Zagazig J. Pharm. Sci., Dec. 2005
Vol. 14, No. 2, pp. 36-40

ISSN 1110-5089

SPECTROFLUOREMETRIC DETERMINATION OF BETAXOLOL IN DOSAGE FORMS AND SPIKED HUMAN PLASMA USING SOLID - PHASE EXTRACTION

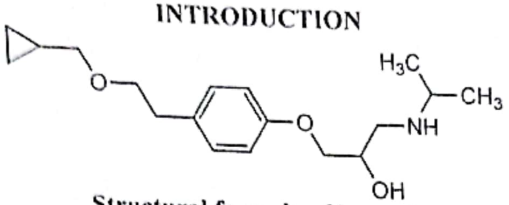
Maha A. Sultan

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, PO Box 22452, Riyadh 11495, Saudi Arabia

ABSTRACT:

The native fluorescence of betaxolol (a potent drug for hypertension and glaucoma) has been studied under different experimental conditions. The highest fluorescence intensity was obtained in methanol at 305 nm using 275 nm for excitation. Surfactants and sensitizers had either negative or no effect on its fluorescence intensity. The fluorescence intensity-concentration plot was rectilinear over the range 0.1-1.0 μg mL^{-1} with lower detection limit of 0.025 μg ml^{-1} (8.1×10^{-8} M). Interference likely to be introduced from co-formulated drugs (such as benzalkonium chloride) or co-administered drugs (such as hydrochlorothiazide), was studied. The method was successfully applied to the determination of the drug in its tablets and ophthalmic solution. The mean % recoveries were in agreement with those provided by the official methods. The method was further extended to the *in-vitro* determination of the drug in spiked human plasma using solid phase extraction, utilizing a C_{18} Bond-Elute column. The mean % recovery (n = 5) was 101.38 ± 2.32.

INTRODUCTION



Structural formula of betaxolol

Betaxolol hydrochloride, is 1-[4-[2-(cyclopropyl-methoxy) ethyl]-phenoxy]-3-[(1-methylethyl) amino]-2-propanol. It is a cardioselective β-adrenergic antagonist. It exhibits high and consistent bioavailability (70-90 %) and a long terminal half-life of (13-20 hr)[1].The drug is highly efficacious for the treatment of hypertension and glaucoma[2].

Reviewing the literature revealed that, only one spectrophotometric method[3] was reported for the assay of betaxolol in pharmaceutical preparations. While all other methods reported for the drug, whether in pharmaceutical preparations or in biological fluids rely on the use of chromatography, *viz*, HPLC[4-7], gas chromatography[8,9], capillary electrophoresis[10] and densitometry[11].

Although chromatographic methods have a high degree of specificity, yet, sample clean up and the instrumentation limitations preclude their use in routine clinical studies.

The literature revealed no reports on the direct fluorometric determination of betaxolol. This led us to study the fluorescence characteristics of the drug as an attempt to develop a simple, sensitive and reliable method for its determination in dosage forms and plasma. The native fluorescence of the compound initiated the present study.

The method was developed as an alternative substitute to the HPLC methods and the results obtained were promising.

EXPERIMENTAL

Apparatus

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

Material and reagents

A reference standard sample of betaxolol hydrochloride was obtained from Sigma Chemical Co. (St.Louis, MO, USA).

Commercial tablets, Kerlone® (20 mg betaxolo / tablet), (Batch # 23076) was obtained from Laboratoires Synthelabo(Le Plessis-Robinson / France) and ophthalmic solution, Betoptic® (containing 0.5 % betaxolol hydrochloride and 0.01 % benzalkonium chloride), (Batch # 4AVM1B) was obtained from laboratories Alcon (Rueil-Malmaison Cedex, France).

Plasma was obtained from King Khalid University Hospital, Riyadh, KSA, and was kept frozen until use after gentle thawing.

A stock solution of betaxolol hydrochloride (1 mg / ml) in methanol was prepared.

The solution was found to be stable for one weak. Working standard solutions were further prepared by dilution with the same solvent as appropriate.

Procedures:

Recommended procedure and calibration curve

Transfer aliquots of the working standard solution into a series of 25 ml volumetric flasks. Complete to the mark with methanol. Measure the fluorescence intensity at 305 nm after excitation at 275 nm. Plot the fluorescence intensity versus the final concentration to get the calibration graph. Alternatively, derive the corresponding regression equation.

Procedure for tablets

Weigh and pulverize 10 tablets. Transfer a weighed quantity of the powder equivalent to 10 mg of betaxolol hydrochloride into a small flask. Add about 60 ml of methanol and sonicate for 30 minutes

E-mail address: mahas20us@yahoo.com (M. Sultan).

Filter into 100 ml volumetric flask. Wash the flask, residue and filter with methanol and add the washing to the same volumetric flask then complete to the mark with the same solvent. Proceed as described under 2.3.1.

Determine the nominal content of the tablets using either the calibration graph or the regression equation.

Procedure for ophthalmic drops

Transfer 2 ml of the ophthalmic solution (0.5 %), into 10 ml volumetric flask and dilute to the mark with methanol. Appropriate dilution with the same solvent was made and proceeds as described under procedure. Determine the nominal content of the ophthalmic solution using either the calibration graph or the regression equation.

Procedure for spiked human plasma

Transfer 1ml aliquots of human plasma spiked with the drug into 2 ml Eppendrof tubes. Sonicate for 5 min. Blank human plasma samples were processed in the same manner using deionized water instead of betaxolol. Sep-Pak C_{18} cartridges were conditioned with 2 x 1 ml methanol and 2 x 1ml deionized water before applying the plasma samples. Care was taken that the cartridges did not run dry. Blank and spiked plasma samples were transferred into the cartridges and vacuum was applied to obtain a flow of 0.5 ml / min. After the entire plasma samples had been aspirated through the cartridges, the cartridges were washed with 2 x 500 µL de-ionized water.

The cartridges were then dried under vacuum for 3 min. All cartridges were eluted with 2 x 500 µL of methanol and then diluted with the same solvent to give the appropriate concentration. Measure the fluorescence of the extracted solution at 275 / 305 nm. Plot the fluorescence intensities versus the final concentration to get the calibration graph. Alternatively, derive the corresponding regression equation.

RESULT AND DISCUSSION

The methanolic solution of betaxolol show a very weak UV absorbance spectrum at 270 nm (ε = 1.7 x 10^3 L.Mole.cm^{-1}). As a consequence poor sensitivity will be achieved by conventional spectrophotometric measurements, and this problem is more aggravated if the compound needs to be estimated in biological fluids. On the other hand, betaxolol solution exhibited intense native fluorescence. Different media such as water, methanol, 0.01M H_2SO_4, ethanol, acetonitrile, chloroform and N,N-dimethylformamide were attempted. As shown in table 1, the highest fluorescence intensity was obtained in methanol, therefore, it was used throughout this study. Figure 1 shows the typical spectra of betaxolol in methanol. The effect of surfactants on the fluorescence intensity of betaxolol was studied by adding 1 ml (containing 100 µg / ml) of each surfactant solution to the drug solution in methanol. The results are abridged in table 2. From the table it is clear that, gelatine caused a dramatic decrease in fluorescence intensity. While β-cyclodextrine, sodium dodecylsulphate and Triton-X 100, on the other hand, caused a negligible decrease in fluorescence intensity.

Analytical performance

The relation between the fluorescence intensity and betaxolol hydrochloride concentration is rectilinear over the range of 0.1 – 1 µg ml^{-1}. Linear regression analysis of the data gave the following equation:

$$R_f = 0.288 + 152.48\ C \ldots\ldots r = 0.9995$$

Where R_f is the relative fluorescence intensity and C is the concentration of betaxolol hydrochloride in µg.ml^{-1} with lower limit of quantification of 0.075 µg ml^{-1}.

The lower limit of detection was experimentally determined (S/N = 2) and was found to be 0.025 µg ml^{-1} (8.1×10^{-8} M). Statistical evaluation of the regression line, regarding standard deviation of the residuals ($S_{y/x}$), standard deviation of the slope (S_b) and standard deviation of the intercept (S_a), gave the following values 5.64×10^{-3}, 1.04×10^{-2} and 1.43×10^{-3}, while the percentage error is 0.25 %.

The small figures obtained point out to the low scattering of the calibration points around the calibration line[12].To test the validity of the method; it was applied to the determination of an authentic sample of betaxolol hydrochloride over the range 0.1–1 µg ml^{-1} (n =7). The results are in good agreement with that given by the United States Pharmacopoeia[13] (Table 3).

Pharmaceutical applications

The proposed method was applied to the determination of betaxolol in tablets and ophthalmic solutions. The results obtained (Table 3), were in agreement with those given by the official methods adopting HPLC methods[13].

Common tablet excipients, such as talc, lactose and magnesium stearate did not interfere with the assay.

Biological application

The high sensitivity of the proposed method renders it applicable to the *in-vitro* determination of betaxolol in biological fluids. Betataxolol was succefully applied in spiked human plasma samples. The assay involved the use of solid phase extraction procedure for human plasma prior to fluoremetric measurements.

The fluorescence – concentration plot is rectilinear over the range of 0.3-1.2 µg ml^{-1}.

Linear regression analysis of the data gave the following equation:

$$R_f = 0.830 + 174.375\ C \ldots\ldots\ldots r = 0.9991$$

Where R_f is the relative fluorescence intensity and C is the concentration of betaxolol in µg ml^{-1}.

The between – day variation was also studied by testing three different concentrations of betaxolol. A summary of the accuracy and precision results is given in table 4.

After establishing the proposed applied to the determination of spiked human plasma samples. The results are given in table 5, and seem to be fairly accurate and precise. Figure 2, shows the fluorescence spectra of spiked human plasma.

Interference study

Interference likely to be introduced either from co-formulated drug (benzalkonium chloride) or co-administered drugs (such as hydrochlorothiazide), were Studied under the same experimental conditions using methanolic solution containing 5 μgml^{-1}. None of the studied drugs showed any interference at the wavelengths of betaxolol.

Effect of light on the stability of betaxolol

The effect of artificial daylight on the stability of betaxolol was studied by measuring the fluorescence intensity of 0.5µg ml^{-1} solution of betaxolol in methanol at different time intervals for 3 hours. No significant decrease of fluorescence intensity was observed. No significant change also was observed upon exposing solution of the same concentration to light emitted from Deuterium lamp; the rate of decrease of fluorescence intensity was negligible. On the other hand, upon exposing a solution of the same concentration to light from Xenon arc lamp, there was a significant decrease in the fluorescence intensity. This behavior is due to the high energy content of the emitted light of Xenon lamp. This necessitates that; the solution should be measured immediately and not left in the light path for more than few minutes.

CONCLUSION

A sensitive spectrofluorimetric procedure has been developed to provide simple, rapid and accurate method for the estimation of betaxolol in bulk samples and in pharmaceutical formulations. The method has been fully validated and is linear down to 1.0 μgml^{-1} with good accuracy and precision. A comparative examination of some pharmaceutical preparations using the proposed and the official methods, demonstrated equivalent results with respect to accuracy and precision. The presence of excipients in the commercial formulations did not affect the accuracy of the method. Moreover the method was successfully applied for the determination of the drug in human plasma using an efficient solid phase extraction procedure for sample clean – up of plasma. It is worthy to mention that the proposed method is regarded as being superior to the official USP XXIV method because it can be used for the determination of the drug in trace amount. Therefore, it could be easily used in quality control laboratories for the analysis of the drug in bulk materials and in pharmaceuticals. Moreover the proposed method was found to be stability indicating assay method.

Table 1: Effect of solvent on the fluorescence intensity of betaxolol (360 $ngml^{-1}$)

Solvent	Fluorescence intensity
0.01 M H_2SO_4	40.4
Methanol	56.0
Ethanol	56.0
Acetonitrile	35.5
Chloroform	33.4
N,N-dimethylformamide	32.5
Water	28.6

Data are the average of 6 independent experiments

Table 2: Effect of surfactants on the fluorescence intensity of betaxolol (360 $ngml^{-1}$)

Surfactant*	Fluorescence intensity
No Surfactant	56.3
β-cyclodextrin	42.5
Gelatine	22.6
Sodium Dodecyl sulphate	49.2
Triton X 100	51.9

*Each surfactant concentration was 100μgml^{-1}.

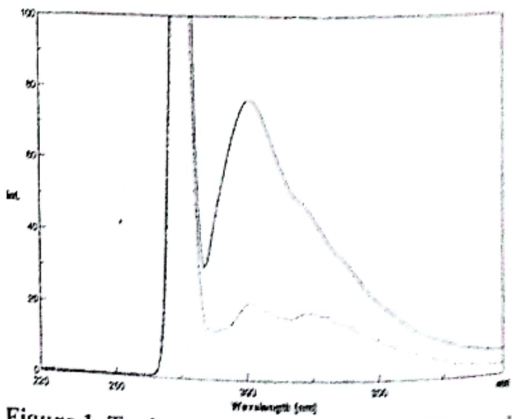


Figure 1. Typical fluorescence spectra of betaxolol in methanol (——) , Blank (---)

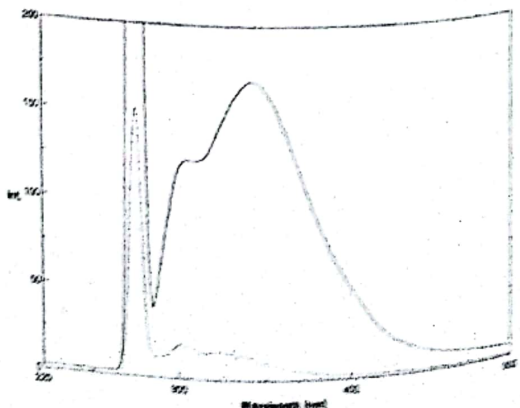


Figure 2. Fluorescence spectra of betaxolol in plasma (——) , Blank (-----)

Table 3: Application of the proposed and official methods to the analysis of betaxolol in pure and dosage forms

Form	Proposed method			Official[13]
	Taken (µg)	Found (µg)	Recovery (%)	Recovery (%)
Pure form	0.1	0.1006	100.62	100.21
	0.3	0.3014	100.48	99.98
	0.5	0.4991	99.82	100.75
	0.8	0.7963	99.54	101.08
	1.0	1.0031	100.31	--
Mean ±C.V.			100.15 ± 0.40	100.51± 0.43
F			1.1556 (6.59)	
t			1.3006 (2.3.6)	
Kerlone tablets® (20 mg betaxolol / tablet)	0.1	0.1010	101.06	99.98
	0.5	0.5078	101.56	101.93
	0.8	0.8038	100.48	100.75
Mean ±C.V.			101.03± 0.44	100.89± 0.80
F			3.3058 (19.00)	
t			0.2711 (2.776)	
Betoptic® Ophth. soln. (0.5mg %. betaxolol)	0.1	0.1009	100.96	100.34
	0.5	0.4977	99.54	101.07
	0.8	0.7946	99.32	100.72
Mean ±C.V.			99.94 ± 0.73	100.71± 0.30
F			5.922 (19.00)	
t			1.702 (2.776)	

C.V.: the coefficient of variation.
F: Variance ratio & t: the student's t- test and the values between brackets are the tabulated values (P = 0.05)[12]

Table 4: Accuracy and precision data for betaxolol in spiked human plasma

Analyte	Actual concentration μgml^{-1}	Recovery (%)	Error (%)	RSD (%)
Intra-day [a]	0.3	101.00	1.0	1.3
	0.6	99.80	0.2	2.0
	0.8	103.25	3.3	1.8
Inter-day [b]	0.3	102.81	2.8	4.5
	0.6	103.33	3.3	3.3
	0.8	98.97	2.1	2.2

a is the mean based on n = 3
b is the mean based on n = 6

Table 5: Application of the proposed method to the determination of betaxolol in spiked human plasma.

Amount added (μgml^{-1})	Amount found (μgml^{-1})	Recovery (%)
0.5	0.512 ± 0.01	102.4
0.6	0.608 ± 0.08	101.33
0.8	0.792 ± 0.110	99.00
1.0	1.052 ± 0.103	105.20
1.2	1.188 ± 0.093	99.00
$\bar{X}$ S.D.		101.386 ± 2.322

REFERENCES

1. Ludden TM, Boyle DA, Gieseker D, Kennedy GT, Crawford MH, Ludden LK and Clementi WA., J. Pharm. Sci.; 77: 779 (1988).
2. Parfitt K, (Ed.) Martindale, The complete Drug Reference, 32 nd ed, The Pharmaceutical Press, Massachusetts (1999).
3. Salem II. Journal of Pharmaceutical and Biomedical Analysis.; 29: 527-538 (2002).
4. Hernando MD, Petrovic M, Fernandez-Alba AR and Barcelo D. Chromatography A;1046: 133-140 (2004).
5. Delamoye M, Duverneuil C, Paraive F, de. Mazancourt P and Alvarez JC. Forensic Science International;141: 23-31 (2004).
6. Dulger B, Basci, N E, Sugdic-yalvac I, Temizer A. Journal of Chromatography B;772: 179-183 (2002).
7. Peter M, Gyeresi A, Fulop F. Journal of Chromatography, A, 910: 247-253 (2001).
8. Ganansia J, Gillet G, Padovani P and Bianchetti G. Journal of Chromatography B: Biomedical Sciences and Applications.; 275: 183-188 (1983).
9. Forsdahl G, Geisendorfer T, Gmeiner G. Chromatographia.; 57: 519-524 (2003).
10. Annecatherine S, Marianne F, Adel MA, Philippe H, Jacques C. Electrophoresis.; 24:363-369 (2003).
11. Hanna H, Arkadiusz P, Tomasz M, Marzena O. Journal of Planar Chromatography-Modern TLC.;16: 280-285 (2003).
12. Miller JC, Miller J N. Statistics for Analytical Chemistry, Wiley, New York, (1984).
13. The United States Pharmacopoeia, USA XXIV NF 19 US Pharmaceutical Convention, Rockville, MD p. 228 (2000).

Received: Nov. 01, 2005
Accepted: Dec. 20, 2005

Zagazig J. Pharm. Sci., Dec. 2005
Vol. 14, No. 2, pp. 36-40

طريقة لصفية لتعيين البيتاكسولول في المستحضرات الصيدلية وبلازما الإنسان باستخدام طريقة الاستخلاص الجافة

مها عبد الرحمن سلطان

قسم الكيمياء الصيدلية – كلية الصيدلة – جامعة الملك سعود – الرياض – المملكة العربية السعودية

يتناول هذا البحث إستنتاج طريقة لصفية كمية لتقدير مركب البيتاكسولول وذلك في حالته النقية وبعض المستحضرات الصيدلية وأيضا في بلازما الإنسان وتعتمد الطريقة على قياس شدة اللصف التلقائية للمركب عند طول موجة ٣٠٥ نانومتر بعد تنشيطه عند طول موجة ٢٧٥ نانومتر وقد أظهرت الطريقة حساسية عالية ($8.1x\ 10^{-8}$ M) وتمت دراسة المؤثرات التجريبية المختلفة التي تؤثر في شدة اللصف بعناية وطبقت الطريقة المقترحة بنجاح لتقدير المركب في المستحضرات الصيدلية وبلازما الإنسان.