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Quantitative estimation of propranolol HCL and hydrochlorothiazide in pharmaceutical dosage form by micellar liquid chromatography

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ABSTRACT

Micellar liquid chromatographic method has been developed for the simultaneous determination of Propranolol hydrochloride and Hydrochlorothiazide from bulk and formulations. Chromatographic separation achieved isocratically on ODS hypersil C18 column (5 µm, 250 mm × 4.6 mm) and micellar mobile phase of 0.07M sodium dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15 % (v/v) 1- propanol as organic modifier in the ratio 70: 30 v/v and ultraviolet detection at 274 nm are used for the determination. In the developed method Propranolol hydrochloride and Hydrochlorothiazide elute at typical retention times of 6.767 min and 2.633 min, respectively at a 1 mL/min flow rate. Parameters such as linearity, precision, accuracy, specificity and robustness are studied as reported in the ICH guidelines. Linearity was observed in the concentration range of 4-24 µg/mL for Propranolol hydrochloride and and 2.5-15 µg/mL for Hydrochlorothiazide. The mean recoveries obtained for Propranolol hydrochloride and Hydrochlorothiazide were 99.33 %and 99.23 % respectively. Developed method was found to be accurate, precise, selective and rapid, can be applied routinely for the analysis of Propranolol hydrochloride and Hydrochlorothiazide in bulk as well as tablet dosage form.

Keywords: Propranolol hydrochloride, Hydrochlorothiazide, HPTLC, Validation

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INTRODUCTION

Propranolol hydrochloride (PHCl), (+)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride, is a non-selective beta blocker mainly used in the treatment of hypertension [1-2]. Hydrochlorothiazide (HCTZ) chemically, 6-Chloro-3, 4-dihydro-2H-l, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, is a thiazide diuretic used as antihypertensive agent [3-4]. Propranolol hydrochloride and Hydrochlorothiazide are official in IP and USP [5-6].Literature survey revealed that there are several methods available to determine PHCl and HCTZ either alone or in combination with other drugs in pharmaceutical formulations and biological fluids using various analytical techniques such as spectrophotometric techniques [7-11], several methods based on separation techniques, including HPTLC [12-14], LC–MS [15-16] and HPLC [17-22] have been also reported. 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. 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. The aim of this work was to develop simple and rapid method for the analysis of pharmaceutical preparation containing PHCl and HCTZ. After thorough survey of literature it was found that no published reports about the simultaneous quantitation of PHCl and HCTZ by MLC in bulk drug and in tablet dosage form were reported. Hence a new MLC method has been developed for

the estimation of PHCl and HCTZ in combined dosage form. The developed method is simple, precise, selective, and rapid and can be used for routine analysis. The structures of the drugs are shown in Fig. 1.

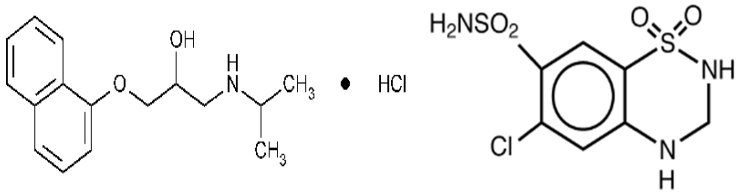

Fig.1.Chemical structures of PHCl (a) and HCTZ (b)

MATERIALS AND METHODS

Chemicals and Reagents:
Reference standards of Propranolol hydrochloride and Hydrochlorothiazide were obtained as gift samples from Piramal Healthcare Ltd. Mumbai and Emcure Pharmaceuticals Ltd., Pune, India. The tablets Ciplar-H (Propranolol HCl-40 mg and Hydrochlorothiazide- 25 mg) of Cipla Ltd. is procured from the local market. For MLC work double distilled water was prepared in the laboratory. 1-propanol, Sodium dodecyl sulphate, disodium hydrogen phosphate and citric acid used were of HPLC grade and analytical grade reagent and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation and chromatographic Conditions:
Chromatographic separation was achieved using MLC System consisted of Intelligent HPLC pump model (Jasco PU 2080 plus), an autosampler and UV/ VIS (Jasco UV 2075 plus) detector. The output signal was monitored and processed using Jasco Borwin version 1.5, LC-Net II/ADC software.

ODS Hypersil C18 (250 mm, 4.6 mm id, 5 µm particle size) was used as the stationary phase. Mobile phase consisting of 0.07M sodium dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15 % (v/v) 1- propanol as organic modifier in the ratio 70: 30 v/v was delivered at a flow rate of 1 mL/min. The detector was set at the wavelength of 274 nm. Injection volume was kept 20 µL.

Preparation of standard and sample solutions
A standard mixed stock solution of PHCland HCTZ was prepared by accurately weighing PHCl(40 mg) and HCTZ (25 mg) into a 100 mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume. The stock solution was further diluted with mobile phase to obtain a solution of PHCL (4 µg/mL) and HCTZ (2.5µg/mL), respectively.

Twenty tablets of the pharmaceutical formulation Ciplar-H (40 mg Propranolol HCl and 25 mg Hydrochlorothiazide) were accurately weighed and average weight determined. They were crushed to a fine powder and an amount of the powder equivalent to 40 mg PHCl and 25 mg HCTZ was weighed and transferred into 100 mL volumetric flask and dissolved in 50 mL of methanol, sonicated for 15 min. and diluted to mark with same solvent and filtered through whatman filter paper no.1. The sample solution was further diluted with mobile phase. The analysis was repeated in triplicate. The possibility of excipients interference with the analysis was examined

System suitability
From the filtered sample solution 4µg/mL for PHCl and 2.5µg/mL for HCTZ were injected into the chromatograph. The analysis was repeated six times to test the system suitability for their retention time, resolution (Rs), theoretical plates number (N) and tailing factors (T).

Analytical Method Validation:
Validation was done as per ICH guidelines [23]. The developed method was validated with respect to parameters such as linearity, LOD and LOQ, precision, accuracy and specificity.

Sample analysis
20 µL of working standard solution and sample solutions were injected into the liquid chromatograph and the chromatograms were recorded. From the peak area of HCTZ and peak area of PHCl the amount of the drugs in the sample were calculated.

Linearityand range
For determining linearity, calibration curves were plotted over a concentration range of 4-24 µg/mL for PHCl and 2.5-15 µg/mL for HCTZ, respectively. A 20 µL of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. Calibration plots were constructed by plotting peak area against the corresponding amount of each drug.

Limit of detection and limit of quantitation:
The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Precision:
Precision studies were carried out to establish the intraday and interday precision of proposed method. 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.

Accuracy
Accuracy of the method was determined by Recovery studies. Recovery studies were carried out by standard addition method. The analysed samples were spiked with extra 80 %, 100 % and 120 % of standard drugs. The mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate and the percentage recovery and relative standard deviation were calculated.

Robustness
To check the robustness of proposed method, small, deliberate changes were made to the chromatographic condition. A study was performed by changing the flow rate and % of propanol in the mobile phase (± 1%). Standard solution prepared as per test method and injected into the MLC system.

RESULTS AND DISCUSSION

Method development
The MLC procedure was optimized for simultaneous determination of PHCl and HCTZ. Good resolution of both the components was obtained with 0.07M sodium dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15 % (v/v) 1- propanol as organic modifier in the ratio 70: 30 v/v. The flow rate of 1 mL/min was optimum. UV detection was made at 274 nm. At this wavelength PHCl and HCTZ can be quantified. Hence, 274 nm determined empirically has been found to be optimum. The average retention times for PHCl and HCTZ was found to be 6.767 min and 2.633 min, respectively (Fig.2).

System suitability
To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1.

Table 1: System suitability parameters (n=6)

Parameters	HCTZ	PHCl
Retention Time in min	2.633	6.767
Resolution (Rs)	0.00	5.421
Theoretical plates number (N)	3765	5174
Tailing Factor	1.007	1.098

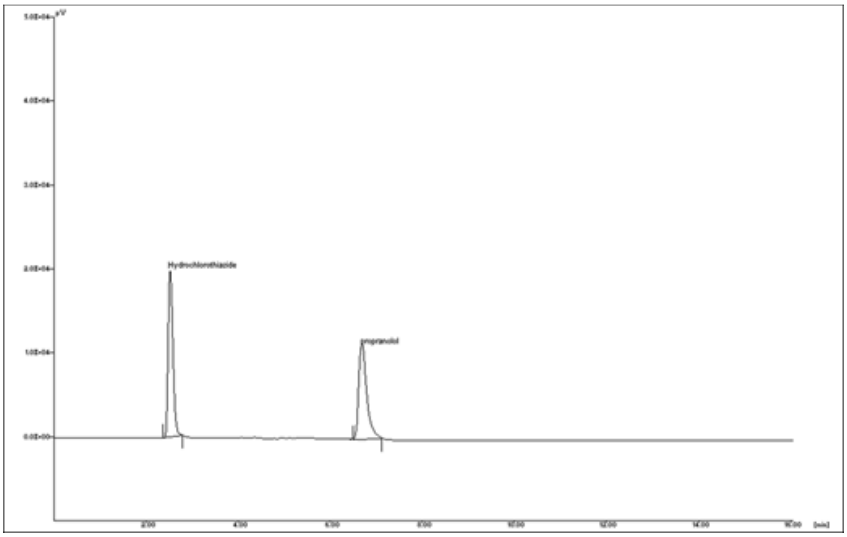

Fig. 2. Chromatogram of HCTZ and PHCl

Validation:
Using optimised chromatographic conditions the MLC method developed was validated in terms oflinearity, LOD and LOQ, precision, robustness and accuracy.

Linearity:
Linearity was accessed by visualizing the calibration graph and plot of the residuals. The points distributed equally above and below the trend line showed linearity. The linear regression equations were Y = 25809X + 319679 (r^2 = 0.9993) for PHCl and Y = 66121X + 389554 (r^2 = 0.9991) for HCTZ. The plots obtained from linear regression are given in fig. 3 for PHCl and fig. 4 for HCTZ, respectively.

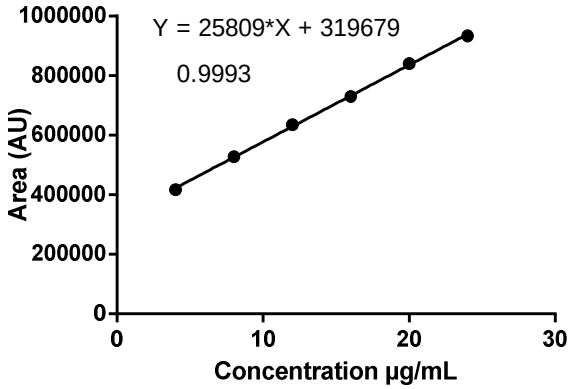

Fig. 3. Calibration curve for PHCl

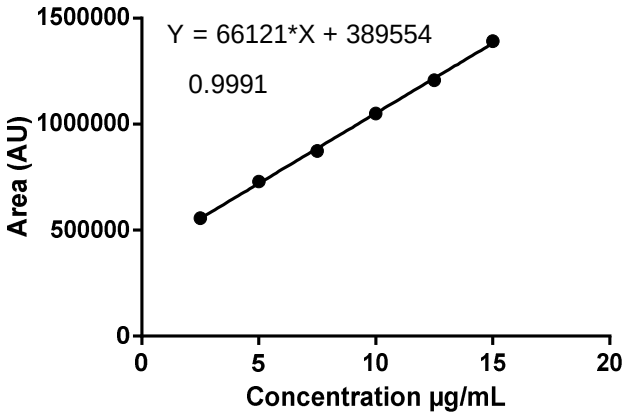

Fig. 4. Calibration curve for HCTZ

Limits of Detection and Quantitation:
The results of the LOD and LOQ were found to be 0.8 µg/mL and 2 µg/mL for PHCl and 0.2µg/mL and 0.6µg/mL for HCTZ, respectively.

Precision:
The precision of the method was expressed as relative standard deviation (RSD, %). Results calculated as % RSD values for intraday and interday precision studies are shown in Table 2 and found to be satisfactory.

Table 2: Precision studies

Conc. (µg/mL)	Intra-day precision (n=3)			Inter-day precision (n=3)		
	Measured Conc. ± SD	(%) RSD	Recovery (%)	Measured Conc. ±SD	(%) RSD	Recovery (%)
Propranolol hydrochloride						
8	7.98 ± 0.089	1.12	99.75	7.95 ± 0.095	1.20	99.38
16	15.94 ± 0.17	1.05	99.63	15.91 ± 0.185	1.16	99.44
24	23.83 ± 0.29	1.21	99.29	23.80 ± 0.274	1.15	99.17
Hydrochlorothiazide						
5	4.96 ± 0.055	1.10	99.20	4.94 ± 0.058	1.17	99.95
10	9.93 ± 0.11	1.06	99.30	9.91 ± 0.11	1.10	99.85
15	14.91 ± 0.17	1.11	99.40	14.90 ± 0.18	1.21	99.33

Accuracy
Accuracy was determined at three levels 80%, 100% and 120% of the target concentration in triplicate and the percentage recovery for the amount added was calculated. The results are presented in Table 3.

Table 3: Recovery studies

Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)	Mean (%) Recovery (± SD)
PHCl 40	80	72	71.65	99.51	99.33 ± 0.215
	100	80	79.50	99.38	
	120	88	87.20	99.09	
HCTZ 25	80	45	44.60	99.11	99.23 ± 0.125
	100	50	49.68	99.36	
	120	55	54.57	99.22	

Robustness:
Robustness of the method was determined by performing same analysis at slightly different parameters from the optimized conditions of selected method. There were no significant changes in the retention times of PHCl and HCTZ when the flow rate (± 0.1 mL/min.) and % of propanol in the mobile phase (± 1%) were changed. The low values of the % RSD indicate the robustness of the method, as shown in Table 4.

Table 4:Robustness evaluation of PHCl and HCTZ

Parameters	Level	PHCl		HCTZ	
		t_R(min)	% RSD	t_R(min)	% RSD
A: Flow rate mL/min.					
0.9	-0.1	6.798	1.12	2.678	1.03
1.0	0.0	6.767	1.17	2.633	1.10
1.1	+0.1	6.718	1.03	2.615	1.08
Mean ± SD		6.761 ± 0.040	1.11 ± 0.071	2.642 ± 0.032	1.07 ±0.036
B: % of propanol in the mobile phase (± 1%)					
% 14	-1.0	6.783	1.15	2.676	1.13
% 15	0.0	6.767	1.04	2.633	1.09
% 16	+1.0	6.714	1.10	2.612	1.05
Mean ± SD		6.755 ± 0.036	1.097 ± 0.055	2.640 ± 0.033	1.09 ± 0.04

Analysis of marketed formulation:

The chromatogram of the sample extracted from conventional tablets showed peaks of HCTZ (Rt2.633 min.) and PHCl (Rt -6.767 min) well resolved from other tablet excipients. The percent contents of PHCl and HCTZ per tablet by proposed method was found to be 99.65 % and 99.68 %, respectively.

CONCLUSION

A simple, rapid MLC method was developed and validated as per ICH guidelines for the simultaneous determination of PHCl and HCTZ in pharmaceutical preparation. The method was validated for linearity, precision, accuracy and specificity. The sample recovery was in good agreement with the respective label claim, which suggested non-interference of formulation additives in its estimation. Many samples can be simultaneously made to run in a shorter span of time. Thus, the developed MLC method is both time and cost effective and can be used for the routine simultaneous analysis of the PHCl and HCTZ in fixed dose combination.

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