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Development of a new HPLC method for simultaneous estimation of Amiloride and Hydrochlorothiazide

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Amiloride and Hydrochlorthiazide in Tablet dosage form. Chromatogram was run through BDS (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrie in the ratio of 45:55 was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Amiloride and Hydrochlorthiazide was 296nm. Retention time of Amiloride and Hydrochlorthiazide were found to be 4.081 min and 2.554 min. %RSD of the Amiloride and Hydrochlorthiazide were and found to be 0.57 and 0.71 respectively. %Recover was Obtained as 100.40% and 99.9% for Amiloride and Hydrochlorthiazide. LOD, LOQ values were obtained from regression equations of Amiloride and Hydrochlorthiazide were 0.06ppm, 0.14ppm and 0.18ppm, 0.41ppm respectively. Regression equation of Amiloride is y = 8186x + 1334, and of Hydrochlorthiazide is y = 3525x + 408.9.

KEY WORDS: Amiloride, Hydrochlorthiazide, RP-HPLC.

1. INTRODUCTION

Amiloride hydrochloride and Hydrochlorothiazide tablets combine the potassium-conserving action of amiloride hydrochloride with the natriuretic action of hydrochlorothiazide and its structural formula is:

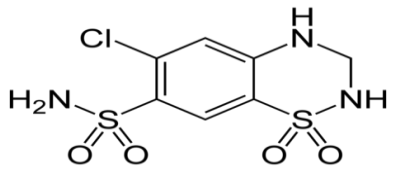

Fig.1.HCTZ

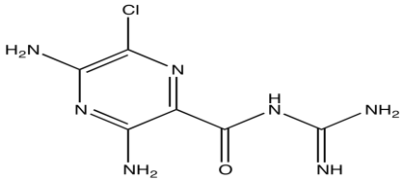

Fig.2.Amiloride

2. MATERIALS AND METHODS

2.1. Materials: Amiloride and Hydrochlorthiazide, Combination of Amiloride and Hydrochlorthiazide tablet dosage forms, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

2.2. Instrument: HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower.

2.3. Preparation of buffer: Buffer: (0.02 Na_2HPO_4): Accurately weighed 2.82gm of disodium hydrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 3.1 with dil. OPA Solution.

2.4. Standard Preparation: Accurately Weighed and transferred 1mg of Amiloride and 10mg of Hydrochlorthiazide working Standards into 10ml clean dry volumetric flasks, add 3/4 ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 2ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 10 mL volumetric flask, 8mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.4ml and 4 was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

2.5. Linearity: Linearity solutions are prepared such that 0.5, 1, 1.5, 2, 2.5, 3ml from the Stock solutions of Amiloride and Hydrochlorthiazide are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 5ppm, 10ppm, 15ppm, 20ppm, 25ppm, 30ppm of Amiloride and 50ppm, 100ppm, 150ppm 200ppm, 250ppm, 300ppm of Hydrochlorthiazide.

2.6. Method Development: There are many trials were done by changing columns and Mobile phases and were reported below.

Trial 1: This trial was run through ODS 250 column with mobile phase composition of 50:50A KH_2PO_4 Buffer and Acetonitrile, Flow rate set at 1ml/min.

Observation: peak splitting was observed.

Trial 2: This trial was run through Altima 150mm column with mobile phase composition of 50: 50 OPA Buffer and Acetonitrile, Flow rate set at 0.8ml/min.

Observation: Peaks eluted in void volume.

Trial 3: This trial was run through BDS 250mm column with mobile phase composition of 50:50 OPA Buffer and Acetonitrile, Flow rate set at 1ml/min.

Observation: In HCTZ peak fronting is observed.

Trial 4: This trial was run through BDS 250mm column with mobile phase composition of 45:45 0.02N NA2PO4 Buffer and Acetonitrile, Flow rate set at 1 ml/min.

Observation: both are eluted with good results.
Optimized Method: Drugs were eluted with good resolution, retention time all the parameters like Plate count and Tailing factor were within the limits.
Mobile phase:
Buffer and Acetonitrile taken in the ratio 45:55A
Chromatographic conditions:

Flow rate	**:**	1ml/min
Column	**:**	BDS 250 x 4.6 mm, 5µ.
Detector wave length	**:**	296nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	20min
Diluent	:	First dissolved in methanol and diluted with buffer

3. RESULTS AND DISCUSSIONS

3.1. System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines

Table.1.System suitability studies of Amiloride and Hydrochlorthiazide method

Property	Amiloride	Hydrochlorthiazide
Retention time (t_R)	4.081± 0.3 min	2.554±0.3min
Theoretical plates (N)	3841 ± 163.48	7944± 163.48
Tailing factor (T)	1.14 ± 0.117	1.18± 0.117

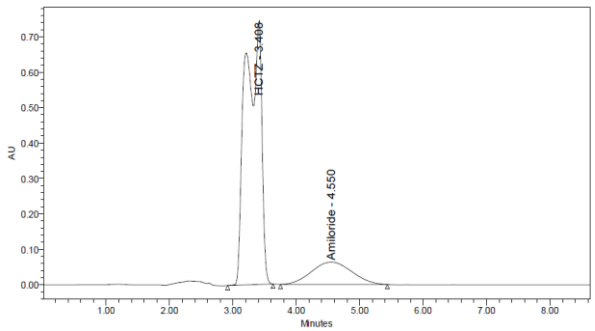

Fig.3.Trial 1 chromatogram

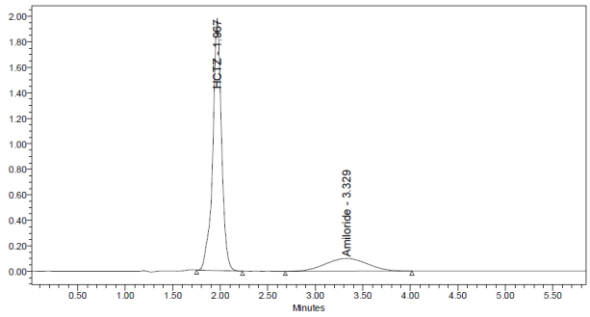

Fig.4.Trial 2 chromatogram

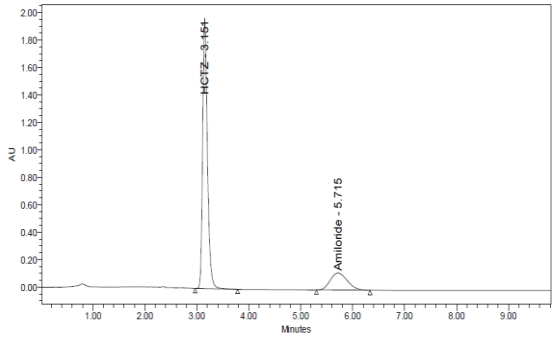

Fig.5.Trial 3 chromatogram

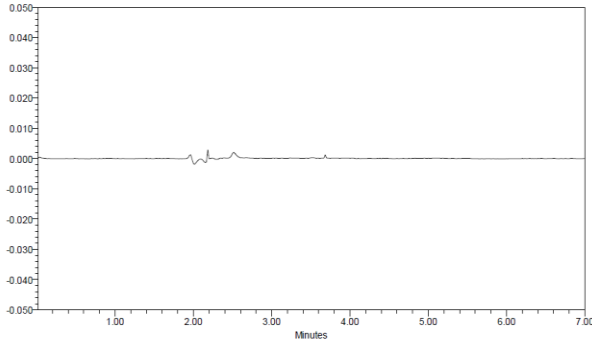

Fig.6.Chromatogram of blank

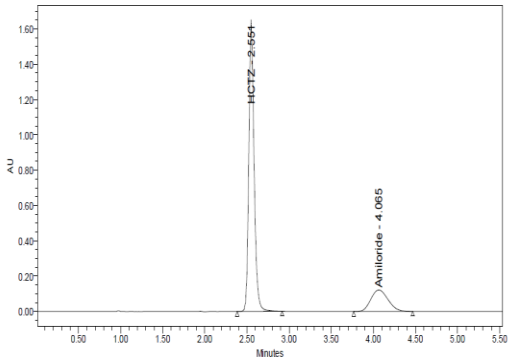

Fig.7.Trial 4 chromatogram

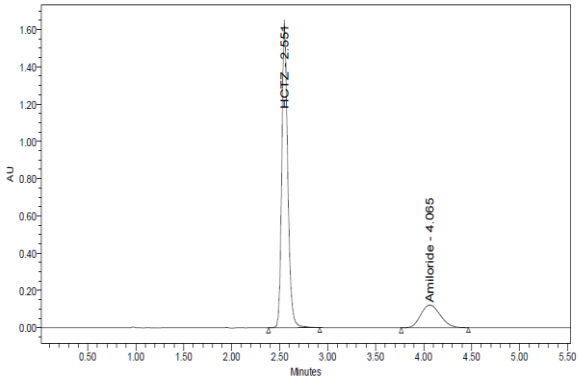

Fig.8.Optimized chromatogram of Amiloride and Hydrochlorthiazide

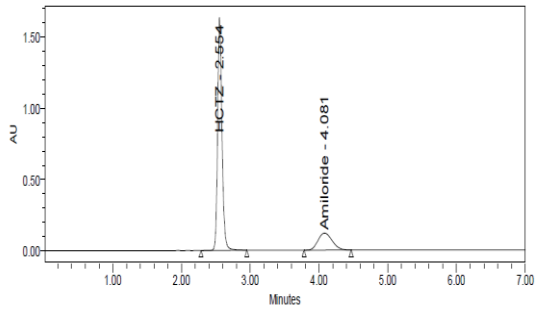

Fig.9.Typical chromatogram of Amiloride and Hydrochlorthiazide

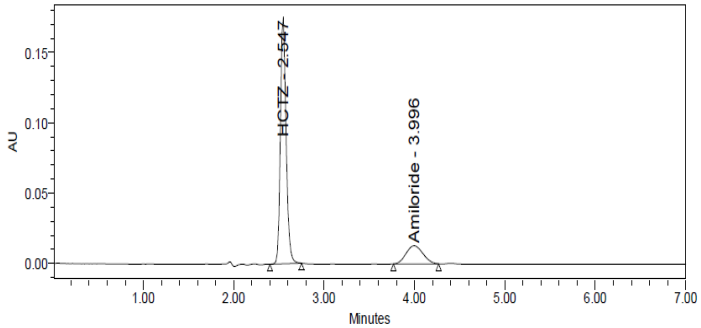

Fig.10.Repeatability Chromatogram of Amiloride and Hydrochlorthiazide method

Table.2.Summary Table

Parameters	Amiloride	Hydrochlorthiazide
Calibration range (mcg / ml)	5-30 ppm	50-300 ppm
Optimized wavelength	296nm	296nm
Retention time	4.081min	2.554min
Regression equation (Y)	y = 8186x + 1334	y = 3525x + 408.9
Correlation coefficient(r^2)	0.999	0.999
Precision (% RSD*)	0.57	0.71
% Recovery	100.40	99.9
Limit of Detection (µg/ ml)	0.06	0.14
Limit of Quantitation (µg / ml)	0.18	0.41

4. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Amiloride and Hydrochlorthiazide in Tablet dosage form. Retention time of Amiloride and Hydrochlorthiazide were found to be 4.081 min and 2.554 min. %RSD of the Amiloride and Hydrochlorthiazide were and found to be 0.57 and 0.71 respectively. %Recover was Obtained as 100.40% and 99.9% for Amiloride and Hydrochlorthiazide. LOD, LOQ values were obtained from regression equations of Amiloride and Hydrochlorthiazide were 0.06ppm, 0.14ppm and 0.18ppm, 0.41ppm respectively. Regression equation of Amiloride is y = 8186x + 1334, and of Hydrochlorthiazide is y = 3525x + 408.9.Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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