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Research Article

SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF AMILORIDE AND HYDROCHLOROTHIAZIDE IN COMBINED DOSAGE FORM

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ABSTRACT

A Simple, precise, accurate and economical spectrophotometric method was developed and validated for simultaneous estimation of Amiloride (AML) and Hydrochlorothiazide (HCZ) in combined dosage form. In simultaneous equation method, AML and HCZ were quantified using their absorptivity values at selected wavelengths, viz., 244nm and 280nm respectively in 0.1 N NaOH. The linearity range was found to be 5-25 µg/ml for AML and HCZ. The accuracy and reproducibility of the proposed method was statistically validated by recovery studies. The simultaneous equation method permits simple, rapid and direct determination of AML and HCZ in commercially available combined dosage form without previous separations and can therefore be used for routine analysis.

Keywords: Amiloride, Hydrochlorothiazide, Spectrophotometric analysis, Simultaneous equation method.

1. INTRODUCTION

Amiloride hydrochloride is a potassium-sparing diuretic. It is chemically 3,5-diamino-N-(diaminomethylene)-6-chloropyrazinecarboxamidemonohydrochloridedihydrate [1, 2] (Fig. 1). It works by inhibiting sodium reabsorption in renal epithelial cells by binding to sodium channels. Inhibition of sodium reabsorption creates a negative voltage in the luminal membranes of principal cells, situated at the distal convoluted tubule and collecting duct. This negative voltage decreases the potassium and hydrogen ion secretion [2, 3]. It is used in conjunction with diuretics to spare potassium loss. Chemical name of Hydrochlorothiazide is 2H-1,2,4-Benzothiadiazine-7-sulfonamide,6-chloro-3,4- dihydro-,1,1-dioxide (Fig. 1). It is slightly soluble in water and sparingly soluble in acetonitrile. Hydrochlorothiazide belongs to a class of drugs called thiazide diuretics antihypertensive [4]. Hydrochlorothiazide binds to and inhibits the enzyme carbonic anhydrase. It is frequently used alone or in combination with other medications for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, hypoparathyroidism, and edema and prevention of kidney stones and used in the treatment of osteoporosis [5]. Literature survey reveals that AML is estimated individually by UV [6] and combine with HCZ forced degradation studies [7], RP-HPLC, Derivative Spectro-photometry [8-10] and HCZ combine with other drugs HPLC [11], UV and HPLC [12], HPLC-UV, LC-DAD and LC-MS [13] UPLC RP-UPLC [14, 15] methods were reported. The purpose of this study was to develop simple, rapid, precise and accurate UV method for the simultaneous estimation of AML and HCZ in pure and in combined tablet dosage form by Simultaneous equation method and validate as per International Conference on Harmonization (ICH) guidelines.

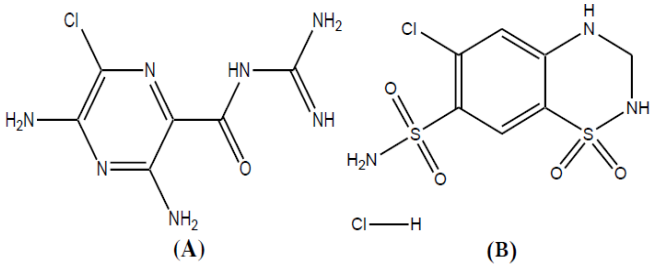

Fig. 1: Chemical structure of (A) Amiloride (B) Hydrochlorothiazide

2. EXPERIMENTAL

2.1. Reagents and chemicals

Working standards of pharmaceutical grade ALM and HCZ were obtained as gift samples from Scan Research Laboratories, Bhopal. The tablet dosage form, Biduret Tablets, manufactured by Glaxo Smith Kline, India (Label Claim: ALM 5mg and HCZ 50 mg), was procured from the local pharmacy. All the chemicals and reagents

used were of HPLC grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

2.2. Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which has a wavelength accuracy ±1 nm, with 1cm quartz cells.

2.3. Method development

2.3.1. Preparation of Standard Stock Solution

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80mL 0.1 N NaOH in 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100 ml with 0.1 N NaOH to get a concentration of 1000 µg/ml (Stock-A) for both drugs. Aliquots of 2.5 ml were withdrawn with help of pipette from standard stock solution A of AML and HCZ and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with Phosphate Buffer (pH 6.8) that gave concentration of 100µg/ml (Stock-B).

2.3.2. Preparation of Working Standard Solution

5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10ml volumetric flask and volume was made up to 10 ml with 0.1 N NaOH. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for HCZ.

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10ml with 0.1 N NaOH. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for AML.

2.3.3. Selection of wavelength for linearity

Solutions of 10 µg/ml of AML and 10µg/ml HCZ were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of AML and HCZwas observed at 244.0 nm and 280.0 nm, respectively. AML and HCZ showed linearity in the concentration range of 5-25 µg/ml and 5-25µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

2.3.4. Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of AML and 10µg/ml of HCZ were scanned in the spectrum mode over the range of 200-400 nm against 0.1 NaOH as blank and the overlain spectra of the two were recorded. AML showed an absorbance peak at 244.0 nm, whereas HCZ at 280.0 nm. The overlain spectra also showed isoabsorptive points at 265.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

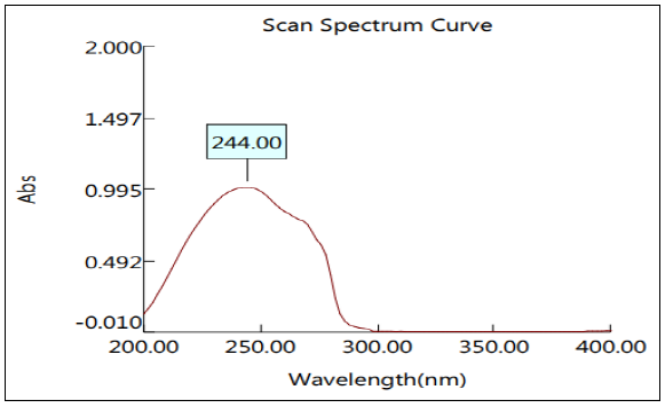

Fig. 2: Determination of λ_{max} of AML

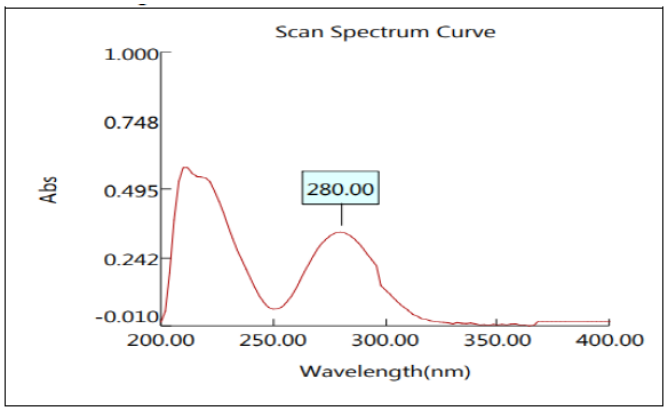

Fig. 3: Determination of λ_{max} of HCZ

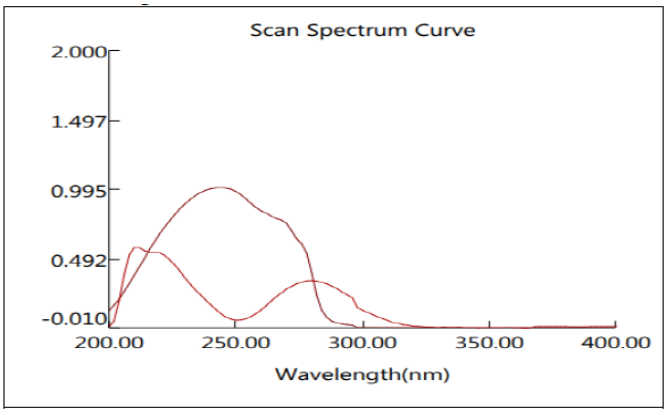

Fig. 4: overlay spectra of ALM and HCZ

2.4. Simultaneous equation method (Vierordt's)

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 244.0 nm and 280.0 nm that are λ_{max} of AML and HCZ respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations:

$$C_{AML} = \frac{A_1 ay_2 - A_2 ay_2}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots \text{Eq. (1)}$$

$$C_{HCZ} = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots \text{Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 244.0 nm and 280.0 nm respectively, ax_1 and ax_2 are absorptivities of AML at λ_1 (244.0 i.e. λ_{max} of AML) and λ_2 (280.0 i.e. λ_{max} of HCZ) respectively and ay_1 and ay_2 are absorptivities of HCZ at λ_1 and λ_2 respectively. C_{HCZ} and C_{AML} are concentrations of AML and HCZ respectively. Fig.5 represent the overlain spectra of both the drugs in 1:1 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the AML and HCZ.

2.5. Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005 [16].

2.5.1. Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in table 1.

2.5.2. Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 2.

2.5.3. Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and % RSD are less than 2 indicate the precision of method. Result of precision are shown in table 3.

2.6. Assay of Tablet Formulation

Mixed blends of AML and HCZ were weighed and ground to a fine powder; amount equal to 50mg of HCZ (5mg AML) was taken in 10 ml volumetric flask. Then 5 ml of 0.1 N NaOH was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with Buffer. After sonication, filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with buffer to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times (Table 4).

Table 1: Results of linearity of Amiloride and Hydrochlorothiazide

Parameter	Amiloride	Hydrochlorothiazide
Concentration (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.045	0.021
Intercept (c)*	-0.007	0.007

** Value of three replicate and five concentrations*

Table 2: Results of recovery study

% Level	% Mean ± SD*	
	Amiloride	Hydrochlorothiazide
80%	98.98±0.749	99.08±0.577
100%	99.03±0.589	99.06±0.991
120%	99.31±0.643	99.44±0.429

** Value of three replicate and five concentrations*

Table 3: Results of Precision

Parameter	% Mean ± SD*	
	Amiloride	Hydrochlorothiazide
Repeatability	99.270±0.105	97.535±0.412
Intermediate precision		
Day to day precision	99.052±0.080	99.053±0.077
Analyst-to-Analyst	99.066±0.093	99.167±0.087
Reproducibility	99.987± 0.071	99.027±0.103

** Value of five replicate and five concentrations*

Table 4: Assay of Tablet Formulation

Conc. Present (µg/ml)		% Conc. Found					
ALM	HCZ	ALM			HCZ		
		Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
5	5	99.00	95.60	100.20	99.80	97.00	97.00
10	10	99.50	99.90	98.40	98.70	99.80	96.50
15	15	99.13	98.53	98.53	99.00	98.53	99.00
20	20	99.80	99.75	99.35	99.70	99.80	98.40
25	25	99.12	99.00	99.48	99.12	99.12	98.60
	Mean	99.31	98.56	99.19	99.26	98.85	97.90
	S.D.	0.300	1.56	0.66	0.421	1.039	0.972
	% RSD	0.298	1.582	0.666	0.424	1.051	0.992

**Average of three replicate and five concentrations*

3. RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis. Thus, for estimation of routine sample of drugs simple, rapid, sensitive and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and use of costly materials. To develop suitable methods of analysis, various solvents were studied. Based on sensitivity of the method and non-toxic behavior, 0.1 N NaOH was selected as a solvent for the methods. Overlain spectra (Fig. 5) shows that at λ_{max} of ALM (244 nm) and λ_{max} of HCZ (280nm) no interference of ALM occurs which suggested development of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with percentage RSD less than 2. The standard deviation, coefficient of variance and standard error were obtained for ALM and HCZ were satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid and accurate way to determine ALM and HCZ simultaneously.

4. CONCLUSION

A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous estimation of ALM and HCZ in their tablet formulation. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Vierordt's method has the advantage of being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed methods can be successfully applied

for determination and dissolution testing of ALM and HCZ in commercial tablet formulations.

Conflict of interest

None declared

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