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Simultaneous UV Spectrophotometric Estimation of Acebutalol Hydrochloride and Hydrochlorothiazide in Bulk and Combined Tablet Dosage Form

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Abstract

There is not a single analytical methods appeared in the literature for combined estimation of Acebutalol Hydrochloride and Hydrochlorothiazide in tablets dosage form. Attempts were made to develop a simple, precise and accurate Simultaneous UV spectroscopic method of Acebutalol Hydrochloride and Hydrochlorothiazide in bulk and Sectrazide tablet dosage form by using simultaneous equation method. UV spectrophotometric method was developed and validated as per ICH guidelines using methanol as mobile phase. Acebutalol Hydrochloride and Hydrochlorothiazide individually follows the Beer-Lamberts law over concentration range 3-18µg/ml and 1-6µg/ml, regression of coefficient was found to be r2=0.9999 and r2=0.9999 respectively. The percentage recovery was found in the range of 98% to 102% at three different levels. The proposed method was successfully applied for the determination of Acebutalol Hydrochloride and Hydrochlorothiazide in tablets dosage form as per ICH guidelines the result of the analysis were validated statistically and were found to be satisfactory.

Keywords: Acebutolol hydrochloride; Hydrochlorothiazide; Simultaneous equation; Validation; UV Spectrophotometer

Introduction

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 sympathomimetric activity (ISA) for use in treating patients with hypertension and ventricular arrhythmias [1-3].

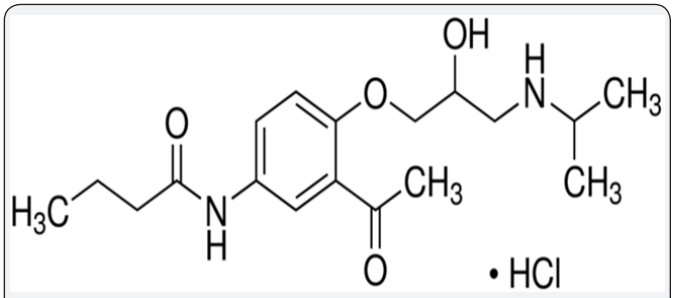

Figure 1: Structure of Acebutalol Hydrochloride

Molecular Formula: $C_{18}H_{29}ClN_2O_4$.

Molecular Weight: 372.9g/mole

Hydrochlorothiazide

Chemically (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide1,1-dioxide) Hydrochlorothiazide 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 [4] (Figure 2).

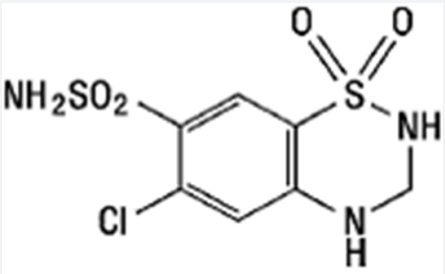

Figure 2: Structure of Hydrochlorothiazide

Molecular Formula: $C_7H_8ClN_3O_4S_2$

Molecular Weight: 372.9g/mole

Objective

The objective of the present study was to develop new analytical UV spectrophotometry method and its validation parameters for the proposed method according to ICH guidelines for the estimation of Acebutolol hydrochloride and Hydrochlorothiazide in tablets dosage form. Attempts were made to develop a simple, precise and accurate Simultaneous UV spectroscopic method.

Materials and methods

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, methanol (AR grade) were purchased from Research lab fine chem. Industries Mumbai and double distilled water was used throughout the analysis.

Instrumentation

A shimadzu 1800UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements [5].

Preparation of standard stock solution

10mg of Acebutalol and 10mg of Hydrochlorothiazide were weighed accurately and transferred to a separate 10ml volumetric flask, dissolved in sufficient quantity of methanol then sonicated for 15min and diluted to 10 ml with the same solvent so as to get the concentration of 1000µg/ml [6].

Determination of absorption maxima

Appropriate dilution of two drugs were prepared separately using standard stock solutions containing Acebutalol Hydrochloride and Hydrochlorothiazide were scanned in the range of 400nm to 200nm to determine the wavelength of maximum absorption for both the drugs. Acebutalol Hydrochloride and Hydrochlorothiazide showed absorbance maxima at 234nm and 224nm respectively. The overlain spectra showed □□max of both drugs (Figure 3) [7-12]

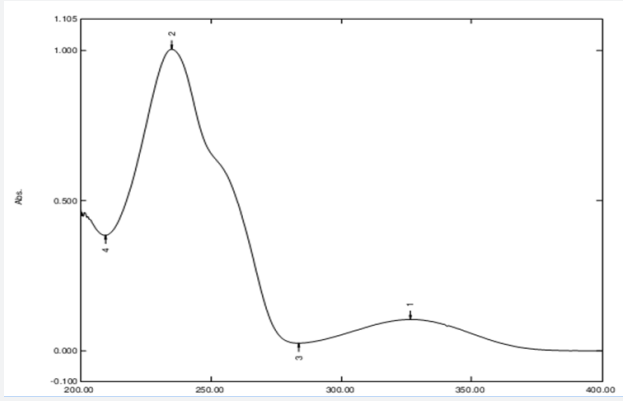

Figure 3: Individual spectra of Acebutalol

Analysis of standard mixture by proposed method

$$c_x = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$c_y = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Where,

Cx = concentration of Acebutalol Hydrochloride

Cy = concentration of Hydrochlorothiazide

ax1 = absorptivity value of Acebutalol Hydrochloride at 234nm.

ax2 = absorptivity value of Acebutalol Hydrochloride at 224nm.

ay1= absorptivity value of Hydrochlorothiazideat 234nm.

ay2= absorptivity value of Hydrochlorothiazide at 224nm.

A1 = absorbance of standard mixture at 234nm.

A2 = absorbance of standard mixture at 224nm.

Analysis of marketed formulation by proposed method

Ten tablets of brand name Sectrazide were used. A quantity of tablet powder equivalent to Acebutalol Hydrochloride (10mg) and Hydrochlorothiazide (10mg) was transferred to 10ml volumetric flask and dissolved in methanol. The aliquot portion of filtrate was further diluted to get Acebutalol Hydrochloride (160ug/ml) and Hydrochlorothiazide (10ug/ml) respectively (Table 1).

Table 1: Result of analysis of Acebutalol Hydrochloride and Hydrochlorothiazide in tablet formulation.

Sr. No	Label claim (mg)		Amount found in mg		% Label claim	
	Acbtl	Hctz	Acbtl	Hctz	Acbtl	Hctz
1	400	25	400.75	25.48	100.18	101.92
2	400	25	396.96	25.00	99.24	100.00
3	400	25	395.68	25.48	98.92	101.92
4	400	25	406.00	24.51	101.50	98.07
5	400	25	399.12	24.51	99.78	98.07
Mean	-	-	-	-	99.92	99.99
SD	-	-	-	-	1.0057	1.9250
%RSD	-	-	-	-	1.0064	1.9250

(Acbtl-Acebutalol Hydrochloride, Hctz Hydrochlorothiazide)

Method validation

The method is developed and validated according to analytical procedure as per the ICH guidelines for validation of analytical procedures. All the parameters such as linearity, precision, LOD, LOQ and accuracy for the analytes were found to be within the limit and satisfactory. The recovery studies showed that the result were within the limit indicating no interference (Table 2 & 3) (Figure 4) [13,14].

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Table 2: Intra-Inter day precision study.

Precision Study		Mean% ± SD	Precision, % RSD
Intra day	Acbtl	0.003	0.470219
	Hctz	0.005132	0.908784
Inter day	Acbtl	0.006245	0.975781
	Hctz	0.005132	0.905577

(Acbtl-Acebutalol Hydrochloride, Hctz Hydrochlorothiazide, S.D.-Standard Deviation, RSD- Relative Standard Deviation)

Table 3: Recovery studies of Acebutalol Hydrochloride and Hydrochlorothiazide.

Level of Recovery % Amount	50%		100%		150%	
	Acbtl	Hctz	Acbtl	Hctz	Acbtl	Hctz
Amount present(µg)	15	5	15	5	15	5
% Recovery	101.4	98.86	99.38	99.64	99.50	99.83

(Acbtl-Acebutalol Hydrochloride, Hctz Hydrochlorothiazide)

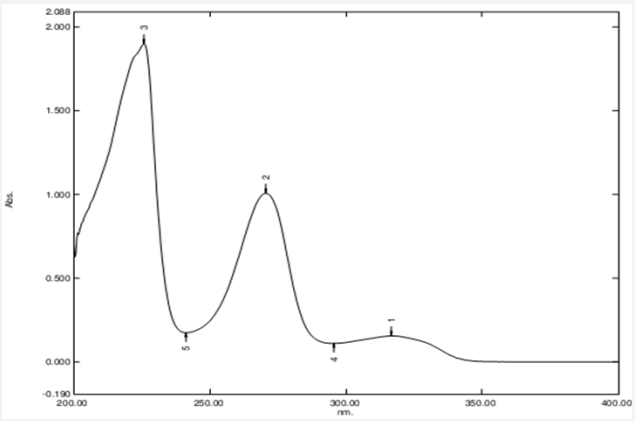

Figure 4: Individual spectra of Hydrochloride Hydrochlorothiazide.

Results and Discussion

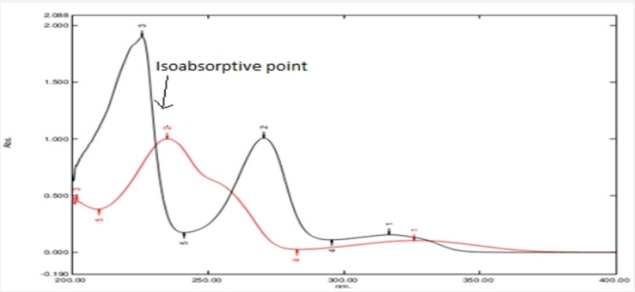

Figure 5: Over In spectra of Mixture of Acebutalol Hydrochloride and Hydrochlorothiazide.

From the individual spectra of Acebutalol Hydrochloride and Hydrochlorthiazide in methanol (Figure 5 & 6) at concentration of 10ug/ml of Acebutalol Hydrochloride and 10µg/ml Hydrochlorthiazide, two wavelengths 234nm and 224nm were selected for simultaneous estimation of drugs respectively. The relation between concentration and absorbance for individual drug was studied. Acebutalol Hydrochloride and Hydrochlorothiazide individually follows the Beer-Lamberts law over concentration range 3-18µg/ml and 1-6µg/ml respectively. The absorptivity values for both the drugs were determined at the selected wavelengths for Acebutalol Hydrochloride and Hydrochlorthiazide respectively. Validation result is shown in the Table 4 [15,16] (Figure 7).

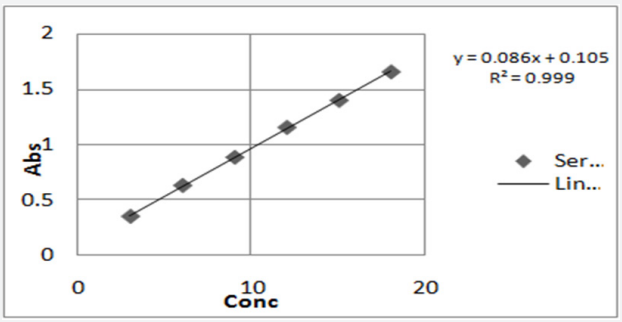

Figure 6: Standard calibration curve of Acebutalol Hydrochloride.

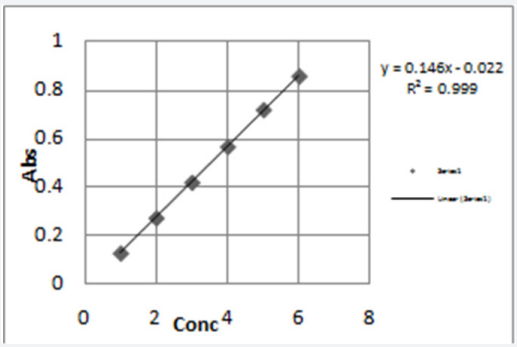

Figure 7: Standard calibration curve of Hydrochlorothiazide.

Table 4: Optical characteristics.

Sr. No.	Parameters	Acebutalol Hydrochloride	Hydrochlorothiazide
1.	Linearity Range (µg/ml)	3-18	1-6
2	Regression Equation(y=mx +c)	y = 0.0867x+0.1053	y = 0.1469x -0.0228
3	Correlation coefficient (r2)	0.9999	0.9999
4	LOD (µg/ml)	0.384912	0.059151
5	LOQ (µg/ml)	1.166401	0.179246
6	Analysis of Tablets (% Assay)	99.92	99.99
7	% Recovery	98-102	98-102
8	Intraday Precision (%RSD)	0.876	0.807
9	Interday Precision (%RSD)	0.804	0.819

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Conclusion

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. The method was found to provide high degree of precision and reproducibility. It can be effectively applied for the routine analysis of Acebutolol hydrochloride and hydrochlorothiazide in bulk drug and in combine tablet dosage form.

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