanil in ethanol. Blank: ethanol.



Scheme 5.

trile solution of TCNQ acceptor (Lewis acid), an intense bluish green color was developed in the visible region showing minor bands at 730, 648 and 668 nm, and the major bands at 840, 825, 762 and 742 nm (Fig. 2). These bands have been attributed to the formation of TCNQ radical anion, which is formed by complete transfer of n-electron from the donor to the electron deficient pi-acceptor TCNQ. The reaction may be suggested in Scheme 2.

3.2.2. Reaction with TCNE

The presence of TCNE radical anion has been detected by optical spectroscopy showing the characteristic two wavelengths at 400 and 420 nm (Fig. 3). In most of these instances, radical formation was attributed to dissociation of the charge-transfer complex with a complete one-electron transfer from the drug donor to TCNE acceptor. The proposed mechanism is illustrated in Scheme 3.

The reaction mixture (donor + acceptor) was essential to attain reproducible results. The period of time allows the complete change of the molecular complex (outer complex) into the inner complex having radical ions formation, which is responsible for the observation of the produced wavelength.

3.2.3. Reaction with chloranil

On studying the absorption curves for β-adrenergic blocking drug, chloranil, and drug–chloranil charge transfer complex, the wavelength was exhibited at 440 nm. Fig. 4 indicates the formation of charge-transfer complex. The formed new band was attributed to an electron transfer complexation reaction between β-adrenergic blocking drug as n-donor and chloranil as electron acceptor followed by formation of radical ions. The proposed mechanism is illustrated in Scheme 4.

3.2.4. Reaction with bromanil

The interaction of any of the investigated β-adrenergic blocking drugs with bromanil acceptor, produce a colored charge-transfer complexes of intensely colored radical ions with high molar absorptivity values at 450 nm (Fig. 5). The proposed mechanism is illustrated in Scheme 5.

3.2.5. Reaction with DDQ

The interaction of all studied drugs with DDQ in acetonitrile at room temperature gave a colored chromogen with a strong absorption maximum at 470 nm (Fig. 6). Different variables were studied and optimized. The proposed mechanism is illustrated in Scheme 6.

3.3. Stoichiometry of the reaction

On studying the molar ratio of the studied β-adrenergic blocking drugs (1–7) with iodine, chloranil, bromanil, DDQ, TCNQ or TCNE, using Job's method of continuous variation [36], it was found to be 1:1. This indicates that only one nitrogen is responsible for the formation of the complex.

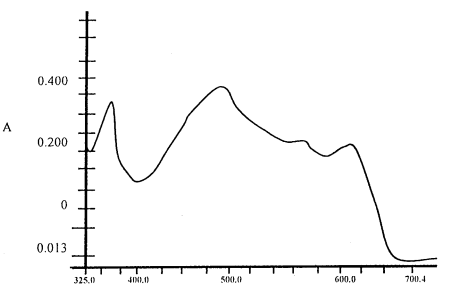
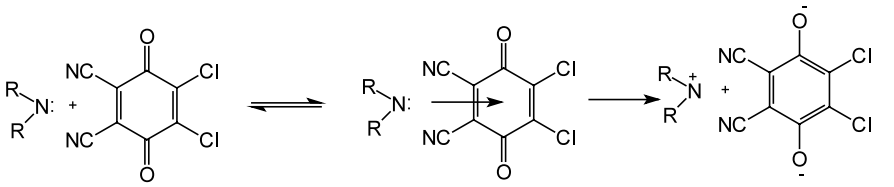


Fig. 6. Absorption spectrum of propranolol hydrochloride (100 μg ml^{-1}) with DDQ in acetonitrile. Blank acetonitrile.



Scheme 6.

3.4. Reagent concentration

It was found that, the most suitable volume for carrying the assays was 1 ml of the above reagents stock solutions (under Section 2.2) of either iodine, chloranil, bromanil, DDQ, TCNQ or TCNE, respectively with the β-adrenergic blocking drugs. The higher concentrations used of the reagents may be useful concentrations for rapidly reaching equilibrium, thus minimizing the time required to attain maximum absorbance readings at the corresponding maxima.

3.5. Reaction time

The reaction time was determined by following the absorbances of the developed color at different time intervals at ambient temperature (25 ± 5 °C). Complete color development was attained instantaneously or after 5–40 min with all compounds investigated (Table 2), and the color remains stable for at least a further 10–30 min.

3.6. Association constants and standard free energy changes

The association constants were calculated for the interaction of each drug with either iodine, chloranil, bromanil, TCNE,TCNQ or DDQ complex using Benesi–Hildebrand equation [37].

$$\frac{[A_O]}{A^{AD}} = \frac{1}{\varepsilon^{AD}} + \frac{1}{K_C^{AD}} \cdot \varepsilon^{AD} \times \frac{1}{[D_O]} \quad (1)$$

where $[A_o]$ and $[D_o]$ are the concentrations of the acceptor and donor respectively, A^{AD} is the absorbance of the complex, ε^{AD} is the molar absorptivity of the complex and K_c^{AD} is the association constant of the complex (1 mol^{-1} mol).

From the above equation, on plotting the values of $[A_o]/A^{AD}$ versus $1/[D_o]$, straight lines were obtained (Table 3). The standard free energy changes of complexation ($\Delta G°$) were calculated from the association constants by the following equation [38].

$$\Delta G° = -2.303RT \log K_C$$

Where $\Delta G°$ is the free energy change of the complex (kJ mol^{-1}), R the gas constant (1.987 cal mol^{-1} deg^{-1}), T the temperature in Kelvin ($273 + °C$) and K_c is the association constant of drug-acceptor complexes (1 mol^{-1}).

The high values of association constants are common in n-electron donors where the intermolecular overlap may be considerable.

3.7. Quantification

At fixed experimental conditions, the intensity of absorption at the specified wavelength was found to be a function of the concentration of the investigated drugs. In all cases studied, Beer's law plots were linear with very small intercepts (0.0089–0.1030). Slopes ranged from 0.0078 to 0.1032 in the general concentration ranges presented in Table 4. The regression equations for the proposed procedures were derived using the least-square method and the correlation coefficient ranged from 0.9991 to 0.9999.

For comparison, the official methods [39] were applied for the determination of the intact drugs (1–6), where drug (7) is not official. Statistical analysis of the results obtained (Table 5) indicated that the proposed procedures were as accurate and precise as the official methods.

3.8. Specificity and interferences

The proposed procedures have the advantage that most of the assays are performed in the visible region far from the UV-absorbing interferences that might be co-extracted from dosage forms. Also, before dealing with the analysis of the pharmaceutical preparations, the effect of

Table 3
Association constants (K_c^{AD}), correlation coefficients and standard free energy changes ($\Delta G°$) of β-adrenergic blocking drugs complexes with iodine (at 365) (I), chloranil (at 440 nm) (II), bromanil (at 450 nm) (III), TCNE (at 420 nm) (IV), TCNQ (at 840 nm) (V) and DDQ (at 470 nm) (VI), obtained from Benesi–Hildebrand plots

Drugs	Acceptors	$\Delta G°$ (kJ mol^{-1})	$K_c^{AD} \times 10^3$ (1 mol^{-1})	Correlation coefficient (r)
Atenolol	I	−3.9	6.3	0.9999
	II	−4.5	2.6	0.9992
	III	−4.7	1.9	0.9999
	IV	−4.7	5.5	0.9997
	V	−5.0	4.4	0.9998
	VI	−4.9	4.1	0.9995
Timolol	I	−5.2	0.9	0.9990
Maleate	II	−4.1	3.7	0.9998
	III	−4.9	2.4	0.9998
	IV	−5.2	3.3	0.9999
	V	−3.9	1.6	0.9999
	VI	−4.7	2.8	0.9999
Propranolol	I	−4.1	5.1	0.9997
Hydrochloride	II	−4.0	1.2	0.9996
	III	−4.1	1.0	0.9994
	IV	−4.2	1.8	0.9999
	V	−4.7	2.1	0.9990
	VI	−4.6	6.2	0.9993
Metoprolol	I	−5.2	7.1	0.9999
Tartarate	II	−4.6	4.3	0.9997
	III	−4.4	5.2	0.9999
	IV	−3.9	2.6	0.9995
	V	−4.1	2.7	0.9998
	VI	−4.4	4.9	0.9997
Betaxolol	I	−3.9	2.8	0.9995
Hydrochloride	II	−4.8	1.9	0.9999
	III	−5.1	3.4	0.9998
	IV	−4.3	2.9	0.9997
	V	−4.4	2.7	0.9999
	VI	−4.1	2.8	0.9992
Bisprolol	I	−3.6	0.9	0.9998
Fumarate	II	−5.8	6.4	0.9999
	III	−4.1	3.9	0.9994
	IV	−4.8	3.5	0.9999
	V	−4.7	1.6	0.9999
	VI	−4.9	1.9	0.9997
Levobunolol	I	−3.9	5.4	0.9996
Hydrochloride	II	−4.2	2.8	0.9996
	III	−5.1	3.0	0.9999
	IV	−4.4	2.8	0.9995
	V	−4.3	2.4	0.9994
	VI	−4.1	4.3	0.9990

Table 4
Quantitative parameters for the reaction of the studied β-adrenergic blocking drugs with iodine (I), chloranil (II), bromanil (III), TCNE (IV), TCNQ (V) and DDQ (VI)

Drugs	Acceptors	Linear range (μg ml^{-1})	[a]	[b]	r[c]	$\varepsilon \times 10^3$ (dm^3 mol^{-1} cm^{-1})
Atenolol	I	6–36	−0.0287	0.0163	0.9999	3.75
	II	4–24	−0.0896	0.0215	0.9995	6.65
	III	20–120	0.0285	0.0124	0.9999	10.55
	IV	6–36	0.0639	0.0163	0.9999	4.88
	V	4–24	−0.0098	0.0311	0.9998	14.01
	VI	30–200	0.0692	0.0121	0.9999	9.25
Timolol maleate	I	6–36	0.0198	0.0290	0.9997	20.52
	II	4–24	0.0287	0.0305	0.9998	10.89
	III	20–120	0.0159	0.0358	0.9994	7.95
	IV	6–36	−0.0984	0.0260	0.9993	2.49
	V	4–24	0.0123	0.0198	0.9992	10.85
	VI	30–200	−0.0452	0.0300	0.9991	11.54
Propranolol hydrochloride	I	6–36	−0.1000	0.0328	0.9999	6.89
	II	4–24	0.0687	0.0268	0.9994	18.63
	III	20–120	0.0296	0.0099	0.9995	10.45
	IV	6–36	0.0852	0.0963	0.9998	3.96
	V	4–24	0.0089	0.1003	0.9997	5.83
	VI	30–200	0.1002	0.0386	0.9999	22.87
Metoprolol tartarate	I	6–36	0.0092	0.0822	0.9994	7.12
	II	4–24	−0.0963	0.0452	0.9995	1.80
	III	20–120	−0.0111	0.0152	0.9999	13.65
	IV	6–36	0.0243	0.0345	0.9999	7.53
	V	4–24	0.0120	0.0745	0.9991	12.97
	VI	30–200	−0.0830	0.0078	0.9991	15.93
Betaxolol hydrochloride	I	6–36	0.1030	0.0951	0.9999	7.21
	II	4–24	0.0103	0.0145	0.9999	18.90
	III	20–120	0.0853	0.0963	0.9996	4.39
	IV	6–36	0.0953	0.1032	0.9999	15.87
	V	4–24	0.0193	0.0098	0.9999	12.34
	VI	30–200	0.0752	0.0750	0.9994	21.35
Bisprolol fumarate	I	6–36	0.0045	0.0341	0.9999	7.36
	II	4–24	0.0790	0.0659	0.9999	16.54
	III	20–120	0.0698	0.0360	0.9998	4.98
	IV	6–36	0.0636	0.0258	0.9992	1.78
	V	4–24	−0.0754	0.0129	0.9999	9.36
	VI	30–200	−0.0701	0.0744	0.9998	10.09
Levobunolol hydrochloride	I	6–36	−0.0154	0.1001	0.9992	11.30
	II	4–24	0.0088	0.0099	0.9998	19.25
	III	20–120	0.0361	0.0151	0.9997	6.89
	IV	6–36	−0.0741	0.0784	0.9997	9.09
	V	4–24	0.0361	0.0129	0.9995	15.91
	VI	30–200	0.0912	0.0196	0.9999	13.56

[a] Intercept.
[b] Slope.
[c] Correlation coefficient.

common additives, adjutants and excipients on the proposed method were experimentally studied.

The results obtained revealed that glucose, lactose, talc powder, magnesium stearate and starch do not interfere.

3.9. Analysis of pharmaceutical dosage forms

The proposed charge-transfer spectrophotometric methods were applied to the determination of the studied β-adrenergic blocking drugs in tablets and ophthalmic solutions. The results were compared statistically with those obtained by applying the official methods.

In the *t* and *F* tests, no significant difference was found between the calculated and theoretical values (95% confidence) of the proposed and official methods. This indicates similar precision and

Table 5
Statistical analysis of the results obtained for assay of authentic β-adrenergic blocking drugs using the proposed methods compared with the official methods [39]

Drugs		Iodine	Chloranil	Bromanil	TCNE	TCNQ	DDQ	Official
Atenolol	X^-	99.98	100.15	100.26	100.09	99.89	100.09	100.20
	±S.D	0.90	0.89	0.94	0.90	0. 9	0.91	0.92
	N	6	6	6	6	6	6	6
	V	0.81	0.79	0.88	0.81	0.83	0.85	0.85
	t (3.85)	0.42	0.10	0.11	0.22	0.62	0.21	
	F (4.28)	1.05	1.08	1.04	1.05	1.02	1.00	
Timolol	X^-	99.92	99.93	99.92	99.79	99.93	100.00	99.90
	±S.D.	0.38	0.37	0.30	0.38	0.41	0.40	0.39
	N	6	6	6	6	6	6	6
	V	0.14	0.14	0.09	0.14	0.17	0.16	0.15
	t (3.85)	0.09	0.14	0.10	0.50	0.13	0.44	
	F (4.28)	1.07	1.07	1.70	1.07	1.13	1.07	
Propranolol	X^-	99.04	98.90	99.10	99.00	98.89	98.99	99.01
	±S.D.	0.50	0.54	0.59	0.65	0.54	0.56	0.55
	N	6	6	6	6	6	6	6
	V	0.25	0.29	0.35	0.42	0.29	0.31	0.30
	t (3.85)	0.10	0.35	0.27	0.03	0.38	0.06	
	F (4.28)	1.20	1.03	1.17	1.40	1.03	1.03	
Metoprolol	X^-	99.10	99.05	99.03	99.06	99.07	99.08	99.03
	±S.D.	1.10	1.01	1.00	1.04	1.05	1.10	1.03
	N	6	6	6	6	6	6	6
	V	1.21	1.02	1.00	1.08	1.10	1.21	1.06
	t (3.85)	0.11	0.03	0.00	0.05	0.07	0.08	
	F (4.28)	1.14	1.04	1.06	1.02	1.04	1.14	
Betaxolol	X^-	100.01	99.99	99.96	99.96	99.98	99.96	100.00
	±S.D.	0.89	0.98	1.00	0.95	0.86	0.95	0.97
	N	6	6	6	6	6	6	6
	V	0.79	0.96	1.00	0.90	0.74	0.90	0.94
	t (3.85)	0.02	0.02	0.07	0.07	0.04	0.07	
	F (4.28)	1.19	1.02	1.06	1.04	1.27	1.04	
Bisprolol	X^-	98.19	99.03	99.00	99.01	99.00	98.90	.....
	±S.D.	0.95	1.11	1.05	1.03	1.03	1.11	
	N	6	6	6	6	6	6	
	V	0.90	1.23	1.10	1.06	1.06	1.23	
Levobunolol	X^-	99.97	99.98	99.98	100.09	99.99	100.07	100.10
	±S.D.	1.09	1.20	1.23	1.15	1.21	1.30	1.20
	N	6	6	6	6	6	6	6
	V	1.19	1.44	1.51	1.32	1.46	1.69	1.44
	t (3.85)	0.20	0.17	0.17	0.01	0.16	0.04	
	F (4.28)		1.21	1.00	1.05	1.09	1.01	1.17

Table 6
Determination of the β-adrenergic blocking drugs in commercial pharmaceutical preparations by the proposed and official methods [39]

Drugs		Iodine	Chloranil	Bromanil	TCNE	TCNQ	DDQ	Official
Betaloc®	X^-	99.02	99.00	99.00	98.80	99.03	99.01	99.03
Tablets	±S.D.	1.02	1.00	0.99	1.01	1.01	1.04	1.03
	N	6	6	6	6	6	6	6
	V	1.04	1.00	0.98	1.02	1.02	1.08	1.06
	t (3.85)	0.02	0.05	0.05	0.39	0.00	0.03	
	F (4.28)	1.02	1.06	1.08	1.04	1.04	1.02	
Concor® tablets	X^-	99.00	99.01	99.04	98.72	98.03	99.01	…….
	±S.D.	0.92	1.04	1.09	1.01	0.98	1.10	
	N	6	6	6	6	6	6	
	V	0.85	1.08	1.19	1.02	0.96	1.21	
Timolol® eye drops	X^-	99.55	99.91	99.84	99.80	99.83	99.91	99.90
	±S.D.	0.32	0.44	0.38	0.41	0.38	0.40	0.39
	N	6	6	6	6	6	6	6
	V	0.10	0.19	0.14	0.17	0.14	0.16	0.15
	t (3.85)	1.73	0.04	0.27	0.43	0.32	0.04	
	F (4.28)	1.50	1.30	1.07	1.13	1.07	1.07	
Betagan® eye drops	X^-	99.99	100.00	99.98	99.90	99.99	99.90	100.10
	±S.D.	1.15	1.15	1.22	1.19	1.16	1.11	1.20
	N	6	6	6	6	6	6	6
	V	1.32	1.32	1.49	1.42	1.35	1.23	1.44
	t (3.85)	0.16	0.15	0.17	0.29	0.16	0.30	
	F (4.28)	1.09	1.09	1.03	1.01	1.07	1.17	
Inderal® tablets	X^-	98.88	99.00	98.52	99.02	99.04	98.99	99.01
	±S.D.	0.60	0.59	0.66	0.75	0.50	0.57	0.55
	N	6	6	6	6	6	6	6
	V	0.36	0.35	0.44	0.56	0.25	0.32	0.30
	t (3.85)	0.39	0.03	1.39	0.03	0.10	0.06	
	F (4.28)	1.20	1.20	1.47	1.89	1.20	1.07	
Tenormin® tablets	X^-	100.22	99.99	100.30	99.96	99.97	100.09	100.20
	±S.D.	0.87	0.88	0.90	0.84	0.95	0.94	0.92
	N	6	6	6	6	6	6	6
	V	0.76	0.77	0.81	0.71	0.90	0.88	0.85
	t (3.85)	0.04	0.40	0.19	0.47	0.43	0.20	
	F (4.28)	1.12	1.10	1.05	1.20	1.05	1.04	
Betoptic® eye drops	X^-	99.89	100.07	100.03	100.06	99.99	100.05	100.00
	±S.D.	0.95	0.99	0.89	0.95	0.85	0.91	0.97
	N	6	6	6	6	6	6	6
	V	0.90	0.98	0.79	0.90	0.72	0.83	0.94
	t (3.85)	0.20	0.12	0.06	0.11	0.02	0.09	
	F (4.28)	1.04	1.04	1.19	1.04	1.31	1.13	

accuracy. Data of Table 6 suggests that the present procedures can be applied to the assay of these drugs in their single dosage forms without interference. Frequently encountered common ingredients of formulations were found not to interfere. Percentage recoveries ranged from 98.03 ± 0.98 to 100.22 ± 0.87 for the applied acceptors.

3.10. Identification on thin-layer chromatograms

The different colors developed from the interaction of the investigated drugs with the different acceptors could be used on thin-layer chromatograms for detection and differentiation of these compounds (from their corresponding R_f

values: 0.37, 0.87, 0.90, 0.70, 0.61, 0.80 and 0.55 for atenolol (1), timolol maleate (2), propranolol hydrochloride (3), metoprolol tartarate (4), betaxolol hydrochloride (5), levobunolol hydrochloride (6) and bisprolol fumarate (7), respectively). Therefore, spraying with different acceptors revealed the coloration of the spots as yellow (iodine and TCNE), orange–yellow (bromanil), red (DDQ), bluish-green (TCNQ) and greenish-yellow (chloranil). The rapid development of colors at room temperature with non-corrosive reagents, the variation of color shades, the sensitivity and the stability of colors suggest obvious use of these acceptor reagents to supplement existing methods for the detection of the studied β-adrenergic blocking drugs on chromatograms. The quantitative determination of the β-adrenergic blocking drugs on thin-layer chromatography using these acceptors is currently investigated.

4. Conclusion

From the aforementioned results, the suggested procedures using sigma and pi-acceptors confirm their suitability for spectrophotometric analysis of named compounds in the micro range. Moreover, they could be applied to the quality control analysis of the investigated β-adrenergic blocking drugs.

References

[1] J.N. Delagado, W.A. Remers (Eds.), Wilson and Gisfold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, ninth ed., Lippincott-Raven Publishers, Philadelphia, NY, 1991, p. 413.
[2] K. Andrejus, Essentials of Medicinal Chemistry, second ed., Wiley, USA, 1988.
[3] N.M. Sanghavi, N.G. Jivani, Talanta 27 (7) (1980) 591.
[4] N.M. Sanghavi, J.T. Vyas, Indian Drugs 29 (7) (1992) 317.
[5] Y.K. Agrowal, K. Raman, S. Rajput, S.R. Menon, Anal. Lett. 25 (8) (1992) 1503.
[6] K. Shreedhar, C.S. Sastry, M.N. Reddy, D.G. Sankar, Indian J. Pharm. Sci. 56 (5) (1994) 190.
[7] C.S. Sastry, K. Sreedhar, M.N. Reddy, D.G. Sankar, East Pharm. 38 (1995) 145.
[8] C.M. Popa, A. Nedelcu, C. Arama, A. Neagu, Farmacia Bucharest 43 (1995) 11.
[9] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, Farmaco 51 (1996) 733.
[10] A. Gupta, A.K. Sharma, East Pharm. 41 (1998) 155.
[11] F.A. El-Yazbi, K.A. Kovar, Sci. Pharm. 66 (1998) 325.
[12] H.H. Abdine, F.A. El-Yazbi, R.A. Shaalan, S.M. Blaih, STP Pharma. Sci. 9 (6) (1999) 587.
[13] G. Alpdogan, S. Sungur, Spectrochim. Acta 55A (13) (1999) 2705.
[14] M.L. Satuf, J.C. Robles, H.C. Goicoechea, A.C. Olivieri, Anal. Lett. 32 (10) (1999) 2019.
[15] S. Gangwal, P. Trivedi, Indian Drugs 36 (4) (1999) 256.
[16] M. Poctova, C.B. Kakac, Cesk. Farm. 34 (6) (1985) 222.
[17] A. Zhang, X. Li, Yaowu Fenixi Zazhi 9 (3) (1989) 129.
[18] M. Gajewska, G. Glass, J. Kostelecki, Acta Pol. Pharm. 49 (3) (1992) 1.
[19] M. Yang, W.W. Zhang, H.N. Wang, Fenxi Huaxue 24 (6) (1996) 740.
[20] M.I. Maguregui, R.M. Alonoso, Chromatogr. J. Biomed. Appl. 674 (1) (1995) 85.
[21] M. Veronico, G. Rango, C. Vetuschi, Spectrosc. Lett. 28 (3) (1995) 60.
[22] P. Lukkari, H. Siren, J. Chromatogr. A 717 (1–2) (1995) 58.
[23] P. Chiap, P. Hubert, P. Boulanger, J. Crommen, Anal. Chim. Acta 391 (2) (1999) 227.
[24] J.C. Wy, H.L. Lord, J. Pawliszyn, H. Kataoka, J. Microcolumn. Sep. 12 (4) (2000) 255.
[25] N.C. Gillott, M.R. Euerby, C.M. Johnson, D.A. Barrett, P.N. Shaw, Chromatographia 51 (3–4) (2000) 167.
[26] S. Svensson, A. Karlsson, O. Gyllenhaal, J. Vessman, Chromatographia 51 (5–6) (2000) 283.
[27] J. Jarzebinski, Z. Szrajber, G. Kolas, Farm. Pol. 34 (1978) 237.
[28] V.N. Pathak, S.R. Shukla, I.C. Shukla, Analyst 107 (1278) (1982) 1086.
[29] A. Alvarez-Lueje, L.J. Nunez-Verqara, J.A. Sequella, Farmaco 46 (4) (1991) 593.
[30] Y.M. Issa, A.S. Amin, Mikrochim. Acta 118 (1–2) (1995) 85.
[31] E. Billiot, I.M. Warner, Anal. Chem. 72 (8) (2000) 1740.
[32] R. Foster, in: Organic Charge Transfer Complexes, Academic Press, London, 1969, pp. 51, 387.
[33] L.R. Melby, in: Patai (Ed.), The Chemistry of the Cyano group, New York, 1970.
[34] C.N.R. Rao, S.N. Bhat, P.C. Dwivedi, in: E.G. Brame (Ed.), Applied Spetroscopy Reviews, vol. 5, Dekker, New York, 1972, pp. 1–170.
[35] P.C. Meier, R.E. Zund, Statistical Methods in Analytical Chemistry, Wiley, New York, 1995.
[36] J. Rose, Advanced Physico-Chemical Experimental, Pittman, London, 1964, p. 54.
[37] H.A. Benesi, J. Hidelbrand, J. Am. Chem. Soc. 71 (1949) 2703.
[38] A.N. Martin, J. Swarbrick, A. Cammarata, Physical Pharmacy, third ed., Lee & Febiger, Philadelphia, PA, 1969, p. 344.
[39] The United States Pharmacopoeia 24, National Formulary 19, US Pharmacopeial Convention, Rockville, MD, 2000, pp. 175, 228, 960, 1101, 1429, 1663.