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Spectrofluorimetric Determination of Labetalol in Pharmaceutical Preparations and Biological Fluids

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ABSTRACT

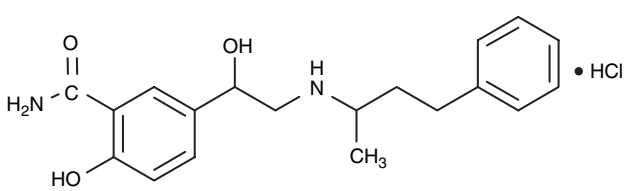
A simple and highly sensitive spectrofluorimetric method has been developed for the determination of labetalol (LBT) in pharmaceutical preparations and biological fluids. The method is based on the reaction between the nitroso-derivative of LBT and 2-cyanoacetamide (2-CAA) in the presence of ammonia to give fluorescent product with excitation wavelength of 335 nm and emission wavelength of 420 nm. The reaction conditions were studied and optimized. The fluorescence intensity-concentration plot is rectilinear over the concentration range of 0.025-0.250 μg/mL with minimum detectability of 1.40 ng/mL (3.6×10^{-9} M). The proposed method was successfully applied to commercial tablets containing LBT; the percentage recoveries agreed well with those obtained using the reference method. The method was further extended to the *in-vitro* determination of LBT in spiked human urine and plasma samples. The percentage recovery was 100.10 ± 3.44 and 101.27 ± 4.97, respectively. A proposed reaction pathway was postulated.

Key words: labetalol-2-cyanoacetamide, spectrofluorimetry, pharmaceutical preparations, biological fluids

INTRODUCTION

Labetalol hydrochloride (LBT): 5-[1-Hydroxy-2-(1-methyl-3-henylpropylamino)ethyl] salicylamide hydrochloride. LBT is a non-cardiovascular β-blocker. It is reported to possess some intrinsic sympathomimetic and membrane stabilizing activity. It has additional selective α1-blocking properties that decrease peripheral vascular resistance. The ratio of α to β blocking activity has been estimated to be about 1:3 following oral administration, and 1:7 following intravenous administration. It is used in the management of hypertension and to induce hypotension during surgery[(1)]. LBT is listed in the British Pharmacopoeia, BP[(2)], the United States Pharmacopoeia USP[(3)], and the European Pharmacopoeia[(4)]. The BP and the European Pharmacopeias recommend non-aqueous titration with potentiometric end point detection for the raw material and spectrophotometric measurement at 302 nm for the tablets and injections. The USP[(3)], on the other hand, recommends HPLC methods for the raw material and its formulations.

The therapeutic importance of LBT initiated several reports on its determination, both in formulations and in biological fluids, viz: spectrophotometry[(5-8,11)], Spectrofluorimetry[(9,11)], HPLC[(12-17)], HPLC-MS(18), LC-MS[(19-20)], micellar liquid chromatography[(23,24)], capillary liquid chromatography[(23)], capillary electrophoresis[(24-26)], capillary isotachophoresis[(27)] and NMR spectroscopy[(28)]. LBT was also determined in pharmaceuticals using an ion selective electrode sensitive to LBT with a liquid membrane[(29)]. All these methods are either not sufficiently sensitive[(2,5-9)] or tedious and require highly sophisticated and dedicated instrumentation[(12-25)]. 2-CAA has been previously utilized for the determination of some pharmaceutical compounds such as 3,4-dihydroxyphenylalanine[(30)], prenalterol HCl[(31)], oxamniquine[(32)] and ascorbic acid[(33)]. The proposed method is based on the reaction of nitroso-derivative of LBT with 2-CAA in the presence of ammonia and the subsequent measurement of the compound formed. The method was applied to its dosage forms and further extended to spiked human urine and plasma samples.



Labetalol hydrochloride

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MATERIALS AND METHODS

I. *Chemicals*

Reference standard sample of labetalol • HCl (LBT HCl) was obtained from Glaxo Welcome, Middlesex, UK. Commercial tablets containing LBT were obtained from the local market. Trandate tablets labeled to contain 100 and 200 mg LBT were obtained from the commercial sources. Urine was obtained from healthy volunteers and kept frozen until use after gentle thawing. Plasma was obtained from El-Esafe center, Mansoura, Egypt.

Other solutions used are described as follow:

- Hydrochloric acid (Merck, Darmstadt, Germany): 0.1 M solution.
- Sodium nitrite (Merck, Darmastadt, Germany): 2% (w/v) aqueous solution.
- Sulphamic acid (Merck, Darmastadt, Germany): 5% (w/v) aqueous solution.
- Ammonia (Merck, Darmastadt, Germany): 33% (w/v) solution.
- 2-CAA (Merck, Darmastadt, Germany): 1% (w/v) aqueous solution.
- Sodium carbonate buffer of pH 9.4: prepared from 2 M sodium carbonate (BDH, UK) solution and adjusted to pH 9.4 using 1 M hydrochloric acid.

II. *Preparation of Standard Solution*

Stock solutions of LBT HCl containing 0.125 mg/mL were prepared freshly every day, in distilled water and were further diluted using distilled water.

III. *Apparatus*

The fluorescence intensities were measured using a Perkin-Elmer Model LS 45 spectrofluorimeter equipped with 20 KW Xenon discharge lamp, excitation, emission gratting monochromators and a 1×1 cm quartz cell. The apparatus was driven by the *hp* computer.

IV. *Construction of Calibration Graph*

Accurately measured portions (0.1-1.0 mL) of the working solution, were transferred into a set of 25-mL volumetric flasks. One milliliter of 0.1 M HCl solution was added followed by 1 mL of 2% sodium nitrite solution, and the mixture was shaken for 2 min then 2 mL of 5% sulphamic acid solution was added and the mixture was shaken well until no more nitrogen was evolved. 2-CAA (1%, 0.5 mL) and 1 mL of ammonia solution were added and the reaction mixture was heated in a boiling water bath for 25 min, then cooled and made up to volume using distilled water. The fluorescence was measured at 420 nm with excitation at 335 nm. The calibration graph was obtained by plotting the fluorescence intensities versus the concentrations of LBT HCl; the regression equation was derived.

V. *Procedures for Commercial Tablets*

Ten tablets were weighed and powdered. An accurately weighed amount of the powder equivalent to 0.01 g of LAB HCl was transferred into a small conical flask and extracted using 3×30 mL portions of water. The extracts were transferred into a 100-mL standard flask and diluted to the mark using distilled water to obtain a solution of 0.1 mg/mL which was further diluted using distilled water to give a working solution of 5.0 μg/mL. The procedure was continued as described under section IV. The nominal content of the tablets was determined either from the calibration graph or from the corresponding regression equation.

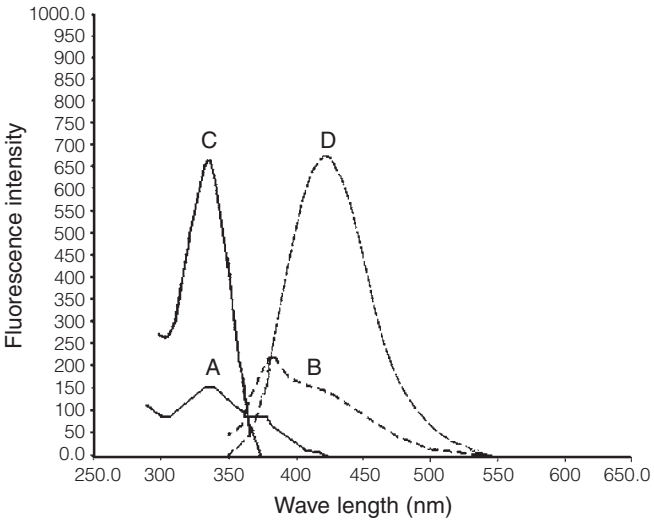

Figure 1. Fluorescence spectra of labetalol HCl (0.225 μg/mL) after reaction of its nitroso-derivative with 0.5 mL of 1% 2-CAA and 1 of 33% ammonia solution.

A & B: Excitation and emmision spectra of blank.
C & D: Excitation and emmision spectra of the reaction product.

VI. *Procedures for Spiked Human Urine Samples*

In a set of 15-mL centrifugation tubes, 1 mL aliquots of plasma or urine with varying amount of labetalol HCl was spiked to give final required concentration. Five milliliter of carbonate buffer of pH 9.4 was added and the solution was mixed well then 5 mL of diethyl ether was added. The mixture was vortexed at 2500 rpm for 10 min then the organic layer was transferred into a conical flask. The extraction was repeated twice with 5 mL of diethyl ether. The ether extract was collected and evaporated to dryness. The residue was dissolved in 1 mL of 0.1 M HCl and transferred into a 25-mL volumetric flask. The procedure was continued as described under IV. Blank experiment was also carried out adopting the above procedure.

RESULTS AND DISCUSSION

Treatment of the nitroso-derivative of LBT HCl with 2-CAA in the presence of ammonia was found to give a fluorescent product. Figure1 shows the excitation and emission spectra obtained. The nitroso-derivative of LBT HCl was obtained rapidly at room temperature within 2 min using 1 mL of 0.1 M hydrochloric acid and 1 mL of 2% sodium nitrite, as shown in Figure 2.

The different experimental parameters affecting the fluorescence intensity were studied. Figure 3 shows that 0.5 ± 0.1 mL of 2-CAA was found to be sufficient to produce the maximum fluorescence intensity; a larger volume

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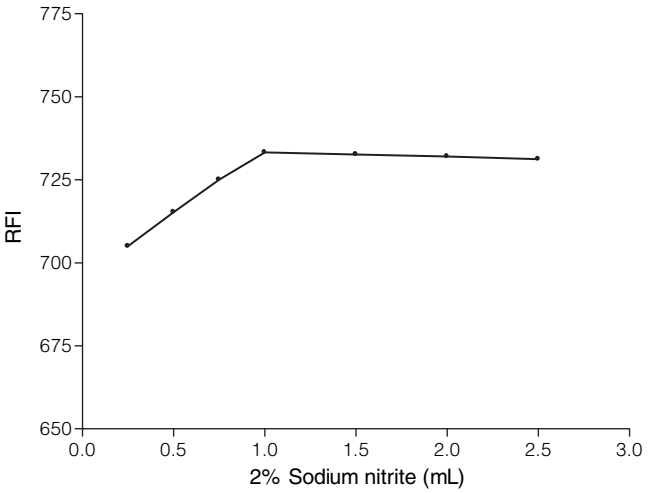


Figure 2. Effect of 2% sodium nitrite on the fluorescence intensity of the reaction product of labetalol HCl (0.25 µg/mL) with 0.5 mL of 1% 2-CAA and 1 mL of 33% ammonia.

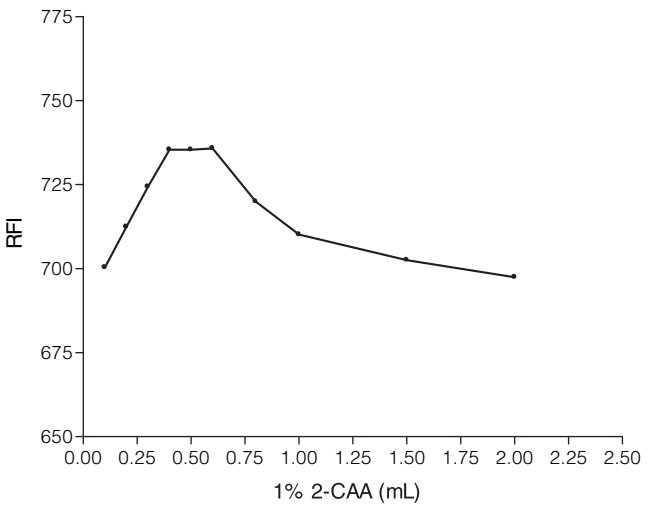


Figure 3. Effect of 1% 2-CAA on the fluorescence intensityof the reaction product of labetalol HCl (0.25 µg/mL) in the presence of 1 mL of 33% ammonia.

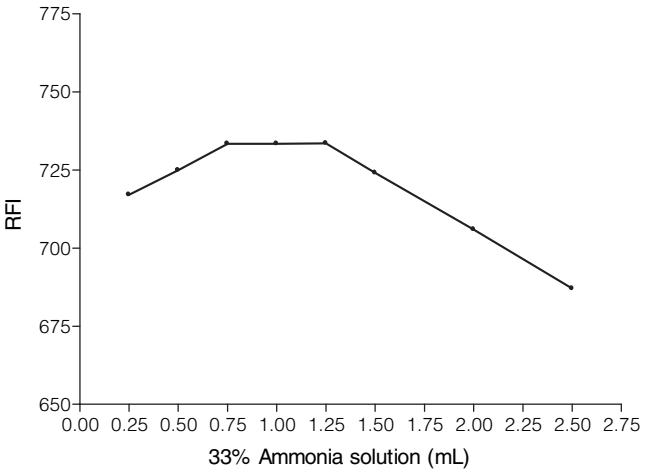


Figure 4. Effect of 33% ammonia solution on the fluorescence intensity of the reaction product of the labetalol HCl (0.25 µg/mL) with 0.5 mL of 1% 2-CAA.

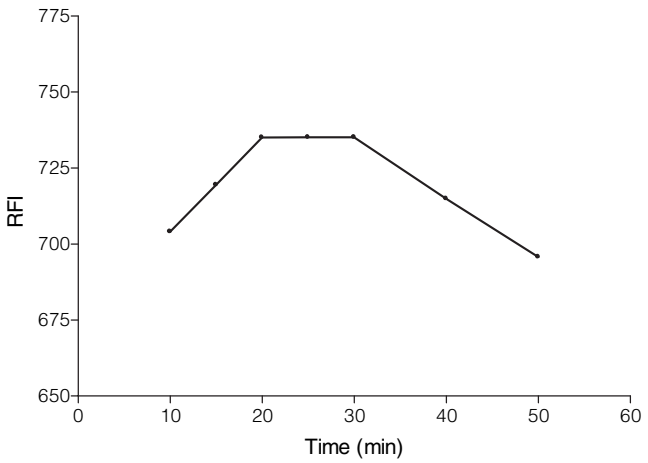


Figure 5. Effect of the boiling time on the formation of fluorescent product of labetalol HCl (0.25 µg/mL) with 0.5 mL of 1% 2-CAA and 1 mL of 33% ammonia.

decreases the fluorescence intensity. As shown in Figure 4, 1.0 ± 0.2 mL of 33% ammonia was enough to produce the maximum fluorescence intensity; a larger volume decreases the fluorescence intensity. It was shown in Figure 5 that the fluorophore is formed after heating in a boiling water bath for 25 min. The formed fluorophore remained stable for at least 120 min at room temperature.

The fluorescence intensity is linearly related to the concentration over the concentration range of 0.025-0.250 µg/mL with minimum detectability of 1.40 ng/mL (3.6×10^{-9} M). Linear regression analysis of the data gave the following equation:

RFI = 20.52 + 2843.24 C r = 0.9999

Where C = Concentration of labetalol HCl in µg/mL

RFI = Relative Fluorescence Intensity.

Statistical evaluation of the regression line gives the following values: standard deviation of the residuals ($S_{y/x}$) is 1.32; standard deviation of the intercept (S_a) is 0.90; standard deviation of the slop (S_b) is 5.80 while relative standard deviation (RSD%) is 0.85% and the percentage error (Er%) is 0.269[34].

I. *The Proposed Mechanism of the Reaction*

Similar to the reaction of 2-CAA with nitroso compounds[35], the reaction is proposed to proceed as shown in Scheme 1. Further cyclization involving the phenolic and the amide groups may occur producing the highly conjugated system.

II. *Validation of the Method*

(I) *Linearity of the Method*

The good linearity of the calibration graphs and negligible scatter of the experimental points, around the straight line, are clearly evident by the values of correlation coefficients and standard deviations.

(II) *Limit of Quantification (LOQ) and Limit of Detection (LOD)*

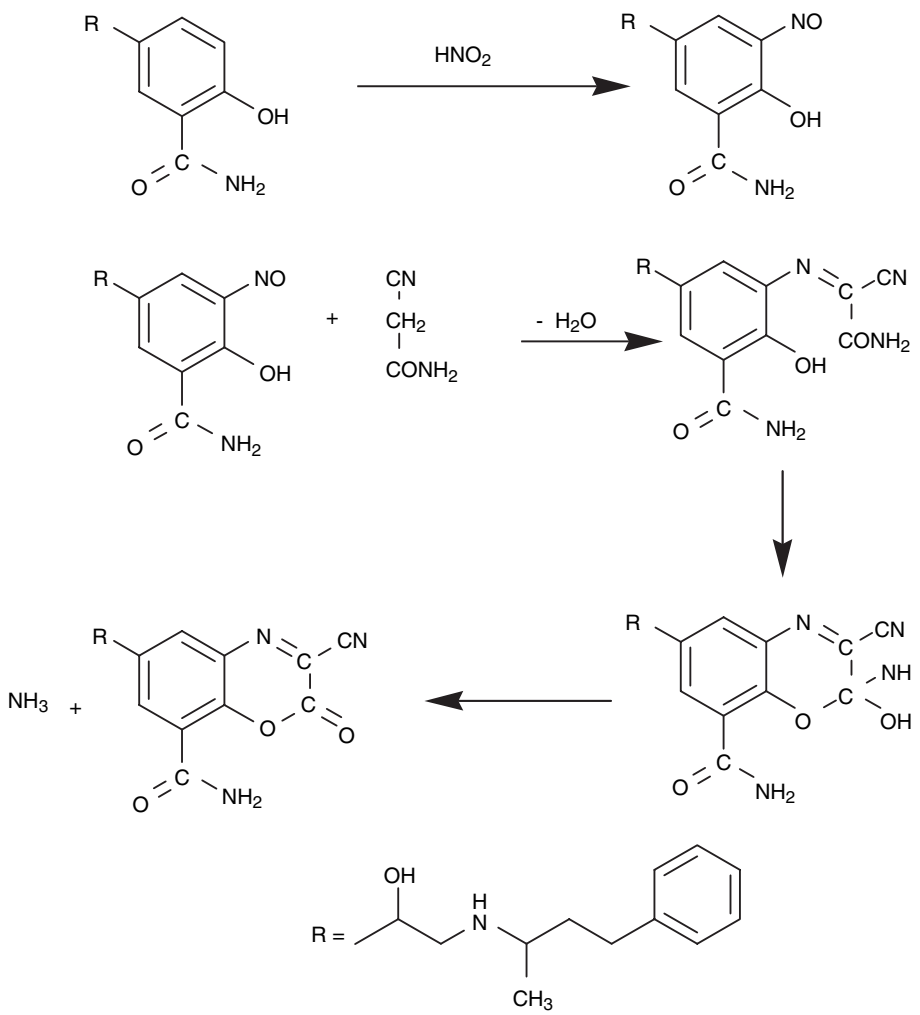
The LOQ was determined by establishing the lowest concentration that can be measured with acceptable accuracy and precision. LBT can be quantified under these conditions at a concentration of 0.0046 μg/mL[34].

Table 1. Evaluation of the accuracy and precision data of the proposed spectrofluorimetric method for the determination of labetalol HCl

Conc. added (μg/mL)	Conc. found (μg/mL)			
	Mean ± SD	%Recovery	RSD%	Er%
Intra-day				
0.075	0.0749 ± 0.003	99.93 ± 0.41	0.41	-0.24
0.150	0.1494 ± 0.001	99.60 ± 0.74	0.74	-0.43
0.250	0.2507 ± 0.001	100.27 ± 0.39	0.39	+0.23
Inter-day				
0.075	0.0743 ± 0.001	99.13 ± 0.48	0.48	-0.34
0.150	0.1505 ± 0.001	99.97 ± 0.81	0.80	+0.47
0.250	0.2486 ± 0.002	99.51 ± 0.81	0.85	-0.49

Table 2. Influence of small variation in the assay conditions on the analytical performance of the proposed spectrofluorimetric method for the analysis of labetalol HCl (n = 3)

Parameter	Recovery (% ± SD)
Volume of 2% $NaNO_2$ (mL)	
0.80	99.00 ± 0.73
1.20	99.65 ± 0.65
Volume of 1% 2-CAA (mL)	
0.40	98.46 ± 0.23
0.06	98.96 ± 0.49
Volume of 33% ammonia solution (mL)	
0.80	98.41 ± 0.84
1.20	100.35 ± 0.28
Boiling time (min)	
22	98.75 ± 0.39
27	99.65 ± 0.04



Scheme 1. The proposed mechanism of the reaction between the nitroso-derivative of labetalol HCl and 2-cyanoacetamide.

The LOD was calculated by establishing the minimum level at which the analyte can be reliably detected and it was found to be 1.40 ng/ml (3.6×10^{-9} M)[34].

(III) *Intermediate Precision and Accuracy*

The results of the inter-day and intra-day accuracy and precision of the method are summarized in Table 1. The inter-day and intra-day precisions were examined by analyzing the three concentrations three times for three consecutive days. The repeatability of the method is fairly high indicated by the low values of SD and RSD. The inter-day and intra-day accuracy was also proved by the low values of Er%.

(IV) *Robustness*

The robustness of the method is demonstrated by its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability[2], as shown in Table 2.

III. *Application of the Proposed Method for the Determination of Labetalol HCl in Dosage Forms and Biological Fluids*

Table 3. Application of the proposed spectrofluorimetric method to the determination of labetalol HCl in pure state

Proposed method			Reference method[11]	
Amount taken (μg/mL)	Amount found (μg/mL)	% Found	Amount taken (μg/mL)	% Found
0.025	0.0257	102.80	0.50	101.57
0.050	0.0500	100.00	1.00	100.40
0.075	0.0749	99.87	1.50	99.77
0.100	0.0998	99.80	2.00	100.10
0.125	0.1245	99.60	2.50	98.96
0.150	0.1494	99.60	3.00	101.06
0.175	0.1753	100.17	3.50	99.14
0.200	0.2000	100.00	4.00	100.36
0.225	0.2248	99.91	4.50	99.50
0.250	0.2507	100.28	5.00	100.48
$\overline{X} \pm SD$		100.20 ± 0.94		100.13 ± 0.82
t		0.27 (2.10)		
F		1.31 (3.18)		

Values between brackets are the tabulated *t* values and F- values at (p = 0.05)[36].
Each result is the mean recovery of three separate determinations (n = 3).

Table 4. Application of the proposed spectrofluorimetric method to the determination of labetalol HCl in pharmaceutical preparations

Preparation	Proposed method			Reference method[11]	
	Amount taken (μg/mL)	Amount found (μg/mL)	% Found	Amount taken (μg/mL)	% Found
Trandate tablets (100 mg labetalol HCl/tablet)	0.08	0.081	101.25	1.50	99.66
	0.12	0.119	99.17	2.00	99.29
	0.14	0.141	100.71	2.50	100.21
	0.20	0.199	99.50	4.00	99.83
$\overline{X} \pm SD$			100.16 ± 0.98		99.75 ± 0.38
t			1.82 (2.45)		
F			6.63 (9.28)		
Trandate tablets (200 mg labetalol HCl/tablet)	0.04	0.040	100.00	1.50	99.20
	0.10	0.101	101.00	2.00	100.97
	0.16	0.161	100.63	2.50	100.62
	0.24	0.238	99.17	3.00	99.43
$\overline{X} \pm SD$			100.20 ± 0.80		100.06 ± 0.87
t			0.32 (2.4)		
F			1.18 (9.28)		

Values between brackets are the tabulated t values and F- values at (p = 0.05)[36].
Each result is the mean recovery of three separate determinations (n = 3).

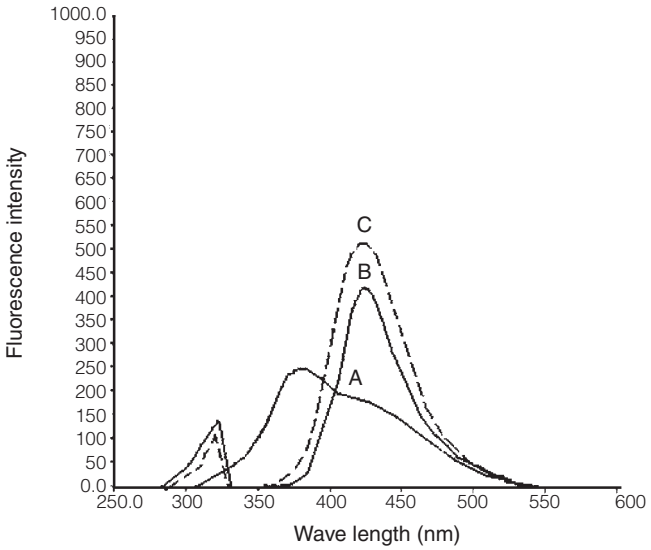


Figure 6. Fluorescence Spectra of labetalol HCl tablet after reaction of its nitroso-derivative with 0.5 mL of 2% 2-CAA and 1 mL of 33% ammonia.

A: Emmision spectrum of blank.

B & C: Emmision spectra of the reaction product using 0.08 and 0.12 µg/mL Labetalol HCl, respectively.

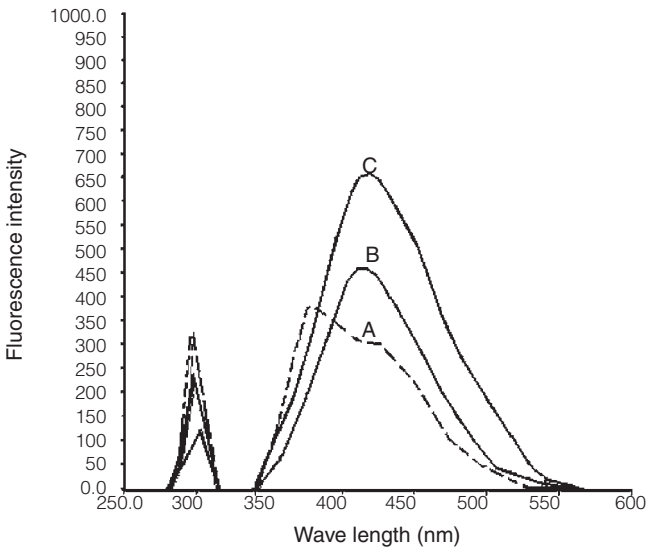


Figure 7. Fluorescence spectra of labetalol HCl in plasma after reaction of its nitroso-derivative with 0.5 mL of 2% 2-CAA and 1 mL of 33% ammonia solution.

A: Emmision spectrum of blank.

B & C: Emmision spectra of the reaction product using 0.025 and 0.15 µg/mL labetalol HCl, respectively.

The precision of the method was evaluated by analyzing standard solutions of LBT HCl. The results in Table 3 were in accordance with those obtained by the reference fluorimetric method[11]. The method was further applied to the determination of LBT in tablets as shown in Figure 6. Table 4 shows that the results obtained are in good agreement with those obtained from the reference fluorimetric method[11]. Statistical analysis of the results obtained shows no significant difference between the performance of the two methods regarding accuracy and precision using Student's *t*-test and Variance ratio F-test.

Table 5. Application of the proposed spectrofluorimetric method to the determination of labetalol HCl in spiked human plasma and urine

Added amount	Plasma		Urine	
(µg)	µg found	% Recovery	µg found	% Recovery
0.025	0.0259	103.41	0.0262	104.89
0.050	0.0548	109.54	0.0508	101.58
0.075	0.0728	97.03	0.0713	95.48
0.100	0.0986	98.58	0.0987	98.74
0.125	0.1283	102.63	0.1221	97.65
0.150	0.1446	96.42	0.1534	102.27
Mean ± SD		101.27 ± 4.97		100.10 ± 3.44

Each result is the mean recovery of three separate determinations (n = 3).

The drug was simply extracted from tablet with water without interference from any soluble excepients such as gelatin, magnesium stearate and lactose.

The high sensitivity of the proposed method, also allowed the in-vitro determination of LBT in spiked human plasma (as shown in Figure 7) and urine samples. LBT is orally administered at doses of 100 or 200 mg two times daily, which results in a urine level of concentration of about 2-4 µg/mL. Thus the proposed method is sufficient for routine estimation of the drugs in human plasma and urine. A prior extraction step, adopting the method of Gergov *et al*.[20], was adopted before application of the method. The results obtained in Table 5 are satisfactorily accurate and precise.

CONCLUSIONS

A specific method was developed for the determination of LBT in dosage forms and spiked human plasma and urine. The method can measure down to 0.025µg/mL with good accuracy. The minimum detectability (3.61×10^{-9} M) is comparable to those values obtained by reference method. The method is simpler, rapid and readily adaptable to routine quality control laboratories.

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