

High Performance Liquid Chromatographic Method for the Simultaneous Determination of Labetalol and Hydrochlorothiazide in Tablets and Spiked Human Plasma

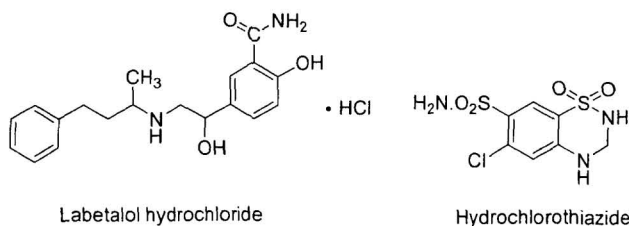
M. Sultan, H. Abdine, N. Zoman and F. Belal*

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Saudi Arabia

Abstract

A reversed-phase HPLC method with spectrophotometric detection was developed for the simultaneous determination of labetalol (LBT) and hydrochlorothiazide (HCD). The chromatographic separation was performed using a Microbondapak C₁₈ column (4.6 i.d. × 250 nm) and paracetamol as internal standard. A mobile phase consisting of 0.05 M phosphate buffer/acetonitrile of pH 4 (7:3) at a flow rate of 0.7 ml/min was used. The detection was affected spectrophotometrically at 302 nm. The working concentration range was 0.3–10 µg/ml with detection limits of 0.05 µg/ml for both drugs. The lower quantitation limit was 0.25 µg/ml in the two cases. The method was successfully applied to tablets, the % recoveries were 99.45±0.68 for LBT and 99.79±0.75 for HCD. The method was extended to the *in-vitro* determination in spiked human plasma. The % recoveries were 91.12±0.33 for LBT and 91.37±0.40 for HCD. The interday and intraday precision and accuracy were evaluated in plasma by calculating the % RSD (n=5) and the % error and were found to be in the ranges of 1.18–4.1% and 0.38–0.36% for both drugs, respectively.

Introduction



Labetalol hydrochloride: 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)-

ethyl]salicylamide hydrochloride. (LBT) is a non-cardiovascular β -blocker drug with selective α_1 -blocking properties which decrease peripheral vascular resistance. It is frequently used in the management of hypertension.^[1]

Hydrochlorothiazide: 6-Chloro-3,4-dihydro-2H,1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide. (HCD) is widely used as a potent diuretic of low toxicity.^[1]

Both drugs are official in the British Pharmacopoeia^[2] (BP) and in the United States Pharmacopoeia^[3] (USP). The BP^[2] described a nonaqueous titration procedure for the determination of LBT-HCl and HCD in bulk and a spectrophotometric method for their determination in tablets. The USP, however, recommended high performance liquid chromatography (HPLC) for the analysis of both LBT-HCl and HCD in bulk and in tablets. Several other methods have been reported in the literature for the determination of LBT, both in formulations and in biological fluids; these include spectrophotometry,^[4-6] NMR spectroscopy,^[7] TLC,^[8] HPLC,^[9-11] LC-MS,^[12-13] micellar liquid chromatography,^[14] capillary electrophoresis^[15] and capillary liquid chromatography.^[16]

Similarly, several methods have been reported for the determination of HCD in bulk, formulations or biological fluids. These include spectrophotometric,^[17-19] HPLC^[20-22] and polarographic methods.^[23,24] Although labetalol and hydrochlorothiazide are frequently co-formulated together in tablets, yet, there is no reported method for the simultaneous analysis of such mixture. This led us to search for a simple, rapid and accurate method for that purpose and HPLC proved to be suitable tool for the analysis of the mixture. The method has been applied for the determination of both components in laboratory-made mixtures, in tablets and was further extended to the *in-vitro* determination in spiked human plasma using the internal standard method. The results obtained were satisfactorily accurate and precise.

Experimental

Reagents and Materials

Reference standard samples of LBT.HCl and HCD were obtained from Glaxo-Welcome, Middlesex, UK, through Drug Control Center, Riyadh, Saudi Arabia, their purity was checked according to the British Pharmacopoeia (BP) method.^[2] Synthesized tablets were prepared in lab to contain 200 mg LBT.HCl and 40 mg HCD/tablet.

Human plasma was obtained from King Khalid University Hospital, blood bank, King Saud University, Riyadh and kept frozen until use after gentle thawing.

Orthophosphoric acid, disodium hydrogen phosphate, ammonia solution and ether were of Analytical Reagent grade, acetonitrile and methanol were HPLC grade; these chemicals were purchased from Merck (Darmstadt, Germany).

HPLC Instrumentation

Optimized Chromatographic Conditions:

Column: μ BondapakTM C₁₈ μ m (4.6 mm id \times 250 mm).
Eluent: (0.05 M) phosphate buffer/acetonitrile (7:3) pH = 4 adjusted with phosphoric acid.
Detector: Attenuation = 16, at 302 nm.
Flow rate: 0.7 mL min⁻¹.
Temperature: Ambient (20–23°C).
Injection volume: 20 μ l sample loop.

Standard Solutions

Individual stock solutions of LBT.HCl, HCD and paracetamol (Internal Standard, IS) were prepared in methanol to give final concentrations of 200 μ g mL⁻¹. Each solution was then diluted as appropriate.

Calibration Curves

Aliquots of the LBT.HCl or HCD stock solutions were transferred into separate 25 ml volumetric flasks to obtain concentrations ranging between 0.3 μ g mL⁻¹–10 μ g mL⁻¹. 1.0 ml of IS solution was added to each flask, then completed to the mark with the mobile phase. Make triplicate 20 μ l injections for each solution. The peak-area ratio of each concentration to the IS against the corresponding standard concentration were plotted to obtain the calibration graphs. Alternatively, the corresponding regression equation was derived.

Analysis of Authentic Mixtures

Volumes of the stock solutions of LBT.HCl and HCD (in the range 0.4–1.0 μ g mL⁻¹) at different ratios (1:1, 5:1 and 2:1) were mixed then 1 ml of IS was added to each solution and completed to the appropriate volume with the mobile-phase. Triplicate 20 μ l injections for each solution were made. Calculate the peak-area ratio of each concentration to the IS. The concentration of each drug is obtained using the calibration graph or the corresponding regression equation.

Analysis of Tablets

The method was applied to the determination of LBT.HCl and HCD in the medicinally recommended ratio (5:1). Samples of about 100 mg LBT.HCl and 20 mg HCD were accurately weighed and mixed with appropriate proportions (5:28) of the drug-matrix components. The mixtures were dissolved and diluted quantitatively to 100 ml using methanol. The solutions were sonicated for 15 min, centrifuged at 4000 rpm, for 10 min and the supernatant was used to prepare solutions of various quantities of the two drugs using the mobile phase as a diluent after addition of the IS solution.

Detection and Determination of LBT.HCl and HCD in Spiked Human Plasma

To 10 × 75 mm glass culture tubes containing 500 µl of human plasma, 20 µl of each LBT.HCl and HCD were added and left to stand for 20 min. A 20 µl aliquot of paracetamol was added as the internal standard, complete to 1 ml with ammonium acetate (pH 9). Each addition included vortexing and centrifugation (4000 rpm for 5–10 min). The aqueous phase was extracted twice with 1 ml of ether by vortexing vigorously for 90 s and then centrifuging at 6000 rpm for 10 min. A fixed volume of the organic phase was removed and evaporated to dryness under a nitrogen steam at ambient temperature. The residue was reconstituted in 1 ml of the mobile phase and triplicate 20 µl injections were made into the liquid chromatographic system.

Blank human plasma samples, which had 60 µl of deionized water instead of the drugs were processed in the same manner.

The percentage recovery of the drug from human plasma was calculated by taking the peak area. From an injection of an extracted sample and dividing it by the peak area from an injection of the corresponding stock solution, X100. Percentage recovery was defined as the experimentally determined plasma concentration of the drug divided by the actual plasma concentration of the drug, X100.

Also, additional samples for the lowest, medium and highest concentrations (0.4, 1.0, 8.0 µg ml⁻¹) were prepared to calculate the inter-day precision and accuracy over 5 consecutive days. On a separate day, a calibration curve and five samples for each of the lowest, medium and highest concentrations were prepared to determine the intra-day precision and accuracy. All samples were extracted and processed as described above.

Results and Discussion

A reversed phase HPLC method was developed to provide a specific procedure suitable for the rapid quality control analysis of binary mixtures containing LBT.HCl and HCD. The method was also extended for their simultaneous determination in spiked human plasma. The method involves the use of an RP-C₁₈ column and a mobile phase consisting of 0.05 M phosphate buffer/acetonitrile (7:3, v/v) of pH 4. The mobile phase was chosen after several trials using various proportions and different pH values. In order to optimize the assay parameters, the effect of acetonitrile and pH on the resolution were studied. At high concentrations of acetonitrile, the peaks of LBT.HCl and HCD overlapped. On using equal ratios of acetonitrile and phosphate buffer, no peaks were observed within the first 15 minutes. Variation of the pH yielded good resolution at pH 4, with reasonable retention times for all peaks.

Ionic strength is an important factor for the resolution of the peaks and the best results were obtained at 0.05 M. Decreasing the ionic strength below 0.05 M decreased the column resolution. Changing the ionic strength of the mobile phase from 0.05 M to 0.01 M resulted in a decrease in the column resolution.

The above described chromatographic system showed an adequate resolution ($R_s = 3.505$) between LBT.HCl ($t_R = 10.227$) and HCD ($t_R = 6.21$) in a reasonable time (Fig. 1) R_s , resolution; t_R , retention time. The proposed method was assessed for linearity, precision, accuracy, sensitivity, specificity and stability.

Linearity

The linearity of the detector responses for both LBT.HCl and HCD were determined by plotting peak area ratios of the drug to the internal standard vs concentrations. The analytical data for the calibration graphs are listed in Tab. 1.

The tab. also shows the values of the standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of the residuals ($S_{y/x}$).

Specificity

The specificity of the method was investigated by observing any interference encountered from the drug matrix. No interference was encountered from common tablet excipients such as talc, starch, lactose, avisil, gelatin and magnesium stearate.

Parameter	Labetalol. hydrochloride	Hydrochlorothiazide
Concentration range (ng/ml)	350–1000	350–1000
Regression equation		
Intercept (a)	– 0.00407	– 0.03231
Slope (b)	0.59642	0.55691
Correlation coefficient (r)	0.9998	0.9998
S_a	2.35×10^{-2}	2.23×10^{-2}
S_b	2.73×10^{-3}	2.60×10^{-3}
$S_{y/x}$	3.20×10^{-2}	3.06×10^{-2}
LOD ($\mu\text{g/ml}$)	0.05	0.05
LOQ ($\mu\text{g/ml}$)	0.25	0.25

Tab. 1. Analytical Data for the Determination of Labetalol. Hydrochloride and Hydrochlorothiazide.

Fig. (1) shows the typical chromatogram of authentic mixture of LBT.HCl and HCD (4.5 $\mu\text{g/ml}$ each) while Fig. (2) shows the chromatogram of LBT.HCl (1.5 $\mu\text{g/ml}$) and HCD (0.85 $\mu\text{g/ml}$) added to plasma. It is clear that plasma components did not interfere with the proposed method.

The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the high values of the correlation coefficients (close to one) and standard deviations.^[25]

Precision and Accuracy

The precision (RSD%) and accuracy (Error%) of the proposed method were assessed by the repeated analysis of plasma samples containing mixtures of LBT.HCl and HCD at different concentrations (Tab. 2). The data indicate that the intra-day precision is in the range of 1.9–3.3% (n=3) and intra-day accuracy is in the range of 1.1–3.7% (n=3) for both LBT.HCl and HCD, and that inter-day precision is in the range of 1.2–4.1% (n=5) and inter-day accuracy in the range of 0.4–3.1% (n=5) for both drugs.

Sensitivity

The limits of detection, LOD and limits of quantification LOQ, were calculated for both LBT.HCl and HCD. The values are given in Tab. 1. The values obtained are comparable to those given with the other chromatographic methods (10–12).

Stability of Analytical Solutions

The stability of the sample solutions at 20°C after preparation was verified by reassaying them (after dilution with the mobile phase). There was no indication of any decomposition of either LBT.HCl or HCD in the samples. Solutions were found to be stable for at least 5 days if kept in the refrigerator.

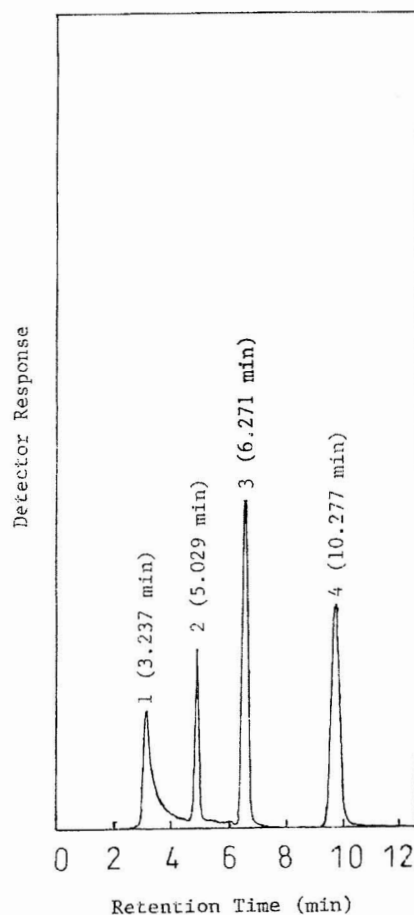


Fig. 1. Typical Chromatogram of Mixture of Labetalol and Hydrochlorothiazide. Conditions: flow rate 0.7 ml/min., UV detection at 302 nm.

- (1): Solvent front (3.937 min)
- (2): Paracetamol (5.029 min).
- (3): Hydrochlorothiazide (6.721 min)
- (4): Labetalol. HCl (10.227 min).

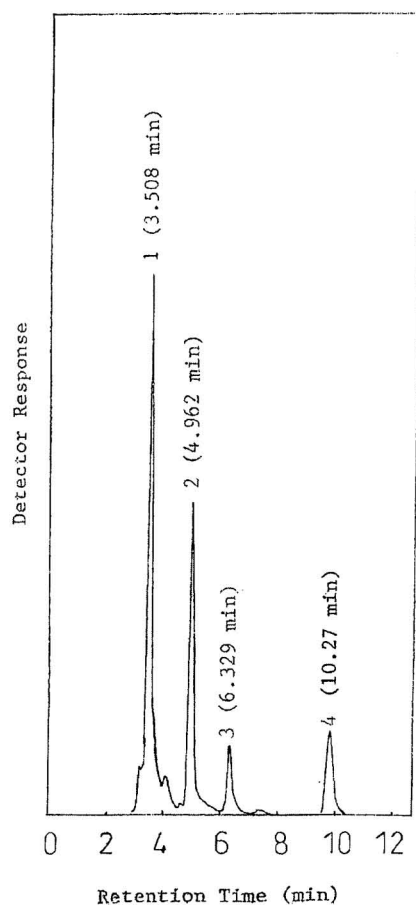


Fig. 2. Typical Chromatogram of Mixture of Labetalol and Hydrochlorothiazide in Plasma. Conditions: flow rate 0.7 ml/min., UV detection at 302 nm.

- (1): Solvent front (3.508 min)
- (2): Paracetamol (4.862 min).
- (3): Hydrochlorothiazide (6.329 min)
- (4): Labetalol. HCl (8.287 min).

Analyte	Conc. Added (ng/ml)	Conc. Found (ng/ml)	Error %	RSD %
Intra-day Labetalol. HCl	500	494.65±14.12 ^a	1.1	2.9
	1000	977.47±28.15	2.3	2.9
	6000	5828.40±159.79	2.9	2.8
Hydrochlorothiazide	500	483.18±9.37	3.4	1.9
	1000	980.00±33.36	2.0	3.3
	6000	5840.60±115.08	2.7	2.0
Inter-day Labetalol. HCl	500	485.39±5.71 ^b	2.9	1.2
	1000	968.58±14.94	3.1	1.5
	6000	5825.88±128.36	2.9	2.2
Hydrochlorothiazide	500	509.99±20.92	2.0	4.1
	1000	986.02±24.59	1.4	2.5
	6000	5977.08±215.83	0.4	3.6

^aMean ± S.D. based on n = 3.

^bMean ± S.D. based on n = 5.

Tab. 2. Accuracy and Precision Data for Labetalol HCl and Hydrochlorothiazide in Plasma.

Applications

Determination of LBT and HCD in Laboratory Prepared Tablets

The validity of the proposed method was studied by assaying laboratory prepared tablets which comprise the binary mixture (200 mg LBT.HCl and 40 mg HCD). Five replicate determinations were made. Satisfactory results were obtained for the recovery of both drugs. The % recoveries ranged from 99.5±0.69 to 100.7±2.4 for LBT and from 99.80±0.88 to 100.4±2.21 for HCD.

The proposed method, however, is not suitable for simultaneously quantify labetalol, hydrochlorothiazide and their possible degradation products, often seen during storage conditions.

Determination of LBT and HCD in Spiked Human Plasma

Plasma sample (n=6) were prepared by spiking blank plasma with LBT.HCl and HCD followed by liquid-liquid extraction and then the solution was injected once on the same day. The absolute recoveries of both drugs were determined by a

comparison of the extracted analyte peak area with the unextracted analyte areas. The results are shown in Tab. 3 are satisfactorily accurate and precise.

Compound	Spiked Concentration ng/ml	Recovery* (%) (Mean \pm S.D. n = 5)	RSD
Labetalol HCl	400	91.58 \pm 2.6	2.8
	2000	90.95 \pm 2.9	3.2
	8000	90.84 \pm 2.9	3.2
Hydrochlorothiazide	400	91.35 \pm 2.5	2.7
	2000	91.87 \pm 2.7	2.9
	8000	90.88 \pm 3.2	3.5

Tab. 3. Determination of Labetalol HCl and Hydrochlorothiazide in Spiked Human Plasma.

*Recoveries were calculated by a comparison of the extracted peak area.

The proposed method, however, is not appropriate to detect the metabolites in plasma.

Conclusion

A reversed phase HPLC method has been developed and validated for the simultaneous determination of LBT.HCl and HCD in tablets and in spiked human plasma. The assay is fast and requires a relatively simple sample preparation. The procedure is suitable for the separation and quantification of each drug in 0.3–10 μ g/ml range. The lower limits of detection are comparable to those given with the other chromatographic methods.

References

- [1] Parfitt K, editor.
In: Martindale, the Complete Drug Reference, 32nd ed. Massachusetts, 1999, 896.
- [2] British Pharmacopoeia, HMSO, Vol. I and II, London, 2000.
- [3] The United States Pharmacopeia XXIV, the National Formulary, 19.
Rockville, Md., USA, 2000.

- [4] Belal F, Al-Shaboury S, Al-Tamrah AS.
Spectrofluorometric Determination of Labetalol in Pharmaceutical Preparations and Spiked Human Urine Through the Formation of Coumarin Derivative.
J. Pharm. Biomed. Analysis 2002;30:1191–1196.
- [5] Belal F, Al-Shaboury S, Al-Tamrah AS.
Spectrophotometric Determination of Labetalol in Pharmaceutical Preparations and Spiked Human Urine.
IL-Farmaco 2002;58:293–299.
- [6] Sastry CSP, Krishna DM.
Spectrophotometric Methods for the Determination of Labetalol-HCl in Pure and Dosage Forms.
Mikrochim. Acta 1996;122:87–93.
- [7] Iorio MA, Mazzeo-Frina A, Doldo A.
Quantitative Analysis of β -adrenergic Blocking Agents by NMR Spectroscopy.
J. Pharm. Biomed. Anal. 1987;5:1–10.
- [8] Witek A, Hopkala H, Matysik G.
TLC-Densitometric Determination of Bisoprolol, Labetalol and Propafenone as Dabsyl Derivatives in Pharmaceutical Preparations.
Chromatographia 1999;50:41–44.
- [9] Zhao H, Li HZ, Qiu ZY.
Separation of Enantiomeric Labetalol by Reversed Phase High-Performance Liquid Chromatography.
Sepu. 1999;17:369–371.
- [10] Bazylak G, Aboul-Enein HY.
Direct Separation of Labetalol Stereoisomers in a High-Performance Liquid Chromatography System Using Helically Self-Assembled Chelate as Chiral Selector in the Mobile Phase.
J. Liq. Chromatogr. Relat. Technol. 1999;22:1171–1192.
- [11] Cenicerros C, Maguregui MI, Jimenez RM, Alonso RM.
Quantitative Determination of the β -blocker Labetalol in Pharmaceuticals and Human Urine by High-Performance Liquid Chromatography with Amperometric Detection.
J. Chromatogr. B. 1998;705:97–103.
- [12] WQ JC, Lord HL, Pawliszyn J, Kataoka H.
Polypyrrole-Coated Capillary in Tube Solid Phase Microextraction Coupled with Liquid Chromatography-Electrospray Ionization Mass Spectrometry for the Determination of β -Blockers in Urine and Serum Samples.
Microcolum. Sep. 2000;12:255–266.
- [13] Gergov M, Robson JV, Duchoslav E, Ojanpera I.
Automated Liquid Chromatography-Tandem Mass Spectrometric Method for Screening β -blocking Agents in Urine.
J. Mass. Spectrom. 2000;35:912–918.

- [14] Carda-Broch S, Rapado-Mortinez R, Estevo-Romero I, Garcia-Alvarez-Coque MC.
Analysis of Urine Samples Containing Cardiovascular Drugs by Micellar Liquid Chromatography with Fluorimetric Detection.
J. Chromatogr. Sci. 1999;37:93–102.
- [15] Wang F, Khaledi MG.
Capillary Electrophoresis Chiral Separations of Basic Compounds Using Cationic Cyclodextrin.
Electrophoresis 1998;19:2095–2100.
- [16] Karlsson C, Wikstrom H, Armstrong DW, Owens PK.
Enantioselective Reversed Phase and Non-aqueous Capillary Electrochromatography Using a Tecoplanin Chiral Stationary Phase.
J. Chromatogr. A. 2000;897:349.
- [17] Abdine HH, El-Yazbi FA, Shaalan RA, Blaih SM.
Direct-differential Solubility and Compensatory Derivative Spectrophotometric Methods for Resolving and Subsequently Determining Binary Mixtures of Sme Antihypertensive Drugs.
STP-Pharm-Sci. 1999;9:587–591.
- [18] Panderi IE.
Simultaneous Determination of Benazepril Hydrochloride and Hydrochlorothiazide in Tablets by Second-order Derivative Spectrophotometry.
J. Pharm. Biomed. Anal. 1999;21:257–265.
- [19] El-Yazbi FA, Abdine HH, Shaalan RA.
Spectrophotometer and Spectrofluorometric Methods for the Assay of Lisinopril in Single and Multicomponent Pharmaceutical Dosage Forms.
J. Pharm. Biomed. Anal. 1999;19:819–827.
- [20] Zecevic M, Zivanovic LJ, Agatonovic-Kustrin S, Ivanovic D, Maksimovic M,
Statistical Optimization of a Reversed-Phase Liquid chromatographic Method for the Analysis of Amiloride and Hydrochlorothiazide in Tablets.
J. Pharm. Biomed. Anal. 2000;22:1–6.
- [21] Panderi IE, Parissi-Poulou M.
Simultaneous Determination of Benazepril Hydrochloride and Hydrochlorothiazide by Micro-Bore Liquid Chromatography.
J. Pharm. Biomed. Anal. 1999;21:1017–1024.
- [22] Kartal M, Erk N.
Simultaneous Determination of Hydrochlorothiazide and Amiloride Hydrochloride by Ratio Spectra Derivative Spectrophotometry and High Performance Liquid Chromatography.
J. Pharm. Biomed. Anal. 1999;19:477–485.
- [23] Martin ME, Hernandez OM, Jimenez AL, Arias JJ, Jimenez F.
Partial Least-Squares Method in Analysis by Differential Pulse Polarography. Simultaneous Determination of Amiloride and Hydrochlorothiazide in Pharmaceutical Preparations.
Anal. Chim. Acta. 1999;381:247–256.

- [24] Liang YN, Sun JH.
Quantitative Determination of Hydrochlorothiazide by First-Derivative
Differential Pulse Polarography.
Fenxi-Shiyanshi. 1994;13:57–59.
- [25] Miller JC, Miller JN, editors.
Statistics for Analytical Chemistry.
Ellis Horwood Chichester, 1984;22:82.

Received July 30th, 2003
Accepted January 23th, 2004