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Il Farmaco 58 (2003) 293–299
IL FARMACO
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Spectrophotometric determination of labetalol in pharmaceutical preparations and spiked human urine

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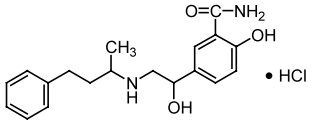
Received 30 March 2002; received in revised form 25 May 2002; accepted 8 June 2002

Abstract

Two simple and sensitive spectrophotometric methods were developed for the spectrophotometric determination of labetaolol (LBT). Both methods are based on the phenolic nature of the drug. The first method (Method I) is based on coupling LBT with diazotized benzocaine in presence of trimethylamine. A yellow colour peaking at 410 nm was produced and its absorbance is linear with the concentration over the range 1–10 μg ml^{-1} with correlation coefficient ($n = 5$) of 0.9993. The molar absorptivity was 2.633×10^4 l mol^{-1} cm^{-1}. The second method (Method II) involves coupling LBT with diazotized *p*-nitroaniline in presence of sodium carbonate. An orange colour peaking at 456 nm was obtained and its absorbance is linear with concentration over the range 1–10 μg ml^{-1} with correlation coefficient ($n = 5$) of 0.99935. The stoichiometry of the reaction in both cases was accomplished adopting the limiting logarithmic method and was found to be 1:1. The developed method could be successfully applied to commercial tablets. The results obtained were in good agreement with those obtained using the official methods. No interference was encountered from co-formulated drugs, such as hydrochlorothiazide. The method was further extended to the in-vitro determination of LBT in spiked human urine. The % recovery ($n = 4$) were 97.7 ± 5.75 and 103.27 ± 5.42 using the Methods I and II, respectively.

Keywords: Labetalol; Diazotized benzocaine; Diazotized *p*-nitroaniline; Dosage forms; Urine

1. Introduction



Structural Formula of Labetalol. HCl

Labetalol (LBT) hydrochloride: 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl] salicylamide hydrochloride, 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–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 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 of the raw material and spectrophotometric measurement at 302 nm for the tablets. The USP [3], on the other hand, recommend 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]. This led us to study the reaction

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0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(02)01291-0

of LBT with diazonium salts in an attempt to develop a simple, sensitive and reliable method for its determination in dosage forms and spiked biological fluids. The results obtained were satisfactorily accurate and precise.

2. Experimental

2.1. Apparatus

Unicam UV–Vis spectrophotometer, Helios alpha, Helioc beta, Cambridge, UK.

2.2. Materials and reagents

All reagents and solvents were of Analytical Reagent (AR) grade, and water was always double distilled.

- Reference standard sample of LBT HCl was obtained from Glaxo Welcome Middlesex, UK through Drug Control Center, Riyadh, Saudi Arabia.
- Tablets containing LBT·HCl were obtained from the local market: Trandate Tablets (Batch # 505641) labeled to contain 100 mg LBT HCl/tablet and Trandate tablets (Batch # 503962) labeled to contain 200 mg LBT·HCl/tablet.
- Urine: was collected from healthy volunteers (males, around 40-year-old) and kept frozen until use after gentle thawing.
- Benzocaine: (Sigma, St. Louis, MO, USA). 1% solution was prepared in MeOH.
- *p*-Nitroaniline: (Sigma, St. Louis, MO, USA).
- Sodium nitrite: (BDH, Poole, UK) 1 and 2% aqueous solutions were prepared.
- Sodium carbonate: 10% (w/v) aqueous solution.
- Acid mixtures: prepared by mixing 86 ml of 15% (w/v) trichloroacetic acid, 20 ml of 2 M H_2SO_4 and 94 ml of distilled water.
- Carbonate buffer, pH 9.4 [3].
- Preparation of diazotized *p*-nitroaniline (DPNA): dissolve 160 mg of *p*-nitroaniline in 8 ml of HCl, cool in ice-bath then add 8 ml of 2% $NaNO_2$ solution. Leave for 10 min then complete to 100 ml with distilled water. The solution is stable for about 5 h.
- Standard drug solution: 0.05% aqueous solution was prepared and further diluted as appropriate to get the working standard solution. The solutions were stable for at least 3 days if kept in the refrigerator.

2.3. Calibration graphs

2.3.1. Diazotized benzocaine (DBC) method

Into a series of 25 ml standard flasks add 1 ml of benzocaine solution, 1 ml of the acid mixture and 1 ml of $NaNO_2$ (1%) solution, mix well and leave to stand for 15 min. Add 2.5 ml of EtOH (95%) followed by an aliquot volume of the working standard solution of LAB·HCl. Mix well and leave for 5 min. Add 4 ml of Et_3N and complete to the mark with distilled water then mix well. Measure the absorbance at 410 nm against a reagent blank. The blank is prepared exactly like the test solution but the sample is omitted. Plot the final concentration to get the calibration graph. Alternatively, derive the regression equation.

2.3.2. Diazotized p-nitroaniline (DPNA) method

Into a series of 25 ml measuring flask transfer 1 ml of DPNA solution followed by 1 ml of EtOH. Transfer aliquot volumes of the working standard solution of LAB·HCl and leave for 5 min. Add 1 ml of Na_2NO_3 solution then complete to the mark with distilled water. Measure the absorbance at 456 nm against a reagent blank. Plot the absorbance versus the final concentration to get the calibration graph. Alternatively, derive the regression equation.

2.4. Analysis of commercial tablets

Weigh and pulverize 20 tablets. Transfer a weighed quantity of the powder equivalent to 50 mg of LAB·HCl into a small flask. Extract with 3×30 ml of water and filter into a 100-ml volumetric flask. Wash the flask and filter and pass the washings into the same measuring flask. Complete to the mark with water and mix well. Proceed as described under Method I or II. Calculate the nominal content of the tablets either from the calibration graph or using the corresponding regression equation.

2.5. Analysis of urine samples

Transfer an aliquot volume of spiked human urine into a small separating funnel. Add 5 ml of carbonate buffer of pH 9.4. Extract with 3×10 ml of ether and collect the ether in an evaporating dish. Evaporate the ether under a stream of nitrogen and dissolve the residue in ethanol. Proceed as under Method I or II. Calculate the concentration of LBT in urine from the corresponding regression equation.

3. Results and discussion

LBT exhibits a low absorption of light in the UV region with $A_{1\,\mathrm{cm}}^{1\%}$ of 85 at 302 nm [26]. 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 diazotized benzocaine (DBC) or DPNA to produce coloured products peaking at 410 nm and 456 nm, respectively

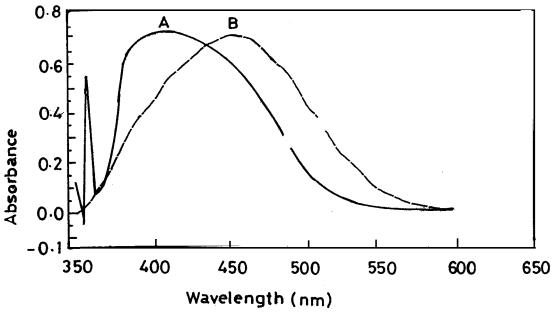


Fig. 1. Absorption spectra of the reaction product of LBT·HCl (6 μg ml^{-1}) with each of: (A) diazotized benzocaine; (B) DPNA.

(Fig. 1). The spectrophotometric properties of the coloured products as well as the different parameters affecting the colour development and its stability were extensively studied in order to determine the optimal conditions for the assay procedures. The reactions were studied as a function of volume of DBC and DPNA, sodium nitrite and the alkalinizing agent (trimethylamine or sodium carbonate). In addition, the effect of time was studied regarding the formation of the diazonium salt, formation of the reaction products and their stability.

4. Study of the experimental conditions

4.1. Method I

The effect of volume of DBC on the colour development was studied. Fig. 2 shows that the volume of DBC is critical, 1.0 ml of the solution gave the highest absorbance reading beyond which the absorbance decreased gradually. Increasing the volume of sodium nitrite solution was found to produce a proportional increase in absorbance up to 1 ml. This volume is also critical. The volume of the acid mixture should not be less than 1 ml, larger volumes also decrease the absorbance readings (Fig. 2). Using either one of the two acids gave lower absorbance readings. The volume of sodium nitrite solution had the same effect; 1 ± 0.1 ml gave the highest absorbance values. The diazocoupling reaction must be conducted in alkaline medium. Trimethylamine, 3.5 ± 0.2 ml gave the highest readings (Fig. 2). The azodye is formed immediately, reaches maximum intensity within 5 min and remains stable for at least 120 min.

4.2. Method II

In this method, the diazonium salt (DPNA) is stable enough to be prepared in stock and used as appropriate. It was found to be stable for about 5 h. The azodye formed is partly soluble in the aqueous medium, therefore, ethanol had to be added to assist its solubility; 1 ml was sufficient (Fig. 3). The diazocoupling reaction must be performed in alkaline medium, therefore, sodium carbonate solution (10%, w/v) was used. One millilitre gave the highest absorbance reading (Fig. 3). The azodye was formed immediately and remained stable for at least 120 min. In both methods, the excess of nitrous acid had no effect on the reaction product, neither its formation nor its stability, therefore, no need to get rid of it.

5. Analytical applications

In both methods, the absorbance–concentration plots are rectilinear over the range 1–10 μg ml^{-1} with minimum detectability of 0.42 μg ml^{-1} (1.15×10^{-6} M). Linear regression analysis of the data gave the regression equations cited in Table 1 with correlation coefficients close to unity in both cases. Statistical evaluation of the regression lines, regarding standard deviation of the residuals ($S_{x/y}$), standard deviation of the slope (S_b) and standard deviation of the intercept (S_a) gave the values given in Table 1. In both cases, the values are very small, pointing out to a highly precise method [27].

The appreciable values of the molar absorptivity (Table 1) and the stability of the reaction products permitted the determination of LBT in its commercial tablets (100 and 200 mg each). The results obtained were favourably compared with those given with the official method [2]. There was no significant difference between the two methods regarding accuracy and precision as revealed by applying the t- and F-test, respectively [27].

Tablet excipients, such as talc, starch, lactose, avisil, gelatin and magnesium stearate did not interfere with the assay. Hydrochlorothiazide, which is frequently co-formulated with LBT also did not interfere. The % relative error due to its presence did not exceed 1% (Table 2).

The proposed method was further extended to the in-vitro determination of LBT in spiked human urine samples using DBC and DPNA (Scheme 1). LBT is orally administered in a dose of 100 or 200 mg three times daily. These doses result in a urine level of concentration of about 2–4 μg ml^{-1}, which lies well within the working range of the method. A prior extraction step, adopting the method of Gergov et al. [19] was conducted. A calibration graph using DBC was first constructed by plotting the absorbance versus the added concentration of LBT. Linear regression analysis of the data gave the following equaton:

$$A = 0.049C - 5.1 \times 10^{-3}, \quad R = 0.99498$$

where C is the concentration of LBT in urine (μg ml^{-1}). Statistical evaluation of the regression line using DBC

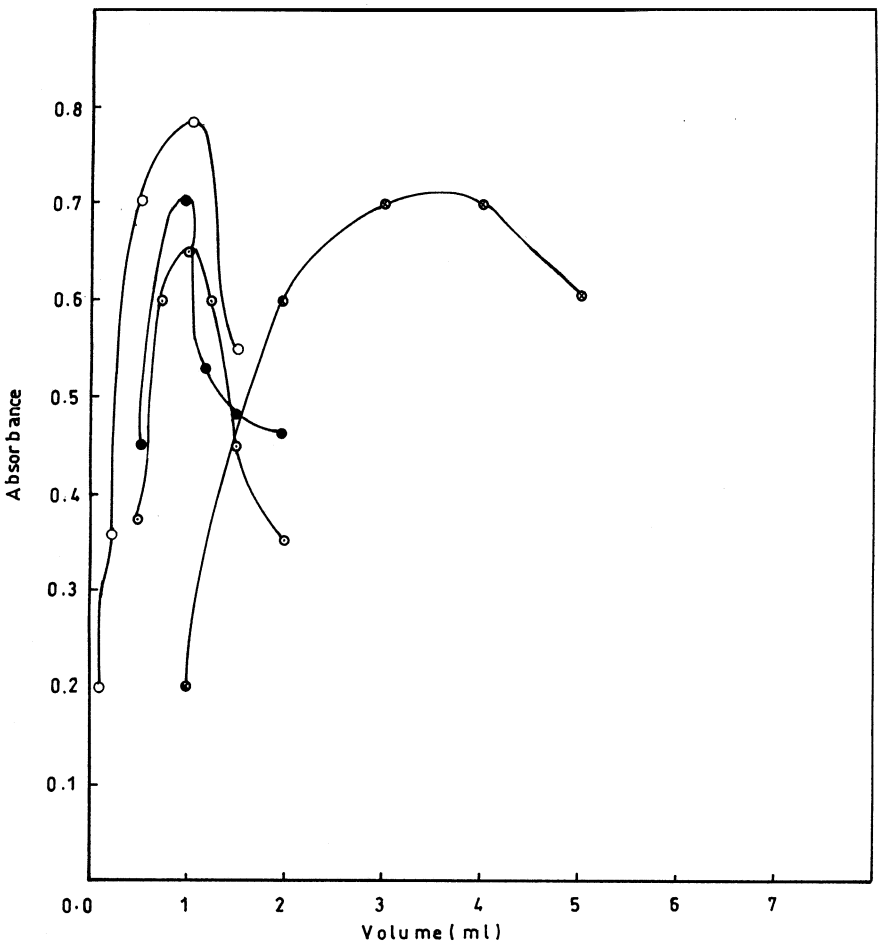


Fig. 2. Effect of reagents on the development of the reaction product (Method I) of LBT·HCl (6 μg ml^{-1}) with each of: ○–○, benzocaine; ●–●, acid mixture; ⊙–⊙, sodium nitrite; ⊗–⊗, trimethylamine.

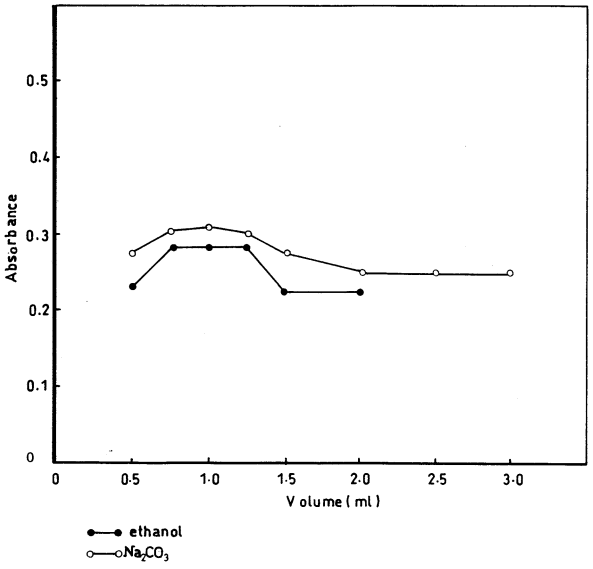


Fig. 3. Effect of reagents on the development of the reaction product (Method II) of LBT·HCl (6 μg/ml) with each of: ●–●, sodium carbonate; ○–○, ethanol.

regarding standard deviation of the residuals ($S_{x/y}$), standard deviation of the intercept (S_a) and standard deviation of the slope (S_b) gave the following values: 0.021, 0.02 and 2.69×10^{-3}, respectively. These small values point out to the high precision of the method [27].

Table 1
Performance data of the proposed methods

Parameter	Method I (DBC)	Method II (DPNA)
λ_{max} (nm)	410	456
Concentration range	1–10 μg ml^{-1}	1–10 μg ml^{-1}
$A^{1\%}_{1\ cm}$	721	630
Molar absorptivity (ε) (l mol^{-1} cm^{-1})	2.73×10^4	2.27×10^4
Minimum detectability	0.42 μg/ml (1.15×10^{-6})	0.42 μg/ml (1.15×10^{-6})
Regression equation	$A = 0.072C - 0.018$	$A = 0.0175 + 0.057C$
Correlation coefficient	0.99949	0.99899
$S_{y/x}$	0.01	7.92×10^{-3}
S_b	1.28×10^{-3}	1.015×10^{-3}
S_a	7.78×10^{-3}	6.16×10^{-3}

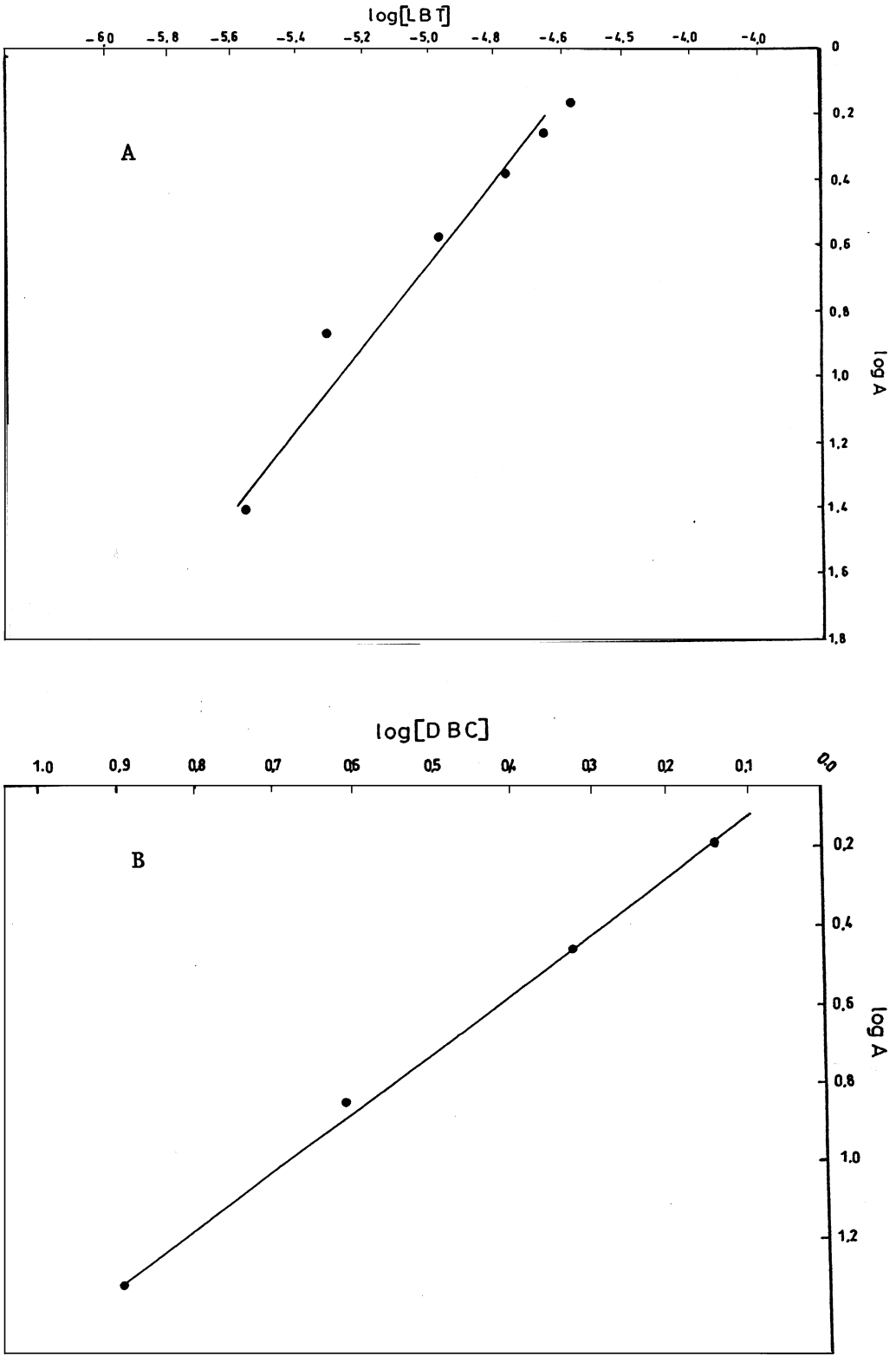


Fig. 4. Limiting logarithmic plots for the molar reactivity of LBT with diazotized benzocaine: (A): log absorbance vs. log[LAB·HCl] which [DBC] kept constant; (B) log absorbance vs. log[DBC] with [LAB·HCl] kept constant.

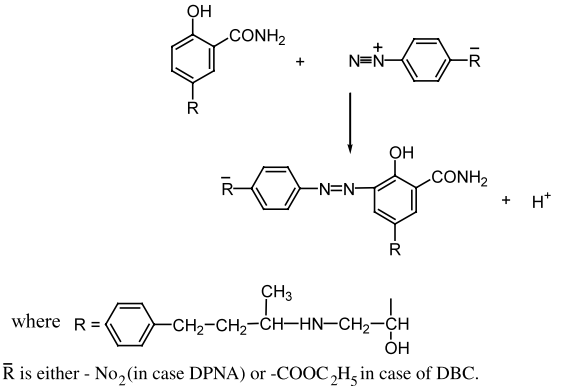
Table 2
Application of the proposed method to the determination of LBT in tablets

Preparation	Proposed method		Reference method [2]
	Method I (DBC)	Method II (DPNA)	
Trandate tablets (LBT·HCl, 100 mg/tablets)	101.56±0.13	101.53±0.15	101.70±0.126
n	5	5	6
t	1.82	2.05	
F	1.062	1.43	
Trandate tablets (LBT·HCl, 100 mg/tablets)	101.7±0.10	101.58±0.151	101.75±0.134
n	5	5	6
t	0.7	1.99	
F	2.05	1.27	
Trandate tablets (LBT·HCl, 200 mg/tablets)	101.8±0.128	101.81±0.152	101.79±0.126
n	5	5	6
t	0.13	0.23	
F	1.06	1.56	

The tabulated values of *t* and *F* at 95% confidence level are 2.262 and 5.19, respectively.

Table 3
Application of the proposed method to the determination of LBT in spiked human urine

Amount added (μg ml^{-1})	Method I (DBC)		Method II (DPNA)	
	Amount found	% Recovery	Amount found	% Recovery
4.0	3.6755	91.89	4.395	109.88
6.0	5.553	92.55	6.4190	106.98
8.0	8.737	109.21	7.705	96.31
10.0	9.716	97.16	9.990	99.90
$\bar{X}$		97.70		103.27
±SD		5.753		5.418



$\bar{R}$ is either - No_2(in case DPNA) or -$COOC_2H_5$ in case of DBC.

Scheme 1. Proposed pathway for the reaction of LBT with each of DBC and DPNA.

The method was then applied to spiked human urine samples. The results obtained using both DBC and DPNA are abridged in Table 3 and are satisfactorily accurate and precise.

6. Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [28]. Two straight lines were obtained upon using increasing concentrations of LBT·HCl while keeping the concentration of DBC constant (Fig. 4A) and upon using increasing concentrations of the reagent while keeping the concentration of the LBT·HCl constant (Fig. 4B). The slopes of the two straight lines are 1.18 and 1.302. This means that the reaction proceeds in a molar ratio of 1.18:1.302, i.e. in a ratio of 1:1. Based on this fact, and depending on the phenolic nature of LBT, the reaction is proposed to proceed as in Scheme 1.

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