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Research Article

MICELLAR LIQUID CHROMATOGRAPHIC ANALYSIS FOR SIMULTANEOUS DETERMINATION OF ATENOLOL AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: The present study was designed with an objective of a simple, fast, precise, selective and accurate Micellar liquid chromatographic method was developed and validated for the simultaneous determination of atenolol and hydrochlorothiazide from bulk and and formulations.

Method: The method uses C18 stationary phases and micellar mobile phases of 0.07M sodium dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15% (v/v) 1- propanol as organic modifier and ultraviolet detection at 225 nm are used for the determination.

Results: Under these conditions, the studied atenolol and hydrochlorothiazide elute between 6.642 ± 0.10 and 2.467 ± 0.01 min at a 1.5 mL/min flow rate. The method showed excellent linearity in the range of 4–48 µg/mL and 1–12 µg/mL with the limit of detection (S/N = 3) 2 µg/mL and 0.1 µg/mL for atenolol and hydrochlorothiazide, respectively. The suggested method was successfully applied to the analysis of the studied atenolol and hydrochlorothiazide in tablet formulation with the respective average recoveries of 99.77 ± 0.20 and 99.55 ± 0.27%.

Conclusion: The method developed can be used for the routine analysis of atenolol and hydrochlorothiazide from their combined dosage form.

Keywords: Micellar liquid chromatography, Atenolol and hydrochlorothiazide

INTRODUCTION

Atenolol, 4-(2-hydroxy-3- [(1-methylethyl) amino] propoxy] benzeneacetamide, is an antihypertensive, antianginal, and antiarrhythmic. Hydrochlorothiazide (HCTZ), chemically 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide with diuretic activity. HCTZ inhibits the absorption of sodium and chloride at the beginning of distal convoluted tubule [1]. Atenolol and hydrochlorothiazide are official in the IP, BP and USP [2-4]. Different procedures have been developed in order to determine atenolol and hydrochlorothiazide either alone or in combination with other drugs in pharmaceutical formulations and biological fluids using several analytical techniques such as Spectrophotometric techniques [5-9]. Several methods based on separation techniques, including HPTLC [10-11], LC–MS [12-13] and mainly HPLC [14-18] in reverse phase mode (RP-HPLC) have been also proposed. Because in conventional RP-HPLC are required high concentrations of organic solvents, buffers and gradient elution are required.

Micellar liquid chromatography (MLC) is an alternative mode to the conventional reversed-phase liquid chromatography, in which an aqueous solution of a surfactant above its critical micellar concentration is used as mobile phase. So the mobile phase is composed by surfactant micelles and monomers and the stationary phase remains constantly and reproducibly modified by the adsorption of surfactant monomers [19]. The technique is an interesting alternative because of the lower cost and toxicity, the often improved selectivity, and the separation of compound mixtures of diverse polarity without requiring gradient elution.

In the present work, a new approach was achieved for the analysis of pharmaceutical preparation containing atenolol and hydrochlorothiazide, by micellar liquid chromatography. Different chromatographic conditions were studied in an attempt to optimize a simple, sensitive and selective method for the evaluation of the studied drugs in bulk and dosage forms. In order to adjust the eluent strength of the micellar mobile phase and reduce the analysis time sodium dodecyl sulfate (SDS) and a small amount of 1-propanol was used [20].

MATERIALS AND METHODS

Reagents and standards

The surfactant was used to prepare the different mobile phases assayed: sodium dodecyl sulphate (SDS, 99%, Merck Chemicals, Mumbai, India) anionic surfactant. Surfactants were dissolved in 0.01M aqueous solutions of phosphate buffer pH 3, prepared with disodium hydrogen phosphate and potassium dihydrogen phosphate (analytical reagent, Merck Chemicals, Mumbai, India) to adjust the pH of the micellar eluent. After that, an adequate amount of 1-propanol (HPLC grade, Merck Chemicals, Mumbai, India) was added to the micellar eluent to obtain the working concentration (v/v).

Atenolol and hydrochlorothiazide were supplied, as a gift, by Emcure Pharmaceuticals Ltd., Pune and Torrent Pharmaceutical Ltd. Ahemdabad, India. Betacard-H tablet containing 50 mg atenolol and 12.5 mg hydrochlorothiazide were obtained commercially within their shelf life.

Stock standard solutions of atenolol and hydrochlorothiazide were prepared by dissolving the compound in 0.07M SDS solution. Working solutions were prepared by dilution of the stock standard solutions in the mobile phase solution used. The solutions were stored in the refrigerator at 4^0C and they were stable at least for 15 days. Double distilled water was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum-filtered through 0.45 µm nylon membrane filter (Pall India Pvt. Ltd.).

Instrumental and measurement

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 Plus). The solutions were injected into the chromatograph through a Rheodyne valve, with a 20 µL loop. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. ODS Hypersil C18 (250 mm, 4.6 mm, 5 µm) column was used. The mobile phase flow rate was 1.5 mL/min. an ultrasonic bath was used to remove the air from the mobile phases.

Sample preparation

Twenty tablets of the pharmaceutical formulation Betacard-H (containing 50 mg atenolol and 12.5 mg hydrochlorothiazide) were assayed. They were crushed to a fine powder and an amount of the powder corresponding to approximately 50 mg atenolol and 12.5 mg hydrochlorothiazide was weighed in a 25 mL volumetric flask. The powder obtained was dissolved in methanol. After that, an adequate volume of aliquot was taken and diluted with 0.07M SDS solution and sonication (for 30 min) the solution was diluted to volume with 0.07 M SDS solution and filtered through 0.45 µm nylon membrane

filter (Pall India Pvt. Ltd). Finally, an aliquot of the clean solution was injected into the chromatograph.

Method Validation

Sample Analysis

From the filtered sample solution 40 µg/mL for atenolol and 10 µg/mL for hydrochlorothiazide were injected into the chromatograph. The analysis was repeated five times

Precision

The intra-day precision (RSD, %) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter- day precision (RSD %) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

Limits of Detection and Quantitation

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × SD/S and LOQ = 10 × SD/S, where SD is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and S is the slope of the corresponding calibration plot.

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently, as shown in Fig. 1a & 1b. The identities for atenolol and hydrochlorothiazide were confirmed by comparing their t_R with those of standards.

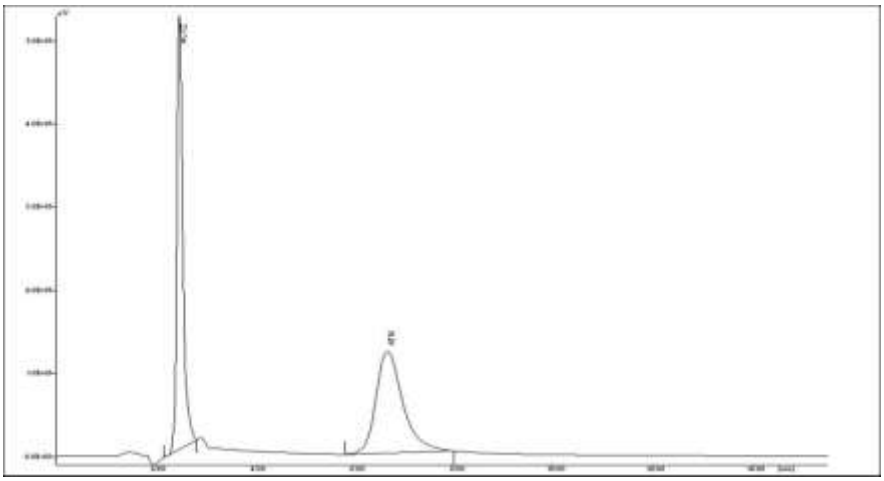


Fig. 1a: Chromatogram of atenolol (t_R = 6.642 min) and hydrochlorothiazide (t_R = 2.467 min) from standard

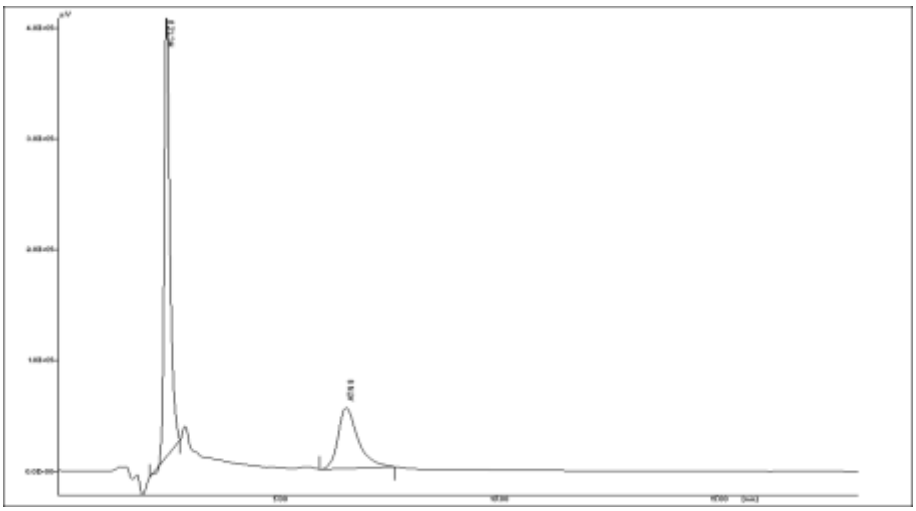


Fig. 1b: Chromatogram of atenolol (t_R = 6.542 min) and hydrochlorothiazide (t_R = 2.458 min) from tablet sample

Accuracy

Analysed samples were overapplied with an extra 80, 100, and 120% of the drugs from standard solutions of atenolol and hydrochlorothiazide and the mixtures were reanalyzed by use of the method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation.

Robustness

Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

RESULTS AND DISCUSSION

Chromatographic efficiencies

The cationic nature of beta blocker and diuretic produces broad asymmetrical peaks in RPLC with aqueous-organic mobile phases and conventional C18 columns, due to the ionic interaction of the charged solutes with the free silanol groups on the alkyl-bonded reversed-phase packings [21]. The use of low pH and the addition of salts or other blocking agents able to bind silanol groups, such as tertiary and quaternary amines, is a common practice to decrease peak tailing of basic drugs. Another alternative is the use of special stationary phases where the silanol groups are base-deactivated [22]. The dissociation

constants (pKa) of atenolol and hydrochlorothiazide in water are in the range 7.9- 9.6 (Table 1).

Although the pH of the mobile phase does not affect their retention, the efficiencies of chromatographic peaks increases and the asymmetries decrease when the pH decreases [23]. For this reason, the experimental work was carried out at low pH (pH 3). Peak efficiency (expressed as theoretical plates, *N*) was estimated at 10% of peak height according to Foley and Dorsey [24]. Asymmetry factors were calculated as the ratio (*B*/*A*) of the distance between the center and the tailing and leading edge of the chromatographic peak, measured also at 10% of peak height. All simulations and optimizations were performed with the software Borwin. Table 2 list the mean values of N and B/A for the atenolol and hydrochlorothiazide eluted from the ODS Hypersil C18 column using micellar SDS–propanol mobile phases. The low efficiencies and highly symmetrical peaks obtained in these conditions are indicative of the presence of free silanol groups in the column, which interact with both the drugs. It should be noted that with the micellar-organic mobile phases, the efficiencies decreased at increasing concentration of the surfactant, and increased with the volume fraction of propanol, which is the expected behaviour. Moreover, both the drugs yielded nearly Gaussian peaks with the SDS–propanol mobile phases.

Table 1: Structures, dissociation constants and (o/w) partition coefficients of the Atenolol and Hydrochlrothiazide

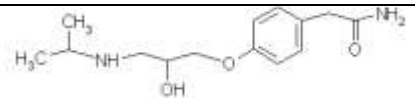
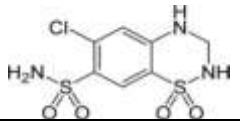
Compound	Structure	pKa
Atenolol		9.6
Hydrochlorothiazide		7.9, 9.2

Table 2: Efficiencies and asymmetry factors for Atenolol and Hydrochlorothiazide eluted with mobile phases of SDS–propanol[a]

Compound	0.07 M SDS		0.1 M SDS		0.15 M SDS	
	N	B/A	N	B/A	N	B/A
Atenolol	3875.57	1.13	3792.83	1.237	3757.24	1.241
Hydrochlorothiazide	2789.49	1.15	2774.60	1.244	2757.24	1.250

[a] Mean values for 15% propanol.

Elution strength

The elution strength was measured for the different micellar-organic system. For MLC, the experimental design consisted of three mobile phases which covered a domain from 0.07 to 0.15M SDS and from 5 to 15% (v/v) propanol. The elution strength (i.e. sensitivity of the retention of solutes) of the surfactant depends on the concentration of organic modifier, and vice versa. The elution strength of propanol changed only slightly for concentrations of SDS between 0.07 and 0.15 M. It decreased at increasing concentration of the surfactant for the most polar drugs. The elution strength of the surfactant usually increased with the concentration of propanol.

Analytical Data

Validation of method

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 4–48 µg/mL for atenolol and 1–12 µg/mL for hydrochlorothiazide. The linear regression equations were Y = 22720X + 721843 (r^2 = 0.9996) for atenolol and Y = 327751X - 180242 (r^2 = 0.9998) for hydrochlorothiazide.

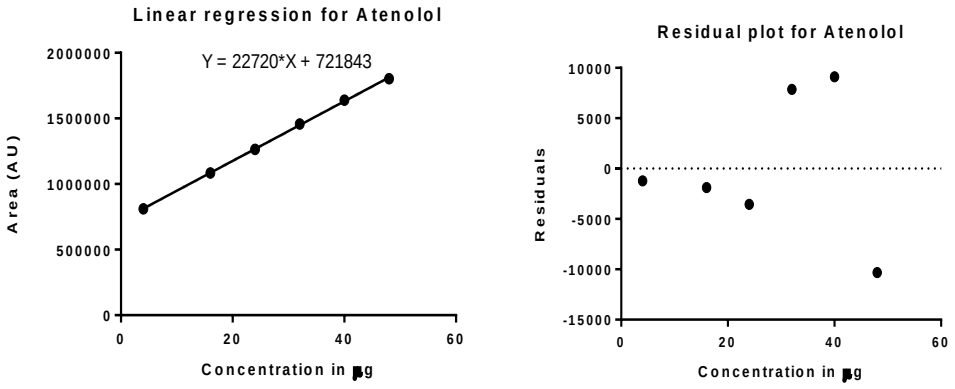


Fig. 2a & 2b: Linear regression for Atenolol and Residual plot for Atenolol

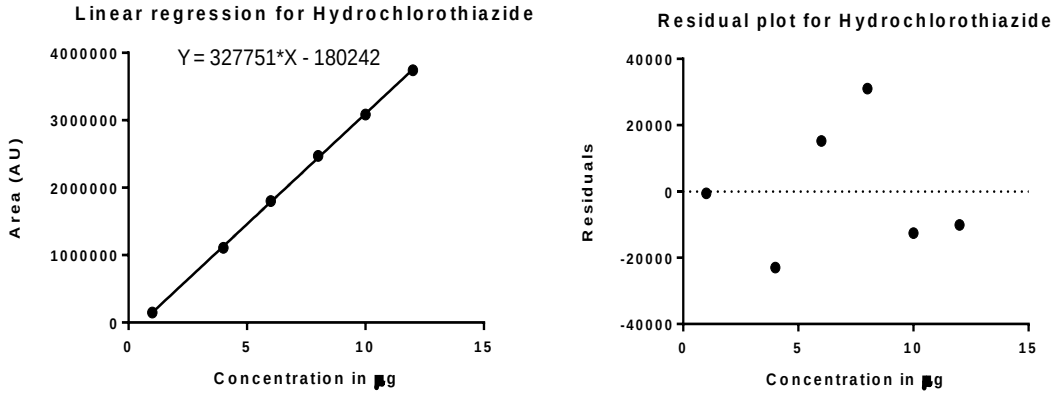


Fig. 3a & 3b: Linear regression for Hydrochlorothiazide and Residual plot for Hydrochlorothiazide

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 3 reveal the high precision of the method.

Limit of Detection and Quantitation

The limit of detection and quantitation calculated were found 2, 5 µg/mL, respectively for atenolol and 0.1, 0.6 µg/mL for hydrochlorothiazide. This indicates the method is sufficiently sensitive.

Accuracy

When the method was used for extraction and subsequent analysis of both drugs from the pharmaceutical dosage forms, and the extract was overapplied with 80, 100, and 120% of additional drug, the recovery was listed in Table 4.

Robustness

The robustness of the proposed method was found after altering the parameters deliberately: the % of propanol in the mobile phase: 14% and 16%, flow rate variants: 1.4 and 1.6 mL/min, the retention time of the compound was evaluated, and the resolution had no significant changes when the parameters were changed. However, there was a change in the retention times with a change in flow rate, but this did not affect the peak symmetry. Each mean value was compared with the mean value obtained by the optimum conditions. The relative standard deviation (% RSD) was found to be less than 2 (Table 5).

Table 3: Precision Study

Concentration (µg/mL)	Intra-day precision			Inter-day precision		
	Measured Conc.	(%) RSD	Recovery (%)	Measured Conc.	(%) RSD	Recovery[a] (%)
Atenolol						
16	15.96	0.55	99.75	15.89	0.93	99.31
32	31.93	0.10	99.78	31.75	0.20	99.22
48	47.83	0.21	99.65	47.51	0.41	98.98
Hydrochlorothiazide						
4	3.97	0.27	99.25	3.95	0.34	98.75
8	7.97	0.70	99.63	7.95	0.93	99.38
12	11.93	0.49	99.42	11.90	0.41	99.17

[a]Mean from three estimates

Table 4: Recovery studies

Drug	Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery[a] (%)
Atenolol	50	80	90	89.58	99.54
		100	100	99.97	99.97
		120	110	109.79	99.81
Hydrochlorothiazide	12.5	80	22.5	22.46	99.82
		100	25.0	24.80	99.20
		150	27.5	27.40	99.64

[a]Mean from five estimates

Table 5: Robustness of the method[a]

Chromatographic factors	Level	Chromatographic changes in % RSD	
		Atenolol	Hydrochlrothiazide
A: Flow rate mL/min.			
1.4	-0.1	1.53	1.26
1.5	0.0	1.39	1.28
1.6	+0.1	1.48	1.35
B: % of propanol in the mobile phase			
% 14	-1.0	1.86	1.76
% 15	0.0	1.64	1.43
% 16	+1.0	1.83	1.59

[a]Mean from three estimates

Sample Analysis

When the Betacard-H tablets were analysed, none of the tablet excipients were found to interfere with the analyte peaks, sharp and well defined peaks for hydrochlorothiazide and atenolol were obtained at t_R 2.458 and 6.542 min, respectively, when scanned at 225 nm. The amount of the label claim measured were 99.59 ± 0.20 % for atenolol and 99.32 ± 0.21 % for hydrochlorothiazide.

CONCLUSION

The proposed micellar chromatographic method has been evaluated over the linearity, precision, accuracy, specificity and proved to be convenient and effective for the quality control of this pharmaceutical formulation. There are certain advantages associated with this method such as high selectivity, sensitivity, economic, less time consuming, less hazardous and low limit of detection. Moreover, the lower solvent consumption along with the short analytical run time leads to a cost effective and environment friendly chromatographic procedure.

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