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Simultaneous Equation Method for the Estimation of Benidipine Hydrochloride and Chlorthalidone by UV Spectrophotometry

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ABSTRACT

The simple, accurate, and precise UV spectrophotometric method has been developed for simultaneous estimation of Benidipine Hydrochloride & Chlorthalidone in bulk tablet formulation. Two wavelengths 237 nm and 275 nm were selected for estimation of Benidipine Hydrochloride & Chlorthalidone by simultaneous equation method respectively. UV spectrophotometric method was developed as per ICH guidelines using Methanol and Water as a Mobile phase. Benidipine Hydrochloride & Chlorthalidone individually follows the Beer-Lamberts Law over concentration range 1-5 & 1.5-7.5 µg/mL, regression of coefficient was found to be r^2=0.998 & r^2=0.999 respectively. The percentage recovery was found to be 98 to 101% at three different levels. The proposed method was successfully applied for the determination of Benidipine Hydrochloride & Chlorthalidone in tablet dosage form as per ICH guidelines the result of the analysis were validated statistically and were found to be satisfactory.

INTRODUCTION:-

Benidipine hydrochloride chemical name 5-*O*-[(3*R*)-1-benzylpiperidin-3-yl] 3-*O*-methyl (4*R*)-2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3,5-dicarboxylate[1]. It was originated in Japan by Kyowa Hakko, it is submitted for FDA approval and it is currently available in some Asian countries like India and Japan [1, 2]. It is a dihydropyridine calcium channel blocker used in treatment of high blood pressure (hypertension) Benidipine hydrochloride is a triple L-, T-, and N-type of calcium channel blocker. It is also used as an anti- mineralocorticoid. Benidipine hydrochloride analysis as an anti-hypertensive drug is of great interest, since hypertension is a very common disorder, particularly in middle and elder age [3]. The mechanism of Benidipine hydrochloride inhibits calcium influx into cells of the Heart and arteries which leads to relax and open blood vessels and allow to blood flow without any pressure [1].

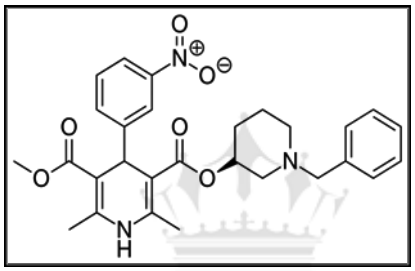
.HCl

Fig. No. 1- Structure of Benidipine hydrochloride

Molecular formula: - $C_{28}H_{32}ClN_3O_6$

Molecular Weight: - 542 g/mol [1]

Chlorthalidone chemical name - 2-chloro-5-(1-hydroxy-3-oxo-2*H*-isoindol-1-yl)benzenesulfonamide [4]. Chlorthalidone (CTD) was first introduced in Switzerland in 1959. It is a thiazide-like diuretic drug used in treatment of multiple disorder including high blood pressure, nephritic syndrome, renal tubular acidosis, diabetes insidious etc. but it is initially preferred in the treatment of high blood pressure [5]. It reduces the reabsorption of sodium and water in tubule which helps in decreasing sodium and water in blood volume and reduces the pressure of blood. Chlorthalidone shows more effective action as compare to hydrochlorothiazide in treatment of hypertension.

Molecular Formula:-$C_{14}H_{11}ClNO_4S$

Molecular Weight: - 338.99g/mol [4]

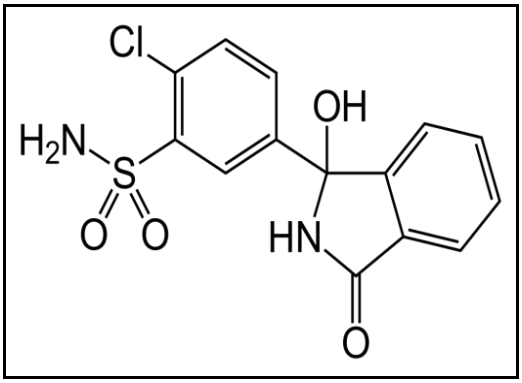

Fig. No. 2- Structure of Chlorthalidone

From the literature survey it was found that no any UV method has been reported on this combination respectively. In this present work, it was proposed to develop and validate a new, simple and accurate UV method for simultaneous estimation of Benidipine hydrochloride and Chlorthalidone in tablet formulation [6,7,8,9].

MATERIALS AND METHODS –

- **Instruments** –

For weighing, a calibrated weighing balance was used. A double beam UV spectrophotometer (Shimadzu-1800) was used. All the glassware which was used made up of Borosilicate glass and they were calibrated.

- **Chemicals -**

Analytical pure sample of Benidipine hydrochloride and Chlorthalidone were received as a gift sample from Akummentis industries and Ipaca laboratories, Mumbai used in study. A pharmaceutical tablet dosage form used in this study was purchased “Benitowa-CH” labeled to contain Benidipine hydrochloride and Chlorthalidone 8:12.5mg per tablet.

Methanol (AR grade) got from the Research lab fine chem industry; Mumbai and Distilled Water were used as a mobile phase in this work.

- **Selection of Wavelength:-**

UV Spectra of Benidipine hydrochloride and Chlorthalidone at 237 nm and 275 nm respectively Mobile phase Methanol: Water (90:10%) was used for good peaks, good absorbance and better sensitivity.

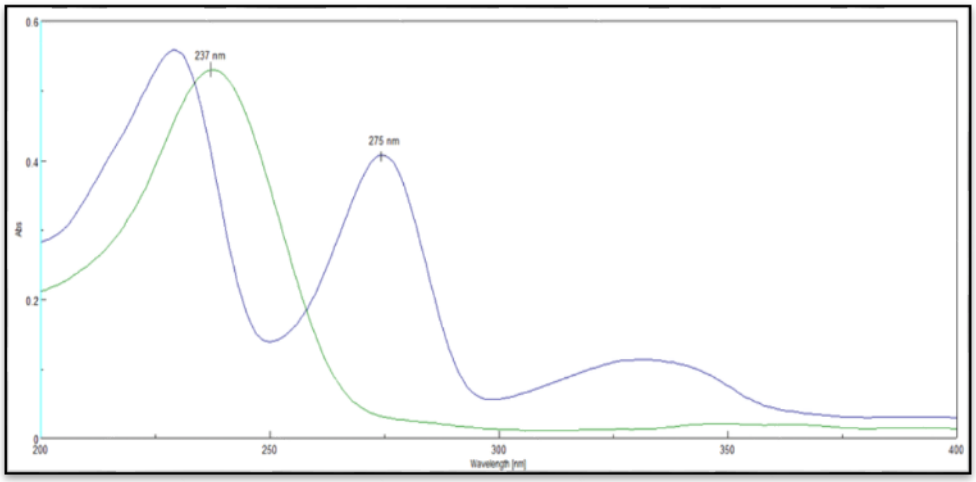

Fig. No. 3: Overlain spectra of Benidipine Hydrochloride & Chlorthalidone

- **Preparation of Mobile phase:-**

500 mL mobile phase was prepared by mixing 400 mL of methanol and 100 mL of water (90:10% v/v).

- **Preparation of Stock solution of Benidipine hydrochloride:-**

Prepare standard solution of Benidipine hydrochloride by adding 10 mg of Benidipne hydrochloride in 100 mL of mobile phase in 100 mL volumetric flask, pipet out 0.1 mL of solution from volumetric flask and add in another 10 mL volumetric flask and makeup volume upto 10mL using mobile phase. So prepared stock solution was 1µg/mL

- **Preparation of Stock solution of Chlorthalidone:-**

Prepare standard solution of Chlorthalidone by adding 10 mg of Chlorthalidone in 100 mL of mobile phase in 100 mL volumetric flask, pipet out 0.15 mL of solution from volumetric flask and add in another10 mL volumetric flask and make up volume up to 10mL using mobile phase So prepared stock solution was 1.5µg/mL.

Simultaneous equation method:-

In order to observe feasibility of proposed method for simultaneous estimation of Benidipine hydrochloride and Chlorthalidone in formulations, the method was tried on standard mixture of different concentrations of both the drug were prepared in mobile phase. The absorbance of Benidipine hydrochloride (1µg/mL) and Chlorthalidone 1.5µg/mL) were recorded at wavelengths 237 nm and 275 nm respectively using a simultaneous estimation method.

$$Cx = \frac{A2ay1 - A1ay2}{Ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{Ax2ay1 - ax1ay2}$$

Where,

Cx= concentration of Benidipne hydrochloride

Cy= concentration of Chlorthalidone

ax1=absorptivity value of Benidipne hydrochloride at 237 nm

ax2= absorptivity value of Benidipne hydrochloride at 275 nm

ay1= absorptivity value of Chlorthalidone at 237 nm

ay2= absorptivity value of Chlorthalidone at 275 nm

A1= absorbance of standard sample at 237 nm

A2= absorbance of standard sample at 275 nm

Analysis of marketed formulation:-

Ten tablet of brand name "Benitowa-CH" were used. From that select 5 tablets and accurately weighed powdered equivalent to single tablet (Benidipine hydrochloride 8mg and Chlorthalidone 12.5 mg) 10 mg of Benidipine hydrochloride and 10 mg of Chlorthalidone were transferred into 100 mL of volumetric flask and make up volume up to 100 mL with mobile phase and pipet out 0.1 mL and transfer in to 10 mL volumetric flask and make up volume up to 10 mL using mobile phase and then sonication for 10-15 min on ultra – sonicator then filtered through Whatman filter paper from this aliquot portion of filtrate was further diluted to get (1µg/mL) of Benidipine hydrochloride and (1.5µg/mL) of Chlorthalidone respectively. The results obtained are shown in table no (1).

Table No. 1: Analysis of marketed formulation

Sr. No.	Benidipine hydrochloride		% Recovery	Chlorthalidone		% Recovery
	Absorbance	Amount recovered in µg/ml		Absorbance	Amount recovered in µg/ml	
1	0.465	0.98	98.384	0.309	1.47	98.299
2	0.463	0.95	95.515	0.308	1.42	94.897
3	0.462	0.93	93.537	0.308	1.42	94.897
4	0.464	0.96	96.766	0.309	1.47	98.299
5	0.463	0.95	95.515	0.308	1.42	94.897
Mean	0.463	0.95	95.943	0.308	1.44	96.257
% RSD	0.24	1.90	1.86	0.17	1.87	1.93

Method Validation:-

Validation of an analytical method is a process to establish the performance characteristics of the developed method to meet the requirement of the intended analytical application. The UV method is validated in terms of linearity, accuracy, precision, LOD and LOQ[10].

1. Linearity:-

Linearity was studied by plotting graph of absorbance v/s concentration and was found to be directly proportional Benidipine hydrochloride was found to be linear in range of 1-5µg/mL and Chlorthalidone was found to be linear in range of 1.5-7.5µg/mL. So series of standard solution of Benidipine hydrochloride were prepared in the concentration range about 1-5µg/mL and chlorthalidone were prepared in a concentration in range of 1.5-7.5µg/mL is shown in below table no. (3).

Table No. 2: Concentration Range & Absorbance

Sr. No.	Concentration of Benidipine hydrochloride in µg/mL	Concentration of Chlorthalidone in µg/mL	Absorbance of benidipine hydrochloride at 237 nm	Absorbance of chlorthalidone at 275 nm
1	1	1.5	0.467	0.325
2	2	3	0.531	0.369
3	3	4.5	0.582	0.409
4	4	6	0.654	0.458
5	5	7.5	0.715	0.501

Table No. 3: Linearity values of Benidipine hydrochloride & Chlorthalidone

Parameter	Benidipine hydrochloride	Chlorthalidone
Range	1-5 µg/mL	1.5-7.5 µg/mL
Slope	0.0619	0.0294
Intercept	0.4041	0.2801
Correlation Coefficient	0.998	0.999

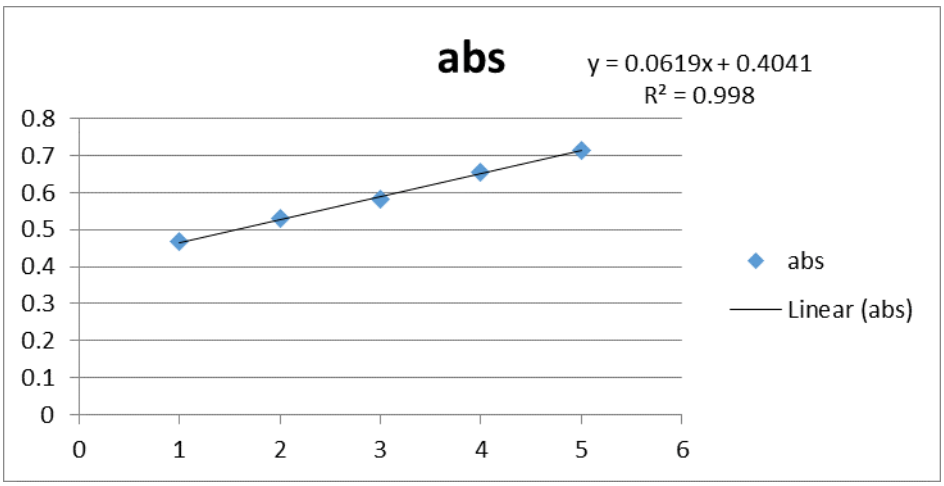

Fig. No. 4: Linearity graph of Benidipine hydrochloride

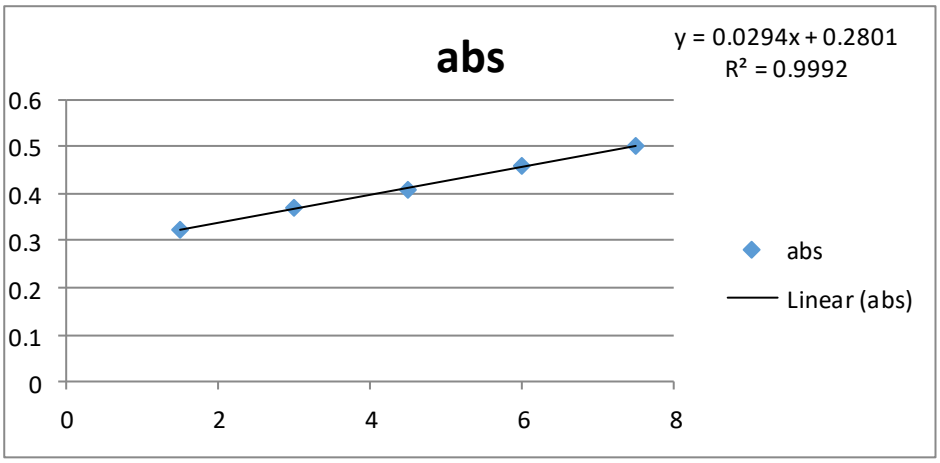

Fig. No. 5- Linearity graph of Chlorthalidone

2. Precision:-

Precision studies were carried in terms of intra-day and inter-day. The % relative standard deviation (% RSD) values were found to be less than 2 which indicate the method is accurate. The result for intra-day precision is shown in below (table no. 4 & 6) and for inter-day precision is shown (table no. 5 & 7).

Table No: 4 -Intra-day precision study of Benidipine Hydrochloride

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
1	0.264	0.271	0.269	0.268	0.003606	1.34
2	0.312	0.319	0.315	0.315.	0.003512	1.11
3	0.498	0.510	0.505	0.504	0.006028	1.28

Table No: 5- Inter-day precision study of Benidipine Hydrochloride

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
1	0.395	0.399	0.389	0.394	0.005033	1.27
2	0.463	0.457	0.465	0.461	0.004163	0.96
3	0.538	0.543	0.548	0.543	0.005000	0.92

Table No: 6- Intra-day precision study of Chlorthalidone

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
1.5	0.199	0.206	0.202	0.202	0.003512	1.73
3	0.277	0.279	0.283	0.279	0.003055	1.09
4.5	0.379	0.382	0.386	0.382	0.003512	0.91

Table No: 7- Inter-day precision study of Benidipine Hydrochloride

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
1.5	0.331	0.326	0.512	0.328	0.002517	0.76
3	0.451	0.459	0.517	0.457	0.005686	1.24
4.5	0.379	0.462	0.509	0.512	0.004041	0.78

3. Accuracy (recovery study)-

To check the accuracy of the developed method and to study the interference of formulation additives, an analytical recovery experiment was carried out by standard addition method. The recovery studies were carried out in three levels i.e. 50%, 100%, 150%, to assure the reliability of the above method recovery studies were carried out by mixing a known quantity of the standard drug with the penalized sample formulation and the contents were reanalyzed by the proposed method. The recovery values were within the limits indicating that the method is accurate. The % recovery values were shown in the below table no. (8&9).

Table No: 8- Accuracy study of Benidipine hydrochloride

Level	Conc. (µg/mL)		Absorbance	% Recovery	Mean % Recovery ±RSD
	sample	Std.			
50%	1	0.5	0.496	98.976	99.29±1.7
			0.498	101.130	
			0.495	97.789	
100%	1	1	0.522	95.234	94.69±0.98
			0.520	93.618	
			0.522	95.234	
150%	1	1.5	0.554	96.865	97.511±0.64
			0.556	98.158	
			0.555	97.512	

Table No: 9- Accuracy study of Chlorthalidone

Level	Conc. (µg/mL)		Absorbance	% Recovery	Mean % Recovery ±RSD
	sample	Std.			
50%	1.5	0.5	0.336	95.068	95.918±1.25
			0.337	96.768	
			0.336	95.068	
100%	1.5	1	0.353	99.183	100.543±1.35
			0.355	101.904	
			0.354	100.544	
150%	1.5	1.5	0.368	98.526	98.903±1.74
			0.366	97.392	
			0.369	100.793	

4. Robustness:-

The analytical technique's robustness is a measure of its ability to remain unaffected by tiny but deliberate modifications in method of parameters, and it gives an indicator of it's depend

ability in routine use. For Benidipine hydrochloride and Chlorthalidone, the method robustness was investigated.

5. LOD & LOQ:-

LOD & LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Whereas σ is the standard deviation of the response (y-intercept) and (S) is the mean of the slop of the calibration plot. The LOD values of Benidipine hydrochloride and Chlorthalidone was found to be 5.22 µg/mL and 7.82µg/mL & LOQ values of Benidipine hydrochloride and Chlorthalidone was found to be 15.82 µg/mL and 23.72 µg/mL respectively.

Table No: 10- LOD &LOQ values

Sr. No.	Name of Drug	LOD in µg/mL	LOQ in µg/mL
1.	Benidipine Hydrochloride	5.22 µg/mL	7.82 µg/mL
2.	Chlorthalidone	15.82 µg/mL	23.72

RESULTS AND DISCUSSION:

The present work provides an accurate, rapid, and sensitive method for the simultaneous estimation of Benidipine hydrochloride and chlorthalidone in bulk tablet formulation. Linear relationship between drug concentrations was obtained over the range of at 1-5 & 1.5-7.5 µg/mL for Benidipine hydrochloride and chlorthalidone respectively. The correlation coefficient, slope and intercept obtained for each drug is shown in table no. (3). The proposed method was also successfully applied to a pharmaceutical formulation. The precision of the method with intra-day and inter-day was found to be good % RSD less than 2 , which indicates that the method was precise and the results presented in table no (4 & 6) & (5 & 7). Recovery studies results are tabulated in table no (8 & 9) for Benidipine Hydrochloride % recovery range from 99.29 to 97.51 with % RSD 1.7 to 0.64. For Chlorthalidone % recovery ranges from 95.91 to 98.90 with % RSD 1.25 to 1.74. The % assay was found to be 95.94 for Benidipine hydrochloride & 96.25 for chlorthalidone respectively. The LOD values of Benidipine hydrochloride and Chlorthalidone was found to be 5.22 µg/mL and 7.82µg/mL & LOQ values of Benidipine hydrochloride and Chlorthalidone was found to be 15.82 µg/mL

and 23.72 µg/mL respectively. No interference was found in the spectrogram of formulation within the absorbance indicating that excipients used in tablet formulation did not interfere with simultaneous estimation of Benidipine Hydrochloride and Chlorthalidone in tablet formulation.

CONCLUSION:

The recently developed UV spectrometric method for determination of Benidipine Hydrochloride and Chlorthalidone simultaneously is simple, specific, accurate, precise, rapid and economical which indicates its competence for routine pharmaceutical analysis of Benidipine hydrochloride and Chlorthalidone in bulk tablet formulation. It is concluded that HPLC method is successfully utilised for the estimation of Benidipine Hydrochloride and Chlorthalidone this new method has been successfully applied for routine analysis.

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