

## SPECTROPHOTOMETRIC DETERMINATION OF AMILORIDE IN BULK AND PHARMACEUTICAL FORMULATIONS

VIJAYA LAKSHMI

Department of Chemistry, Sri Krishnadevaraya University, Kurnool, A.P, India. Email: Vijaya82.hari@gmail.com

Received: 17 Jan 2013, Revised and Accepted: 16 Mar 2013

### ABSTRACT

**Introduction:** Simple, sensitive, rapid and accurate spectrophotometric methods have been developed for the determination of Amiloride in pure and pharmaceutical preparation.

**Methods:** The method is based on the formation of ion-association complex of Amiloride with Wool fast blue(WFB) and Bromo cresol green(BCG) in acidic buffer followed by its extraction into organic solvent (chloroform). The absorbance of organic layer was measured at their respective wavelength of maximum absorbance against the reagent blank. The chemical reaction between Amiloride and dyes (WFB,BCG) have been studied by uv-visible spectrophotometric analysis.

**Results:** The calibration curves were linear with correlation coefficient of 0.999 over the concentration range of 20-100 µg/mL for both the methods with maximum absorbance at 590 nm, 420 nm for WFB and BCG respectively. Different experimental parameters affecting the color development and stability of colored product are carefully studied and optimized. The results of analysis validated statistically and by recovery studies.

**Conclusion:** The proposed methods are simple, rapid, accurate, precise and economical and can be used in the estimation of Amiloride in bulk and pharmaceutical formulations.

**Keywords:** Amiloride (AML), Spectrophotometry, Dosageforms, Wool fast blue (WFB), Bromo cresol green (BCG).

### INTRODUCTION

Amiloride chemically, 3,5-diamino-6-chloro-N-(diaminomethylene) pyrazine-2-carboxamide which 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-amilofrusse). Due to its potassium-sparing capacities, hyperkalemia (high blood potassium levels) are occasionally observed in patients taking amiloride. The risk is high in concurrent use of ACE inhibitors or spironolactone is soluble in methanol. The structure of Amiloride is as follows in Fig.1.

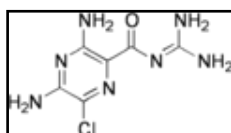


Fig. 1: Amiloride

It is used in the management of hypertension and congestive heart failure. Amiloride is available in the market as combination drug with furosemide such as Amifru tab, Amimide, Exna-k tab, It is also available in combination with Atenelol & Hydrochlorothiazide namely Beta-Biduret cap, BP-Loride tab, Hipres-Dcap. It has adverse effects like Nausea, diarrhea, dizziness, photosensitivity hypotension bone marrow depression fatig muscle cramps raised CPK level.

### Structure of dyes

#### 1) Wool Fast Blue (WFB)

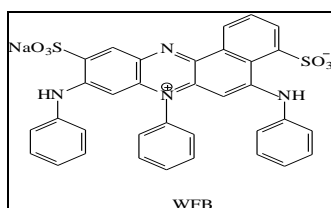


Fig. 2: WFB

#### 2) Bromo cresol green (BCG)

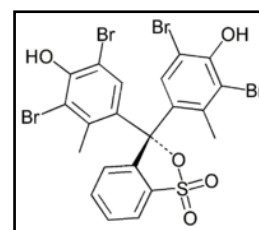


Fig. 3: BCG

Only a few methods viz, HPLC, Spectrofluorimetry, electrophoresis, UV-visible spectrophotometry appeared in the literature for the determination of AML in bulk and pharmaceutical formulations. There is a need for simple spectrophotometric method for the analysis of Amiloride in pharmaceutical formulations. No extractive spectrophotometric methods have been reported in the literature for amiloride analysis. In this paper, simple and sensitive extractive spectrophotometric methods for the analysis of amiloride were described. The methods are based on the formation of chloroform soluble ion-association complexes of amiloride with Wool fast blue (WFB) in acidic buffer at pH 1.5 and Bromo cresol green (BCG) in acidic buffer at pH 3.5. No interference was observed in the analysis of amiloride from common excipients in levels found in pharmaceutical formulation. These proposed methods are not rapid but 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. All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Reagents were prepared a fresh for every method.

#### Preparation of Standard solution of drug

An accurately weighed 50 mg of amiloride was dissolved in methanol. The volume was adjusted to 50 ml with methanol in 50 ml

standard flask. One ml of this solution contains 1mg/ml. The stock solution was further diluted to get desired concentration.

#### Preparation of Reagents

##### For Method - A: Wool fast blue solution: (0.2% W/V)

Wool fast blue solution was prepared by dissolving 200mg of wool fast blue (Flaka) in 100 ml of distilled water.

##### Buffer solution (pH 1.5)

Buffer solution was prepared by mixing 289 ml of glycine solution (37.52 gm of glycine and 29.24 gm of sodium chloride are dissolved in 500ml of distilled water) with 711ml of 0.1 M HCl.

##### For Method - B: BCG solution (0.5% w/v)

It was prepared by dissolving 500 mg of Bromo cresol green (Loba) in 100 ml of distilled water.

##### Buffer solution pH 3.5 (potassium acid phthalate - HCl)

It was obtained by diluting a mixture of 50 ml of 0.2M potassium acid phthalate and 8.4 ml of 0.2M HCl to 200 ml with distilled water and the pH is adjusted to 3.5.

#### Procedures

##### Method A

The wavelength of maximum absorbance of the amiloride drug treated with wool fast blue solution was ascertained by the following procedure.

Into a 10 ml volumetric flasks, the standard drug solution (1 mg/ml) in chloroform were transferred and diluted with same so

as to give several dilutions to obtain 20µg/ml of amiloride. 1 ml of amiloride solution was transferred into a separating funnel. To this solution 1 ml of wool fast blue reagent, 1 ml of 1.5pH buffer solution are added. Reaction mixture was shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer was separated out and absorbance was measured in the wavelength range of 500 to 650 nm, against the reagent blank.

From fig, it was clear that amiloride drug treated with wool fast blue solution has maximum absorbance at 590 nm. Hence further all studies are made at 590 nm.

##### Assay Procedure

To study the effect of drug concentration on the absorbance of the ion pair complex under optimal conditions now arrived was studied by the following method to know the suitability of the method for the assay of amiloride.

Various aliquots of the standard amiloride solution ranging from 1-5 ml are transferred into a series of separating funnel. To each flask, 2.5 ml of wool fast blue solution, 2.5 ml of buffer solution and 5 ml of chloroform are added. Reaction mixture in each funnel was shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer was separated out and absorbance was measured at 590 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration graph was obtained by plotting absorbance values against the concentration of amiloride solution. The calibration curve was found to be linear over a concentration range of 20 to 100 µg/ml of amiloride. The linearity of the curve obtained indicates that it obeys Beer's law. The amount of amiloride present in the sample was estimated from the calibration graph.

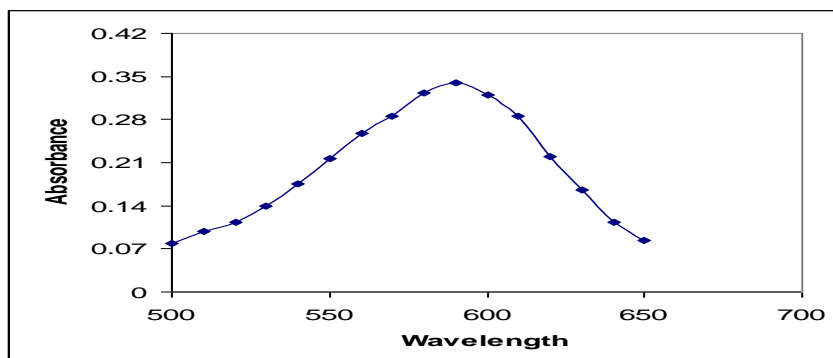


Fig. 4: Absorption Spectrum of amiloride - Wool fast blue

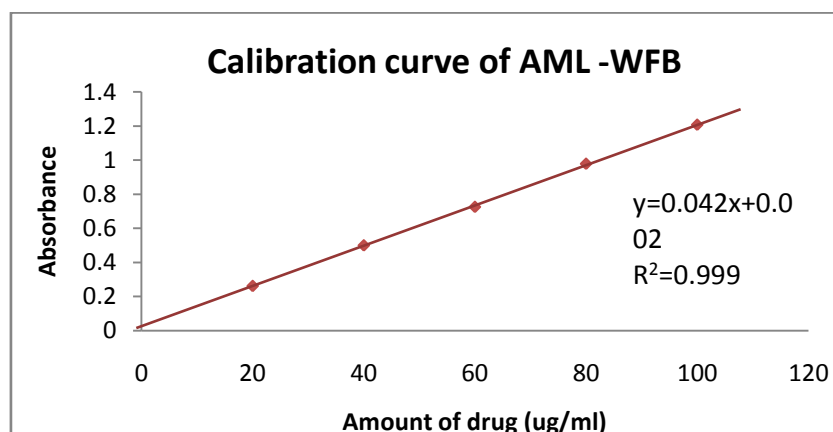


Fig. 5: Calibration curve of Amiloride-Wool fast blue

##### Method B

The wave length of maximum absorbance of the AML drug treated with BCG (Bromo Cresol Green) dye was ascertained by the

following procedure. Into a 10 ml volumetric flasks, the standard drug solution(1 mg/ml) in chloroform were transferred and diluted with same so as to give several dilutions to obtain 20 µg/ml of

amiloride solution. To 1 ml of drug solution, 1.0 ml of BCG (dye solution) and 1.0 ml of 3.5 pH (Buffer Solution) are added. The final volume is adjusted to 10 ml with distilled water. Again 10ml of chloroform was added and the reaction mixture was shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous

and chloroform layer. The chloroform layer was separated out. This chloroform extractable solution possesses light orange color, for that absorption was scanned in the wavelength range of 350 to 500nm against reagent blank (prepared in similar manner, devoid of drug solution). The absorption spectrum was given in fig.

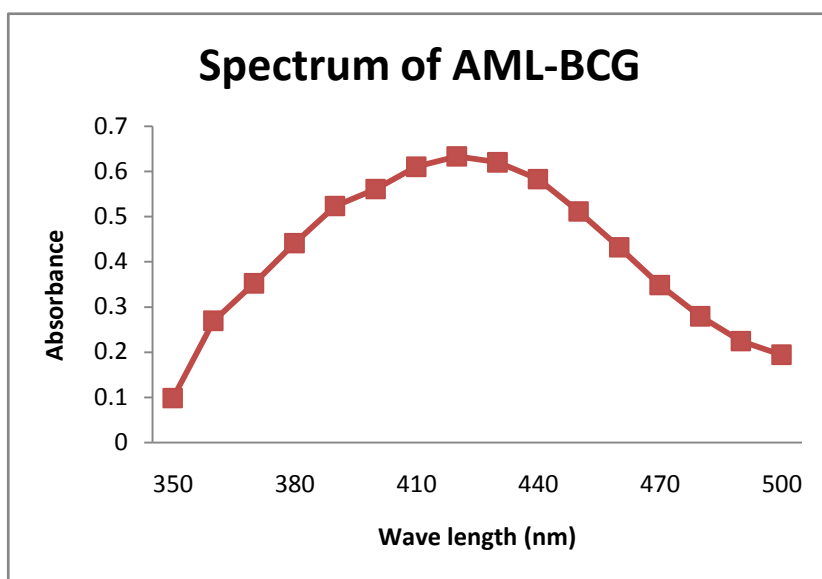


Fig. 6: Absorption spectrum of Amiloride-Bromo cresol green

The absorption spectrum was presented in the figure. From fig it was clear that the amiloride drug solution treated with BCG dye solution has maximum absorbance at 420nm. Hence all further studies are made at 420nm.

#### Assay Procedure for Amiloride (AML) drug:

To study the effect of drug concentration on the absorbance of the ion association complex under optimal conditions now arrived was studied by the following method to know the suitability of the method for the assay of AML.

Various aliquots of the standard AML drug solution ranging from 1ml to 5 ml are transferred in to a series of separating funnels. To each funnel 2.0 ml of BCG dye solution and 1.0 ml of 3.5 pH (Buffer

Solution) are added. The contents are made up to 8 ml with distilled water. To this 10 ml of chloroform was added, the reaction mixture in each separating funnel was shaken gently for 5 min and allowed to stand for 5 min, so as to separate aqueous and chloroform layers. The chloroform layer was separated out and absorbance was measured at 420 nm against the reagent blank (prepared in a similar manner, devoid of drug solution).

The drug calibration graph was constructed by plotting absorbance values against the concentration of AML drug solution. The calibration curve was found to be linear over the concentration range of 20 - 100 µg/ml for AML drug. The linearity of the curve shows that it obeys Beer's law. The amount of AML present in the sample was estimated from the calibration graph.

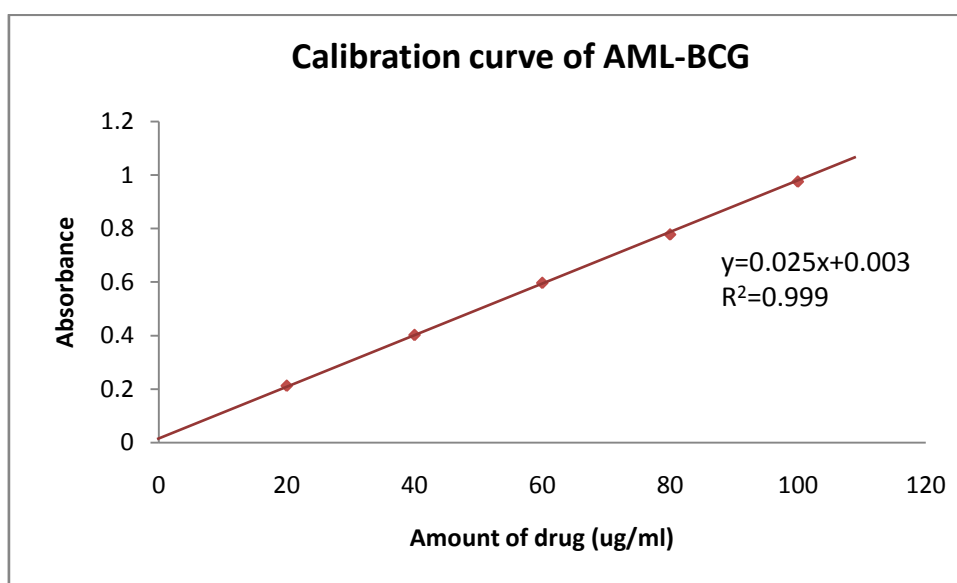


Fig. 7: Calibration curve of Amiloride-Bromo cresol green

Table 1: Optical characteristics

Parameters	Method-A Value	Method-B Value
$\lambda_{max}$ (nm)	590	420
Beer's law limit ( $\mu\text{g/ml}$ )	20-100	20-100
Correlation coefficient ( $r^2$ )	0.999	0.999
Regression Equation ( $y=mx+c$ )	$y=0.025x+0.003$	$y=0.042x+0.002$
Intercept (c)	0.003	0.002
Slope (m)	0.025	0.042

### Analysis of tablets

The proposed procedure for the assay AML was applied for its determination in commercial tablets.

For analysis of tablet formulation, 20 tablets of AML are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of AML was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 min.

The resultant solution was filtered through whatman filter paper no: 41 in to another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol. 5 ml filtrate of the sample solution was diluted to 10 ml with methanol and treated as per the procedure of calibration curve. Amount of the drug present in the sample was computed from respective calibration curve.

Table 2: Assay of Amiloride by ion association method-A in dosage forms

Sample	Labelled amount (mg)	Amount found by Reference method $\pm$ S.D*	Amount found by proposed method $\pm$ S.D*	% of Label Claim	RSD%	$t_{cal}$	F
Tablet1	10	10.019 $\pm$ 0.004	10.018 $\pm$ 0.005	100.07	0.05	0.373	0.713
Tablet2	40	39.527 $\pm$ 0.075	39.610 $\pm$ 0.075	99.01	0.191	0.386	0.994
Tablet3	50	49.526 $\pm$ 0.075	49.508 $\pm$ 0.077	99.0	0.155	0.397	0.947

Average  $\pm$  Standard deviation of six determinations, the t & F-values refer to comparison of the proposed method with reference method.

Table 3: Assay of Amiloride by ion association method-B in dosage forms

Sample	Labelled amount (mg)	Amount found by Reference method $\pm$ S.D*	Amount found by proposed method $\pm$ S.D*	% of Label Claim	RSD%	$t_{cal}$	F
Tablet1	10	10.019 $\pm$ 0.0042	10.018 $\pm$ 0.006	100.08	0.067	0.256	0.394
Tablet2	40	39.527 $\pm$ 0.075	39.667 $\pm$ 0.0735	99.16	0.187	0.911	1.046
Tablet3	50	49.526 $\pm$ 0.075	49.542 $\pm$ 0.088	99.08	0.177	0.342	0.713

Average  $\pm$  Standard deviation of six determinations, the t & F-values refer to comparison of the proposed method with reference method

## RESULTS AND DISCUSSION

Amiloride forms ion-association complexes in acidic buffers with Wool fast blue (WFB), Bromo cresol green (BCG). The complexes were extracted quantitatively into chloroform and exhibiting absorption maxima at 590 nm and 420 nm with WFB and BCG respectively. The experimental factors affecting the development and stability of the ion-association complexes were studied and optimized. Such factors include pH, concentration of reagents, extraction time and organic solvent for extraction. Various organic solvents used for extraction like chloroform, carbon tetrachloride, xylene, toluene, dichloromethane and ethyl acetate. Chloroform was found to be the most suitable solvent for the quantitative extraction of ion-association complex while partial extraction of the complex was achieved with other solvents. The color product was due to ion pair formation of the drug with the dye in acidic medium. The optimum conditions were established by varying one parameter at a time and keeping other parameters fixed by observing the effect produced on the absorbance of the colored species. Investigations were carried out to establish most favourable conditions for the formation of colored product. The influence of AML with different amounts of pH1.5 buffer for method A, pH 3.5 buffer for method B on the reaction has been studied. It was observed that the absorbance started decreasing above 2.5 ml of pH 1.5 for method A and 1 ml of pH 3.5 for method B, so 2.5 ml of buffer for method A and 1 ml of buffer for method B were used for further study. The effect of changing the concentration of wool fast blue over the range 1.5 ml to 4.0 ml for method A and bromo cresol green over the range of 0.5 ml to 3.0 ml was examined and it was observed that the absorbance started decreasing above 2.5 ml with wool fast blue and 2.0 ml with bromo cresol green. Hence 2.5 ml of 0.2% w/v wool fast

blue dye solution for method A and 2.0 ml of 0.5% bromo cresol green dye solution for method B were used for further studies.

### Analytical characteristics

The calibration curve (concentration vs absorbance) was linear over the range of 20-100  $\mu\text{g/ml}$  of amiloride for both the methods. The Beer's law limits, regression equations and correlation coefficient values were evaluated. The linearity of calibration graphs were proved by the high values of the correlation coefficient (R). Accuracy and precision of the two methods were determined by analyzing three different concentrations in six replicates. The percent of relative standard deviation and percent of label claim are calculated for the developed methods. To

determine the accuracy of these methods, three different amounts of bulk samples within the linearity limits are prepared and analyzed by the developed methods. The percent recoveries of the drug by these methods are found to be within the range which indicates that the developed methods are accurate. The values of standard deviation and coefficient of variation are low, indicates high accuracy and reproducibility of the method. The 't' calculated values were well compared with the theoretical value of 2.78 there by indicating that the precision of the method. There was no effect of additive and excipients such as starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

### CONCLUSION

The proposed method was found to be simple, precise, accurate, time saving and economic can be conveniently adopted for routine

analysis of estimation of amiloride in bulk drug samples and pharmaceutical formulations as seen from the agreement of the amount of amiloride in the present methods and the labeled amount of the pharmaceutical preparation.

#### ACKNOWLEDGEMENTS

The authors are grateful to BAL Pharma Ltd, Bangalore, for providing pure drug samples, Department of Chemistry, S.K. University, Kurnool, A.P. for their continuous support and encouragement for providing the necessary infrastructure and facilities for executing this work.

#### REFERENCES

1. Smita Patil, Y. V. Pore, B. S. Kuchekar, Aruna Mane And V. G. Khire; Indian Journal of Pharmaceutical 1; 58- 61, 2009.
2. Sanjay Bari, Shital Sathe, Pritam Jain and Sanjay surana. Spectrophotometric method for simultaneous estimation of atenolol in combination with losartan potassium and hydrochlorothiazide in bulk and tablet formulation. Journal of pharmacy and Bioallied Sciences. 2010; 2(4): 372-375.
3. Olga C. Lastra, Igor G. Lemus, Hugo J. Sanchez and Renato F. Perez. Development and validation of an uv derivative spectrophotometric determination of losartan potassium in tablets. Journal of pharmaceutical and Biomedical Analysis. 2003; 33(2): 175-180.
4. K. Srinivasa Rao, K. Srinivas. RP-HPLC method for the determination of losartan potassium and ramipril in combined dosage form. Indian Journal of pharmaceutical Sciences. 2010; 72 (1): 108-111.
5. G. Carlucci, G. Palemo, P. Mazzeo and M.G.Quaglia. Simultaneous determination of losartan and hydrochlorothiazide in tablets by high performance liquid chromatography. Journal of pharmaceutical and Biomedical Analysis. 2000; 23(1): 185-189.
6. Deanne L. Hertzog, Jennifer Finnegan Mc Caffertyb, Xueguang Fangb, R. Jeffrey Tyrrellc and Rober. Development and validation of a stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide and their degradation products. Journal of pharmaceutical and Biomedical Analysis, 2002; 30(3): 747-760.
7. Silverstein RM, Webster FX. Spectrometric Identification of Organic Compounds. 6th ed. New York : Jhon Wiley and Sons ; 2002.
8. Balasekaran, Prameela Rani, Development and Validation of spectrophotometric method for the determination of DPP4 inhibitor, Sitagliptin, in its pharmaceutical dosage forms, Int J Pharm PharmSci, 2010, 2 (4).
9. Mehta RS, Patel DM, UV and Visible Spectrophotometric Analysis of Pioglitazone Hydrochloride In Bulk And Tablets, IJPS, 2005.
10. S.Shanti kumar\*a, Y. Krishna veni a, G. Rameshb simultaneous estimation of sitagliptin and pioglitazone by uv-spectroscopic method and study of interference of various excipients on this combination of drugs Int J Curr Pharm Res, Vol 4, Issue 2, 2012
11. Subahshree sahooa\*, Chandra kanti chakraborti1, Pradipta kumar behera2
12. Spectroscopic investigations of a ciprofloxacin / HPMC mucoadhesive suspension International journal of applied pharmaceuticals volume 4, issue 3, 2012.
13. Balram choudhary\*1, Anju goyal 1, Sukhbir L.khokra 2 New visible spectrophotometric method for estimation of Itopride hydrochloride from tablets formulations using methyl orange reagent International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Issue 1, July-Sep. 2009.
14. Dube D, Vyas SP, Simultaneous estimation of lamivudine and silymarin in mixture. Int J Pharm Pharm Sci. 2009; 1:108-111.
15. Sivasubrahmanian L, Lakshmi, KS, Tintu T, Simultaneous Spectrophotometric estimation of paracetamol and lonoxicam in tablet dosage form. Int J Pharm Pharm Sci. 2010; 2:166-168.