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Research Article

UTILITY OF COMPLEXATION REACTION FOR THE DETERMINATION OF SOME CARDIOVASCULAR DRYGS

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ABSTRACT

Two simple, sensitive and accurate extraction spectrophotometric methods were developed for the determination of propafenone HCl,naftidrofuryl oxalate and bisoprolol hemifumarate both in pure form and in pharmaceutical preparations. The first method based on the formation of ion -pair complexes between the cited drugs and two dyes namely tropeolin OOO or chromazurol S using extractive procedure . The calibration plots were rectilinear over concentration range of 6-28 ,6-32 and 6-28 µg/mL with limits of detection of 1.45,1.38 and 0.99 µgmL^{-1} for propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate respectively in case of tropeolin OOO; 28-100, 28-68 and 10-46 µg mL $^{-1}$ with limits of detection of 5.1,5.4,2.7 µg mL $^{-1}$ in case of chromazurol S. The second method based on the formation of ternary complex between the cited drugs and the binary inorganic complex Mo(V)-thiocyanate followed by extraction with methylene chloride . Beer's law was obeyed in the concentration range of 4-32, 6-32 and 4-32 µg ml^{-1} with limits of detection of 1,1.11,1.13 µgmL^{-1} for propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate respectively. The different experimental parameters affecting the development of the complex were studied and optimized. Statistical comparison of the results was performed using Student's t-test and variance ratio F-test at 95% confidence level and there was no significant difference between the proposed methods and the official or reference methods. Rapid and short analysis time, simplicity, sensitivity, low cost compared with other techniques, make the method attractive for analysis of the cited drugs in pure forms and in pharmaceutical preparations.

Keywords: Cardiovascular, Extractive, Spectrophotometric, Tropeolin OOO, Chronazurol S, Mo(V)- thiocyanate

INTRODUCTION

Propafenone hydrochloride [2-(2-hydroxy-3-propylaminopropoxy)-3-phenylpropiophenone hydrochloride] 1. It is used in the treatment of cardiac arrhythmias 2. Several methods including Spectrophotometric 3,spectrofluorimetric 4, capillary electrophoresis 5-7, Conductometric8, HPLC methods 9-10 , LC-MS 11-12, adsorptive stripping voltammetry13, gas chromatography using chemical ionization mass spectrometry (CIMS)14 and TLC-densitometric determination15 were reported for propafenone determination .

Naftidrofuryl oxalate is also known as nafronyl oxalate,(2-(diethylaminoethyl)-2-[(naphthalene-1-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate hydrogen oxalate) 16. It is used as a vasodilator in the treatment of peripheral and cerebral vascular disorders. It is claimed to enhance cellular oxidative capacity thereby protecting cells against results of ischaemia 2. Various analytical methods were reported for the determination of naftidrofuryl in biological fluids and/or pharmaceutical preparations. Most of these studies foccused on kinetic spectrophotometry 17, derivative spectrophotometry, spectrofluorimetry, differential-pulse voltammetry 18, HPLC19-21 . Others include phosphorimetric analysis 22-23, potentiometric method with nafronyl ion-selective electrodes24 and flow injection analysis with fluorescence optosensor 25.

Bisoprolol Hemifumarate , 1-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl) amino2-Propanol fumarate (2:1), is a highly selective_1-receptor blocking agent used for the treatment of hypertension and angina pectoris 2. Several methods were used including Spectrophotometric26-27 , volttametric 28, capillary electrophoresis29 , GC-MS 30, LC-chemilumesence nitrogen detection31 , Densitometric32, potentiometric33, flourometric34 and HPLC methods 35,36. Bisoprolol were determined with Hydrochlorothiazide or Amlodipine besylate in combined pharmaceutical dosage forms using RP-HPLC37 , HPLC-MS38, HPLC39 and Spectrophotometric methods 40-43.

The present study deals with development and validation of visible extractive spectrophotometric methods for the determination of the cited drugs in bulk form and pharmaceutical preparations. The methods in addition to being selective, is sensitive, simple, cost effectiveness, there is no need of heating or expensive device. An inspection of the performance characteristics of the reported visible spectrophotometric methods for the studied drugs ,some of them suffer from some drawbacks as law sensitivity , too many steps and heating at higher temperatures hence the present work aims to demonstrate ,simple and sensitive spectrophotometric method using simple steps and ecofriendly chemicals for estimation of the mentioned drugs in pure and pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

A Shimadzu UV and visible recording spectrophotometer (UV 260) with two 10-20 mm matched quartz cells were employed for all absorbance measurements.

Materials and reagents

All solutions were prepared with analytical-reagent grade chemicals and water was always doubly distilled. The studied drugs were of pharmaceutical grade.

In the first method 0.1% aqueous solution of Tropeolin OOO (Prolabo) was prepared, while Chromazurol S was prepared as 0.1% and 0.3% aqueous solutions. In the second method, 8% and 12% aqueous solutions of ammonium thiocyanate,2% and 2.5% aqueous solutions of sodium molybdate (Fluka AG,Buchs SG) were used . Acetate buffers pH 2.7 and 3.7 16, buffer solution of pH 1.1 44, ,10%aqueous solution of ascorbic acid , 5M HCl were used.

Standard solutions

Solutions of 0.4 mg mL -1 of Naftidrofuryl oxalate(drug (Mina Pharm, under licence of Merck Santé France), Propafenone HCl(Kahira pharm, and chem. Ind. Co., under the licence from Abott Laboratories) and Bisoprolol hemifumarate(Amoun Pharm, under licence of Merk Santé France) were prepared by dissolving 10 mg of drug in 25 mL distilled water.

Pharmaceutical preparations

Rytmonorm® tablets containing 150mg Propafenone HCl per tablet (Kahira pharm, and chem. Ind. Co., under the licence from Abott Laboratories).

Praxilene® tablets containing 200 mg Naftidrofuryl oxalate per tablet (Mina Pharm, under licence of Merck Santé France)

Cerebromap® capsules containing 200 mg Naftidrofuryl oxalate per capsule(Multi-Apex pharm)

Concor® tablets containing 10 mg Bisoprolol hemi fumarate (Amoun Pharm, under licence of Merk Santé France)

General Procedures

Method A

Into a series of 125 mL separating funnels , aliquots of each standard drug solution were transferred, appropriate volumes of dye and buffer were added, table(1).The content was mixed thoroughly, the complex was extracted with 10mL appropriate organic solvent Table (1). The organic layer was then passed over anhydrous sodium sulfate, collected in10mL volumetric flask and absorbance was measured at suitable wavelength against reagent blank treated similarly.

Table 1: Analytical parameters for determination of Propafenone HCl, Naftidrofuryl oxalate and Bisoprolol hemifumarate using tropeolin OOO and chromazurol S.

	Tropeolin OOO method			Chromazurol S method		
Item	Propafenone HCl	Naftidrofuryl oxalate	Bisoprolol hemifumarate	Propafenone HCl	Naftidrofuryl oxalate	Bisoprolol hemifumarate
Conc of dye	1ml 0.1%	1ml 0.1%	1ml 0.1%	1ml 0.1%	2ml 0.1%	1ml 0.3%
Buffer	3ml pH 2.7	2ml pH 2.7	1ml pH3.7	---*	2ml pH 1.1	1ml pH 1.1
Solvent	CH_2Cl_2	CH_2Cl_2	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$
No. of extraction	one time	one time	one time	one time	one time	one time
Wave length	486	486	485	501	501	505
Beer's law range(µg ml^{-1})	6-28	6-32	6-28	28-100	28-68	10-46

*** No buffer is needed.**

Method B

Into a series of 100 mL separating funnels, appropriate volumes of sodium molybdate, 5M HCl, ammonium thiocyanate and ascorbic acid were mixed and left for 10 minutes. Aliquots of each standard drug solutions were added and left for another proper time. The formed complex was extracted by methylene chloride twice with 5 mL portions after shaking for 30 seconds, organic layer was filtered over anhydrous sodium sulfate, collected in10mL volumetric flask. Absorbance of the colored complexes was measured at suitable wavelength against reagent blank treated similarly. Table(2).

Table 2: Analytical parameters for determination of Propafenone HCl, Naftidrofuryl oxalate and Bisoprolol hemifumarate using Mo(V)-thiocyanate.

Item	Propafenone HCl	Naftidrofuryl oxalate	Bisoprolol hemifumarate
Conc. of Na_2MoO_4	2 ml 2.5%	2 ml 2.5%	2 ml 2%
Acid concentration	3 ml 5M	2 ml 5M	3 ml 5M
Conc. of NH_4SCN	4 ml 8%	3 ml 12%	2 ml 12%
Conc. Of ascorbic acid	2 ml 10%	2 ml 10%	0.5 ml 10%
Time before adding drug	10 minutes	10 minutes	10 minutes
Time after adding drug	10 minutes	5 minutes	5 minutes
Solvent	CH_2Cl_2	CH_2Cl_2	CH_2Cl_2
No. of extractions	Twice	Twice	Twice
Wave lengh	469	467	469
Beer's law range(µg ml^{-1})	4 - 32	6-32	4-32

Assay of pharmaceutical preparations

Assay of tablets

Ten tablets were weighed, pulverized into fine powder, specific quantity of powdered drugs equivalent to 10 mg pure drug were dissolved in distilled water, solutions were filtered and diluted to 25mL with distilled water. Procedures were completed as in general procedures applying standard addition technique.

Assay of capsules

The contents of ten capsules were emptied, pulverized. An accurately weighed amount equivalent to 10 mg pure drug was dissolved in distilled water, solutions were filtered and diluted to 25mL with distilled water. Procedures were completed as in general procedures applying standard addition technique.

RESULTS AND DISCUSSION

Method A

Acidic dyes were widely used for colorimetric determination of various pharmaceutical compounds. The principle of this method is based on allowing the drug which has basic cationic center to react with the anionic dye at selected pH value. A highly colored ion-pair chromogen was formed and determined by extraction with organic solvent. Fig.(1,2)

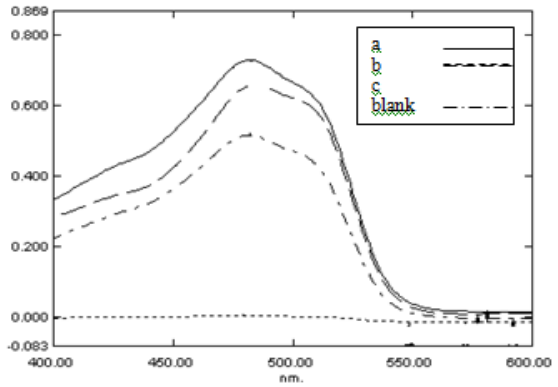


Fig 1:Absorption spectra of tropeolin ooo complexes with:a) Bisoprolol hemifumarate (20µg ml^{-1}), b)Propafenone HCl(20µg ml^{-1}) and c) Naftidrofuryl oxalate(20µg ml^{-1})

Optimum conditions affecting the reaction were studied

Effect of reagent concentration

1mL of 0.1% tropeolin OOO was sufficient to give maximum absorbance for all cited drugs, while 1mL and 2 mL of 0.1%, and 1mL of 0.3% of chromazurol S were sufficient for propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate respectively.

Effect of buffer

In order to establish the optimum pH value, wide range of buffers pH (1-10) were tried, for tropeolin OOO maximum absorbance values were obtained using 3mL and 2mL pH2.7 and 1mL pH3.7 for propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate.

For chromazurol S, 1mL and 2 mL pH 1.1 were sufficient to give maximum results with bisoprolol hemifumarate and naftidrofuryl oxalate respectively, while propafenone didn't need any buffer addition and it was found that pH of its reaction media was 6.3.

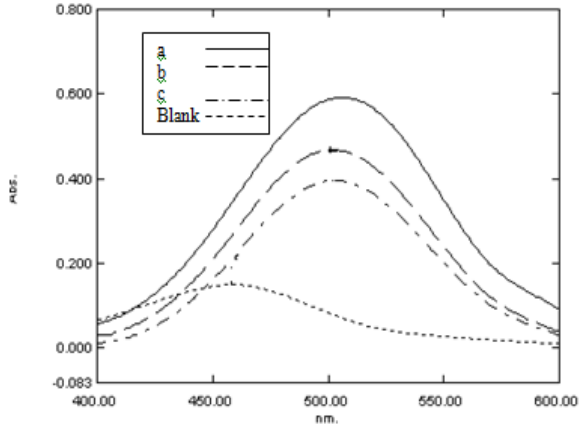


Fig 2:Absorbtion spectra of Chromzurol S complexes with a) Bisoprolol hemifumarate (40µg ml^{-1}), b)Propafenone HCl(40µg ml^{-1}) and c) Naftidrofuryl oxalate(40µg ml^{-1}).

Effect of organic solvent

Many organic solvents e.g. (Chloroform, methylene chloride, ethylene chloride, benzene, ether and ethyl acetate) were tried to find most appropriate solvent that give maximum absorbance and has lower extraction ability of reagent.

For tropeolin OOO, methylene chloride was chosen for propafenone HCl and naftidrofuryl oxalate, while chloroform was used for bisoprolol hemifumarate.

For chromazurol S, chloroform was the appropriate solvent for the three cited drugs.

Effect of reaction time

The reaction time required for complete color development of ion-pair complex was studied. It was found that maximum absorbance was attained immediately.

Effect of shaking time and number of extractions

The shaking time required to allow all ion-pair complex to be extracted into the organic phase was also studied. It was found that 30 seconds was enough for complete extraction.

Also there was no difference in results between single, double or triple extraction with organic solvent.The ion-pair formed was stable for at least 30 minutes.

Stoichiometry of the reaction

The composition of the ion-pair was studied applying Job's method of continuous variation 45 . Results indicate that the stoichiometry ratio of (drug: dye) was (1:1). Fig. (3, 4).

Method B

The ion pairs are formed between the amino group of drugs and Mo (V)- thiocyanate binary complex via protonated nitrogen atom of these drugs.

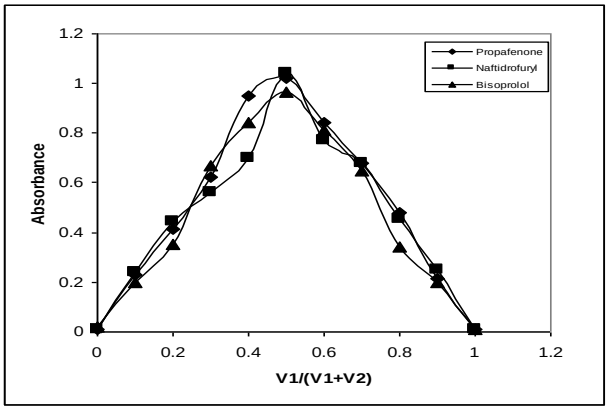


Fig 3. Determination of the stiochiometry of the reaction of 5×10^{-4}M Propafenone, Naftidrofuryl and bisobrolol with 5×10^{-4} M tropeolin OOO.

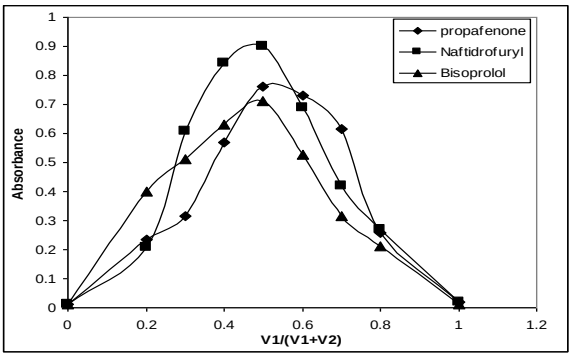


Fig 4. Determination of the stiochiometry of the reaction of 5×10^{-4}M Propafenone, Naftidrofuryl and bisobrolol with 5×10^{-4} M Chromzurol S.

Molybdenum (V) is formed by the reduction of molybdenum (VI) with ascorbic acid in the presence of 5M HCl, then combines with thiocyanate to form red binary molybdenum(V)-thiocyanate complex which is not extractable with methylene chloride. On adding the drug solution, an orange red ternary complex is formed in the same acid media which is extractable with methylene chloride. Fig. (5)

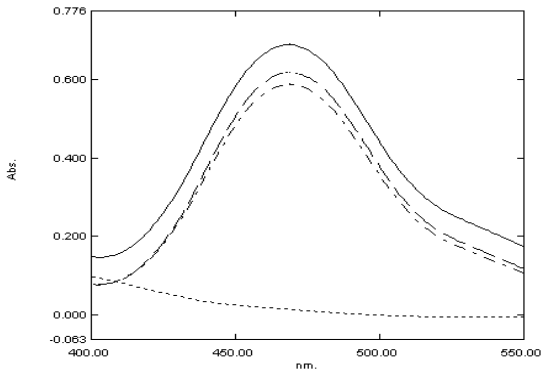


Fig 5:Absorbtion spectra of Mo(V)-thiocyanate complexes with a) Bisoprolol hemifumarate (20µg ml^{-1}), b)Propafenone HCl(20µg ml^{-1}) and c) Naftidrofuryl oxalate(20µg ml^{-1}).

The optimum conditions for the reaction were established:

Effect of acidity

Ternary complex formation was only established in acidic media, so different acids were tried like (hydrochloric, sulfuric, nitric and acetic acids).It was found that the complex was not formed in acetic

acid media, but formed only in hydrochloric, sulfuric and nitric acids.It was found that maximum absorbance readings were obtained on using 2-3 mL 5M HCl where pH of reaction media was 1.32- 1.65.

Effect of sodium molybdate concentration

It was found that maximum absorbance was obtained using 2 mL 2.5% sodium molybdate for propafenone HCl and naftidrofuryl oxalate, while bisoprolol hemifumarate needs 2 mL 2% sodium molybdate to complete the reaction.

Effect of ammonium thiocyanate concentration

4 mL 8% thiocyanate was sufficient to obtain maximum absorbance values for propafenone HCl, while 3mL and 2 mL 12% thiocyanate was required for naftidrofuryl oxalate and bisoprolol hemifumarate.

Effect of ascorbic acid concentration

2mL 10% ascorbic acid for propafenone HCl and naftidrofuryl oxalate, 0.5 mL 10% for bisoprolol hemifumarate were sufficient to complete the reaction.

Effect of reaction time

Mo (V) thiocyanate binary complex was completely formed after 10 minutes. Mo (V) thiocyanate drug ternary complex required 10 minutes for complete formation in case of bisoprolol hemifumarate and propafenone HCl, while 5 minutes was sufficient for naftidrofuryl oxalate.

Effect of organic solvent

Different solvents like chloroform, methylene chloride, ethylene chloride, benzene, ether and ethyl acetate) were tried. It was found that methylene chloride was the best solvent to be used for the three cited drugs.

Effect of time of shaking and times of extraction

It was found that maximum absorbance was obtained after double extraction with 10 mL methylene chloride (5 mL for each one) and shaking for 30 second each time.

The formed complex was stable for more than 24 h at 25ºC in the organic solvent.

Effect of aqueous to organic phase ratio

Varying the ratio from (0.8:1) to (2:1) showed that the increasing the aqueous phase decreasing the absorbance readings, so no dilution of aqueous phase was required.

Stoichiometry of the reaction:

Applying Job's method of continuous variation45, the stoichiometry of the Mo(V)to each drug in the presence of excess amounts of ammonium thiocyanate was (1:1)for all cited drugs. Fig (6).

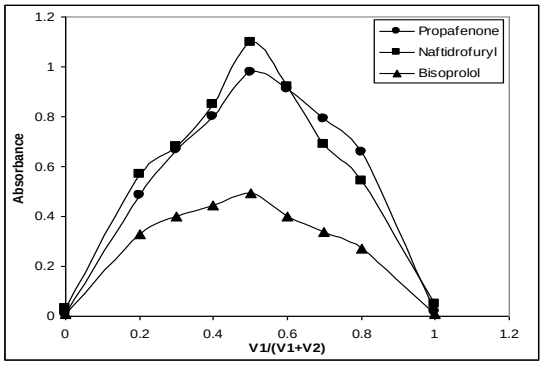
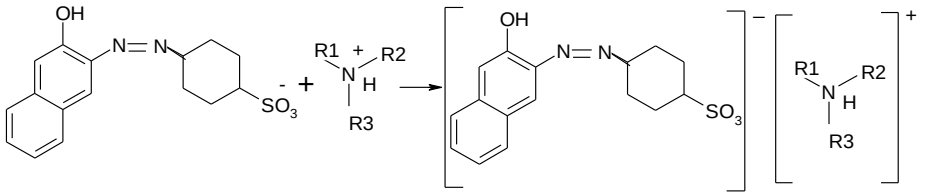


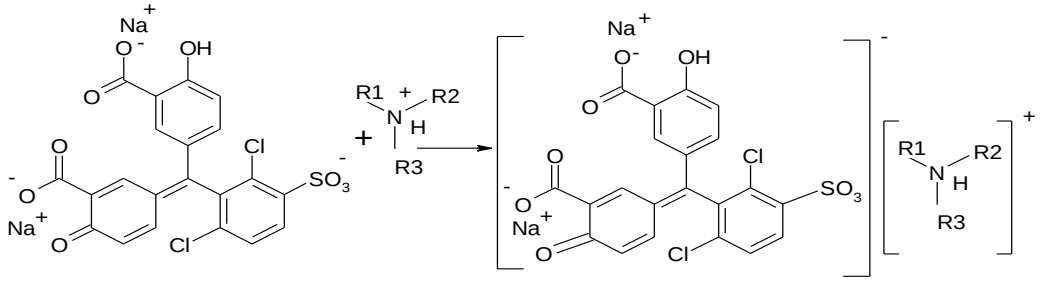
Fig 6. Determination of the stiochiometry of the reaction of 5×10^{-4}M Propafenone, Naftidrofuryl and bisobrolol with 5×10^{-4} M Sodium molybdate in the presence of excess ammonium thiocyanate.

Reaction

a- Proposed reaction mechanism of the cited drugs with tropeolin OOO

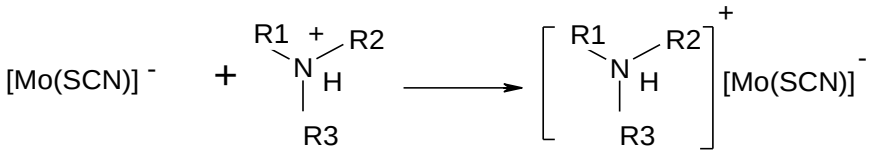


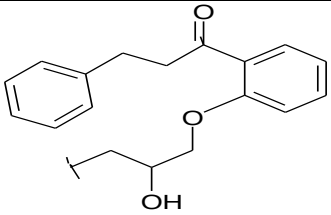
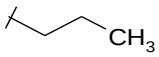
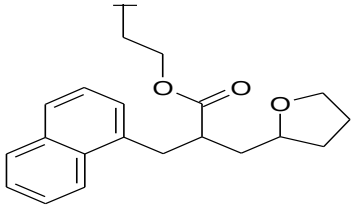
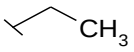
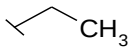
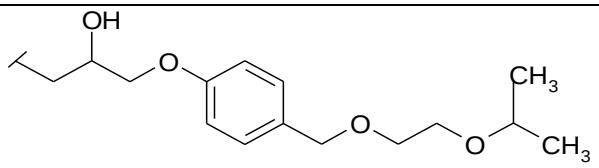
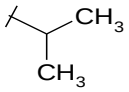
b- Proposed reaction mechanism of the cited drugs with chromazurol S



c- Proposed reaction mechanism of the cited drugs with Mo(V)-thiocyanate

$$Mo(VI) \xrightarrow[5M\ HCl]{\text{ascorbic acid}} Mo(V) \xrightarrow{6(SCN)^-} [Mo(SCN)]^-$$



Drug name	R1	R2	R3
Propafenone HCl			H-
Naftidrofuryl oxalate			
Bisoprolol hemifumarate			-H

Method validation

Under the described experimental conditions standard calibration curves with good linearity for propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate with tropeolin OOO, chromazurolS and Mo(V)-thiocyanate were constructed by plotting absorbance against concentration.The standard deviation , molar absorbtivity, correlation coeffiecient, intercept and slope for the calibration curve were calculated in tables(3,4)

Table3: Spectral data for Determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate using tropeolin OOO and chromazurol S.

	Tropeolin OOO method			**Chromazurol S method**		
Parameters	**Propafenone HCl**	**Naftidrofuryl oxalate**	**Bisoprolol hemifumarate**	**Propafenone HCl**	**Naftidrofuryl oxalate**	**Bisoprolol hemifumarate**
Beer's law range, µg/mL	6-28	6-32	6-28	28-100	28-68	10-64
Apparent molar absorpitivity, $mol^{-1}L\ cm^{-1}$	8.73×10^{3}	1.3×10^{4}	1.45×10^{4}	2.6×10^{3}	5.1×10^{3}	5.58×10^{3}
Sandell's sensitivity µg/ml per 0001A	2.58×10^{-3}	2.74×10^{-3}	3.79×10^{-3}	7.74×10^{-4}	1.09×10^{-3}	1.45×10^{-3}
Regression equation						
intercept(a)	-0.0375	-0.0439	-0.0537	-0.0277	-0.3694	-0.0327
Slope(b)	0.0286	0.0301	0.0417	0.0082	0.01923	0.0155
Correlation Coefficient®	0.9998	0.9998	0.9998	0.9998	0.9996	0.9998
Detection limit	1.45	1.38	0.99	5.1	5.46	2.7
Quantification limit	4.4	4.18	3.02	15.45	16.58	8.17
N°. of expeiments	9	8	8	7	9	7

Table4: Spectral data for determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate using Mo(V)- thiocyanate.

Parameters	**Propafenone HCl**	**Naftidrofuryl oxalate**	**Bisoprolol hemifumarate**
Beer's law range, µg/mL	4-32	6-32	4-32
Apparent molar absorpitivity, $mol^{-1}L\ cm^{-1}$	1.15×10^{4}	1.47×10^{4}	1.17×10^{4}
Sandell's sensitivity µg/ml per 0001A	3.39×10^{-3}	3.11×10^{-3}	3.06×10^{-3}
Regression equation			
intercept(a)	0.0292	-0.0112	0.0331
Slope(b)	0.032	0.0318	0.0282
Correlation Coefficient®	0.9999	0.9998	0.9998
Detection limit	1	1.11	1.13
Quantification limit	3.03	4.52	3.44
N°. of expeiments	8	8	7

Also relative standard deviation, analytical standard error were calculated,The limit of quantification (LOQ) was determined by establishing the least concentration that can be measured below which the calibration range is non linear. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analytes that can be readily detected .(Tables 5-7).

Fig 5: Determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate through complexation with Tropeolin OOO

Statistics	Propafenone HCl		Naftidrofuryl oxalate		Bisoprolol hemifumarate	
	Taken, µg/mL	Recovery	Taken, µg/mL	Recovery	Taken, µg/mL	Recovery
	6	100.52	6	99.06	6	100.99
	8	100.74	10	99.30	8	99.13
	10	99.83	12	99.36	12	101.46
	12	100.09	16	100.48	16	99.48
	16	101.29	20	100.65	18	100.41
	18	98.39	24	101.32	20	98.76
	20	99.74	28	98.83	24	100.39
	24	99.87	32	99.87	28	100.35
	28	100.59				
Mean*±SD		100.12±0.824		99.85±0.877		100.12±0.926
N		9		8		8
SD		0.824		0.877		0.926
RSD		0.823		0.879		0.925
V		0.679		0.77		0.858
SE		0.27		0.31		0.33

*** Mean of three different experiments**

Table 6: Determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate through complexation with chromazurolS.

Statistics	**Propafenone HCl**		**Naftidrofuryl oxalate**		**Bisoprolol hemifumarate**	
	Taken, µg/mL	**Recovery**	**Taken, µg/mL**	**Recovery**	**Taken ,µg/mL**	**Recovery**
	28	99.17	28	100.69	10	99.16
	40	100.82	32	100.46	20	99.26
	48	101.04	36	100.28	36	99.05
	60	100.34	40	100.00	40	100.60
	80	99.19	48	99.04	48	100.77
	88	99.60	52		56	
			56	98.62		99.85
	100	100.94	64	100.87	64	99.16
			68	100.20		
				100.35		
Mean*±SD		100.15±0.825		100.06±0.748		99.69±0.726
N		7		9		7
SD		0.825		0.748		0.726
RSD		0.824		0.747		0.728
V		0.681		0.56		0.526
SE		0.31		0.25		0.27

*** Mean of three different experiments**

Table 7: Determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate through complexation with Mo(V)-thiocyanate.

Statistics	**Propafenone HCl**		**Naftidrofuryl oxalate**		**Bisoprolol hemifumarate**	
	Taken, µg/mL	**Recovery**	**Taken, µg/mL**	**Recovery**	**Taken, µg/mL**	**Recovery**
	4	100.63	6	100.73	4	99.20
	8	101.09	8	100.71	8	98.80
	12	99.17	12	99.90	12	100.74
	16	100.35	16	98.70	16	101.26
	20	99.19	20	100.50	20	98.92
	24	99.32	24	99.21	24	99.87
	28	99.98	26	100.53	32	99.94
	32	100.27	32	100.06		
Mean*±SD		100.00±0.717		100.04±0.744		99.82±0.929
N		8		8		7
SD		0.717		0.744		0.929
RSD		0.717		0.744		0.93
V		0.514		0.554		0.862
SE		0.25		0.26		0.35

*** Mean of three different experiments**

. The validity of proposed methods was assessed by its application to the determination of the cited drugs in their pharmaceutical preparations, Tables(8-10) .To prove the accuracy of the proposed method, the results of the assay of the studied drugs in pharmaceutical preparations were compared with the official methods for propafenone HCl, naftidrofuryl oxalate 1,16 and reference method for bisoprolol hemifumarate 27, the statistical analysis of the results using student's t-test and variance ratio F-test showed no significant differences between them regarding accuracy and precision, table(11).

Table 8: Application of standard addition technique for determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate in their pharmaceutical formulations using tropeolin OOO.

Rytmonorm tablets			Praxilene tablets			Cerepromap capsules			Concor tablets		
Taken	Added	Recovery* %	Taken	Added	Recovery* %	Taken	Added	Recovery* %	Taken	Added	Recovery* %
g ml^{-1}			g ml^{-1}			g ml^{-1}			g ml^{-1}		
8	--	102.05	12		98.81	6		100.72	8		98.23
	6	99.36		6	99.61		6	100.17		6	99.80
	8	100.31		10	98.31		12	101.30		8	100.63
	12	98.63		12	99.92		16	99.44		10	101.61
	14	98.77		16	99.23		20	99.82		12	101.06
	16	101.29		20	101.31		24	101.73		14	101.69
	18	98.97					26	101.44		16	99.18
Mean ±S.D.		99.56 ±1.040		99.61±0.977			100.65 ± 0.961			100.66 ± 1.007	
N		6		5			6			6	
S.D		1.040		0.977			0.961			1.007	
V.		1.083		0.954			0.923			1.014	
S.E.		0.425		0.437			0.392			0.411	

*** Mean of three different experiments**

Table 9: Application of standard addition technique for determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate in their pharmaceutical formulations using chromazurol S.

Rytmonorm tablets			Praxilene tablets			Cerepromap capsules			Concor tablets		
Taken	Added	Recovery* %	Taken	Added	Recovery* %	Taken	Added	Recovery* %	Taken	Added	Recovery* %
g ml^{-1}			g ml^{-1}			g ml^{-1}			g ml^{-1}		
28	--	101.79	28		99.91	28		100.65	20		98.94
	28	100.50		28	100.18		28	100.36		10	99.81
	40	98.69		30	102.51		30	102.17		20	101.19
	48	98.25		32	100.66		32	100.82		24	101.26
	60	100.55		36	100.31			100.79		36	101.56
	68	98.40		40	99.77			99.08		40	99.47
	80	99.34						99.00			
Mean ±S.D.		99.29 ±1.023		100.69±1.068			101.12 ± 0.940			100.66 ± 0.950	
N		6		5			3			5	
S.D		1.023		1.068			0.940			0.950	
V.		1.047		1.141			0.884			0.902	
S.E.		0.418		0.478			0.543			0.425	

*** Mean of three different experiments**

Table 10: Application of standard addition technique for determination of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate in their pharmaceutical formulations through ternary complex formation with molybdenum(V)-thiocyanate

Rytmonorm tablets			Praxilene tablets			Cerepromap capsules			Concor tablets		
Taken	Added	Recovery* %	Taken	Added	Recovery* %	Taken	Added	Recovery* %	Taken	Added	Recovery* %
g ml^{-1}			g ml^{-1}			g ml^{-1}			g ml^{-1}		
4	--	101.48	6		99.69	6		100.21	4		100.09
	4	99.84		6	101.26		6	100.21		8	99.25
	8	100.31		8	99.92		8	100.71		12	100.74
	12	99.69		12	100.16		12	99.90		16	101.04
	16	101.52		20	101.13		16	100.67		20	98.56
	20	100.28		24	99.21		20	101.13		24	99.42
	24	101.80		26	101.5		26	98.72		28	101.05
Mean* ±S.D.		100.57 ±0.880		100.53 ± 0.903			100.22 ± 0.852			100.01 ± 1.067	
N		6		6			6			6	
V		0.774		0.815			0.726			1.140	
S.D.		0.880		0.903			0.852			1.067	
S.E.		0.36		0.37			0.35			0.436	

*** Mean of three different experiments**

Table 11: Statistical analysis of the results obtained by the proposed methods and the official or reference methods of propafenone HCl, naftidrofuryl oxalate and bisoprolol hemifumarate.

Drug	Official methods	Tropeolin OOO	Chromazurol S	Mo(V)-Thiocyanate
Propafenone HCl				
Mean±SD	100.29± 0.63	100.12±0.824	100.15±0.825	100.00±0.717
varience	0.395	0.679	0.681	0.514
N	3	9	7	8
t-value		0.323 (2.228)	0.259 (2.306)	0.613 (2.262)
F-value		1.719 (4.46)	1.72 (5.14)	1.301 (4.74)
Naftidrofuryl oxalate				
Mean±SD	100.05±0.42	99.85±0.877	100.06 ±0.748	100.04±0.744
varience	0.175	0.77	0.559	0.554
N	3	8	9	8
t-value		0.37 (2.262)	0.022 (2.228)	0.022 (2.262)
F-value		4.4 (4.74)	3.194 (4.46)	3.166 (4.74)
Bisoprolol hemifumarate				
Mean±SD	100.24± 0.782	100.12± 0.926	99.69± 0.726	99.82± 0.929
variance	0.612	0.858	0.526	0.862
N	6	8	7	7
t-value		0.256 (2.179)	1.315 (2.201)	0.872 (2.201)
F-value		1.402 (3.97)	1.163(4.39)	1.408(4.39)

***Theoretical values for *t* and *F* at p = 0.05**

Intraday and interday precisions were assessed using six determinations of three different concentrations of the three 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 were calculated.

The results of accuracy and precision,Table (12) show that the proposed methods have good repeatability and reproducibility.

Table 12:The intra-day and inter-day accuracy and precision data for Propafenone, Naftidrofuryl and Bisoprolol obtained by Tropeolin ooo, Chromazurol S and Mo-thiocyanate methods.

		Propafenone HCl					Naftidrofuryl oxalate					Bisoprolol hemifumarate				
		Taken µg/mL	Found µg/mL	Recovery %	RSD %	Er %	Taken µg/mL	Found µg/mL	Recovery %	RSD %	Er %	Taken µg/mL	Found µg/mL	Recovery %	RSD %	Er %
Tropeolin ooo	Intraday	6	6.01	100.23	0.610	0.23	6	5.97	99.42	0.975	-0.57	6	6.05	100.87	0.738	0.87
		8	8.089	101.11	0.882	1.11	10	9.96	99.58	0.713	-0.42	8	7.93	99.13	0.690	-0.87
		10	9.95	99.48	0.994	-0.52	12	11.97	99.78	0.627	-0.22	12	12.17	101.43	0.473	1.43
		12	11.96	99.65	0.606	-0.35	16	16.08	100.48	0.292	0.48	16	15.91	99.45	0.322	-0.55
	Interday	6	6.01	100.14	0.876	0.14	6	5.95	99.24	0.762	-0.76	6	6.05	100.80	0.963	0.80
		8	8.05	100.60	0.809	0.60	10	9.93	99.30	0.763	-0.70	8	7.93	99.08	0.945	-0.92
		10	9.95	99.53	0.844	-0.47	12	11.96	99.69	0.754	-0.32	12	12.16	101.29	0.743	1.29
		12	11.97	99.75	0.725	-0.25	16	16.01	100.62	0.446	0.62	16	15.93	99.55	0.528	-0.45
	Intraday	28	27.74	99.03	0.454	-0.97	28	28.22	100.80	0.223	0.80	10	9.94	99.38	0.977	-0.62
		40	40.33	100.82	0.506	0.82	32	31.80	99.37	0.423	-0.62	20	19.85	99.26	0.741	-0.74
		48	48.40	100.83	0.665	0.83	36	36.41	101.15	0.155	1.15	36	35.70	99.17	0.438	-0.83

		60	60.41	100.68	0.330	0.68	40	39.80	99.50	0.142	-0.49	40	40.25	100.62	0.371	0.62
Chromazurol S	Interday	28	27.74	99.10	0.757	-0.90	28	28.18	100.64	0.355	0.64	10	9.93	99.27	0.957	-0.73
		40	40.39	100.98	0.626	0.98	32	31.79	99.34	0.553	-0.66	20	19.86	99.31	0.951	-0.69
		48	48.50	101.04	0.656	1.04	36	36.43	101.20	0.315	1.20	36	35.67	99.08	0.599	-0.92
		60	60.21	100.35	0.641	0.34	40	39.83	99.57	0.226	-0.43	40	40.27	100.68	0.462	0.68
	Intraday	4	4.03	100.63	1.098	0.63	6	6.04	100.73	0.736	0.73	4	3.98	99.50	0.920	-0.50
		8	8.08	100.96	0.836	0.96	8	8.04	100.51	0.950	0.51	8	7.90	98.80	0.941	-1.20
		12	11.91	99.25	0.824	-0.75	12	12.00	100.03	0.857	0.03	12	12.83	100.69	0.818	0.69
		16	16.07	100.42	0.659	0.42	16	15.82	98.87	0.425	-1.13	16	16.20	101.23	0.322	1.23
Mo-thiocyanate	Interday	4	4.04	100.89	1.058	0.89	6	6.04	100.65	0.767	0.65	4	3.97	99.20	0.799	-0.80
		8	8.09	101.09	1.008	1.09	8	8.08	100.84	1.006	0.84	8	7.91	98.88	0.718	-1.13
		12	11.92	99.34	0.872	-0.66	12	11.98	99.85	0.869	-0.15	12	12.08	100.64	0.661	0.64
		16	16.05	100.29	0.636	0.29	16	15.80	98.77	0.411	-1.23	16	16.22	101.37	0.453	1.37

The robustness of the procedure adopted in the proposed methods is demonstrated by the constancy of the absorption intensity with minor changes in the experimental parameters such as the change of the pH of

buffer(buffer of pH 2.7 ± 0.2,buffer of pH 3.7 ±0.1 and buffer of pH 1.1±0.1mL) and volume of reagent(reagent volume± 0.2). These minor changes that may take place during the experimental operation did not affect the absorption intensity indicating the excellent robustness of the proposed methods.

Elucidation of the structure of bisoprolol- Mo(V)-thiocyanate ternary complex by means of IR:

The reaction product was isolated and subjected to structural elucidation by means of infra red (IR). Bisoprolol gave principal peaks at 3422.99 of -OH, 3045.05 of –NH and 1571.1 for carboxylate salt(C=O). When the complex was isolated, it gave peaks at 3426.89 of –OH and 2921.63 of ammonium ion, in addition to the appearance of a peak at 2031.64 due to the presence of –CN group in the products and disappearance of carboxylate salt beak. (figs. 7-9).

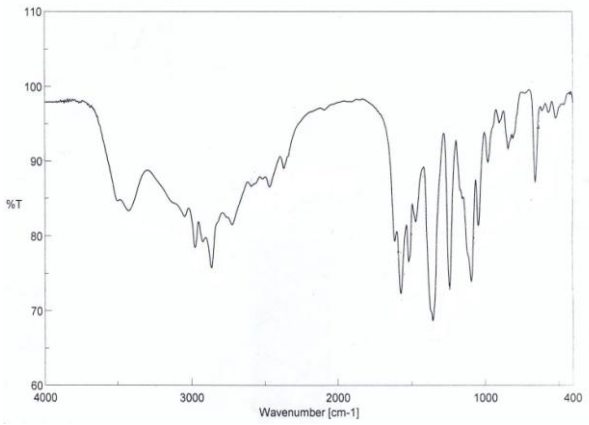


Fig 7:IR charts of bisoprolol hemifumarate.

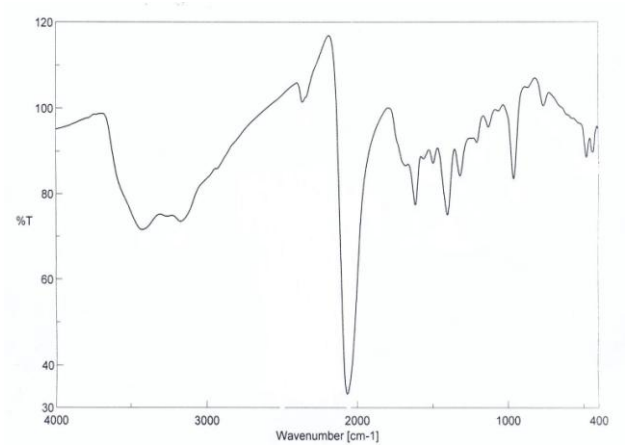


Fig 8.:IR charts of molybdenum thiocyanate binary complex.

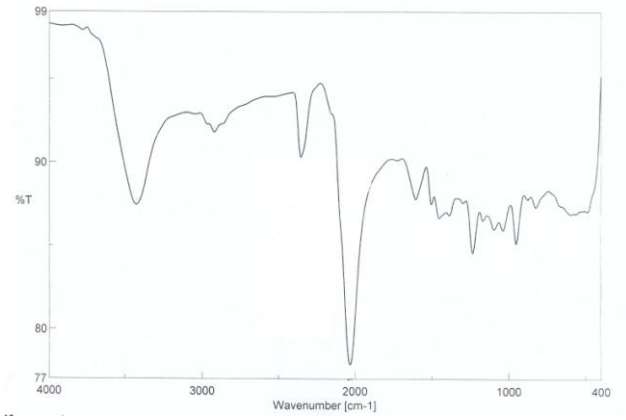


Fig 9: IR charts of bisoprolol- Mo-thiocyanate ternary complex.

CONCLUSION

In conclusion the proposed methods have the advantages of high sensitivity, rapidity and simplicity over the official methods as they require about (6µg mL -1) only of drug. Also they are precise and accurate methods for determination of the investigated drugs either in pure form or in pharmaceutical preparations with low costs due to availability of chemicals and equipments. In addition to the satisfactory sensitivity and reproducibility as well as the convenience and simplicity.

So the suggested methods can be used for routine analysis of these cardiovascular drugs because of the above mentioned advantages.

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