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Estimation of Amiloride and Hydrochlorothiazide in Bulk and Tablet Dosage Form by Using RP-HPLC Method

Ravikanti Amulya^{*1}, K. Usha²

Department of Pharmaceutical Analysis, Princetone College of Pharmacy, Pocharam, Hyderabad, Telangana

*Corresponding Author: **Ravikanti Amulya**

Email: princeton.pharmacy@gmail.com



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Abstract: A simple, accurate, and reliable reverse phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Amiloride and Hydrochlorothiazide in bulk and tablet dosage forms. Chromatographic separation was achieved using a Waters HPLC system equipped with an autosampler and PDA detector (996 model), employing an Altima C18 column (4.6 × 150 mm, 5 µm). The mobile phase consisted of Methanol and Acetonitrile in a ratio of 65:35 v/v, delivered at a flow rate of 1.0 ml/min. The detection wavelength was set at 234 nm, with a column temperature maintained at 35°C and an injection volume of 10 µl. The total run time was 14 minutes. The method provided good resolution between the two drugs and exhibited excellent linearity, precision, and accuracy. This validated RP-HPLC method is suitable for routine quality control and quantitative analysis of Amiloride and Hydrochlorothiazide in pharmaceutical formulations.

Keywords: RP-HPLC, Amiloride, Hydrochlorothiazide, Altima C18 column, simultaneous estimation, validation.

INTRODUCTION

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively and quantitatively [1], for analysing the drug sample in bulk, pharmaceutical formulations and biological fluids, many analytical techniques are used [2]. Chromatography may be regarded as an analytical technique employed for the purification and separation of organic and inorganic substances. It is also very useful for the fractionation of complex mixtures, separation of closely related compound like isomers. Chromatography relatively new technique which was 1st developed by M. Tswett, a botanist in 1906 in Warsaw. Basically, this technique is based on differences in the rate at which component of a mixture move through a porous medium (Stationary phase) under the influence of some solvent or gas (mobile phase) at a high pressure [3]. The instrumental method like High Performance Liquid Chromatography (HPLC) was derive from the classical column chromatography and, is widely used tool of analytical chemistry now a days. High Performance Liquid Chromatography is more versatile than gas chromatography since (a) it is not limited to volatile and thermally stable samples, and (b) the choice of mobile and stationary phases is wider.[4]

1. METHOD DEVELOPMENT

Analytical methods development and validation has important roles in the analysis such as discovery, development, and manufacture of pharmaceutical products. The product containing one or more drug called combination product.

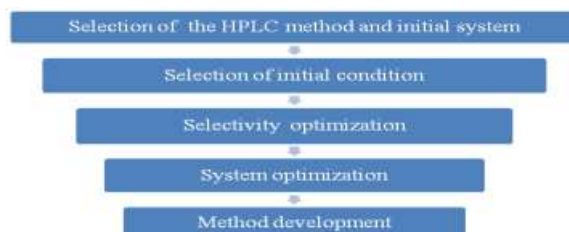


Fig 1: Steps in method development

These combination products create a different challenge to the analytical chemist responsible for the development and validation of analytical methods. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products [5].

1.1 Basic criteria for new method development of drug analysis

- If the drug or drug combination is not be added in any pharmacopoeias,
- If a proper analytical procedure is not available due to patent problems
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients or stability problems
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,

The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable [6,7].

1. Chromatographic principles 3

1.1 Retention

The retention of a drug with a given packing material and eluent can be expressed as a retention time or retention volume. Retention or elution volume is the quantity of the mobile phase required to pull the sample through the column. Retention time is defined as how long a component is retaining the column by the stationary phase relative to the time it resides in the mobile phase. The retention is best described as column capacity ratio (k'), which can be used to evaluate the efficiency of columns. The longer a component is retained by the column, more is the capacity factor. The column capacity ratio of a compound (A) is defined by the following equation:

$$k' = \frac{t_A - t_0}{t_0}$$

Where, V_A is the elution volume of component A and V_0 is the elution volume of a non-retained compound. At constant flow rate, retention times (t_A and t_0) can be used instead of retention or elution volumes.

1.2 Resolution

Resolution is the ability of the column to separate peaks on the chromatograph. Resolution (R) is expressed as the ratio of the distance between two peak maxima to the mean value of the peak width at the base line

$$R = \frac{t_B - t_A}{w_A + w_B}$$

Where,

t_B is the retention time of component B,

t_A is the retention time of component A.

w_A is the peak width of component A and w_B is the peak width of component B.

If R is equal to or more than 1, then components are completely separated, but if R is less than 1, then components overlap.

1.3 Sensitivity

Sensitivity is a measure of the smallest detectable level of a component in a chromatographic separation and is dependent on the signal-to-noise ratio in a given detector. Sensitivity can be increased by derivatization of the compound of interest, optimization of chromatographic system or miniaturization of the system [8].

2. Physicochemical properties of drug

Physicochemical properties of a drug molecule play an important role in method development. For Method development has to consider the physical properties like solubility, polarity, pKa and pH of the drug molecule. Polarity helps an analyst, to decide the solvent and composition of the mobile phase. In a nonpolar covalent bond, the electrons are shared equally between two atoms. A polar covalent bond is one in which one atom has a greater attraction for the electrons than the other atom. The solubility of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. In general, like dissolves like i.e., materials with similar polarity are soluble in each other. Selection of diluents is based on the solubility of analyte. The analyte must be soluble in the diluents and must not react with any of the diluent components. The diluent should match to the starting eluent composition of the assay to ensure that no peak distortion will occur, especially for early eluting components.

The solubility of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. In general, like dissolves like i.e., materials with similar polarity are soluble in each other. Selection of diluents is based on the solubility of analyte. The analyte must be soluble in the diluents and must not react with any of the diluent components. The diluent should match to the starting eluent composition of the assay to ensure that no peak distortion will occur, especially for early eluting components. pH and pKa plays an important role in HPLC method development.

The pH value is defined as the negative of the logarithm to base 10 of the concentration of the hydrogen ion.

$$\text{pH} = -\log_{10}[\text{H}_3\text{O}^+]$$

The acidity or basicity of a substance is defined most typically by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC. Sharp, symmetrical peaks are necessary in quantitative analysis in order to achieve low detection limits, low relative standard deviations between injections, and reproducible retention times. The acidity of an aqueous solution is determined by the concentration of $[\text{H}_3\text{O}^+]$ ions. Thus, the pH of a solution indicates the concentration of hydrogen ions in the solution. The concentration of hydrogen ions can be indicated as $[\text{H}^+]$ or its solvated form in as $[\text{H}_3\text{O}^+]$ whose value normally lies between 0 and 14. The lower the pH, the more acidic is the solution. The pH of a solution can be changed simply by adding acid or base to the solution. The pKa is characteristic of a particular compound, and it tells how readily the compound gives up a proton.

An acid dissociation constant is a particular example of equilibrium constant. For the specific equilibrium between a monoprotic acid, HA and its conjugate base A^- ,



The position of equilibrium is measured by the equilibrium constant, K eq.

$$K_{\text{eq}} = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{H}_2\text{O}][\text{HA}]}$$

Now, in dilute solutions of acid, $[\text{H}_2\text{O}]$ stay roughly constant. Therefore, define a new equilibrium constant- the acidity constant K_a .

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}]}$$

This is also in logarithmic form are follows:

$$\text{pK}_a = -\log_{10} K_a.$$

It turns that the pKa of an acid is the pH at which it is exactly half dissociated. This can be shown by rearranging the expression for Ka:

$$\text{pH} = \text{pKa} - \log\left(\frac{[\text{AH}]}{[\text{A}^-]}\right)$$

At half-neutralization $[\text{A}^-] / [\text{HA}] = 1$; since $\log(1) = 0$, the pH at half-neutralization is numerically equal to pKa. Conversely, when $\text{pH} = \text{pKa}$, the concentration of HA is equal to the concentration of A^- . The buffer region extends over the approximate range $\text{pKa} \pm 2$, though buffering is weak outside the range $\text{pKa} \pm 1$. At

$$\text{pKa} \pm 1, [\text{A}^-]/[\text{HA}] = 10 \text{ or } 1/10.$$

If the pH is known, the ratio may be calculated. This ratio is independent of the analytical concentration of the acid [9].

4. HPLC CONDITIONS

4.1. Buffer

Obtaining satisfactory peak shape for your analytes requires the proper choice of a mobile phase buffer. Buffers improve peak shape of basic compounds and can help modify the band spacing (or selectivity) and retention of acidic or basic compounds. We strongly recommend 10 - 20 mM of an ammonium salt of your choice as a starting buffer (i.e. acetate, formate, carbonate, or phosphate salts) for electrolyte solutes.

Table 1: Merck Index and CRC Handbook of Chemistry and Physics [10]

pKa (25°C)	Compound
0.3	Trifluoroacetic acid ²

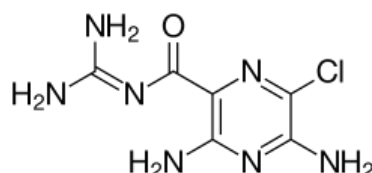
MATERIALS AND METHODS

AMILORIDE

Drug: Amiloride

Drug Category: Potassium-sparing Diuretic; Antihypertensive

Structure:



Chemical Name / IUPAC Name:

3,5-diamino-6-chloro-N-(diaminomethylene)pyrazinecarboxamide

Molecular Formula: C₆H₈ClN₇O

Molecular Weight: 229.63 g/mol

Official Pharmacopoeia: USP

PHYSICOCHEMICAL PROPERTIES

Description: Yellow crystalline powder

Solubility: Slightly soluble in water, soluble in dilute acids

Stability: Stable under recommended storage conditions

Storage Conditions: Store at 15–30°C; protect from light

Melting Point: ~278–284 °C (with decomposition)

pKa: ~8.7

Log P: ~-1.5

PHARMACOKINETIC PROPERTIES

Bioavailability: ~15–25%

Half-life: ~6–9 hours

Absorption: Variable, not significantly affected by food

Volume of Distribution: ~5 L/kg

Protein Binding: ~23%

Metabolism: Minimal

Excretion: Primarily renal (unchanged)

PHARMACODYNAMICS

Mechanism of Action: Blocks epithelial sodium channels (ENaC) in the distal convoluted tubule and collecting ducts → ↓ sodium reabsorption and ↓ potassium excretion → mild diuretic and potassium-sparing effect

Therapeutic Indications:

- Hypertension (in combination)
- Edema associated with CHF, hepatic cirrhosis
- Hypokalemia prevention (with other diuretics)

Adverse Reactions: Hyperkalemia (potentially severe), nausea, vomiting, headache, muscle cramps

Contraindications: Hyperkalemia, anuria, severe renal impairment, use with potassium supplements or other K⁺-sparing agents

INTERACTIONS

Drug Interactions: ACE inhibitors, ARBs, potassium supplements → ↑ risk of hyperkalemia, NSAIDs → ↓ renal function, Lithium → ↑ toxicity

Food Interactions: Food may delay absorption slightly, not clinically significant

DRUG FORMULATION

S. No	Drug Name	Label Claim	Brand Name	Company
1	Amiloride	5 mg Tablet	Midamor	Merck

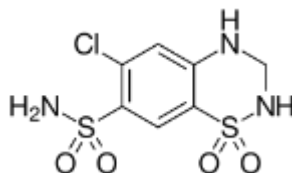
HYDROCHLOROTHIAZIDE

Drug: Hydrochlorothiazide

Synonym: HCTZ

Drug Category: Thiazide Diuretic; Antihypertensive

Structure:



Chemical Name / IUPAC Name:

6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide

Molecular Formula: C₇H₈ClN₃O₄S₂

Molecular Weight: 297.74 g/mol

Official Pharmacopoeia: USP, BP

PHYSICOCHEMICAL PROPERTIES

Description: White to off-white crystalline powder

Solubility: Slightly soluble in water; soluble in alkaline solutions

Stability: Stable under recommended conditions; photosensitive

Storage Conditions: Store at 15–30°C; protect from light

Melting Point: ~270 °C (with decomposition)

pKa: ~7.9 and 9.2

Log P: ~0.1

PHARMACOKINETIC PROPERTIES

Bioavailability: ~60–70%

Half-life: 6–15 hours

Absorption: Moderately well absorbed

Volume of Distribution: ~3.6–7.8 L/kg

Protein Binding: ~40–70%

Metabolism: Minimal

Excretion: Primarily renal (unchanged)

PHARMACODYNAMICS

Mechanism of Action:

Inhibits Na^+/Cl^- symporter in the distal convoluted tubule $\rightarrow$ $\uparrow$ excretion of sodium, chloride, water $\rightarrow$ $\downarrow$ blood volume and blood pressure

Therapeutic Indications: Hypertension, Edema associated with CHF, liver cirrhosis, or renal disease

Adverse Reactions: Hypokalemia, hyponatremia, hyperuricemia, hyperglycemia, hypotension, dizziness

Contraindications: Anuria, hypersensitivity to sulfonamides or thiazides

INTERACTIONS

Drug Interactions: Lithium ($\uparrow$ toxicity), Corticosteroids ($\uparrow$ K^+ loss), Antidiabetics ($\downarrow$ efficacy), NSAIDs ($\downarrow$ diuretic effect)

Food Interactions: Food may enhance absorption slightly

DRUG FORMULATION

S. No	Drug Name	Label Claim	Brand Name	Company
1	Hydrochlorothiazide	25 mg Tablet	Microzide	Mylan

RESULTS AND DISCUSSION

Trails for Method Development

Trail 1:

Mobile phase : Acetonitrile: Water (63:37%v/v)
 Column : Inertsil C18 (4.6mm $\times$ 150mm, 5 μ m particle size)
 Flow rate : 1.1ml/min
 Wavelength : 213 nm
 Column temp : 40°C
 Injection Volume : 10 μ l
 Run time : 4 minutes

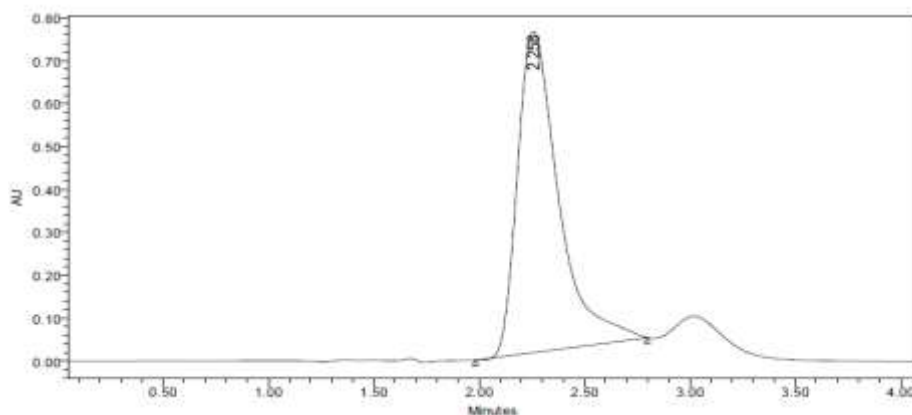


Fig 7.1: Chromatogram for Trail 1

Table 7.1: Peak Results for Trail 1

S.No.	Peak Name	R _t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Amiloride	2.250	12658584	865845		1.06	1268

Observation: In a separation of Amiloride and Hydrochlorothiazide peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

Trail 2:

Mobile phase : Acetonitrile: Methanol (30:70v/v)

Column : Develosil C18 (4.6mm×250mm) 5μm

Flow rate : 1.0 ml/min

Wavelength : 213 nm

Column temp : 40°C

Injection Volume: 10 μl

Run time : 2.20 minutes

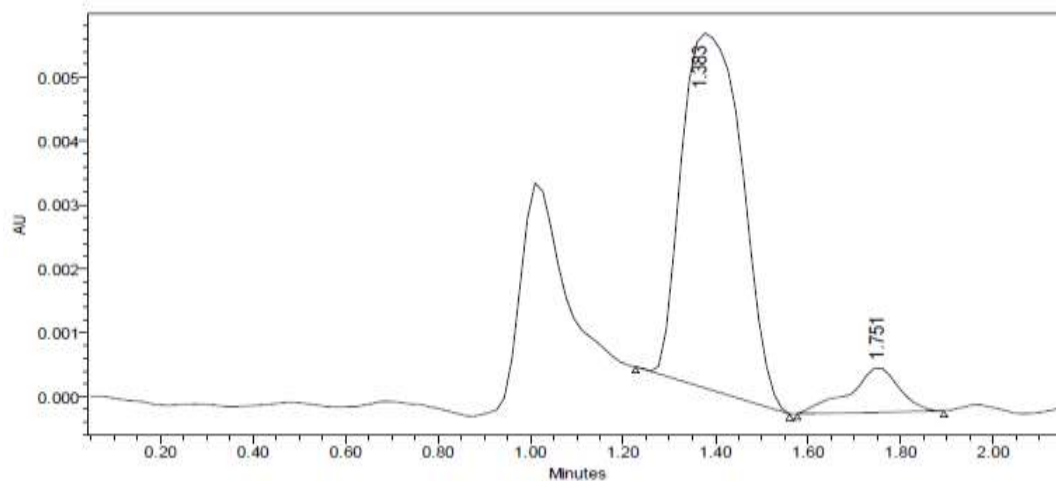


Fig 7.2: Chromatogram for Trail 2

Table 7.2: Peak Results for Trail 2

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amiloride	1.383	6584582	68854		1.04	1147
2	Hydrochlorothiazide	1.751	3568	4582	0.68	0.93	1365

Observation: The separations of two compounds were obtained but improper separation is obtained and resolution is not good. So, we go for further trails.

Trail 3:

Mobile phase : Methanol: TEA Buffer (40:60% v/v)
 Column : Zorbax C18 (4.6mm×250mm, 5µm)
 Flow rate : 0.9 ml/min
 Wavelength : 213 nm
 Column temp : 40°C
 Injection Volume : 20 µl
 Run time : 8.5 minutes

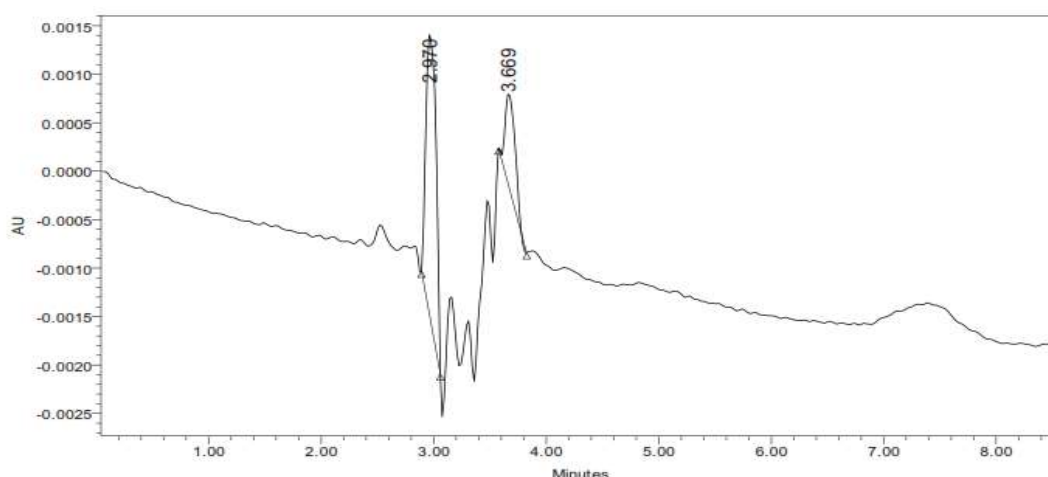


Fig 7.3: Chromatogram for Trail 3

Table 7.3: Peak Results for Trail 3

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amiloride	2.970	658254	85487		2.96	1365
2	Hydrochlorothiazide	3.669	42658	5365	1.48	2.41	2568

Observation: The separations of two compounds were obtained but base line noise was very high, peak symmetry was not available. We go for further trail as the void peaks are obtained.

Trail 4:

Mobile phase : Acetonitrile: Water (40:60 v/v))
 Column : Symmetry ODS C18 (4.6mm×150mm, 5µ Particle Size)
 Flow rate : 1 ml/min
 Wavelength : 213 nm
 Column temp : 40°C
 Injection Volume : 10 µl
 Run time : 7 minutes

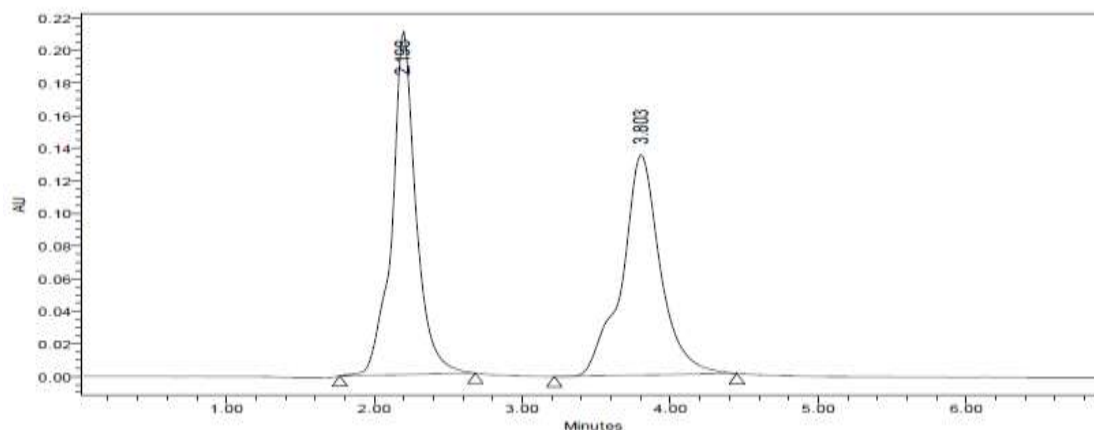


Fig 7.4: Chromatogram for Trail 4

Table 7.4: Peak Results for Trail 4

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amiloride	2.198	58784785	784587		1.41	1352
2	Hydrochlorothiazide	3.803	3568586	56525	3.48	1.12	1587

Observation: After repeating the same conditions got proper peaks, base line is good and we can go for repeatability. Therefore, separations of two peaks, base line, peak symmetry, resolution are proper.

Optimized Chromatogram (Standard)

Column : Altima C18 (4.6×150mm, 5μ)
 Mobile phase : Methanol: ACN (65:35v/v)
 Flow rate : 1ml/min
 Wavelength : 234 nm
 Injection volume : 10 μl
 Run time : 10 min

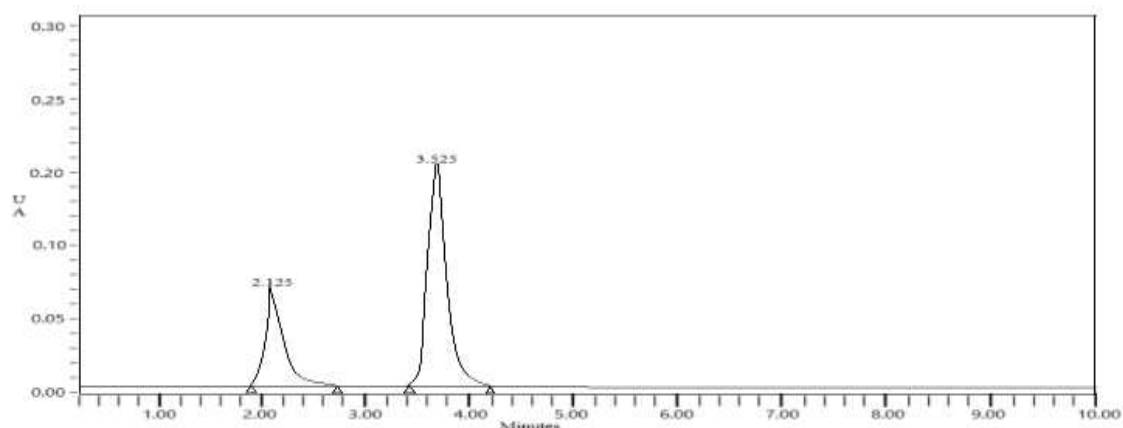


Fig 7.5: Optimized Chromatogram

Table 7.5: Peak Results for Optimized Chromatogram

S. No.	Peak Name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amiloride	2.125	658748	67824		1.06	6852
2	Hydrochlorothiazide	3.525	8695825	841253	4.65	1.18	8548

Observation: From the above chromatogram it was observed that the Amiloride and Hydrochlorothiazide peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So, it's optimized trial.

Optimized Chromatogram (Sample)

Column : Altima C18 (4.6×150mm, 5μ)

Mobile phase : Methanol: ACN (65:35v/v)

Flow rate : 1ml/min

Wavelength : 234 nm

Injection volume : 10 μl

Run time : 10 min

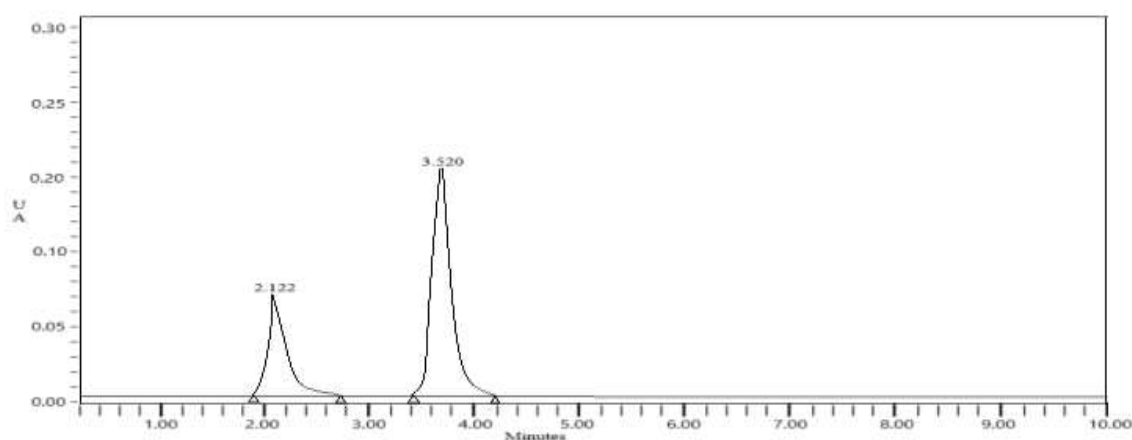


Fig 7.6: Optimized Chromatogram (Sample)

Table-7.6: Optimized Chromatogram (Sample)

S. No.	Peak Name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amiloride	2.105	668547	68476		1.07	6985
2	Hydrochlorothiazide	3.552	8758985	856485	4.66	1.19	8659

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank:

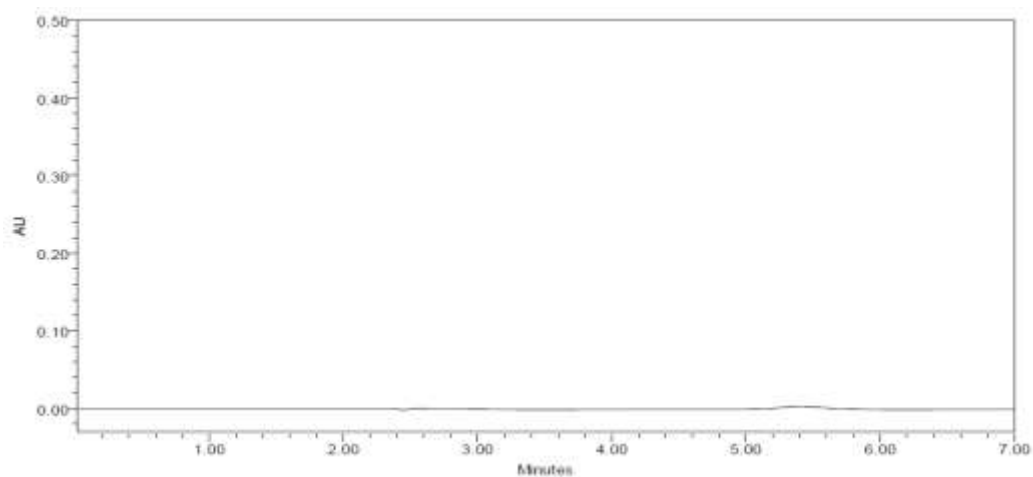


Fig 7.7: Chromatogram showing blank (mobile phase preparation)

System Suitability:

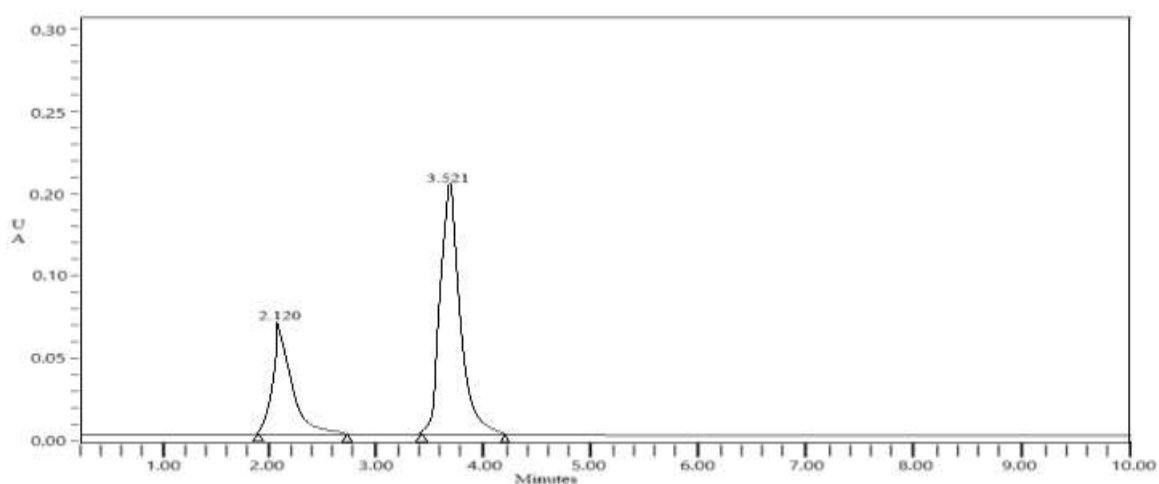


Fig 7.8: Chromatogram showing injection -1

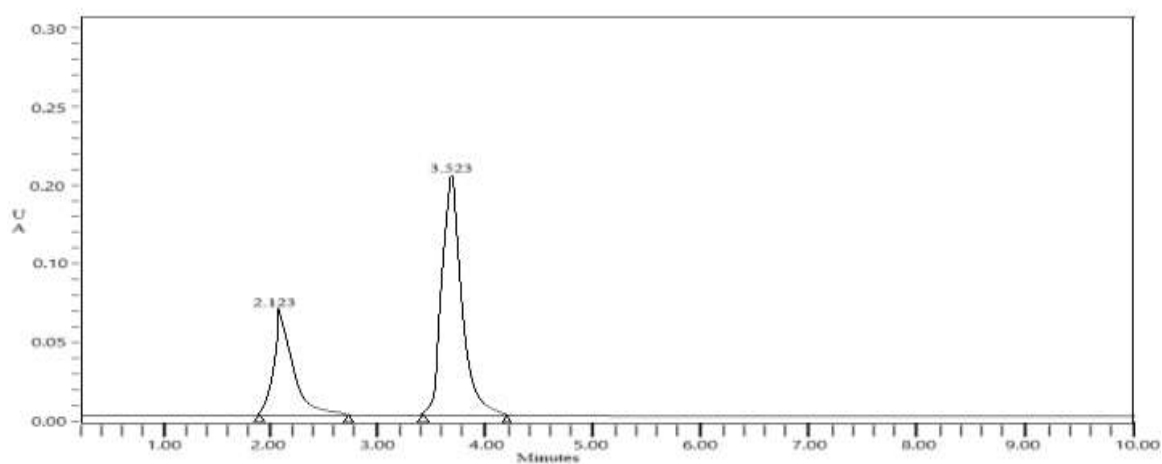


Fig 7.9: Chromatogram showing injection -2

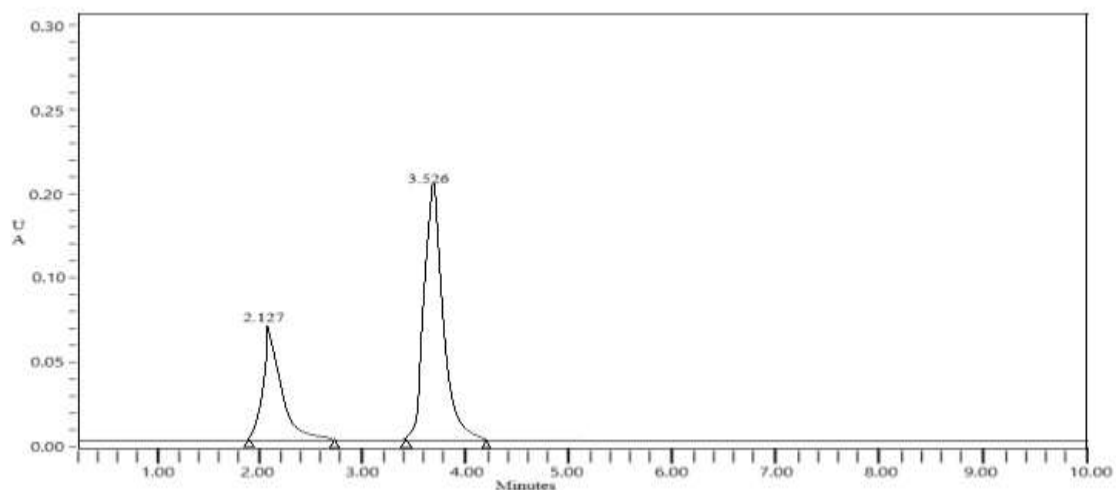


Fig 7.10: Chromatogram showing injection -3

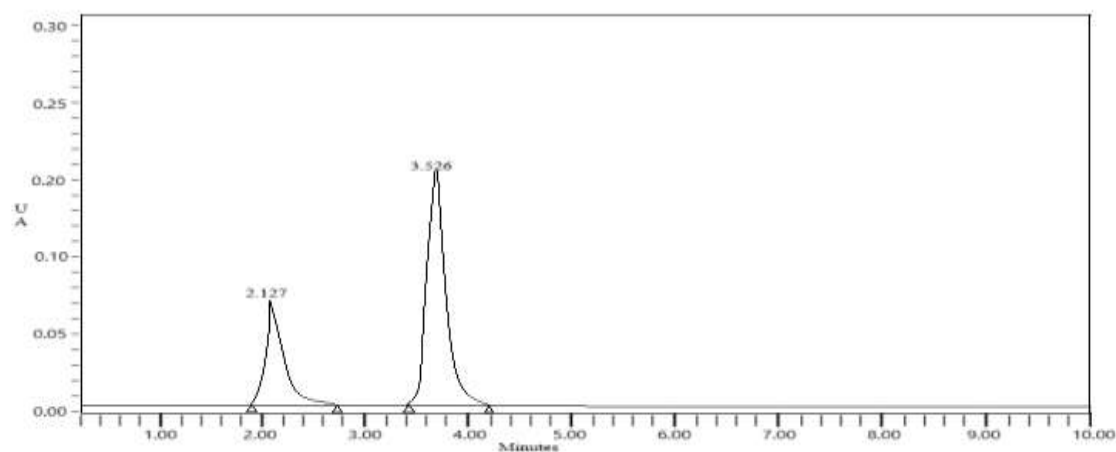


Fig 7.11: Chromatogram showing injection-4

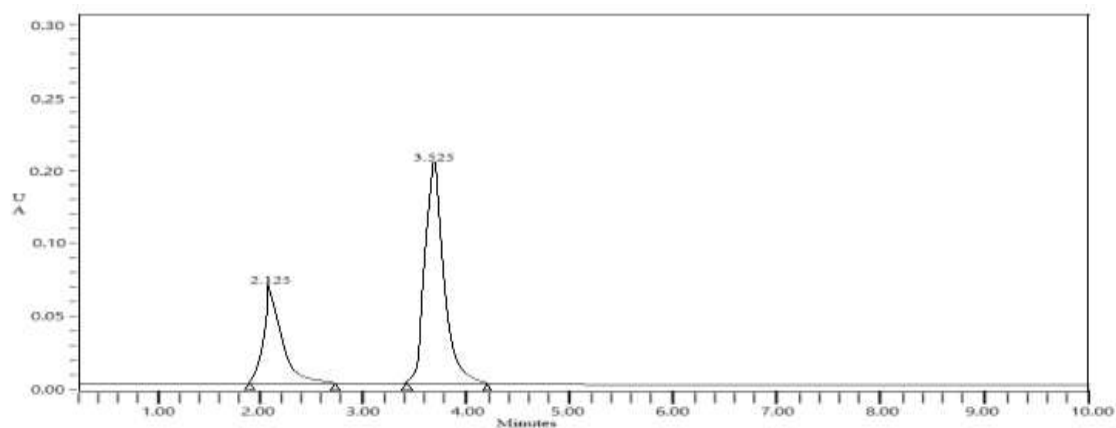


Fig 7.12: Chromatogram showing injection -5

Table 7.7: Results of system suitability for Amiloride

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Amiloride	2.120	658658	67854	6895	1.06
2	Amiloride	2.123	657893	67582	6847	1.07
3	Amiloride	2.127	658985	67895	6875	1.06
4	Amiloride	2.122	659863	67852	6845	1.06

5	Amiloride	2.125	658784	67456	6865	1.07
Mean			658836.6			
Std. Dev			707.2067			
% RSD			0.107342			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table 7.8: Results of system suitability for Hydrochlorothiazide

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Hydrochlorothiazide	3.521	8658485	845250	8542	1.18	4.65
2	Hydrochlorothiazide	3.523	8695847	847584	8574	1.19	4.66
3	Hydrochlorothiazide	3.526	8657474	847612	8569	1.18	4.65
4	Hydrochlorothiazide	3.520	8625698	846985	8532	1.18	4.65
5	Hydrochlorothiazide	3.525	8675842	847526	8541	1.19	4.66
Mean			8662669				
Std. Dev			25911.66				
% RSD			0.299119				

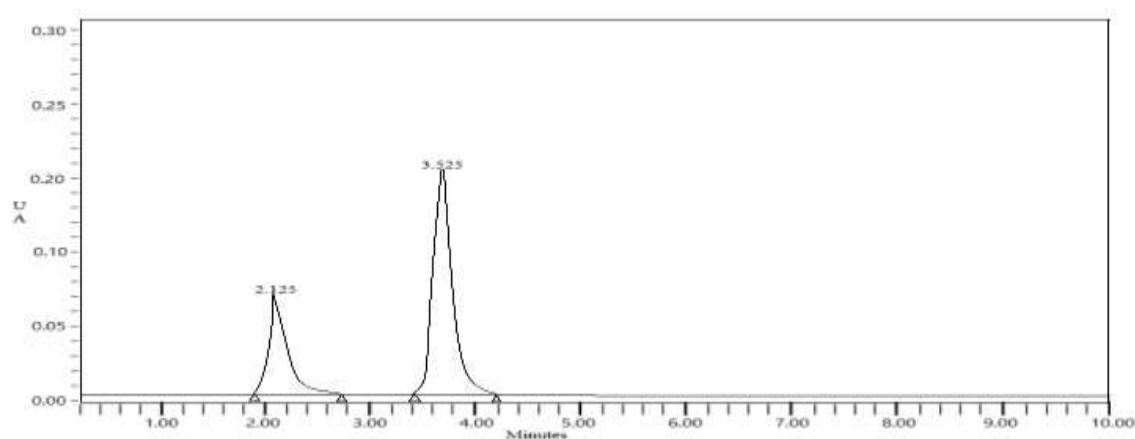
Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Amiloride and Hydrochlorothiazide in drug product.

Assay (Standard):**Fig 7.13: Chromatogram showing assay of standard injection -1**

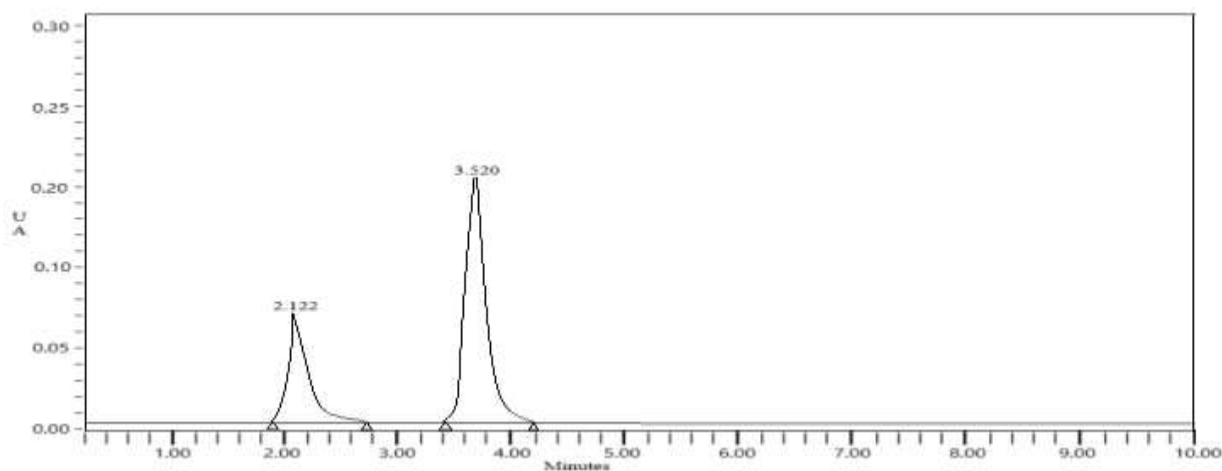


Fig 7.14: Chromatogram showing assay of standard injection -2

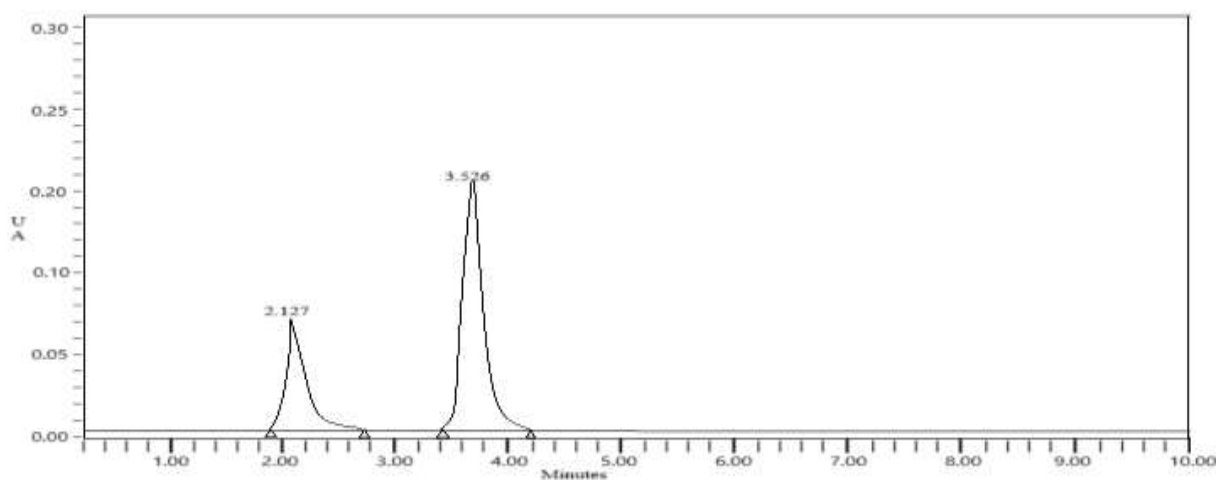


Fig 7.15: Chromatogram showing assay of standard injection -3

Table 7.9: Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Amiloride	2.125	658985	67854		1.06	6859	1
2	Hydrochlorothiazide	3.525	8659852	845798	4.68	1.18	8643	1
3	Amiloride	2.122	657542	67259		1.07	6874	2
4	Hydrochlorothiazide	3.520	8652874	846354	4.69	1.19	8596	2
5	Amiloride	2.127	658935	67823		1.06	6982	3
6	Hydrochlorothiazide	3.525	8659875	849653	4.68	1.18	8569	3

Assay (Sample):

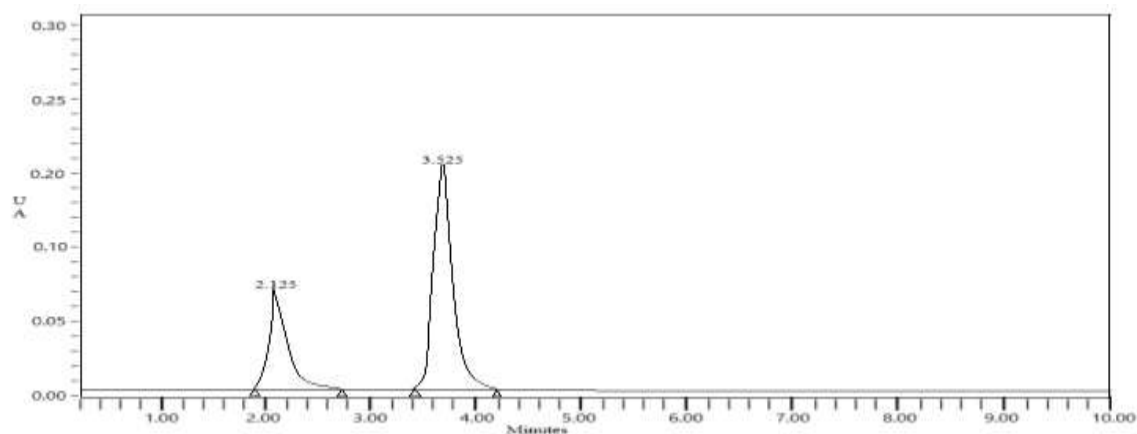


Fig 7.16: Chromatogram showing assay of sample injection-1

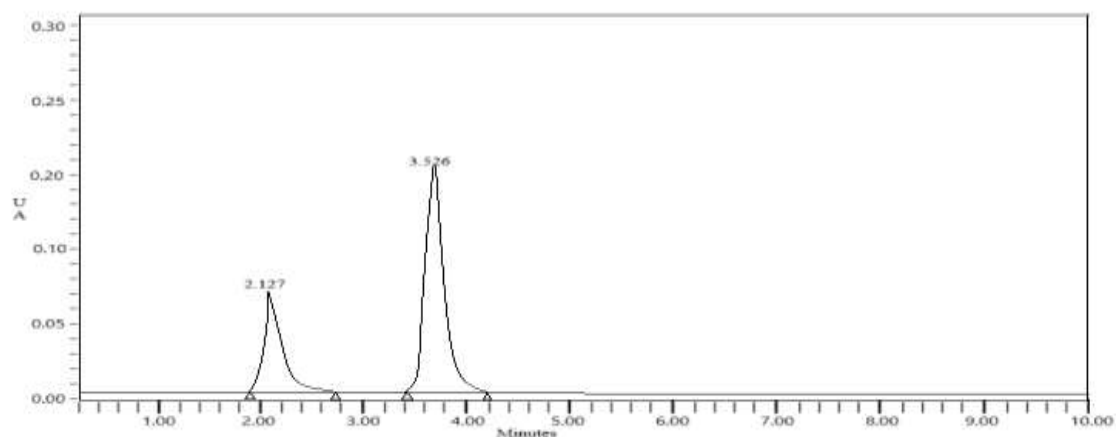


Fig 7.17: Chromatogram showing assay of sample injection-2

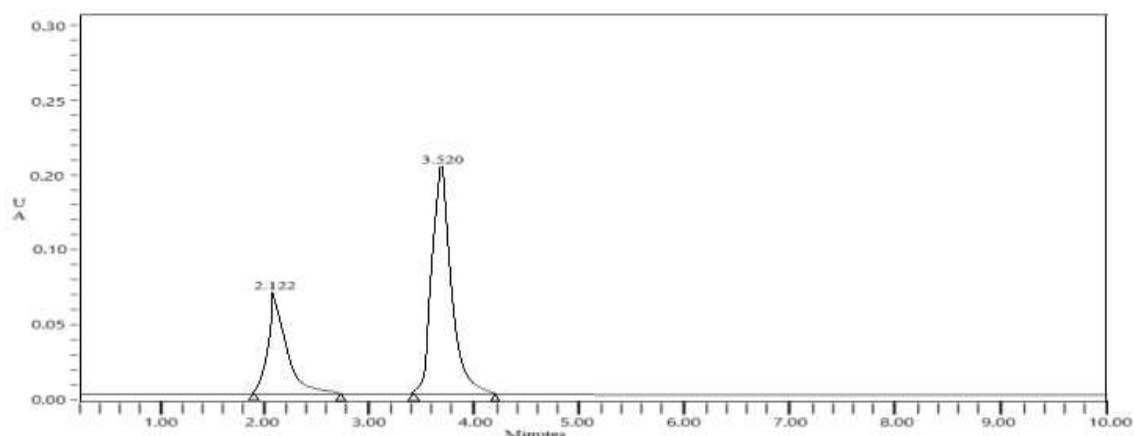


Fig 7.18: Chromatogram showing assay of sample injection-3

Table 7.10: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Amiloride	2.125	665985	685983	4.68	1.06	6965	1
2	Hydrochlorothiazide	3.525	8758985	8546985		1.19	8659	1
3	Amiloride	2.127	662974	685986	4.69	1.07	6928	2
4	Hydrochlorothiazide	3.526	8759864	8569854		1.18	8647	2

5	Amiloride	2.122	669852	685482		1.06	6975	3
6	Hydrochlorothiazide	3.520	8756598	8569853	4.68	1.18	8624	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Amiloride and Hydrochlorothiazide in pharmaceutical dosage form was found to be 99.86%.

LINEARITY

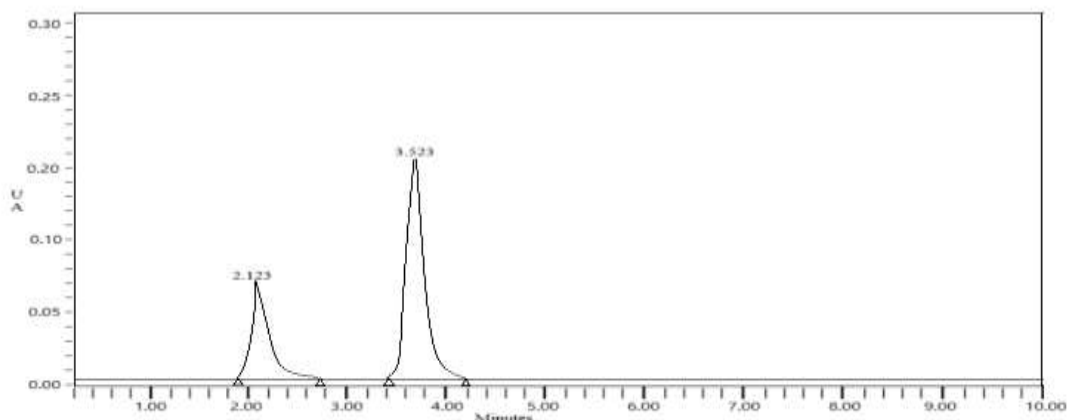


Fig 7.19: Chromatogram for linearity concentration-20 µg/ml of Amiloride & 30 µg/ml of Hydrochlorothiazide

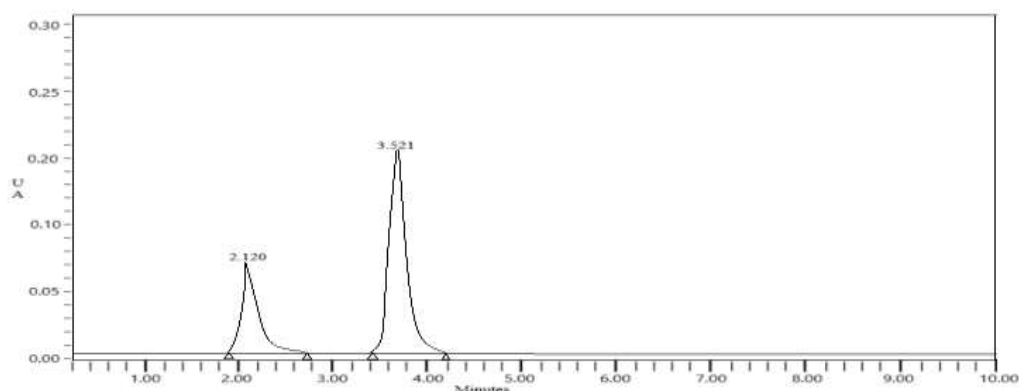


Fig 7.20: Chromatogram for linearity concentration-40 µg/ml of Amiloride & 40 µg/ml of Hydrochlorothiazide

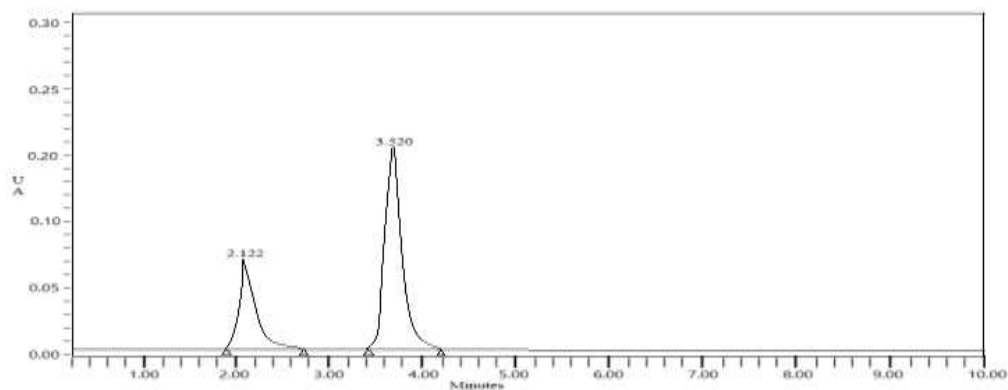


Fig 7.21: Chromatogram for linearity concentration-60 µg/ml of Amiloride & 50 µg/ml of Hydrochlorothiazide

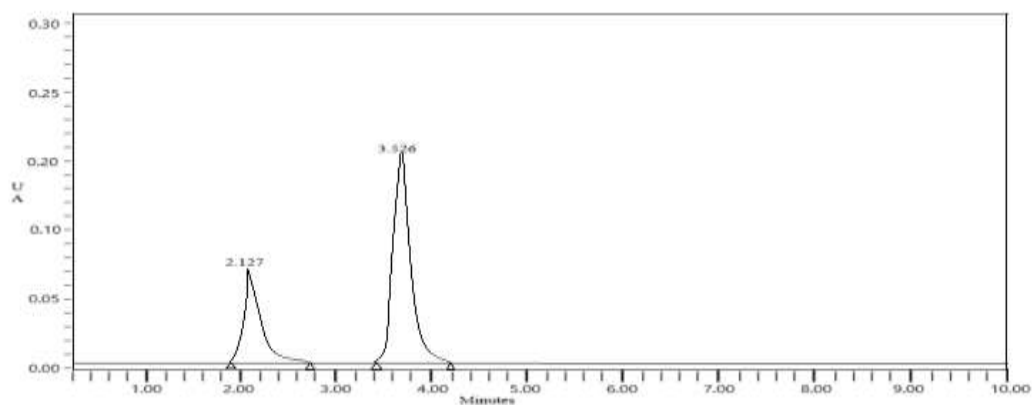


Fig 7.22: Chromatogram for linearity concentration-80 µg/ml of Amloride & 60 µg/ml of Hydrochlorothiazide

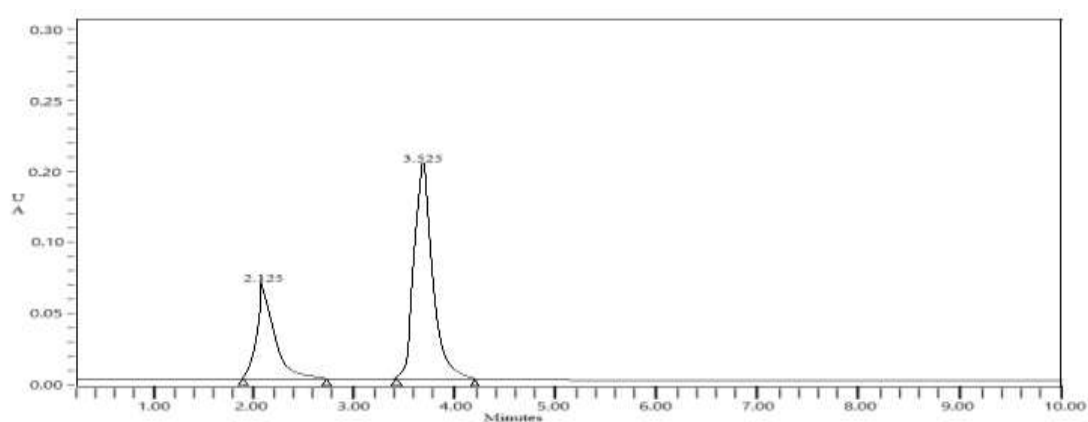
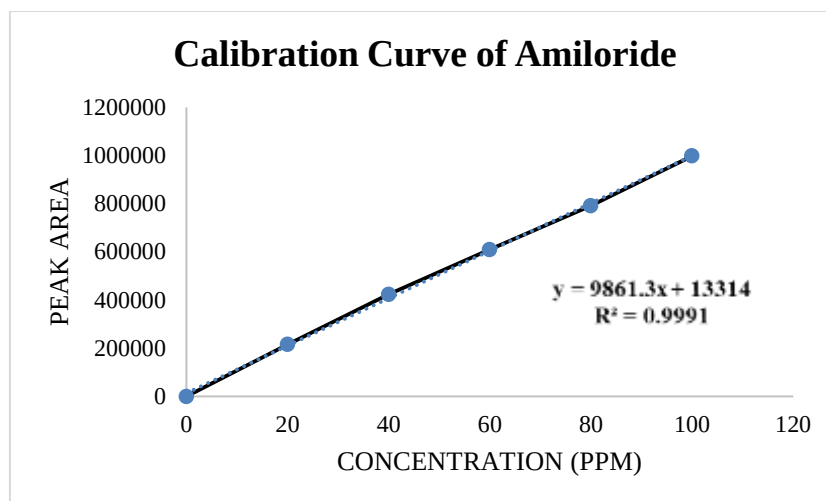


Fig 7.23: Chromatogram for linearity concentration-100 µg/ml of Amloride & 70 µg/ml of Hydrochlorothiazide

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Amlodipine:

Concentration µg/ml	Average Peak Area
0	0
20	215687
40	423568
60	609748
80	791286
100	997987



CONCLUSION

A rapid, precise, and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Amloride and Hydrochlorothiazide in bulk and tablet dosage forms. The method employed a Waters HPLC system equipped with an autosampler and PDA detector (996 model), and separation was achieved using an Altima C18 column (4.6×150 mm, 5 µm particle size). The mobile phase consisted of Methanol and Acetonitrile in a 65:35 v/v ratio, delivered at a flow rate of 1.0 ml/min. The detection wavelength was set at 234 nm, and the column temperature was maintained at 35°C. The injection volume was 10 µl with a total run time of 14 minutes.

Both drugs were well resolved under the optimized conditions, and the method demonstrated good linearity, precision, and recovery. This analytical technique proved to be effective for routine quality control and analysis of Amloride and Hydrochlorothiazide in combined pharmaceutical formulations.

The developed RP-HPLC method is simple, sensitive, and robust for the simultaneous estimation of Amloride and Hydrochlorothiazide in bulk and tablet forms. It offers excellent resolution, acceptable retention times, and high accuracy with minimal sample preparation. Given its reliability and efficiency, this method is highly suitable for routine analysis in pharmaceutical industries.

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