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Spectrofluorometric determination of labetolol in pharmaceutical preparations and spiked human urine through the formation of coumarin derivative

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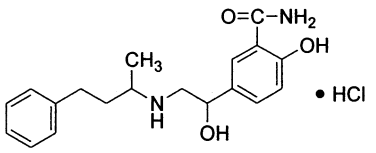
Abstract

A simple, sensitive and specific spectrofluorimetric method has been developed for the determination of labetalol (LBT). The method is based on the reaction between LBT and ethylacetoacetate in the presence of sulphuric acid to give yellow fluorescent product with excitation wavelength of 312 nm and emission wavelength of 432 nm. The reaction conditions were studied and optimized. The fluorescence intensity-concentration plot is rectilinear over the range 1–15 μg/ml with minimum detectability limit of 0.8 μg/ml (2.16×10^{-6} M). The proposed method was successfully applied to commercial tablets containing LBT, the percentage recoveries agreed well with those obtained using the official methods. Hydrochlorothiazide, which is frequently co-formulated with LBT did not interfere with the assay. The method was further extended to the in-vitro determination of LBT in spiked human urine samples. The percentage recovery was 101.50 ± 6.18 ($n = 6$). A proposal of the reaction pathway was postulated.

Keywords: Labetalol; Ethyl acetoacetate; Fluorimetry; Tablets; Urine

1. Introduction



Labetalol hydrochloride: 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl] salicylamide hydrochloride. Labetalol (LBT) is a non-cardiovascular β-blocker. It is reported to possess some intrinsic sympathomimetic and membrane stabilizing activity. It has—in addition—selective α_1-blocking properties which 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 hypo-

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tension during surgery [1]. LBT is the subject of a monograph in each of the British Pharmacopoeia, BP [2]; the United States Pharmacopoeia, USP [3] and the European Pharmacopea [4]. Both the BP and European Pharmacopoeas recommend non-aqueous titration for the raw material and spectrophotometric measurement at 302 nm for the tablets and injections. The USP [3], on the other hand, recommends HPLC method 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 [5–9], NMR spectroscopy [10], TLC [11–13], HPLC [14–17], LC-MS [18,19], GC [20,21], micellar liquid chromatography [22], capillary electrophoresis [23,24] and capillary liquid chromatography [25]. All these methods are either not sufficiently sensitive [2,5–9] or tedious and require highly sophisticated and dedicated instrumentation [10–25]. The proposed method is based on the reaction of LBT with ethylacetoacetate in the presence of a dehydrating agent (sulphuric acid) according to von Pechmann–Duisberg condensation [26,27] and the subsequent measurement of the compound formed. Only one report was published on the use of this reaction in the field of pharmaceutical analysis, that is the determination of phenolic antibiotics [28]. In this piece of work, a simple spectrofluorometric method for LBT has been developed adopting the above condensation reaction. The method was applied to its dosage forms and further extended to spiked human urine samples. The results obtained were promising.

2. Experimental

2.1. Apparatus

The fluorescence intensities were measured using a Perkin–Elmer Model LS-50B spectrofluorimeter equipped with 20 kW Xenon discharge lamp, excitation, emission grating monochromators and a 1×1 cm quartz cell. The apparatus is driven by PC computer.

2.2. Materials and reagents

Reference standard sample of labetalol · HCl (LBT · HCl) was obtained from Glaxo Welcome, Middlesex, UK, through Drug Control Center, Riyadh, Saudi Arabia. Its purity was checked according to the British Pharmacopoeia (BP) method [2] and was found to be 100.67 ± 0.88. Commercial tablets containing LBT were obtained from the local market. Trandate tablets (Batch # 505641) labeled to contain 100 mg LBT · HCl/tablet and trandate tablets (Batch # 303962) labeled to contain 200 mg LBT · HCl/tablet.

Urine was obtained from healthy volunteers (males, around 40 years old) and kept frozen until use after gentle thawing.

- Ethylacetoacetate (Prolabo, Paris, France): 2% v/v solution was freshly prepared in absolute ethanol.
- Sulphuric acid (BDH, Poole, UK) labeled to contain 98% (w/v).
- Ether (BDH, Poole, UK).
- Carbonate buffer pH 9.4 [3].

2.3. Preparation of standard solution

Stock solution was prepared by dissolving 50.0 mg of LBT · HCl in 100.0 ml measuring flask. Working standard solution was obtained by serial dilution with the same solvent. The solutions were stable for at least 3 days if kept in the refrigerator.

2.4. Construction of calibration graph

To a set of 10 ml volumetric flasks, aliquot solutions of the working standard solution were quantitatively transferred. To each flask 0.5 ml of each of ethylacetoacetate (2% v/v solution) and sulphuric acid were added. The volume was completed to the mark using absolute ethanol. The fluorescence was measured at 432 nm after excitation at 312 nm. The calibration graph was obtained by plotting the fluorescence intensity versus the final concentration of LBT · HCl. Alternatively, the regression equation was derived.

2.5. Procedure for commercial tablets

Twenty tablets were weighed and pulverized. A weighed amount of the mixed powder equivalent to 50.0 mg of LBT · HCl was transferred into a 100 ml measuring flask. About 60 ml of absolute ethanol were added and the mixture agitated for 10 min using a magnetic stirrer. The volume was completed to the mark using the same solvent. The solution was filtered and the first 5 ml of the filtrate were discarded. The clear filtrate was then diluted with absolute ethanol to suite the procedure (Section 2.4).

2.6. Procedure for spiked human urine samples

Aliquot volumes of spiked human urine samples were transferred into small separating funnel. Five ml of carbonate buffer pH 9.4 were added and the solution was mixed well. The solution was then extracted with 3 × 5 ml of diethylether. The ether extract was collected and evaporated. The residue was dissolved in 5 ml of absolute ethanol. The procedure was then applied as under Section 2.4. The nominal content of LBT · HCl was determined from the corresponding regression equation.

3. Results and discussion

LBT exhibits a low absorption of light in the UV region with $A^{1\%}_{1\ cm}$ of 85 at 302 nm [29]. As a consequence, poor sensitivity will be achieved by conventional UV spectrophotometric measurements, and this problem is more aggravated if it is needed to estimate this drug in biological fluids. However, due to the phenolic nature of the drug, it can readily couple with ethylacetoacetate in the presence of sulphuric acid as a dehydrating agent to produce strongly fluorescent compound (Fig. 1). At the same time, ethylacetoacetate has no fluorescence under the reaction conditions.

3.1. Optimization of the reaction conditions

The reaction conditions with respect to the reaction medium, the reagent concentration, the acidity or the volume of sulphuric acid and reaction time and temperature were optimized to achieve maximum sensitivity. The first goal was to choose the most appropriate solvent for this type of condensation. The fluorescence intensities of the solutions as function of the solvent were compared. Solvents investigated include: dioxan, acetonitrile, methanol, dimethylsulphoxide, acetone, isopropanol, water and ethanol. Of all the solvents, the highest fluorescence intensity was obtained upon using absolute ethanol (Table 1). This proves that this type of condensation reactions has to be carried out in a completely anhydrous medium.

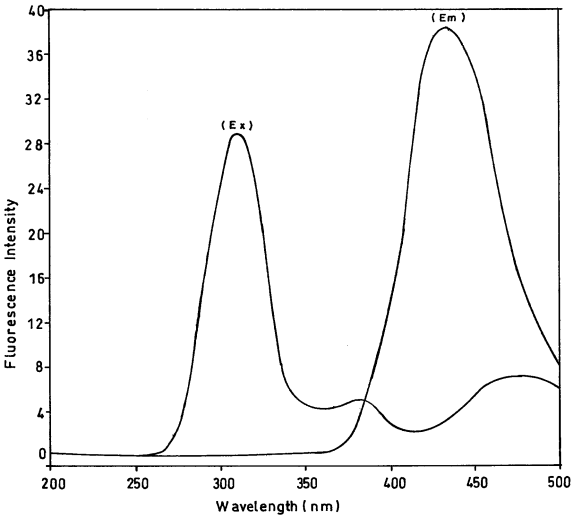


Fig. 1. Fluorescence spectra of the reaction product of labetalol · HCl (8.0 μg/ml) with ethylacetoacetate. Ex, excitation spectrum; Em, emission spectrum.

Table 1
Effect of solvent on the fluorescence-intensity of the reaction product of labetalol with ethylacetoacetate

Solvent	Fluorescence intensity
1. Dioxan	38.2
2. Acetonitrile	33.7
3. Methanol	28.2
4. Dimethylsulphoxide	37.6
5. Isopropanol	29.4
6. Acetone	0.0
7. Water	6.0
8. Ethanol	44.1

The fluorescence of the solutions was investigated over the sulphuric acid volume range of 0.3–2.5 ml. The optimum fluorescence was achieved at 0.5 ml (Fig. 2). Similarly the effect of the volume of ethylacetoacetate on the fluorescence intensity was studied over the range 0.3–3 ml. The highest fluorescence intensity was obtained over the range 0.3–1 ml, beyond which the fluorescence decreased gradually (Fig. 2).

In order to examine the effect of temperature on the fluorescence of the formed coumarin, the condensation reaction was conducted at different temperatures: ambient (25 °C) 40, 60, 80 and 100 C using a thermostated water bath for period of 10 min. From the results in Table 2, it is evident that the formed coumarin is decomposed at higher temperatures and room temperature is appropriate to carry out the reaction.

Finally, the effect of time on the development of the fluorophore and its stability was studied. The reaction product is formed immediately, reaches maximum development after 10 min and remains stable for at least 2 h.

3.2. Validation

The method was tested for linearity, specificity, precision and reproducibility. By using 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 concentration of the drug in the range of 1–15 μg/ml with lower limit of detection of 0.8 μg/ml (2.16×10^{-6} M). Statistical evaluation of the experimental data regarding standard deviation of the residuals ($S_{x/y}$), standard deviation of the slope (S_b) and standard deviation of the intercept gave the following values: 1.12, 0.083 and 0.062, respectively. These small values point out to the high precision of the method [30]. The good linearity of the calibration graph and the negligible scatter of the experimental points are clearly evident by the correlation coefficients.

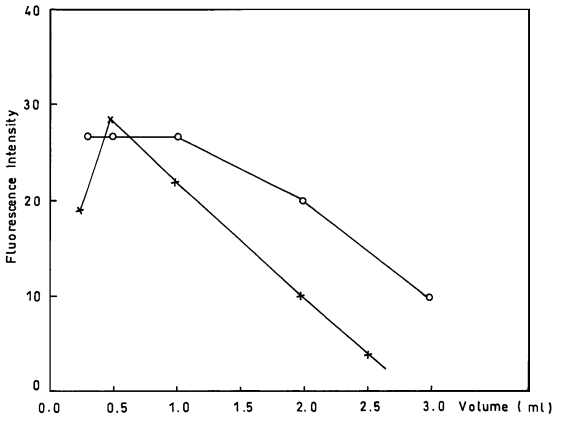


Fig. 2. Effect of sulphuric acid (× – ×) and ethylacetoacetate (○ – ○) on the fluorescence intensity of the reaction product of labetalol · HCl (8.0 μg/ml) with ethylacetoacetate.

Table 2
Effect of temperature on the development of the fluorophore of LBT with ethylacetoacetate

Temperature, °C	Fluorescence intensity
25	40.4
40	36.1
60	25.9
80	17.6
100	0.0

The validity of the method could be proved by analyzing an authentic sample of LBT · HCl. The results obtained (Table 3) are in good agreement with those given by the official method [2].

Table 3
Application of the proposed spectrofluorometric method to the determination of labetalol · HCl in pure form

Proposed method			Official method [2]
μg taken	μg found	% Recovery	
4.0	3.93	98.25	100.1
			100.0
5.0	4.90	98.00	101.5
			100.2
6.0	5.92	98.67	101.5
7.0	7.01	100.14	
9.0	9.00	100.00	
10.0	10.17	101.70	
14.0	14.70	101.43	
$\bar{X}$		99.74	100.67
±S.D.		1.63	0.88
T		1.57 (2.228)	
F		3.45 (6.16)	

Statistical analysis of the results obtained by both methods, by applying the Student t-test and the variance-ratio, F-test, reveals no significant difference between the performance of the two methods, regarding accuracy and precision, respectively [30].

The specificity of the method was investigated by observing any interference encountered from common tablet excipients or from the frequently co-formulated diuretic, hydrochlorothiazide. No interference was introduced by any of them.

3.3. Analytical applications

Under the described experimental conditions, the fluorescence intensity-concentration plot was rectilinear over the range 1–15 μg/ml. Linear regression analysis of the data gave the following equation:

$$F = 4.227C - 0.33 \quad R = 0.99899$$

where F is the fluorescence intensity and C is the concentration in μg/ml.

The simplicity of the method and the stability of the reaction product permitted the determination of LBT in its commercial tablets (100 and 200 mg, each). The results obtained (Table 4) were statistically comparable with those given using the official method [2]. Common tablet excipients, such as talc powder, maize starch, hydrogenated vegetable oil, avisil, lactose, magnesium stearate 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 LBT in spiked human urine samples. LBT is orally administered in doses of 100 or 200 mg two times daily. This results in a urine level of concentration of about 2–4 μg/ml. This concentration range lies within the working range of the method. A prior extraction step, adopting the method of Gerov et al. [19] was necessary before application of the method. A calibration graph was first constructed by plotting the fluorescence intensities versus increasing concentration of LBT in spiked human urine sample over the range 2–12 μg/ml. Linear regression analysis of the data gave the following equation:

$$F = 3.99C - 1.22 \quad R = 0.9969$$

Table 4
Application of the proposed method for the determination of labetalol · HCl in commercial tablets

Preparation[b]	Recovery %	
	Proposed method	Reference method [2]
1. Trandate™ tablets (100 mg labetalol · HCl/tablet)	99.73 ± 0.151 ($n = 6$)	99.70 ± 0.143 ($n = 5$)
t	0.334 (2.262)[a]	
F	1.111 (6.26)[a]	
2. Trandate™ tablets (100 mg labetalol · HCl/tablet)	99.59 ± 0.193 ($n = 6$)	99.61 ± 0.127 ($n = 5$)
t	0.497 (2.262)	
F	2.24 (6.26)[a]	
3. Trandate™ tablets (200 mg labetalol · HCl/tablet	99.59 ± 0.146 ($n = 6$)	99.61 ± 0.118 ($n = 5$)
t	0.246 (2.26)[a]	
F	1.5 (6.26)[a]	

[a] The figures in brackets are the tabulated values of t and F at 95% confidence limits.
[b] Products of Glaxo Wellcome, Middlesex, UK.

where F is the fluorescence intensity; C is the concentration in μg/ml.

The lower detection limit was 1.27 μg/ml (3.43×10^{-6} M). The proposed method was then applied to spiked human urine samples. The results abridged in Table 5 are satisfactorily accurate and precise.

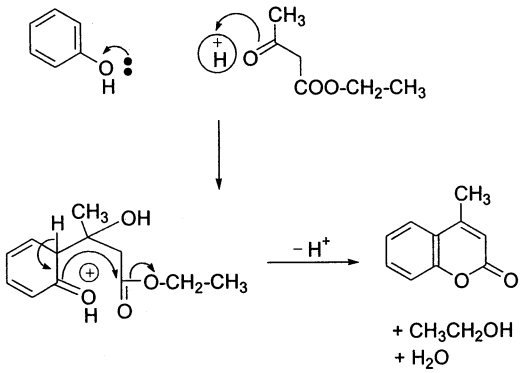
Table 5
Application of the proposed spectrofluorometric method to the determination of labetalol in spiked human urine

Added amount (μg/ml)	Found	% Recovery
2.0	2.18	109.0
4.0	4.14	103.50
6.0	5.89	98.17
8.0	7.62	95.25
10.0	9.58	95.80
12.0	12.58	104.83
$\bar{X}$		101.59
±S.D.		6.18

3.4. Mechanism of the reaction

The general synthesis of coumarins involves the interaction of phenol with a β-ketoester (ethyl acetoacetate) in the presence of a condensing agent or dehydrating agent. Sulphuric acid is generally used as condensing agent for simple monohydric phenols [31]. The highly reactive ethylacetoacetate reacts with LBT in the presence of sulphuric acid to cyclize into a coumarin ring giving yellowish green fluorescence.

The mechanism is thought to involve the initial formation of a β-hydroxyester, which then cyclizes and dehydrates to yield the coumarin.



The above pathway was postulated by analogy to the reported mechanism for other monohydric phenols [26,28].

4. Conclusion

A specific method was developed to the determination of LBT in dosage forms and spiked human urine. The method can measure up to 1.0 μg/ml with good accuracy. The minimum detectability (2.16×10^{-6} M) is comparable to those obtained by chromatographic methods. The method is simple, rapid and readily adaptable to routine quality control laboratories.

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