Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Spectrofluorometric determination of some β-blockers in tablets and human plasma using 9,10-dimethoxyanthracene-2-sodium sulfonate

H. Abdine, M. A. Sultan, M. M. Hefnawy, F. Belal

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Prof. Dr. F. Belal, Department of Pharmaceutical Chemistry, College of Pharmacy, P.O.Box 2457, King Saud University, Riyadh 11451, Saudi Arabia
ffbelal@yahoo.com

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A simple and sensitive spectrofluorometric method was developed for the quantitative determination of some β-blockers, namely arotinolol, atenolol and labetalol as hydrochloride salts. The method is based on the reaction of these drugs as n-electron donors with the fluorogenic reagent 9,10-dimethoxy-2-anthracene sulfonate (DMAS) as π-acceptor in acidic medium. The obtained ion-pairs were extracted into chloroform and measured spectrofluorometrically at 452 nm after excitation at 385 nm. The fluorescence intensity-concentration plots are rectilinear over the ranges of 0.5–5 μg · ml^{1}, 1.0–11.0 μg · ml^{1} and 0.6–6.4 μg · ml^{1} for labetalol, atenolol and arotinolol, respectively. The different parameters affecting the reaction pathway were thoroughly studied and optimized. No interference was observed from the common pharmaceutical excipients. The proposed method was successfully applied to the analysis of tablets and the results were statistically compared with those obtained by reference methods. The method was further extended to the *in vitro* determination of the drugs in spiked human plasma, the% recoveries (n = 3) ranged from 96.98 ± 1.55 to 98.28 ± 2.19. A proposal of the reaction pathway was postulated.

1. Introduction

Several analytical methods have been described for the quantitative determination of β-blockers. However, the development of a highly sensitive analytical methods for determining β-blockers in plasma samples is still needed, in particular since β-blockers which are misused as doping agents in sports, have been added to the list of the forbidden drugs by the International Olympic Committee (IOC) (Ceniceros et al. 1988). The United States Pharmacopoeia (USP 1995) and British Pharmacopoeia (BP 1998) described titrimetric, spectrophotometric and chromatographic methods for the assay of these drugs in raw materials and formulations. Other methods in common use for determining β-blockers involve the use of spectrophotometry (Hesham 2002; Amin et al. 2002; Belal et al. 2002), fluorimetry (Belal et al. 2002a; Murillo-Pulgarin et al. 1998) high performance liquid chromatography (Park et al. 2002; Ranta et al. 2002; Badaloni et al. 2003; Aboul-Enein and Hefnawy 2003) gas chromatography (Siren et al. 1993; Black et al. 1996), potentiometry (Hassan et al. 2003), polarography (Arranz et al. 1999) and capillary electrophoresis (Hefnawy 2003).

The method proposed here is based on the formation of a highly fluorescent ion-pair between the studied drugs through their amino group and 9,10-dimethoxyanthracene-2-sodium sulphonate (DMAS) through its counter ion.

DMAS was used for the determination and detection of some basic drugs either by direct fluorimetric measurement (Riley et al. 1989) or by HPLC through on or post column derivatization (Frenandez et al. 1996; Haas et al. 1997).

2. Investigations, results and discussion

All the drugs studied possess secondary or tertiary amino groups which in acidic medium can react with DMAS through its counter (sulphonate) ion with the formation of a highly fluorescent ion-pair product. These products show maximum fluorescence intensity at 452 nm upon excitation at 385 nm as shown in Fig. 1.

The factors affecting the ion-pair formation were studied and optimized. It was found that 2 ml of 0.04% aqueous solution of DMAS were sufficient to give the highest fluorescence intensity. Further increase in volume of DMAS showed higher background fluorescence. Also 1 ml of glacial acetic acid gave the optimum fluorescence

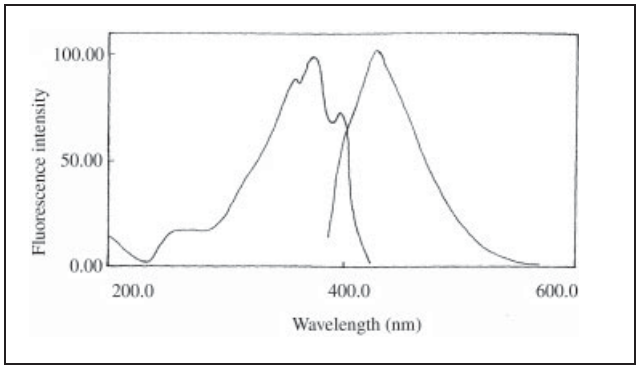


Fig. 1: Excitation and emission spectra of the ion-pair produced from labetalol HCl (5 μg/ml) with DAMS (0.04%) in chloroform

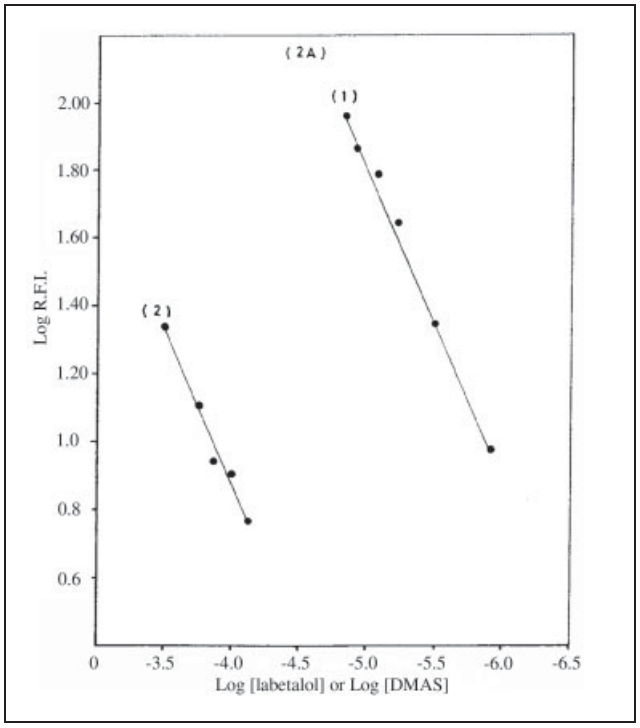


Fig. 2A: Limiting logarithmic plots for the molar reactivity of labetalol HCl (Fig. 2A) with DAMS: 1 log fluorescence vs log [labetalol HCl] with DAMS kept at 1.1810^3 M, 2: log fluorescence vs log [DAMS] with labetalol HCl kept at 4.810^3 M

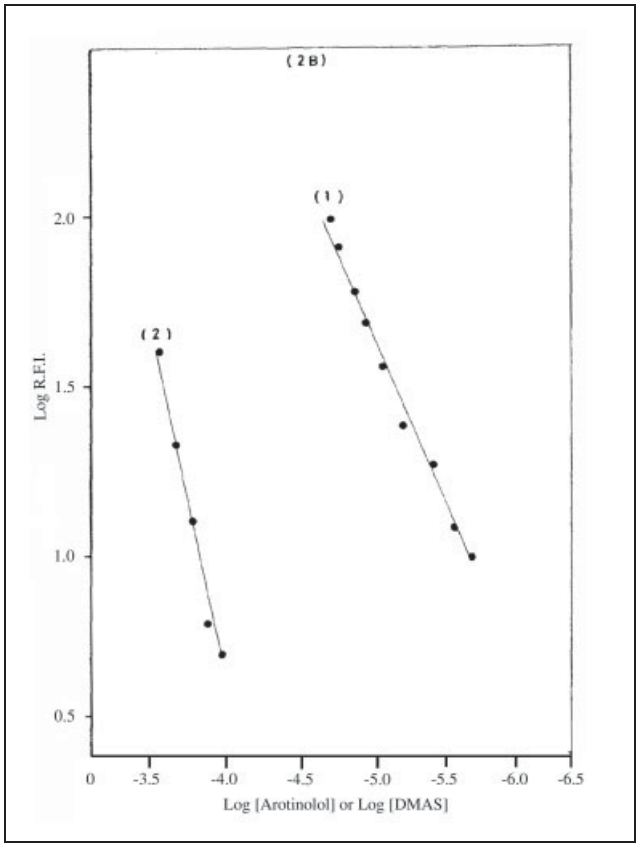
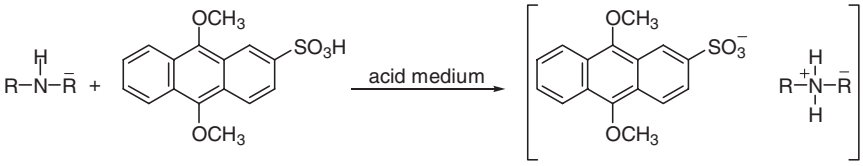


Fig. 2B: 1 log relative fluorescence vs log (arotinolol HCl) with DAMS kept at 1.18×10^{-3} M; 2: log fluorescence vs log (DAMS) with arotinolol HCl kept at 4.8×10^{-3} M

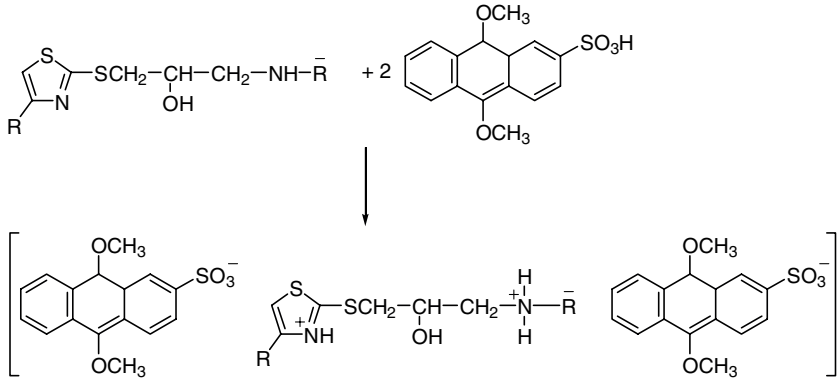
intensity. Chloroform was found to be the most proper solvent for the extraction of the ion-pair formed, showing the highest fluorescence intensity with the minimum background fluorescence. The ion-pair produced was found to be stable for at least 90 min at room temperature.

The stoichiometry of the reaction was studied adopting the limiting logarithmic method (Rose 1964) and it was found that the reaction proceeds in the ratio of 1 : 1 for labetalol and atenolol as revealed by the plot of log FI against log concentrations (Fig. 2A). The ratio of the slopes of the 2 straight lines is 0.989 to 0.875 i.e. the ratio is 1 : 1 (Scheme 1).

Scheme 1



Scheme 2



As for arotinolol, the ratio of the slopes of the two straight lines (Fig. 2B) is 2.097 to 0.9891, thus pointing out to a ratio of 2 : 1 (Scheme 2). Both labetolol and atenolol contain one secondary amino group and this explains the ratio of 1 : 1.

As for artinolol, it contains, in addition to the secondary amino group, basic nitrogen in the thiazole ring; therefore, the ratio is 2 : 1. Based on the above observations, the reaction pathway is suggested to proceed as shown in Scheme 2.

The method was tested for linearity, specificity, precision and reproducibility. With the above fluorometric method, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the relative fluorescence intensity on the concentrations of the studied drugs in the ranges listed in Table 1. Statistical evaluation of the experimental data regarding standard deviation of the residuals ($S_{y/x}$), standard deviation of the slope (S_b) and standard deviation of the intercept (S_a) were calculated (Table 1). The small values point out to high precision of the method (Miller and Miller 1983). The good linearity of the calibration graph and the negligible scatter of the experimental points are clearly evident by the correlation coefficients (close to 1 in all cases).

The validity of the method could be proved by analyzing authentic samples of the drugs. The results obtained are in good agreement with those given by the comparison methods (BP 1998 for atenolol, and Hefnawy 2003 for arotinolol).

The specificity of the method was investigated by observing that no interference was encountered from common tablet excipients. The simplicity of the method and the stability of the reaction product permitted the determination of the studied drugs in commercial tablets. The results obtained (Table 2) were statistically comparable with those given using the previously mentioned comparison methods (BP 1998 and Hefnawy 2003). Common tablet excipients, such as talc powder, lactose, avisil, maize starch, hydrogenated vegetable oil, lactose and gelatin did not interfere with the assay.

The high sensitivity attained by the proposed method allowed its extension to the *in vitro* determination of the studied drugs in spiked human plasma samples, the results are abridged in Table 3 and seem to be satisfactorily accurate and precise.

The major advantage of the proposed method over the reported chromatographic methods as applied to human plasma is that is does not require a prior extraction step. Thus, it is more simple and time saving.

Table 3: Application of the proposed method to the determination of the studied drugs in spiked human plasma

Compd.	Nominal content (μg/ml)	% Found (μg/ml)	% Recovery
Arotinolol	1.00	0.986	98.60
	2.00	1.90	95.00
	4.00	4.05	101.25
$\bar{X} \pm SD$		98.28	
		2.19	
Atenolol	1.50	1.47	98.00
	5.00	4.76	95.20
	8.00	7.82	97.75
$\bar{X} \pm SD$		96.98	
		1.55	
Labetalol	1.0	9.87	98.70
	2.0	1.95	97.50
	3.0	2.87	96.67
$\bar{X} \pm SD$		97.29	
		1.25	

Table 1: Analytical data for the determination of the β-blockers studied

Parameter	Arotinolol HCl	Atenolol	Labetalol HCl
Concentration range (μg ml^{1})	0.6–6.4	1.0–11.0	0.5–5.0
Regression equation: intercept (a)	0.188	−0.288	0.243
S_a	0.073	0.064	0.0110
Slope (b)	14.979	8.98	18.47
S_b	0.166	9.5 ± 10^3	2.66 ± 10^3
$S_{y/x}$	0.0610	0.0796	0.01
Correlation coefficient	0.9998	0.9999	0.9997
LOD (μg ml^{1-})	0.12 (3.22 ± 10^{-7} M)	0.20 (7.5 ± 10^{-7} M)	0.08 (1.22 ± 10^{-6} M)
LOQ (μg ml^{1-})	0.55 (1.47 ± 10^{-6} M)	0.88 (3.3 ± 10^{-6} M)	0.45 (2.16 ± 10^{-7} M)

S_a = Standard deviation of the intercept
S_b = Standard deviation of the slope
$S_{y/x}$ = Standard deviation of residuals

Table 2: Application of the proposed method to the analysis of the studied drugs in tablets

Sample and Manufacturer	Nominal content (mg/tablet)	% Recovery	
		Proposed method	Reference method
Arotinolol, laboratory made tablets	50	99.97 ± 0.67 t-value 2.13, F-value 1.64	99.16 ± 0.52
Atenolol tablets Hypoten[1]	50	100.66 ± 1.29 t-value 1.02, F-value 3.32	101.50 ± 0.71
Tenormin[2]	50	99.64 ± 1.26 t-value 1.25, F-value 2.56	100.5 ± 0.791
Labetalol tablets Trandate[3]	100	99.80 ± 2.1 t-value 1.80, F-value 2.47	98.4 ± 3.3

Each result is mean of five determinations ± SD
Theoretical t- and F- values at p 0.05 are 2.31 and 6.39, respectively
[1] Product of Hikma Pharmaceutical Co. Amman, Jordan. (Batch # 4678)
[2] Product of Kahira Pharmaceutical Co., Cairo, Egypt. (Batch # 0210578)
[3] Product of Glaxo Welcome, Middlesex, UK. (Batch # 303962)

3. Experimental

3.1. Apparatus

The fluorescence intensities were measured using a spectrofluorimeter (Jasco, model FP 6200; Japan), equipped with Xenon arc discharge lamp, excitation, emission grating monochromators and a 1 cm quartz cell, at low sensitivity. The apparatus was driven by a Pentium IV PC Computer.

3.2. Reagents and materials

A reference standard sample of atenolol was obtained from Sigma, St. Louis, MO, USA, while arotinolol hydrochloride was a gift from Sumitomo Pharmaceutical Co. (Osaka, Japan). Labetalol hydrochloride was obtained from Glaxo-Welcome, Middlesex, UK. Commercial tablets containing the studied drugs were obtained from the local market. Human plasma was obtained from King Khalid University Hospital, Riyadh, Saudi Arabia, and was kept frozen until use after gentle thawing. Acetic acid, glacial, extra pure, 99.5% (BDH, Poole, UK). 9,10-Dimethoxyanthracene-2-sulphonic acid, sodium salt (Fluka, Switzerland), 0.04% w/v aqueous solution was freshly prepared daily. Chloroform (Honil Limited, London, UK).

3.3. Procedures

3.3.1. Standard solutions

Stock solutions were prepared by dissolving 40 mg of each of the studied drugs in 100.0 ml of methanol and were further diluted with the same solvent as appropriate. The solutions were stable for at least 4 days if kept in the refrigerator.

3.3.2. Calibration graphs

Accurately measured aliquots from each stock solution equivalent to 4–44 μg/ml of atenolol, 2.5–25.0 μg/ml arotinolol HCl and 2–20 μg/ml of labetalol HCl were transferred into a series of 50-ml separating funnels. 1 ml of glacial acetic acid followed by 1 ml of 0.04% aqueous solution of DMAS were added and mixed well. The reaction mixture was shaken for about 5 min with three successive portions of chloroform (7, 5, 5 ml). The organic layers were collected on phase separator filter paper (1 PS) Whatmann type into 25 ml measuring flasks. The paper was washed several times with chloroform and the combined filtrate and washings were completed to volume with chloroform. The fluorescence intensity was measured against a reagent blank similarly prepared using chloroform instead of the drugs at 385/452 nm. The calibration graphs were obtained by plotting the fluorescence intensity *versus* the final concentration. Alternatively, the corresponding regression equations were derived.

3.3.3. Procedure for tablets

Weigh and pulverize 10 tablets. Transfer a weighed quantity of the powder equivalent to 40 mg of the drug into 100-ml volumetric flask; add about 60 ml of methanol and sonicate for 30 min. Complete to the mark with the same solvent. Centrifuge the mixture for 5 min and transfer the clear centrifugate into a small volumetric flask. Proceed as described under 3.3.2. Determine the nominal content of the tablets either from the previously plotted calibration graph or using the corresponding regression equation.

3.3.4. Procedure for spiked human plasma

Transfer 1 ml of human plasma previously spiked with the drug into 50-ml separating funnels. Add 1 ml of glacial acetic acid and 1 ml of 0.04% DMAS solution and mix well. Shake the reaction mixture for about 5 minutes with three successive portions of chloroform (7, 5, 5 ml). Collect the organic layers on phase separator filter paper into a 25-ml measuring flask and complete to the volume with chloroform. Measure the fluorescence at 385/452 nm.

The nominal content of each of the studied drug in plasma was determined from the corresponding regression equation.

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