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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD OF ACEBUTALOL HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

There is not a singal analytical methods appeared in the literature for the determination of Acebutalol Hydrochloride and Hydrochlorothiazide in combine tablet dosage form. Attempts were made to develop RP-HPLC method of Acebutalol Hydrochloride and Hydrochlorothiazide in bulk drug and Sectrazide tablet formulation. RP-HPLC method was developed and validated as per ICH guidelines using Column C18 (4.6mm x 250mm) and Acetonitrile:Phosphate buffer (pH 2.5) in the ratio 85:15 as mobile phase. Retention time of Acebutalol Hydrochloride and Hydrochlorothiazide was found to be 3.07 and 4.43 mins respectively at the wavelength 230nm and flow rate 1.0ml/min. Acebutalol Hydrochloride (Acbtl) and Hydrochlorothiazide (Hctz) individually follows the Beer-Lamberts law over concentration range 5-25µg/ml and 2-10 µg/ml regression of coefficient was found to be r^2=0.998 and r^2=0.999 respectively. The percentage recovery was found in the range of 98% to 102% at three different levels. The method was found to simple yet accurate, precise and reproducible and successfully applied for the routine quality control analysis of Acebutalol Hydrochloride and Hydrochlorothiazide in bulk drug as well as in tablet formulation as per ICH guidelines. The result of the analysis were validated statistically and were found to be satisfactory.

Keywords: Acebutolol Hydrochloride, Hydrochlorothiazide, Simultaneous equation, HPLC, UV Spectrophotometer

INTRODUCTION:

Objective:

The objective of the present study was to develop new analytical HPLC method and its validation parameters according to ICH guidelines for the estimation of Acebutolol hydrochloride and Hydrochlorothiazide in tablets dosage form.

MATERIALS AND METHODS:

ACEBUTOLOL HYDROCHLORIDE: Chemically (N-[3-Acetyl-4-[2-hydroxy-3[(1-methylethyl) amino] propoxy] phenyl] butanamide) Acebutolol hydrochloride **(Figure 1)** is a cardioselective, hydrophilic β-adrenoreceptor blocking agent with mild intrinsic sympathomimetic activity (ISA) for use in treating patients with hypertension and ventricular arrhythmias **[17, 18]**.

Molecular Formula: $C_{18}H_{29}C_lN_2O_4$.

Molecular weight: 372.9 g/mole

HYDROCHLOROTHIAZIDE: Chemically (6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide) Hydrochlorothiazide **(Figure 2)** is a thiazide class of diuretics used to reduces blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule **[17, 18]** .

Molecular Formula: $C_7H_8C_lN_3O_4S_2$

Molecular weight: 297.7 g/mol

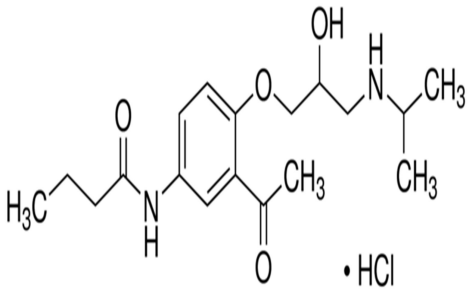

Figure 1: Structure of Acebutalol Hydrochloride

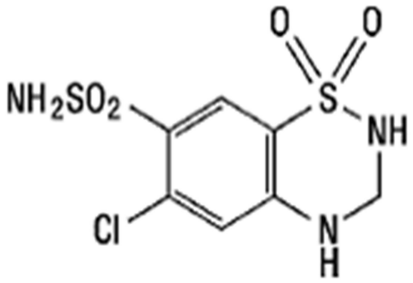

Figure 2: Structure of Hydrochlorothiazide

Chemical and reagents:

Acebutolol hydrochloride and Hydrochlorothiazide [bulk drug] used were of analytical reagent grade purchased from Marksons Pharmaceutical Industry, Pvt. Ltd. Verana, Goa, India, Acetonitrile: phosphate Buffer (HPLC & AR grade) were purchased from Research lab fine chem. Industries Mumbai and double distilled water was used throughout the analysis.

Instrumentation:

Systronic HPLC (Innertsil C18 Column (4.6mm x 250mm), UV visible Detector 2489) was used for all spectral measurements.

Preparation of standard stock solution:

10 mg of Acebutalol and 10 mg of Hydrochlorothiazide were weighed accurately and transferred to a seperate 10 ml volumetric flask, dissolved in sufficient quantity of Acetonitrile: phosphate Buffer then sonicated for 15min and diluted further so as to get the the final sample solution 16µg/ml and 1µg/ml of Acebutalol Hydrochloride and Hydrochlorothiazide respectively **(Table 1)**.

Identification of peaks

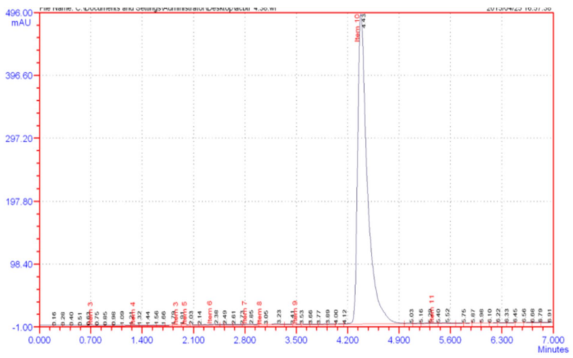

Figure 3: Chromatogram of standard Acebutalol Hydrochloride

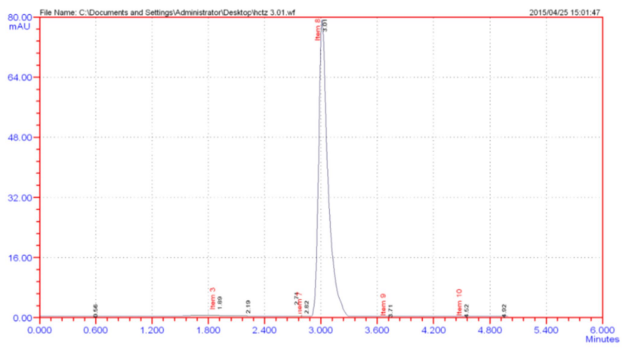

Figure 4: Chromatogram of standard Hydrochlorothiazide

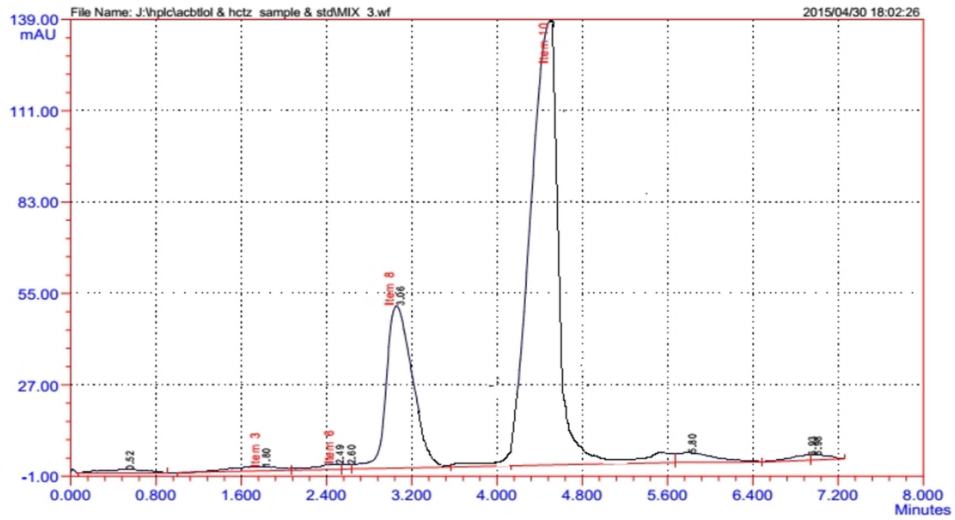

Figure 5: Chromatogram of Tablet (mixture of Acebutalol Hydrochloride and Hydrochlorothiazide

Table 1: Results of Optimization of Chromatographic Condition

Drug	Acbtl	Hctz
RT	4.43	3.06
Area	359899.1	69184.7

Table 2: Assay Results of Marketed Formulation

Sr. No.	Sample wt. taken in mg		Area Found		Assay %	
	Acbtl	Hctz	Acbtl	Hctz	Acbtl	Hctz
1.	400	25	39258	7542	100.21	99.11
2.	400	25	38640	7574	98.62	99.57
3.	400	25	38521	7682	98.31	101.10
4.	400	25	38956	7492	99.44	98.41
5.	400	25	38606	7740	98.53	101.93
Mean			38796.2	7606	99.02	100.02
SD			-		0.7900	1.4523
%RSD			-		0.7978	1.4519

Assay of Marketed Formulation:

The final sample solution contains Acebutalol Hydrochloride and Hydrochlorothiazide in the conc. 16µg/ml and 1µg/ml respectively. In this way six sample solutions were prepared and injected to HPLC system with 10µl injection volume **(Table 2)**.

VALIDATION [15, 16]:

The optical characteristics such as Beer's law limits, percent relative standard deviation and percent range of error were determined. All of the analytical validation parameter for the proposed method was determined according to ICH guidelines. The method was found to provide high degree of precision and reproducibility.

Table 3: Intra-Inter day precision study

	Intra day		Inter day	
	Acbtl	Hctz	Acbtl	Hctz
Mean% ± S.D.	0.003	0.005132	0.005859	0.661837
Precision, %RSD	0.470219	0.908784	0.005132	0.905577

Table 4: Recovery studies of Acebutalol Hydrochloride and Hydrochlorothiazide

Level of Recovery% Amount	50%		100%		150%	
	Acbtl	Hctz	Acbtl	Hctz	Acbtl	Hctz
Amount present (µg)	25 25 25	10 10 10	25 25 25	10 10 10	25 25 25	10 10 10
% Recovery	101.49 100.27 98.10	99.59 98.86 98.26	99.38 101.35 99.72	99.64 98.74 100.80	99.50 99.86 100.81	99.83 99.11 100.80

The recovery studies showed that the result were within the limit indicating no interference.

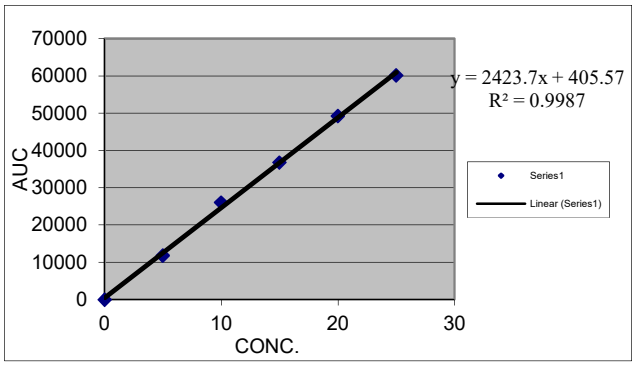

Figure 6: Standard calibration curve of Acebutalol Hydrochloride

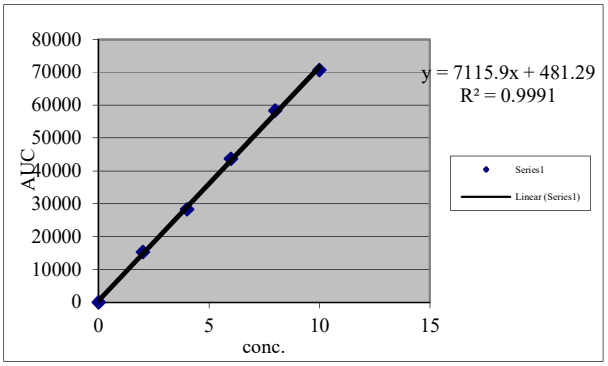

Figure 7: Standard calibration curve of Hydrochlorothiazide

RESULT AND DISCUSSION

RP-HPLC method was developed and validated as per ICH guidelines using Acetonitrile: Phosphate buffer (pH 2.5) in the ratio 85:15 as mobile phase. The chromatograph for both the drugs and mixture were developed at the selected isoabsorptivity point. Retention time of Acebutalol Hydrochloride and Hydrochlorothiazide was found to be 3.07 and 4.43mins respectively at the wavelength 230nm and flow rate 1.0ml/min. The method was found to simple accurate, precise and reproducible. Acebutalol Hydrochloride and Hydrochlorothiazide individually follows the Beer-Lamberts law over concentration range 5-25 µg/ml and 2-10 µg/ml respectively. Validation result is shown in the **Table 5**.

Table 5: Optical characteristics

Sr. No.	Parameters	Acebutalol Hydrochloride	Hydrochlorothiazide
1.	Linearity Range (µg/ml)	5-25	2-10
2.	Regression Equation(y = mx+c)	y = 2423x+405.57	y = 7115x + 481.29
3.	Correlation coefficient (r2)	0.998	0.999
4.	LOD (µg/ml)	1.35	0.40
5.	LOQ (µg/ml)	4.11	1.23
6.	Analysis of Tablets (%Assay)	0.7978	1.4519
7.	% Recovery	98-102	98-102
8.	Intraday Precision (%RSD)	1.789819	1.495855
9.	Interday Precision (%RSD)	1.843931	1.7332

CONCLUSION:

There is not a singal analytical methods appeared in the literature for the determination of Acebutalol Hydrochloride and Hydrochlorothiazide. The proposed method is simple, accurate, precise and selective for the estimation of Acebutalol Hydrochloride and Hydrochlorothiazide.

The method is economical, rapid and do not require any sophisticated instruments contrast to chromatographic method. it can be effectively applied for the routine analysis of Acebutolol hydrochloride and hydrochlorothiazide in bulk drug and tablet dosage form.

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