Original Article

Spectrophotometric method for estimation of amiloride in bulk and tablet dosage form

Abstract

Introduction: Amiloride chemically, 3,5-diamino-6-chloro-N-(diaminomethylene) pyrazine-2-carboxamide. It is used in the management of congestive heart failure, available as Amifru tab, Amimide. It causes adverse effects like Nausea, diarrhea and dizziness. **Materials:** 0.1 N Hydrochloric acid, 0.1 N Sodium hydroxide and 1 mg/ml amiloride drug solution were required. Spectral and absorbance measurements were made using ELICO UV-160 double beam Spectrophotometer. **Method:** Amiloride drug solution concentration range of 25 to 125ug/ml in 0.1N HCl medium was scanned over the wave length range of 235-320 against blank prepared in 0.1N NaOH solution. Two wavelengths are selected one at positive peak 245 nm and another at negative peak 290 nm, the amplitude is calculated from these values. **Results and Discussion:** The sum of the absolute values at these wavelengths is called amplitude. The amplitude is proportional to the amount of drug. High accuracy, reproducibility and low t-values were reported from the calibration curve plotted with the amplitude verses amount of drug. So the proposed method is simple, less time consuming and it can be successfully adopted for the estimation of amiloride.

Key words:
Acid medium (0.1 M hydrochloric acid), amiloride, basic medium (0.1 M sodium hydroxide)

Introduction

Amiloride

AML is chemically, 3,5-diamino-6-chloro-N-(diaminomethylene) pyrazine-2-carboxamide. AML works by directly blocking the epithelial sodium channel there by inhibiting sodium reabsorption in the distal convoluted tubules and collecting ducts in the kidneys (this mechanism is the same for triamterene). This promotes the loss of sodium and water from the body, but without depleting potassium. The drug is often used in conjunction with thiazide (e.g., co-amilozide) or loop diuretics (e.g., co-amilofruse). Due to its potassium-sparing capacities, hyperkalemia (high blood potassium levels) are occasionally observed in patients taking AML. The risk is high in concurrent use of angiotensin converting enzyme inhibitors or spironolactonet. It is soluble in methanol. The structure of AML is as shown in Figure 1.

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It is used in the management of hypertension and congestive heart failure. It is available in the market as a combination drug with furosemide such as amifru tablet, amimide and exna-k tablet. It is also available in combination with atenelol and hydrochlorothiazide namely Beta-Biduret cap, BP-loride tablet, hipres-D cap. It causes adverse effects such as nausea, diarrhea, dizziness, photosensitivity hypotension; bone marrow depression fatigue muscle cramps raised creatine phosphokinase level.

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Only a few methods viz., high-performance liquid chromatography (HPLC), spectrofluorimetry, electrophoresis, ultraviolet (UV)-visible spectrophotometry appeared in the literature for the determination of AML in bulk and pharmaceutical formulations.[1-4] There is a need for simple spectrophotometric method for the analysis of AML in pharmaceutical formulations. No spectrophotometric methods in UV regions are reported in the literature for AML analysis. In this paper, simple and sensitive UV spectrophotometric method for the analysis of AML was described. The method is based on the difference absorbance of AML in acid and base medium. No interference was observed in the analysis of AML from common excipients found in pharmaceutical formulation. The proposed method is economic when compared with HPLC methods.

Materials and Methods

Instrumentation

After due calibration of the instrument, spectral and absorbance measurements are made using ELICO UV-160 a double beam spectrophotometer manufactured by M/S ELICO private Limited, Hyderabad, India. Pure AML sample was kindly gifted by BAL Pharma, Bangalore. All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Reagents were prepared afresh for every method.

Since the drug was stable in 0.1 M sodium hydroxide and 0.1 M hydrochloric acid, freshly prepared 0.1 M sodium hydroxide and 0.1 M hydrochloric acid were selected as the solvents for the developed method.[5-7]

Preparation of reagents

Hydrochloric acid (0.1 N)

Hydrochloric acid solution (0.1 N) is prepared by diluting the requisite volume of concentrated AR hydrochloric acid (Ranbaxy make) with distilled water and standardized by usual procedure.

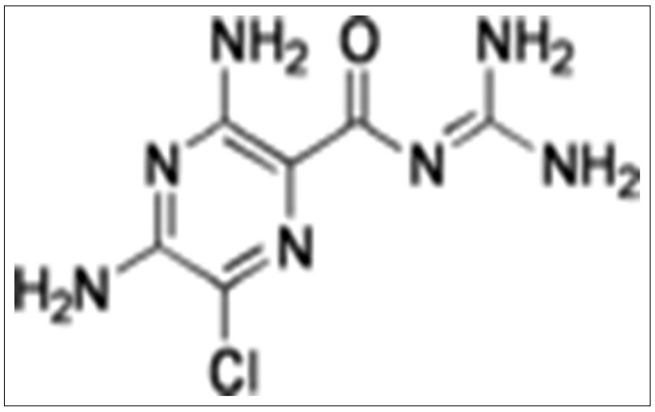

Figure 1: Amiloride

NaOH solution (0.1 N)

It is prepared by dissolving 4 g of sodium hydroxide (Merck) to 1000 ml with distilled water.

Preparation of standard solution of drug

AML solution

AML 50 mg was weighed separately and dissolved in 0.1 M hydrochloric acid and 0.1 M sodium hydroxide separately. The solutions were made up to the volume 50 ml in volumetric flasks with 0.1 M hydrochloric acid and 0.1 M sodium hydroxide separately. 1 ml of this solution contains 1000 µg/ml. 2.5-12.5 ml of stock solutions were transferred into separate 100 ml volumetric flasks and diluted with 0.1 M hydrochloric acid and 0.1 M sodium hydroxide separately to get concentration ranging from 25 to 125 µg/ml.

Method

AML drug solutions ranging from 2.5 to 12.5 ml are taken in to a series of 100 ml standard flasks and to each flask 1 ml of 0.1 N hydrochloric acid is added. These flasks are scanned over the wave length range of 235-320 nm against reagent blank prepared by taking AML drug solution ranging from 2.5 to 12.5 ml in 100 ml volumetric flasks containing 1 ml of 0.1 M sodium hydroxide solution. From the absorbance values two wavelengths 245 and 290 nm are selected one at positive peak and another at negative peak, the amplitude is calculated from these values.[8-11] The results are presented in Figure 2.

Calibration and amount of drug

The calibration curve is plotted between amplitude values and amount of drug (concentration of drug). The calibration curve is found to be linear over a concentration range of 25-125 µg/ml of AML. The amount of AML present in the sample is estimated from the calibration graph. The data and results are presented in Table 1 and Figure 3.

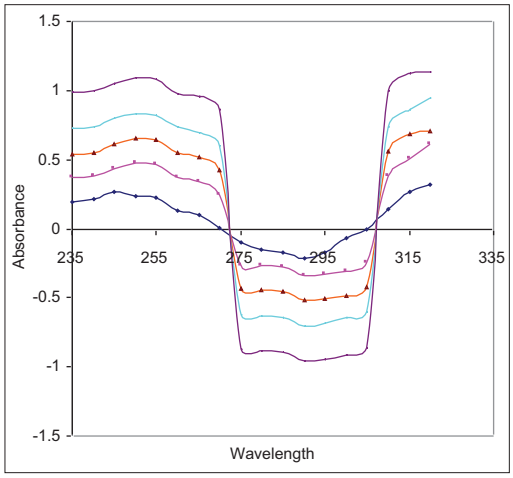

Figure 2: Spectrum of amiloride

Pharmaceutical analysis

Tablets powdered equivalent to 50 mg of the drug is weighed accurately and transferred into 50 ml standard flask and shaken well with 30 ml of methanol for 5 min. The solution is filtered into 50 ml standard flask and the volume is adjusted to 50 ml with methanol. One of the drug solutions is further diluted to 5 ml with distilled water to obtain the working concentration. The procedure described above is followed for the taken samples and the drug content is estimated from the calibration curve. The results are present in Table 2.

Average ± standard deviation (SD) of six determinations, the *t* and *F* values refer to comparison of the proposed method with the reference method.

Results and Discussion

The method is applied for the estimation of AML in pharmaceutical formulation. The drug solution in acid medium is scanned over the UV region by taking the basic drug solution as blank. From the absorbance values, spectrum is constructed. Two wave lengths are selected one at 245 nm and another at 290 nm. The sum of the absolute values at these wavelengths is called amplitude. The amplitude is proportional to the amount of drug. The calibration curve was plotted with the amplitude values verses amount of drug. The precession of the method was evaluated using the SD of the results and the coefficient of variation between the six values of the AML content in the tablets found by performing the assay of the powder blend in six replicates. The values of relative standard deviation (RSD) were found to be 0.145, 0.284 and 0.068, respectively. These low values of RSD indicate that the method is precise in estimating the content of AML from the tablet. The accuracy of the method to estimate the correct amount of the drug added was ascertained by using the *t*-test at each level. The *t*-values are found to be 0.184, 0.794 and 0.685 respectively. These values are lower than the "*t*" theoretical values with 4 ($n - 1 = 5 - 1$) degrees of freedom at 5% level of significance indicates that there is no significant difference between proposed method and standard method. Thus, the method is accurate in estimating the AML content.

The proposed method is simple, less time consuming and it can be successfully adopted for the estimation of AML in tablets. Optical characteristics are presented in Table 3.

Table 1: Calibration table of amiloride

Amount of drug (μg/ml)	Absorbance (+ve) at 245 nm	Absorbance (−ve) at 290 nm	Amplitude
25	0.158	−0.256	0.414
50	0.446	−0.356	0.802
75	0.758	−0.451	1.209
100	0.893	−0.708	1.601
125	0.997	−1.112	2.109

Conclusion

The SD, % of RSD and standard error calculated for the method are low and it indicates the high degree of precision of the method. The % RSD is also less than 2% as required by International Conference on Harmonization guidelines. The results of the recovery studies shows the high degree of accuracy of the proposed method.[12] Hence, the developed method is simple, rapid, precise, accurate, cost-effective and can be employed for the routine analysis of AML in both bulk and tablet dosage form.

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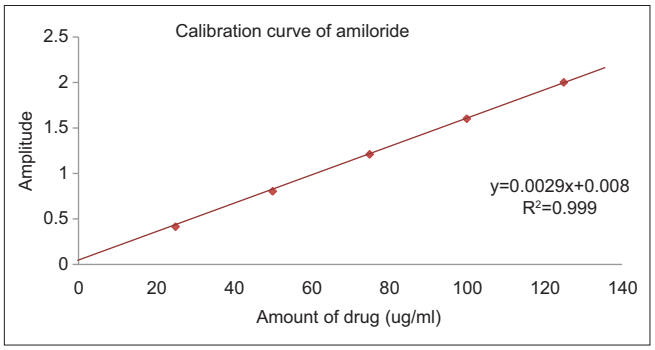

Figure 3: Calibration curve of amiloride

Table 2: Assay of amiloride by difference absorbance method in pharmaceutical formulations

Sample	Labeled amount (mg)	Amount found by reference method±SD	Amount found by proposed method±SD	% of label claim	RSD%	t_{cal}	*F*
Tablet 1	50	49.526±0.075	49.518±0.072	99.02	0.145	0.184	1.063
Tablet 2	50	49.522±0.084	49.575±0.141	99.14	0.284	0.794	0.357
Tablet 3	10	9.952±0.007	9.949±0.006	99.48	0.068	0.685	1.195

RSD – Relative standard deviation; SD – Standard deviation

Table 3: Optical characteristics

Parameters	Method value
λ_{max} (nm)	245 and 290
Beer's law limit (μg/ml)	25–125
Correlation coefficient (r^2)	0.999
Regression equation ($y=mx+c$)	$y=0.0029x+0.008$
Intercept (c)	0.008
Slope (m)	0.0029

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Conflicts of interest
There are no conflicts of interest.

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