

## Spectrophotometric Methods for the Determination of Alfuzosin-HCl and Carvedilol in their Formulations

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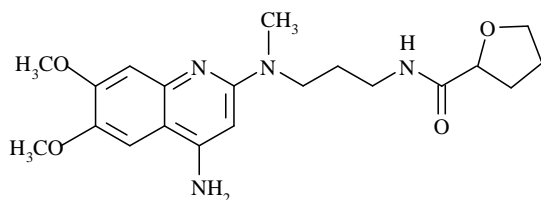
**Summary:** Simple and sensitive visible spectrophotometric methods were developed for the analysis of alfuzosin-HCl and carvedilol in bulk powder and in pharmaceutical formulations. Alfuzosin-HCl was determined by three methods, the first one was based on its reaction with 1,2-naphthoquinone-4-sulphonate in alkaline medium at room temp to give a yellow product with  $\lambda_{\max}$  352 nm. The second method involved bromination of alfuzosin-HCl with N-bromosuccinimide in aqueous solution at room temperature to give a soluble yellow product measured at 348 nm. The third one included the oxidative coupling of the drug with 3-methylbenzothiazolinone hydrazone in acid medium at 70°C in presence of  $\text{Ce}^{+4}$  to yield a coloured product with  $\lambda_{\max}$  448 nm. Carvedilol was determined through its charge transfer complex with dichlorodicyanobenzoquinone at  $\lambda_{\max}$  460 nm in methanol-acetonitrile medium. The optimum reaction conditions were evaluated and reaction mechanisms were suggested. Good linearities were achieved in the range of 10-80, 20-180, 2.5-95  $\mu\text{g/ml}$  alfuzosin-HCl and 40-280  $\mu\text{g/ml}$  carvedilol with mean recoveries of 100.0 $\pm$ 1.52, 100.2 $\pm$ 1.04, 99.7 $\pm$ 1.51 and 100.0 $\pm$ 1.43%, respectively. The proposed methods were evaluated according to the ICH and successfully applied to analyse both drugs in their tablets and the results obtained were in accordance with those obtained from reported methods.

### Introduction

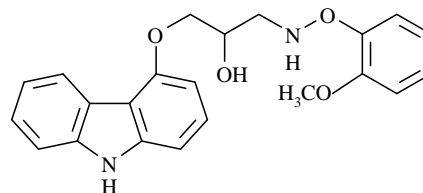
Alfuzosin HCl, N-[3-[(4-amino-6,7-dimethoxy-quinazolin-2-yl)-methyl-amino] propyl] oxolane-2-carboxamide hydrochloride, is an alpha-1-adrenoreceptor blocker. Carvedilol; 1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethyl]amino]-2-propanol is a beta-blocker<sup>(1-3)</sup>. Both drugs are introduced as antihypertensive drugs<sup>(1-3)</sup>, however alfuzosin-HCl was subsequently used for treatment of benign prostatic hyperplasia<sup>(2,3)</sup>.

The two drugs and their formulations are officially determined in BP 2008 by non-aqueous titration with  $\text{HClO}_4$ <sup>(4)</sup>. The reported methods for determination of alfuzosin-HCl were HPLC<sup>(5-9)</sup>, spectrophotometry<sup>(10-12)</sup> and voltammetry<sup>(13)</sup>. Carvedilol was determined by several techniques including HPLC<sup>(14-20)</sup>, electrophoresis<sup>(21-23)</sup>, TLC<sup>(24)</sup>,

GC(25), spectroscopy(26-32), voltammetry(33) and chemiluminescence(34). The aim of this study was to develop simple valid spectrophotometric methods to determine the two drugs in their formulations.



Alfuzosin



Carvedilol

## Experimental

### Apparatus

Unicam UV-Vis spectrophotometer, Helios alpha- Helios beta model with 1 cm cuvette (Biochrom, England).

### Materials and reagents

All chemicals and solvents used were of spectroscopic grade and distilled water was used throughout this work. Pure alfuzosin HCl and carvedilol were kindly supplied from Saudi Pharmaceutical Industries and Medical Appliances Cooperation (Al-Qassim, Saudi Arabia) at purity 99.9% and 99.7%, respectively. Xatral SR tablets, 5 mg alfuzosin HCl per tablet, BN 23508 (Synthelabo group, France) and Dilatrend tablets, 25 mg Carvedilol per tablet, BN M1001 (Roche S.P.A, Italy under license of Hoffmann-La Roche, Switzerland) were purchased from the local market in Saudi Arabia. Methanol (BDH, England), and acetonitrile (99.9% , BDH-England) were used. Solutions of 0.24% Methylbenzothiazolin 2-one hydrazone (MBTH) in water, 1 M H<sub>2</sub>SO<sub>4</sub> (BDH, England) and 0.63% ceric ammonium sulphate (NH<sub>4</sub>)<sub>2</sub>Ce(SO<sub>4</sub>)<sub>3</sub>.2H<sub>2</sub>O (Fluka, UK) in 1M H<sub>2</sub>SO<sub>4</sub> were prepared. Also 0.05% 1,2-Naphthoquinone-4-sulphonate sodium salt, NQS (BDH, England), 0.7% N-bromosuccinimide, NBS (Riedel, Germany) in water and 0.2% 2,3-Dichloro-5,6-dicyano-p-benzoquinone, DDQ (Fluka, UK) in methanol were freshly prepared

and used within 2, 6 and 4 h, respectively. NaOH (BDH, England), 0.1 M solution in water was used.

### **Standard solutions**

Alfuzosin HCl solution in water (0.5 mg/ml) and Carvedilol methanolic solution (1 mg/ml) were prepared and used in the four methods.

### **General procedures**

#### **Determination of alfuzosin-HCl**

##### **NQS method**

Into a series of 10-ml volumetric flasks, aliquots (0.4-3.6 ml) of alfuzosin HCl standard solution (0.5 mg/ml) were introduced. 0.5 ml of 0.1 M NaOH and of NQS solutions were added then each mixture was diluted to volume with water. The absorption was measured at 352 nm against a reagent blank and plotted against the corresponding concentration in  $\mu\text{g/ml}$  to obtain the calibration curve.

##### **NBS method**

0.05-1.9 ml of standard alfuzosin HCl solution in water were transferred into a series of 10-ml volumetric flasks, each is mixed with 1ml of NBS solution. The flasks were completed to the mark with water and the absorption of the yellow product was measured at 348 nm against a reagent blank. A calibration curve was obtained by relating absorbance at the relevant maximum against drug concentration in  $\mu\text{g/ml}$ .

##### **MBTH method**

Aliquots (0.2-0.6 ml) of standard alfuzosin HCl solution were transferred into a series of 20 ml test tubes, 1.6 ml of  $\text{Ce}^{+4}$  solution (in 1 M  $\text{H}_2\text{SO}_4$ ) was added to each tube followed by 2.5 ml of MBTH solution. The tubes were heated at  $70 \pm 5^\circ\text{C}$  for 50 min, then cooled to room temperature and their constituents transferred quantitatively to a series of 10-ml volumetric flasks. The volume was completed with water and absorbance was measured at 448 nm against a reagent blank similarly prepared. The calibration curve was plotted by relating the absorbance against drug concentration in  $\mu\text{g/ml}$ .

#### **Determination of carvedilol**

Accurately measured volumes (0.4-2.8 ml) of carvedilol standard solution in methanol (1gm/ml) were transferred into a series of 10-ml volumetric flasks. 2 ml

of 0.2% DDQ solution in methanol were added and the volume was completed to 5 ml with the same solvent. Then each solution was adjusted to 10 ml with acetonitrile. The absorbance of the reddish orange product was measured at 460 nm and related to the concentration to obtain the calibration curve.

### **Application to pharmaceutical formulations**

Ten Xatral tablets were finely ground and mixed. An amount of the fine powder equivalent to 25mg alfuzosin HCl was weighed and extracted with 40 ml water by sonication for 30 min. The aqueous suspension was filtered into 50-ml volumetric flask and completed to volume with water. The obtained solution labeled to contain 0.5 mg/ml alfuzosin HCl was analysed by the three proposed methods as detailed above.

Similarly, Dilatrend tablets were extracted with methanol using a weight of the fine powder equivalent to 25 mg carvedilol and 25 ml solvent to prepare alcoholic solution claimed to contain 1 mg/ml carvedilol to be analysed through complexation with DDQ as described above.

### **Results and discussion**

The problem of overcoming irrelevant absorbances of excipients, flavours and additives in pharmaceutical preparations will remain an everlasting problem for chemists. In this work, alfuzosin HCl and carvedilol were tried to be analysed in their tablet formulations by rapid sensitive and accurate spectrophotometric method selectively through their reaction with different chromogenic reagents. Alfuzosin-HCl in water exhibited three peaks at 210, 244 and 332 nm, whereas carvedilol in methanol shows four peaks at 224, 243, 284, 332 nm, Fig. 1.

### **Determination of alfuzosin HCl using NQS**

Alfuzosin HCl was found to react with naphthoquinone-4-sulphonate sodium salt (NQS) in aqueous alkaline medium to yield a yellowish-orange coloured product with  $\lambda_{\max}$  352 nm, Fig 2. The molar ratio method was applied and found to be 1:1 drug-reagent. As previously reported<sup>(35,36)</sup> alfuzosin was suggested to react with NQS in alkaline medium by nucleophilic displacement of its sulfonic group by the drug anion after the removal of acidic hydrogen of its amide group.

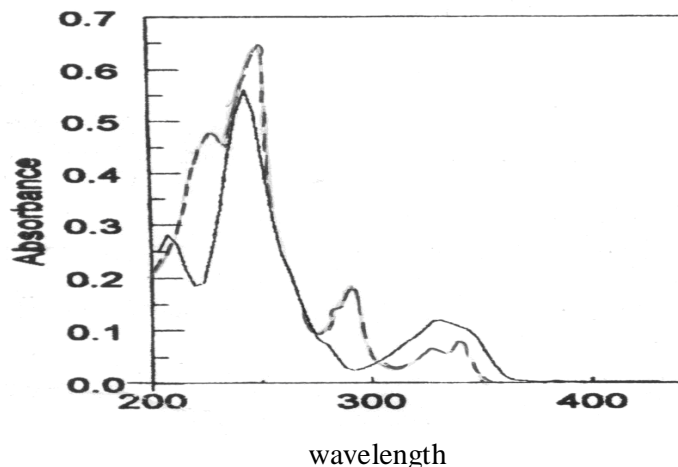


Fig:1 UV absorption spectra of alfuzosin-HCl, 5 µg/ml in water (—) and carvedilol, 5 µg/ml in methanol (---).

#### Determination of alfuzosin HCl using NBS

N-Bromosuccinimide (NBS) was extensively used to determine organic and pharmaceutical compounds through its reactive cationic bromine by different mechanisms<sup>(37)</sup>. Studying the structural formula of alfuzosin HCl, it was found to be promising for electrophilic substitution on the dimethoxyquinazolinyl moiety. This was devoted to analyse the drug by its reaction with NBS to yield a soluble yellow dibrominated product that was picked at 348 nm (Fig. 2.), as proved by applying the molar ratio method.

#### Determination of alfuzosin HCl using MBTH

Methylbenzothiazolinone (MBTH) plays a prominent role in the spectrophotometric analysis of numerous pharmaceuticals. Upon oxidation with a suitable oxidant, it loses 2 electrons producing an intermediate electrophile which is the coupling species<sup>(38-40)</sup>. This was applied to the spectrophotometric determination of alfuzosin HCl using MBTH in acid medium in presence of  $Ce^{+4}$  to give a red coloured product absorbing maximally at 448 nm (Fig. 3.) and proposed to have the structure indicated in scheme (1).

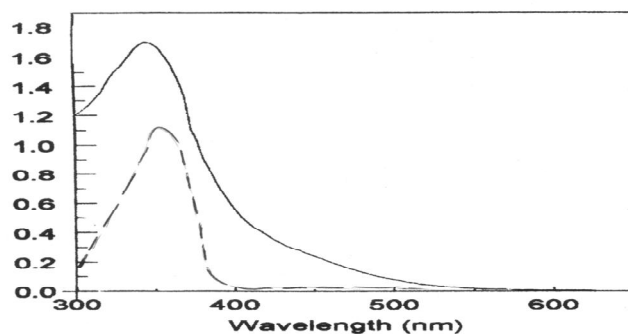


Fig 2. UV-Vis spectra of the reaction product of: 100 µg/ml alfuzosin HCl with NQS in aqueous alkaline medium (---) 60 µg/ml alfuzosin HCl with NBS in water (—).

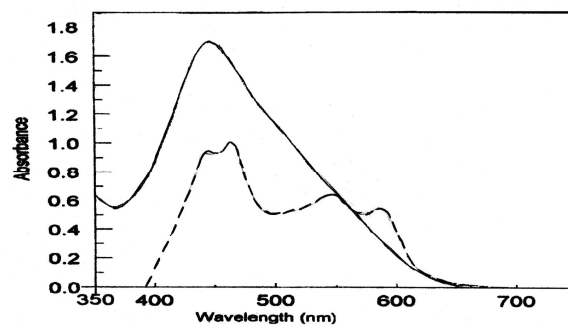
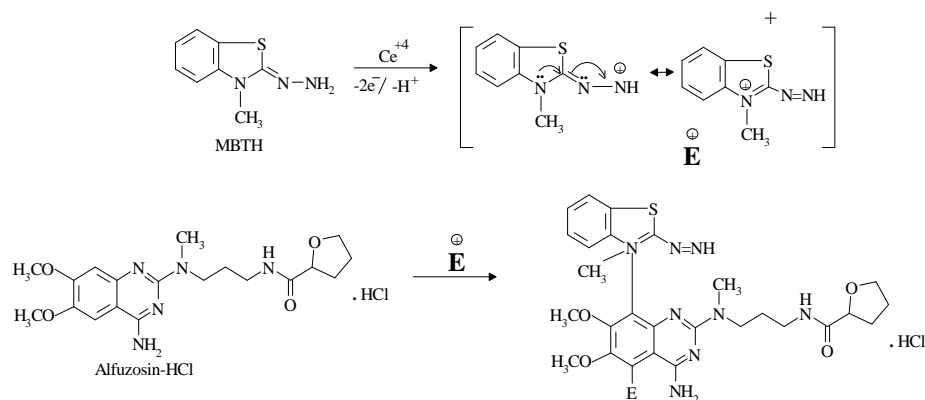


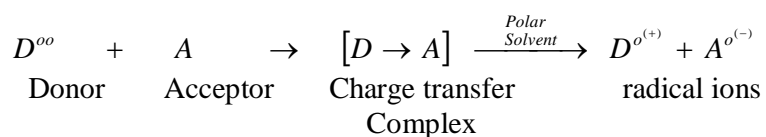
Fig.3. Absorption spectra of the reaction product of : alfuzosin-HCl (70ml/µg) with MBTH/Ce<sup>4+</sup> system in aqueous acid medium (—) and carvedilol (140ml/µg) with DDQ in mixture methanol-acetonitrile 1:1 (---).



Scheme (1): Proposed Mechanism of the reaction between Alfuzosin-HCl and MBTH/Ce<sup>4+</sup> in acid medium.

### Carvedilol complexation with DDQ

$\pi$ - Acceptors react with basic nitrogenous compounds as n-donors to form charge transfer complexes or radical anions according to the solvent used.<sup>(41,42)</sup> Carvedilol contain 2 secondary amino groups, the more basic aliphatic one represents the electron donor group that was supposed to react with 2,3- Dichloro- 5,6 dicyano- p-benzoquinone (DDQ) in 1:1 methanol –acetonitrile to give an orange–red colour having three absorption maxima at about 460, 540 and 580 nm (Fig. 3), the first at 460 nm was chosen for the quantitation of the drug as it gave the most reproducible results. The formed colour may be attributed to the formation of DDQ radical anion as presented in the following equation<sup>(42)</sup> :



### Experimental parameters

Different experimental parameters affecting the reaction using a reaction mixture of 10 ml in each of the four proposed methods were studied with respect to the reagents concentration, acidity or alkalinity of the medium, temperature, reaction time and stability of colours and the optimum condition were indicated in Table (1). In addition, the reaction mixture of alfuzosin HCl with each of NQS, NBS and MBTH reagents was diluted with MeOH, EtOH, acetonitrile or water and highest sensitivity was obtained with water as diluents which adds to the benefits of the proposed methods. However, carvedilol was found to be insoluble in acetonitrile, thus its solution in methanol (200  $\mu\text{g/ml}$ ) was allowed to react with DDQ in methanol and the reaction mixture was diluted with different solvents namely methanol, ethanol, acetonitrile, acetone and dimethylsulfoxide. The later gave nil absorbance, while acetonitrile gave the highest sensitivity at 460nm. So acetonitrile was used to achieve a solvent mixture of 1:1 methanol acetonitrile.

Table (1): The optimum experimental parameters for the determination of alfuzosin – HCl and carvedilol by the proposed methods

Experimental parameter	Alluzosin HCl			Carvedilol DDQ
	NQS	NBS	MBTH	
1.Reagent conc.	0.5- 2ml of 0.05% NQS solution in	1.0-1.3 ml of 0.7% freshly prepared NBS solution	2.5 ml of 0.24% MBTH and 1.6 ml of 0.63% of $(\text{NH}_4)_2\text{Ce}(\text{SO}_4)_3$ in $1\text{MH}_2\text{SO}_4$	2.0-2.5 ml of 0.2% DDQ in methanol
2. Acidity or alkalinity of the medium	0.5-0.6 ml of 0.1M NaOH	No effect	0.8-1.0M $\text{H}_2\text{SO}_4$ - as solvent for $\text{Ce}^{4+}$	--
3. Temperature	Room temp. as no effect for heating at 40-60°C	Room temp. as at 50-100°C lower A was obtained	$70 \pm 2^\circ\text{C}$	Room temp.
Reaction time	Spontaneous	Spontaneous	50-60 min	30 min
4. Sequence of reactant additions	NaOH to the drug, then NQS	No Effect	$\text{Ce}^{4+}$ added to drug solution then MBTH	No effect
5. Stability of colour	60 min	20 min	60 min	30 min

### Validation of methods

Under the above optimum conditions prescribed, the four methods were validated according to the ICH and USP 2005 guide lines<sup>(43)</sup>.

### Linearity

Beer's law was obeyed in the ranges of 20-180, 2.5-95 and 10-80  $\mu\text{g/ml}$  alfuzosin HCl by the three reagents; NQS, NBS and MBTH, respectively, and in the range of 40-280  $\mu\text{g/ml}$  carvedilol by charge–transfer complexation with DDQ. The regression parameters, LOD and LOQ are illustrated in Table (2).



### Accuracy and precision

Good accuracies were obtained for the four procedures which ranged between 99.7 and 100.2%, Table (2). The precision was assured by triplicate analysis of three different concentrations within the linearity range in the same day and on three consecutive days. The intraday RSD ranged between 0.41 and 0.55%, while the interday RSD were 0.21– 1.55%, Table (2).

Table ( 2): Characteristic parameters of the regression equations and assay validation results of the proposed spectrophotometric methods for alfuzosin-HCl and carvedilol.

Parameters	Alfuzosin-HCl			carvedilol
	NQS	NBS	MBTH	DDQ
$\lambda$ max nm	352	348	448	460
Linearity (ppm)	20-180	2.5-95	10-80	40-280
Regression				
Slope	0.011	0.0289	0.0254	0.0085
Intercept	0.02	0.03	-0.11	-0.24
Correlation coff	0.9996	0.9998	0.9999	0.9998
LOD ( $\mu$ g/ml)	4.25	1.25	2.5	10
LOQ ( $\mu$ g/ml)	15.0	2.5	7.0	35
Accuracy (mean $\pm$ SD%)	100.2 $\pm$ 1.04	99.7 $\pm$ 1.51	100.0 $\pm$ 1.52	100.0 $\pm$ 1.43
Precision (RSD%)				
Intraday	0.152	0.53	0.41	0.55
interday	0.21-0.72	0.83-1.38	0.66-1.35	0.22-1.55

### Selectivity

It was evaluated by the successful application of the four proposed methods to the analysis of alfuzosin-HCl and carvedilol in Xatral and Dilatrend tablets, respectively. The mean recoveries obtained were 99.4 $\pm$ 1.75, 99.8 $\pm$ 1.52 and 99.5 $\pm$  0.94% for alfuzosin HCl, using NQS, NBS and MBTH, respectively or 98.7 $\pm$ 1.32% for carvedilol using DDQ. These results when statistically compared with those obtained from the reported methods<sup>(1,30)</sup>, no significant difference was found between them with respect to precision and accuracy at 95%, Table (3).

Table (3): Determination of alfuzosin-HCl and carvedilol in their formulations by the proposed and reported methods<sup>(1,30)</sup>.

Xatral SR tablets (BN 23508) 5 mg				Dilatrend tablets (BN M1001) 25 mg	
NQS method	NBS method	MBTH method	Reported method	DDQ method	Reported method
99.4±1.75%	99.8±1.52%	99.5±1.94%	100.1±1.7%		
N= 8	N= 9	N= 7	N = 5		
t = 0.74	T = 0.35	t = 0.58			
(2.201)	(2.179)	(2.228)			
F = 1.05	F = 1.25	F= 1.30			
(4.12)	(3.84)	(4.53)			
				98.7±1.32	99.7±1.42
				N = 8	N = 8
				t = 1.5	
				(2.145)	
				F = 1.16	
				(3.79)	

Ref (1) determined alfuzosin HCl by measuring the absorbance of its aqueous solution at 245 nm.

Ref (30) determined Carvedilol by measuring the absorbance of its reaction product with chloranilic acid in methanol at 530 nm.

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