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Application of 4-chloro-7-nitrobenzofurazan for the analysis of propafenone and diltiazem hydrochlorides using kinetic spectrophotometric and spectrofluorimetric methods

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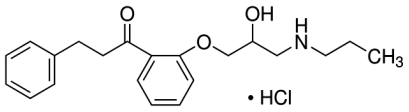
ABSTRACT

Several simple, sensitive, accurate and inexpensive spectrophotometric and spectrofluorimetric methods were developed for the determination of propafenone HCl and diltiazem HCl using 4-chloro-7-nitrobenzofurazan (NBD-Cl) accompanied with kinetic study, either in pure form or in pharmaceutical preparations. In this work, the cited drugs react with (NBD-Cl) in presence of borate buffer of pH = 7.6 at a fixed time of 30 minutes on thermostated water bath at (75-80 °C). The absorbance was measured using spectrophotometric technique at 489 and 481 nm for propafenone HCl and diltiazem HCl, respectively, or by using spectrofluorimetric technique after dilution at the specific wavelength of excitation and emission.The calibration curves were linear in the range of 4-44, 16-96 µg/mL when using spectrophotometric method, and 0.4-3.6, 1.6-8.8 µg/mL when spectrofluorimetric method was applied for propafenone HCl and diltiazem HCl, respectively. The limit of quantitation and the limit of detection were also calculated. The methods were applied successfully to commercial dosage form and can be further applied for their determination on a large scale in quality control laboratories. The obtained results statistically agreed with those obtained by reference methods. The determination of the studied drugs by the fixed concentration and rate constant methods is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable.

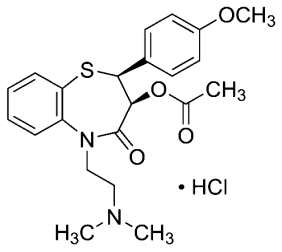
1. Introduction

Propafenone hydrochloride, chemically known as 1-[2-[(2*RS*)-2-hydroxy-3-(propylamino)propoxy]phenyl]-3-phenyl-propan-1-onehydrochloride (Scheme 1) [1]. It is a class IC (Na^+ channel block) antiarrhythmic with some negative inotropic and beta-adrenoceptor blocking activity. It is used in the management of supraventricular and ventricular arrhythmias [2]. Propafenone HCl is official and can be determined in British Pharmacopoeia (BP) [1] and in United States Pharmacopoeia (USP) [3] by non-aqueous titration using perchloric acid determining end-point potentiometrically.

A spectrofluorimetric method was described for the determination of propafenone hydrochloride using *N*'-methyl nicotinamide chloride (NMNCl) in the presence of alkali, followed by addition of formic acid, where highly fluorescent reaction products were produced and measured quantitatively at λ_{em} 409 nm (λ_{ex} = 310 nm) [4]. HPLC techniques were extensively used for determination of propafenone, propafenone enantiomers and propafenone metabolites inbiological fluids using UV [5], fluorimetric [6] or electrochemical detections [7]. LC-flourescence detection [8] and LC-UV [9] detection were adopted for determination of propafenone. LC-MS [10], HPLC-MS [11], GC-MS [12], GC-electron-capture detection [13], TLC [14] and capillary electrophoresis [15] were also used for determination of the drug and its metabolites in pharmaceutical formulations and biological fluids. Propafenone was determined in blood serum using adsorptive-stripping voltammetry [16].



$C_{21}H_{27}NO_3$.HCl
Propafenone hydrochloride, 1-[2-(2-Hydroxy-3-(propylamino)propoxy)phenyl]-3-phenyl-1-propanone hydrochloride



$C_{22}H_{26}N_2O_4$ S. HCl
Diltiazemhydrochloride, (2S,3S)-(+)-*cis*-3-Acetoxy-5-(2-dimethylaminoethyl)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one hydrochloride

Scheme 1

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A simple differential oscillographic voltammetric method was reported for the determination of propafenone hydrochloride in tablets [17]. Diltiazemhydrochlorideis (2*S*,3*S*)-5-[2-(dimethylamino) ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetatehydrochloride [1]. Diltiazem is a benzothiazepine calcium-channel blocker. It is a peripheral and coronary vasodilator with limited negative inotropic activity. Diltiazeminhibits cardiac conduction, particularly at the sino-atrial and atrioventricular nodes. It is used in the management of angina pectoris and hypertension [2]. The BP specifies non-aqueous titration technique detecting the end point potentio-metrically for its determination [1], while USP describes liquid chromatographic method [3].

Ion-pair complex formation reactions were used for assaying diltiazem in its dosage forms using methyl orange, eriochrome black I, mordant black II, tropeolin, picric acid [18] ,orange II, alizarin red S [19], bromothymol blue, bromophenol blue, bromocresolgreen [20], chromotrope 2R and rosebengal [21]. Also diltiazem was determined by its reaction with cobalt thiocyanate at pH = 3-5 forming ternary complex which was extracted and measured at 627 nm [22]. Diltiazem hydrochloride was determined simultaneously using first-order and second-order derivative UV- spectrophotometric methods [23].Also it was determined in commercial dosage forms through the reaction of the tertiary amino group of the drug with sodium hypochlorite to form the chloro drug derivative, which reacts with starch and potassium iodide in sodium bicarbonate forming blue color measured at 540 nm [24]. Spectrophotometric determination of diltiazem with kinetic study was reported [25]. The method was based on two-stage reaction of the drug with hydroxylamine and ferric salt with the formation of red colored complex. Oxidation-reduction reactions were widely used for diltiazem assay in pure and pharmaceutical dosage forms. Diltiazem was oxidized using sodium metavanadate measuring absorbance at 750 nm [26]. Also it was assayed through oxidation of the drug with iron(III) in acidic medium where the liberated iron(II) reacts with 1,10-phenanthroline or 2,2-bipyridyl to form stable complexes measured at 510 and 520 nm, respectively [27]. El-Didamony described three spectrophotometric methods based on oxidation of diltiazem with *N*-bromosuccinimide (NBS) and determination of unconsumed NBS by measuring the decrease in absorbance of amaranth dye or by cerric ammonium sulfate and subsequent determination of the unconsumed oxidant by a decrease in the color of chromotrope 2R or rhodamine 6G [28]. Also diltiazem was oxidized by copper(II) in buffered solution (pH = 7.0) where the copper(I) produced reacts with 4,4′-dicarboxy-2,2′-biquinoline acid and the complexes formed are spectrophotometrically measured at 558 nm [29]. HPLC was extensively described for determination of diltiazem HCl in pharmaceutical preparations [30], biological fluids [31] using UV or flourimetric [32] detections. HPLC was also used for determination of diltiazem with its metabolites either in serum [33] or plasma [34].HPLC was used for enantiomeric separation of diltiazem enantiomers in pharmaceuticals [35]. RP-HPLC [36], LC [37], HPTLC [38], GC [39] and capillary electrophoresis [40] were also applied for determination of diltiazem in biological fluids, tissues and pharmaceutical formulations. Tetraphenylborate was used to form ion-pair complex with the drug as an electroactive material to prepare four types of ion selective electrodes [41]. Ion-selective electrodes were also applied for determination of diltiazem HCl using PVC membrane electrodes based on ion pair complexes formation with sodium Dinonylnaphthalene sulphonic acid [42] or tetraphenylborate [43]. Diltiazem was also determined using Nafion-modified glassy carbon electrode [44]. Diltiazem can be measured by adsorptive stripping voltammetry [45].

In this research, spectrophotometric, spectrofluorimetric methods were developed for the determination of propafenone HCl and diltiazem HCl using NBD-Cl accompanied with kinetic study.

2. Experimental

2.1. Instrumentation

A Shimadzu recording spectrophotometer UV 1800 equipped with 10 mm matched quartz cells was employed for all absorbance measurements. Perkin-Elmer LS45 fluorescence spectrophotometer equipped with a pulsed xenon lamp and 10 mm matched quartz cells.

2.2. Materials and reagents

Chemicals of analytical grade and double distilled water were used throughout the work. Propafenone HCl and Diltiazem HCl obtained from Kahira Pharm. and Chem. Ind. Co., under the license from Abott Laboratories, Egypt and EPICO, Egypt, respectively.

4-Chloro-7-nitrobenzen-2-oxa-1,3-diazole (NBD-Cl) (Aldrich), a fresh solution (0.1% w/v) in methanol, was prepared by dissolving 0.1g in 100 mL methanol.

Borate buffer pH = 7.6 was prepared by dissolving 2.5 g of sodium chloride, 2.85 g of sodium tetraborate and 10.5 g of boric acid in sufficient water to produce 1000 mL [1].

2.3. Standard solutions

0.4 mg/mL solutions of propafenone HCl and diltiazem HCl were prepared by dissolving 10 mg of the drugs in 25 mL distilled water.

2.4. Pharmaceutical preparations

-Rytmonorm® tablets (Kahira pharm, and Chem. Ind. Co., under the license from Abott Laboratories, Egypt) labeled to contain 150mg Propafenone HCl per tablet.

-Altiazem® tablets (EIPICo, Egypt) labeled to contain 60 mg diltiazem HCl per tablet.

-Delay-tiazem® capsules (Galaxo Smith Kline, Egypt) labeled to contain 120 mg diltiazem HCl per capsule.

2.5. General procedure

2.5.1. Spectrophotometric procedure

Accurately measured aliquots of standard solutions were transferred into a series of 10 mL volumetric flasks, and then specified amounts of borate buffer pH = 7.6 and NBD-Cl solution (0.1% w/v) were added. The mixtures were heated for 30 minutes in water bath (75-80 °C). Cool the solutions and acidify with 1 M HCl. The reaction products were diluted to 10 mL with methanol and the absorbance was measured at 489 and481 nm for propafenone HCl and diltiazem HCl, respectively, against a reagent blank prepared in the same manner, Table 1.

2.5.2. Spectroflourimetric procedure

Aliquots of standard solutions were treated as in spectrophotometric procedure and 1 mL of the previously derivatized solutions were transferred into another set of 10 mL volumetric flasks and diluted up to the mark with methanol. The fluorescence intensity was measured at the specific wavelength of excitation and emission against a reagent blank treated similarly, Table 1.

Table 1. Analytical parameters for determination of propafenone HCl and diltiazem HCl using NBD-Cl.

Parameter	Propafenone HCl	Diltiazem HCl
Linearity range for spectrophotometric method (μg/mL)	4-44	16-96
Linearity range for spectroflourimetric method (μg/mL)	0.4-3.6	1.6-8.8
Buffer pH = 7.6 volume (mL)	2	3
Volume of 0.1% w/v NBD-Cl (mL)	1	2
Temperature (°C)	75-80	75-80
Time of heating (min.)	30	30
Volume of 1M HCl (mL)	0.5	1
λ_{max}(nm)	489	481
λ_{ex} (nm)	481	476
λ_{em}(nm)	540	536

2.5.3. For pharmaceutical formulations

For tablets: Ten tablets were powdered and an amount equivalent to 10 mg propafenone HCl and diltiazem HCl were shaken with 10 mL distilled water, then filtered and diluted to 25 mL with distilled water.

For capsules: An accurately weighted amount of the mixed contents of 10 capsules equivalent to 10 mg of diltiazem HCl, were shaken with 10 mL distilled water, then filtered and diluted to 25 mL with distilled water

The assay was completed as under general procedure by applying standard addition technique.

3. Results and discussion

4-Chloro-7-nitrobenzofurazan which is also known as 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl), is an electroactive halide reagent; it has been used as a fluorogenic or chromogenic reagent in pharmaceutical analysis.

The analysis of different compounds or drugs either in pharmaceuticals or in biological samples was performed after derivatization with NBD-Cl followed by measuring the resulted product by means of spectrophotometry [46-49], Spectrofluorimetry [50-52], HPLC as in determination aliphatic amines [53].

It has also been used in charge transfer reactions due to its electrophilic properties, where it acts as π-acceptor for determination of some β-blockers [54], some skeletal muscle relaxant and antihistaminic drugs [55].

NBD-Cl was also applied for the kinetic determination of clindamycin phosphate [56], tramadol hydrochloride [57], dothiepin hydrochloride [58], trimetazidinedihydrochloride [59], befunolol hydrochloride [60], isoxsuprine [61], ciprofloxacin hydrochloride, norfloxacin [62], acetylcysteine, carbocisteine [63], fluvastatin [64], betahistinedihydrochloride and etilefrine hydrochloride [65].

In this work, spectrophotometric, spectrofluorimetric methods were developed for the determination of propafenone HCl and diltiazem HCl using NBD-Cl accompanied with kinetic study.

The presence of nitro group in 6 or 7 position in NBD-Cl molecule induces a direct and/or indirect electrophilic reactivity. This can be also attributed to the strongly unsaturated character of nitro-benzofurazone system [66]. Propafenone HCl and diltiazemHCl reacted directly with NBD-Cl as they already contain amino groups. The reaction product has yellowish brown color with λ_{max} at 489 and481 nm, for propafenone HCl and diltiazem HCl, respectively. Moreover the reaction product exhibited strong fluorescence at λ_{em} 540 nm (λ_{ex} = 481 nm) and λ_{em} 536 nm (λ_{ex} = 476 nm) for propafenone HCl and diltiazem HCl, respectively, Figure 1 and 2. The suggested mechanism of reaction can be interpreted in Scheme 2.

3.1. Method development

Investigations were carried out to establish the most favorable conditions.

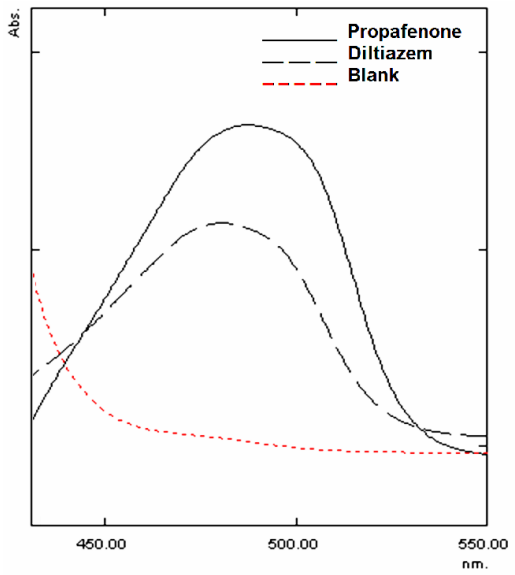


Figure 1. Absorption spectra of the reaction between NBD-Cl (0.1% w/v) and 40 μg/mL propafenone HCl, 56 μg/mL diltiazem HCl.

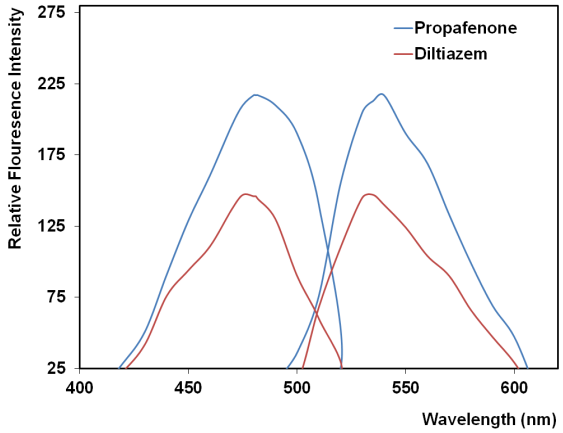
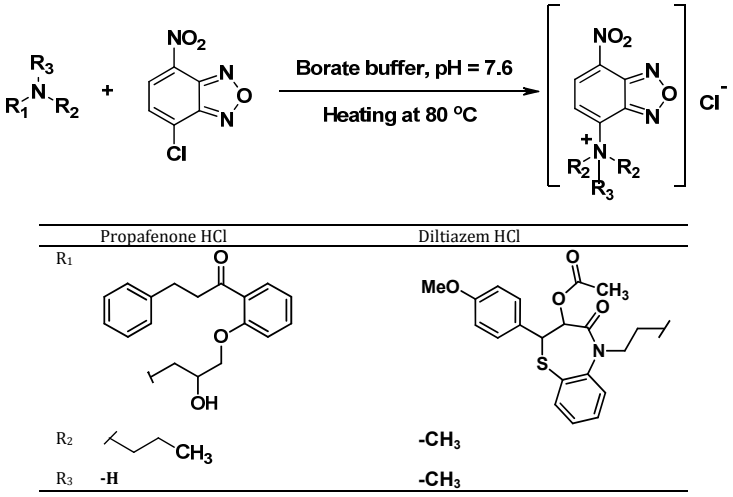


Figure 2. Excitation and emission spectra of the reaction between (0.1% w/v) NBD-Cl and 2.4 μg/mL propafenone HCl, 4.8 μg/mL diltiazem HCl.

3.1.1. Effect of pH

Different borate buffers in a pH range of 7-9 and different bases such as disodium hydrogen phosphate, sodium bicarbonate, sodium acetate, and borax were tried. The best results were obtained on using 2 and 3 mL borate buffer of pH = 7.6.



Scheme 2

Table 2. Spectral data for determination of propafenone HCl and diltiazem HCl using NBD-Cl.

Items	Spectrophotometric method		Spectrofluorimetric method	
	Propafenone HCl	Diltiazem HCl	Propafenone HCl	Diltiazem HCl
Linearity range (µg/mL)	4-44	16-96	0.4-3.6	1.6-8.8
Apparent molar absorptivity* ($mol^{-1}cm^{-1}$)	7.894×10^3	3.524×10^3		
Sandell's sensitivity (mg/mL per 0001A)	2.336×10^{-3}	7.814×10^{-4}		
Limit of detection (µg/mL)	1.21	4.71	0.115	0.407
Limit of quantification (µg/mL)	3.66	14.29	0.349	1.23
Regression equation**				
Slope (b)	0.0197	0.0070	84.353	23.846
Intercept (a)	0.0827	0.0428	15.897	33.500
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9997

* Calculated on the basis of the molecular weight of the drug.
** A = a + bC

3.1.2. Effect of NBD-Cl concentration

1 and 2 mL 0.1% w/v NBD-Cl was sufficient for production of maximum and reproducible color intensity for propafenone HCl and diltiazem HCl, respectively.

3.1.3. Effect of HCl molarity and volume

The reaction products must be acidified to pH = 2 using HCl before measurement, in order to change NBD-OH to NBD-OCH_3 [66,46]. It was found that maximum absorbance readings were obtained on using 0.5 and 1.0 mL 1 M HCl for propafenone HCl and diltiazem HCl, respectively.

3.1.4. Effect of temperature and time

Following the color development from ambient temperature (25 ±5 °C) to 100 °C, It was found that the reaction rate was very slow at room temperature and complete color development was attained after 30 minutes in water bath at (75-80 °C) for both drugs. Above 80 °C is not preferable due to precipitation of the product on walls of tubes [67]. The color remains stable for up to 2-3 hours for both drugs.

3.1.5. Effect of diluting solvents

Several organic solvents such as methanol, ethanol, distilled water, acetone and acetonitrile were investigated. Methanol was found to be the most appropriate solvent for both drugs to give the highest absorbance and more stability.

3.2. Method validation

3.2.1. Quantification, accuracy and precision

Beer's law was obeyed over a concentration range of 4-44 and 16-96 µg/mL for propafenone HCl and diltiazem HCl, respectively. Besides, the fluorescence intensity showed linear relationship from 0.4-3.6 and 1.6-8.8 µg/mL for the two drugs, respectively.

Molar absorptivity, correlation coefficient, intercept and slope for the calibration curve, detection and quantification limit were calculated, Table 2. Also relative standard deviation, analytical standard error and variance were calculated and listed in Table 3. The proposed methods were applied for determination of the selected drugs in their pharmaceutical preparations using the standard addition technique, Tables 4 and 5. Results obtained were compared with official methods [1,3] of both drugs and no significant difference was found between the proposed and reference methods, Table 6.

3.2.2. Accuracy and precision

Accuracy and precision were carried out by six determinations at four different concentrations of both drugs in the same day (intra-day), and in six different days (inter-day). Percentage relative standard deviation (R.S.D. %) as precision and percentage relative error (Er %) as accuracy of the suggested method was calculated. The percentage relative error calculated using the following equation:

$$Er\% = [(found - added) / added] \times 100 \quad (1)$$

The results of accuracy and precision show that the proposed methods have good repeatability and reproducibility, Table 7.

Table 3. Determination of propafenone HCl and diltiazem HCl using NBD-Cl.

Statistics	Spectrophotometric method				Spectrofluorimetric method			
	Propafenone HCl		Diltiazem HCl		Propafenone HCl		Diltiazem HCl	
	Taken µg/mL	Recovery * %	Taken µg/mL	Recovery * %	Taken µg/mL	Recovery * %	Taken µg/mL	Recovery * %
	4	99.37	16	98.39	0.4	101.07	1.6	100.91
	16	100.67	32	100.98	0.8	100.92	2.4	100.47
	20	99.06	40	99.00	1.2	99.88	3.2	100.25
	24	100.95	56	100.31	1.6	99.36	4.8	99.16
	28	99.76	60	100.52	2.4	99.33	5.6	98.47
	32	99.67	80	99.50	2.8	100.39	7.2	101.64
	44	100.06	88	99.38	3.6	100.14	8.0	100.38
			96	100.18			8.8	99.36
Mean±S.D.	99.93 ± 0.680		99.73 ± 0.915		100.16 ± 0.690		100.08± 1.023	
N	7		8		7		8	
V	0.462		0.837		0.476		1.046	
S.D.	0.680		0.915		0.690		1.023	
R.S.D.	0.680		0.918		0.689		1.022	
S.E.	0.257		0.324		0.263		0.362	

* Mean of three different experiments.

Table 4. Application of standard addition technique for the determination of propafenone HCl and diltiazem HCl in their pharmaceutical formulations using spectrophotometric method.

	Propafenone HCl			Diltiazem HCl					
	Rytmonorm tablets			Altiazem tablets			Delay-tiazem capsules		
	Taken µg/mL	Added	Recovery* %	Taken µg/mL	Added	Recovery* %	Taken µg/mL	Added	Recovery* %
	8	--	99.81	16	--	100.18	16	--	101.96
		8	100.44		16	101.07		16	101.07
		24	99.668		32	100.08		32	100.54
		26	98.07		40	99.00		44	99.41
		28	99.22		44	101.36		52	100.33
		32	99.19		48	100.36		56	99.54
		36	98.32		52	99.51		64	98.92
Mean±S.D.	99.15±0.874			100.23±0.901			99.97±0.805		
N	6			6			6		
V	0.765			0.813			0.647		
S.D.	0.874			0.901			0.805		
S.E.	0.357			0.368			0.329		

* Mean of three different experiments.

Table 5. Application of standard addition technique for determination of propafenone HCl and diltiazem HCl in their pharmaceutical formulations using spectroflourimetric method.

	Propafenone HCl			Diltiazem HCl					
	Rytmonorm tablets			Altiazem tablets			Delay-tiazem capsules		
	Taken µg/mL	Added	Recovery* %	Taken µg/mL	Added	Recovery* %	Taken µg/mL	Added	Recovery* %
	0.8	--	99.43	1.6	--	100.91	1.6	--	103.52
		0.4	98.11		1.6	100.91		1.6	98.29
		1.6	100.10		2.4	98.72		2.4	100.47
		2	98.46		3.2	98.94		4	99.07
		2.2	97.59		4	99.07		5.6	100.12
		2.4	99.34		4.8	100.03		6.4	99.97
		2.8	99.54		5.6	100.72		7.2	100.58
Mean±S.D.	99.33±0.957			99.73±0.951			99.75±0.894		
N	6			6			6		
V	0.915			0.905			0.799		
S.D.	0.957			0.951			0.894		
S.E.	0.391			0.388			0.365		

* Mean of three different experiments.

The proposed methods were simple, accurate and the spectrofluorimetric method was more sensitive than the spectrophotometric one and both of them are suitable for analysis of the selected drugs with good recoveries.

3.3. Kinetic study of the reaction with NBD-Cl

The rate of the reaction was found to be concentration dependent. The rate of the reaction was followed with various concentrations of studied drugs in the range of 4-44 and 16-96 µg/mL for propafenone HCl and diltiazem HCl, respectively. The graph shown in Figure 3 and 4 indicate that the reaction rate of propafenone HCl and diltiazem HCl obeys the following equation:

$$Rate=K`[drug]^n \quad (2)$$

where K` is the pseudo-order constant of the reaction and n is the order of the reaction.

The rate of the reaction may be estimated by the variable-time method [68]. In this method the reaction rate was followed by measuring the change of absorbance at different time intervals Taking logarithms of rates and concentration (Table 8) Equation 2 is transformed into:

$$\log (rate)=\log \Delta A/\Delta t = \log K` + n \log [drug] \quad (3)$$

where A is the absorbance and t is the time in seconds.

Regression of log (rate) versus log (drug) gave the regression equations:

Table 6. Statistical data for determination of propafenone HCl and diltiazem HCl using NBD-Cl.

ITEMS	Propafenone HCl			Diltiazem HCl		
	Reference method [3]	Spectrophotometric method	Spectrofluorimetric method	Reference method [1]	Spectrophotometric method	Spectrofluorimetric method
Mean ±S.D.	100.29± 0.630	99.93± 0.680	100.16± 0.690	99.38± 1.070	99.73 ± 0.915	100.08 ± 1.022
N	3	7	7	4	8	8
V	0.395	0.462	0.476	1.140	0.837	1.046
S.D.	0.630	0.680	0.690	1.070	0.915	1.022
t		0.782 (2.306)*	0.279 (2.306)*		0.593 (2.228)*	1.103 (2.228)*
F		1.170 (5.140)*	1.205 (5.140)*		1.362 (4.350)*	1.090 (4.350)*

* Theoretical values of t and F at P = 0.05.

Table 7. The intra-day and inter-day accuracy and precision data for propafenone HCl and diltiazem HCl obtained using NBD-Cl.

		Propafenone HCl					Diltiazem HCl				
		Taken µg/mL	Found µg/mL	Recovery %	RSD %	Er %	Taken µg/mL	Found µg/mL	Recovery %	RSD %	Er %
Spectrophotometric method	Intraday	4	3.98	99.58	2.723	-0.42	16	15.74	98.39	2.223	-1.61
		16	16.08	100.51	1.984	0.51	32	32.22	100.68	1.956	0.68
		20	19.85	99.23	1.044	-0.77	40	39.62	99.06	1.801	-0.94
		24	24.18	100.74	0.994	0.74	56	56.005	100.01	1.190	0.01
	Interday	4	3.97	99.15	2.735	-0.85	16	15.84	98.99	2.530	-1.01
		16	16.16	100.98	1.447	0.98	32	32.12	100.39	2.060	0.39
		20	19.81	99.06	1.840	-0.94	40	39.70	99.24	1.773	-0.76
		24	24.15	100.63	1.102	0.63	56	56.27	100.48	1.587	0.48
Spectroflourimetric Method	Intraday	0.4	0.404	101.07	2.623	1.07	1.6	1.608	100.47	3.050	0.47
		0.8	0.799	99.93	2.026	-0.07	2.4	2.404	100.18	2.039	0.18
		1.2	1.199	99.88	1.532	-0.12	3.2	3.215	100.47	1.525	0.47
		1.6	1.592	99.48	1.283	-0.52	4.8	4.767	99.31	1.796	-0.69
	Interday	0.4	0.404	101.07	2.623	1.07	1.6	1.615	100.91	3.285	0.91
		0.8	0.797	99.68	2.382	-0.32	2.4	2.418	100.76	3.366	0.76
		1.2	1.197	99.72	2.539	-0.28	3.2	3.201	100.03	2.099	0.03
		1.6	1.594	199.61	2.033	-0.39	4.8	4.781	99.60	2.271	-0.40

Table 8. Logarithms of the rates for different concentrations of propafenone HCl and diltiazem HCl.

Propafenone HCl		Diltiazem HCl	
Log (rate), log $\Delta A/\Delta t$	Log [conc.] (M)	Log (rate), log $\Delta A/\Delta t$	Log [conc.] (M)
-4.429	-4.927	-4.311	-4.450
-4.017	-4.325	-4.062	-4.149
-3.933	-4.228	-3.956	-4.052
-3.861	-4.149	-3.799	-3.906
-3.790	-4.082	-3.764	-3.876
-3.749	-4.024	-3.671	-3.751
-3.574	-3.885	-3.619	-3.710
-	-	-3.588	-3.672

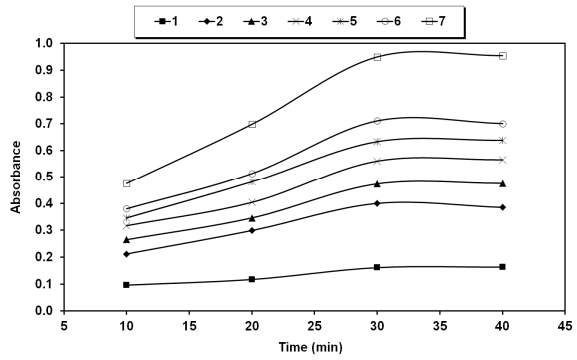


Figure 3. Absorbance versus time graphs for the reaction between propafenone HCl and NBD-Cl at different concentrations of propafenone: (1) 1.183×10^{-5} M.(2) 4.735×10^{-5} M. (3) 5.919×10^{-5} M. (4) 7.103×10^{-5} M. (5) 8.287×10^{-5} M. (6) 9.470×10^{-5} M.(7) 1.302×10^{-4} M.

log(rate)= 0.2763 +0.9955 log C(r=0.9963), K` = 1.889 S^{-1} for propafenone HCl (4)

log (rate) = -0.1126 + 0.9462 log C (r = 0.9985), K` = 0.772 S^{-1} for diltiazem HCl (5)

hence the reaction is first order (n ≈ 1) with respect to drug concentration.

3.3.1. Evaluation of the kinetic methods

The quantitative determination of the studied drugs under the optimized experimental conditions outlined before, would result in a pseudo-first order reaction with respect to their concentration. However, the rates will be directly proportional to drug concentration in a pseudo-first order rate equation as follows:

$$Rate = K` [M_{drug}] \quad (6)$$

where K` is the pseudo-first order constant. Equation 6 was the basis for several experiments, which were run to obtain drug concentration using the rate data. Rate constant, constant concentration and fixed-time [69,70] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (r) and the intercept.

3.3.2. Rate-constant method

Graphs of log (absorbance) versus time for the studied drugs concentrations in the range 1.184×10^{-5} to 1.302×10^{-4} and 3.547×10^{-5} to 2.129×10^{-4} M for propafenone HCl and diltiazem HCl, respectively, were plotted and all appeared to be rectilinear. Pseudo-first order rate constants corresponding to different drugs concentrations (C) were calculated from the slopes multiplied by -2.303 and are presented in (Table 9), (Figure 5 and 6).

Table 9. Values of *K`* calculated from slopes of log A *vs.* t graph multiplied by -2.303 for different concentrations of propafenone HCl and diltiazem HCl.

Propafenone HCl		Diltiazem HCl	
K` (S^{-1})	(M)	K` (S^{-1})	(M)
-3.18×10^{-4}	1.18×10^{-5}	-4.98×10^{-4}	3.55×10^{-5}
-3.47×10^{-4}	4.74×10^{-5}	-5.49×10^{-4}	7.09×10^{-5}
-3.44×10^{-4}	5.92×10^{-5}	-5.61×10^{-4}	8.87×10^{-5}
-3.43×10^{-4}	7.10×10^{-5}	-5.88×10^{-4}	1.24×10^{-4}
-3.52×10^{-4}	8.29×10^{-5}	-6.01×10^{-4}	1.33×10^{-4}
-3.61×10^{-4}	9.47×10^{-5}	-5.99×10^{-4}	1.77×10^{-4}
-4.01×10^{-4}	1.30×10^{-4}	-6.11×10^{-4}	1.95×10^{-4}
-	-	-6.01×10^{-4}	2.13×10^{-4}

Table 10. Values of reciprocal of time taken at fixed absorbance for different rates of variable concentration of propafenone HCl and diltiazem HCl.

Propafenone HCl		Diltiazem HCl	
1/t (S^{-1})	(M)	1/t (S^{-1})	(M)
5.56×10^{-4}	5.92×10^{-5}	6.94×10^{-4}	7.09×10^{-5}
6.67×10^{-4}	7.10×10^{-5}	9.26×10^{-4}	8.87×10^{-5}
8.33×10^{-4}	8.29×10^{-5}	1.21×10^{-3}	1.24×10^{-4}
9.25×10^{-4}	9.47×10^{-5}	1.27×10^{-3}	1.33×10^{-4}
1.67×10^{-3}	1.30×10^{-4}	1.50×10^{-3}	1.77×10^{-4}
-	-	1.59×10^{-3}	1.95×10^{-4}
-	-	1.67×10^{-3}	2.13×10^{-4}

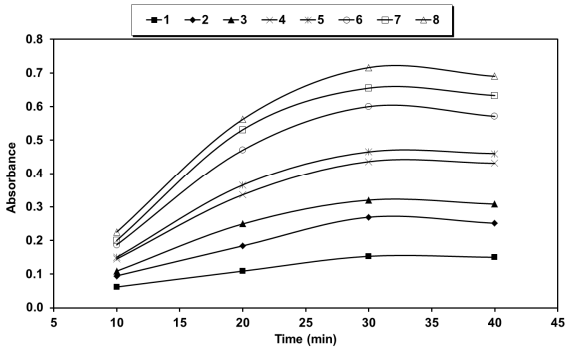


Figure 4. Absorbance versus time graphs for the reaction between diltiazem HCl and NBD-Cl at different concentrations of diltiazem: (1) 3.547×10^{-5} M.(2) 7.095×10^{-5} M. (3) 8.869×10^{-5} M. (4) 1.242×10^{-4} M. (5) 1.330×10^{-4} M. (6) 1.774×10^{-4} M.(7) 1.951×10^{-4} M. (8) 2.129×10^{-4} M.

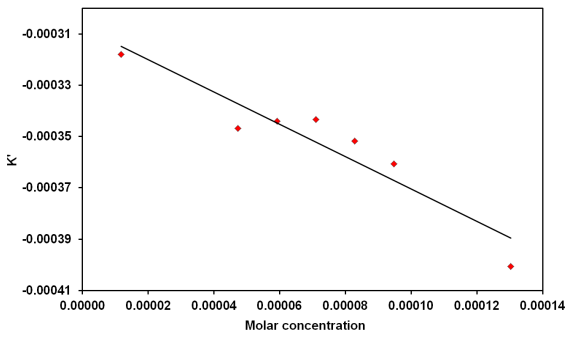


Figure 5. Values of k`(s^{-1}) calculated from slopes of different log absorbance *vs* t graph multiplied by -2.303 for different concentrations of propafenone HCl: (1) 1.183×10^{-5} M. (2) 4.735×10^{-5} M. (3) 5.919×10^{-5} M. (4) 7.103×10^{-5} M.(5) 8.287×10^{-5} M.(6) 9.470×10^{-5} M.(7) 1.302×10^{-4} M.

Regression of (C) versus K` gave the equation:

K`= - 0.0003 - 0.6317 C (r=0.9443) (for propafenone HCl) (7)

K`= - 0.0005 - 0.5438 C (r=0.8974) (for diltiazem HCl) (8)

The value (r) indicates poor linearity, which is probably due to inconsistency of K`.

3.3.3. Fixed-concentration method

Reaction rates were determined for different concentrations in the range 1.184×10^{-5} to 1.302×10^{-4} and 3.547×10^{-5} to 2.129×10^{-4} M for propafenone HCl and diltiazem HCl, respectively. A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time (i.e. 1/t) versus the initial concentration of the studied drugs (Table 10) was plotted (Figure 7 and 8). The following equations for calibration graphs were worked out by linear regression:

1/ t = 0.0003+ 15.759 C (r=0.9843) for propafenone HCl (9)

1/ t = 0.0003+ 6.5346 C (r=0.9847) for diltiazem HCl (10)

The range of the concentration of the studied drugs giving the most acceptable calibration graph with the above equations was very limited, which could be disadvantage.

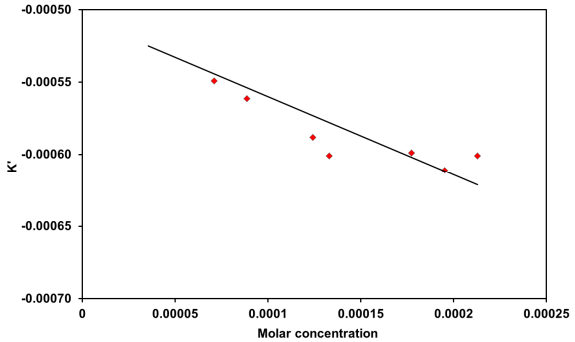


Figure 6. Values of k`(s^{-1}) calculated from slopes of different log absorbance *vs* t graph multiplied by -2.303 for different concentrations of diltiazem HCl: (1) 3.547×10^{-5} M.(2) 7.095×10^{-5} M. (3) 8.869×10^{-5} M. (4) 1.242×10^{-4} M. (5) 1.330×10^{-4} M. (6) 1.774×10^{-4} M.(7) 1.951×10^{-4} M. (8) 2.129×10^{-4} M.

3.3.4. Fixed time method

Reaction rates were determined for different concentration of the studied drugs. At a pre-selected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of the absorbance versus initial concentration of the studied drugs were obtained at fixed times of 10, 20, 30 and 40 min with the calibration equation shown in (Table 11).

Table 11. Calibration equations at different fixed time over the range of 1.18 x 10^{-5} - 1.30 x 10^{-4} and 3.55 x 10^{-5} - 2.13 x 10^{-4} M for propafenone HCl and diltiazem, respectively.

Time (min)	Calibration equation	Correlation coefficient (r)
Propafenone HCl		
10	A = 0.0675 + 0.0096 C	0.9949
20	A = 0.0615 + 0.0145 C	0.9989
30	A = 0.0827 + 0.0197 C	0.9999
40	A = 0.0791 + 0.0198 C	0.9994
Diltiazem HCl		
10	A = 0.0311 + 0.0197 C	0.9986
20	A = 0.0118 + 0.0058 C	0.9989
30	A = 0.0428 + 0.0070 C	0.9999
40	A = 0.0426 + 0.0067 C	0.9990

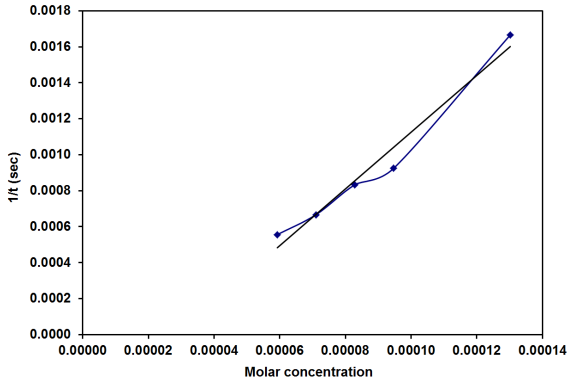


Figure 7. Values of reciprocal of time taken at fixed absorbance for different rates of variable concentrations of propafenone HCl.

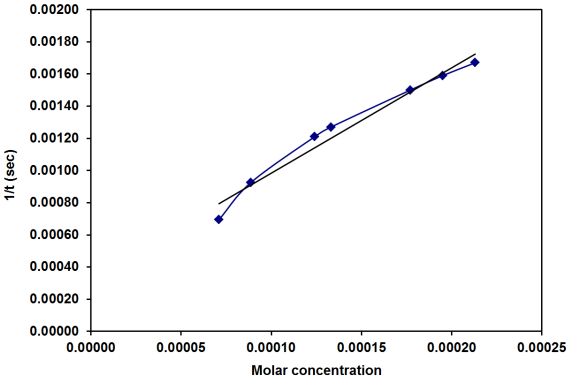


Figure 8. Values of reciprocal of time taken at fixed absorbance for different rates of variable.

It is clear that, both the slopes and intercepts increase with time. The most acceptable values of the correlation coefficient and more reaction products (indicated by higher absorbance readings) as shown in (Figure 3 and 4) were obtained for a fixed time of 30 min, which was, therefore chosen as the most suitable time interval for measurements.

After optimizing the reaction conditions, the fixed time method was applied to the determination of propafenone HCl and diltiazem HCl in pure form and in pharmaceutical formulation over the concentration range of (4-44 and 16-96 µg/mL) for both drugs respectively.

Analysis of the date gives the following regression equation:

A =0.0827 + 0.0197 C(r= 0.9999) for propafenone HCl (11)

A = 0.0428 + 0.0070 C(r = 0.9999) for diltiazem HCl (12)

The fixed time method was applied to the determination of the studied drugs in the pure form and in the supplied drug formulations. The concentrations of the studied drugs were calculated using the corresponding calibration equation at fixed time of 30 min. The developed analytical method was validated. Under the described experimental conditions, calibration graphs were constructed for all of the studied drugs; linear relationship was found between the absorbance at λ_{max} and the concentration of the drug (Table 1). The recoveries, standard deviations, relative standard deviations, standard errors, variances for the two studied drugs applying the previously stated methods were listed in (Table 3). The proposed methods were applied for determination of the selected drugs in their pharmaceutical formulations using the standard addition method. The results of analysis of the commercial dosage forms and the recovery study are shown in (Table 4 and 5). The average percent recoveries obtained, indicated good accuracy of the methods.

The result obtained for the analysis of the studied drugs in drug formulations employed was compared with those obtained with the official and reference methods [1,3], (Table 6). The Student t-test and F-test values of 95% confidence level did not exceed the theoretical values indicating no significant difference between the accuracy and the precision of the two methods.

4. Conclusion

The proposed method holds several advantages when compared to other previously reported methods, such as sensitivity and selectivity. In contrast to HPLC, there is no need for special hardware, or expensive solvent. In conclusion, the given data reveal that the proposed methods are accurate and sensitive, with good precision and accuracy. With these methods, one can do accurate analysis at low cost without losing accuracy. The proposed methods can be used for the routine analysis of the cited drugs in the pure form and in pharmaceutical formulations, in quality control laboratories, or on smaller scale in small laboratories.

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