# Chapter III
# Analytical Method

## III.1 TEST PROCEDURE FOR ASSAY OF ALFUZOSIN HCl IN ALFUZOSIN ER TABLETS (10 mg) (BY HPLC)

**Chromatographic Conditions:**

| | | |
|---|---|---|
| Mobile phase | : | Buffer: Acetonitrile: Tetrahydrofuran<br>80: 20 : 1 |
| Buffer | : | 5ml of Perchloric acid was diluted in 900 ml water and pH was adjusted to 3.5 ± 0.05 with dilute sodium hydroxide solution and volume was made upto 1000 ml with water. |
| Column | : | Inertsil ODS 2; 150mm x 4.6mm, 5µm |
| Column temp. | : | Ambient |
| Flow rate | : | 1.5 ml/min |
| Wave length | : | 244nm |
| Inj. Volume | : | 20 µl |
| Run Time | : | 15 minutes |
| Diluent | : | Water: Acetonitrile (70:30) |

**Blank Preparation:**

5 ml Methanol was transferred to a 25ml volumetric flask, diluted upto the mark with diluent and mixed.

**Standard Preparation:**

About 50mg of Alfuzosin HCl working standard was acceurately weighed into a 250ml volumetric flask, 10 ml of methanol was added to it and sonicated to dissolve the standard. The volume was made up to 250 ml with diluent and mixed. 5 ml of this solution was diluted to 25 ml with diluent and mixed.

**Sample Preparation:**

10 tablets were accurately weighed and transferred into a 500ml volumetric flask. About 350ml of methanol was added and was allowed to stand for about 10 min with intermittent shaking to disperse the tablets. It was further sonicated for 30 minutes with intermittent shaking, allowed to cool to room temperature and the volume was made up to 500ml with methanol and further mixed. Solution was filtered through 0.45µ filter and the filtrate was used after discarding 3 ml. 5ml of the filtrate was diluted to 25 ml with diluent and mixed.

**System Suitability Test:**

*To meet the system suitability criteria, following parameters were fulfilled:*

- Number of theoretical plates for the analyte peak in standard preparation were kept not less than 3000.
- Tailing factor for the analyte peak in standard preparation was kept not more than 2.0.
- Relative standard deviation for five replicate injections of standard preparation was kept not more than 2.0%.

**Procedure:**

- Blank preparation was injected.
- Standard preparation was injected five times and compliance for system suitability test was checked.
- Sample preparation was injected in duplicate.

**Calculation:** Assay was calculated by using the following formula

$$\% \text{ Assay} = \frac{\text{Av. Area of Sample}}{\text{Av. Area of Std.}} \times \frac{\text{Std. wt. taken}}{50} \times \frac{\text{sample wt. to be taken}}{\text{sample wt. taken}} \times \text{Potency of std}$$

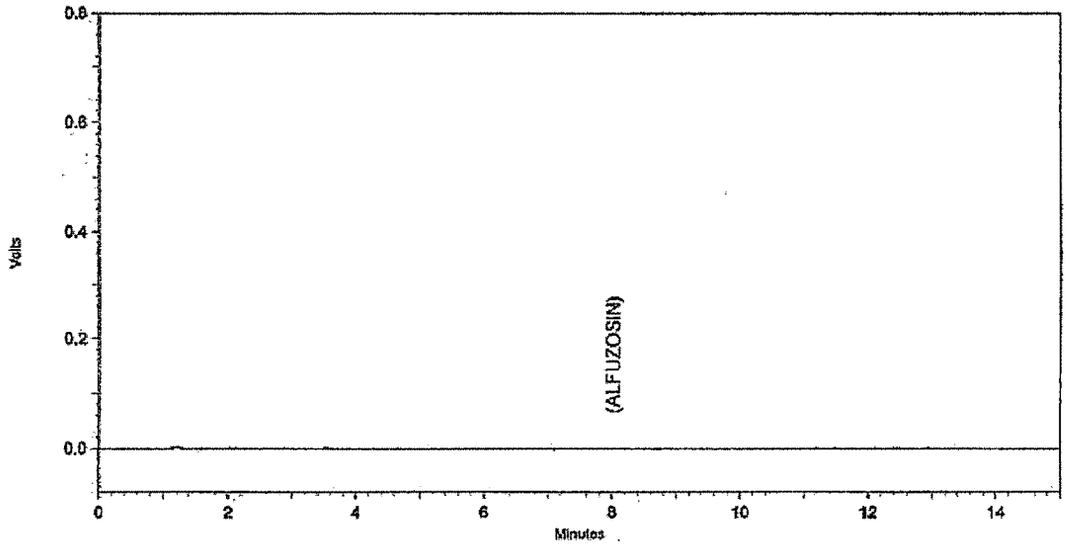


**Figure III. 1 Chromatogram of the blank sample for determination of assay of Alfuzosin Hydrochloride**

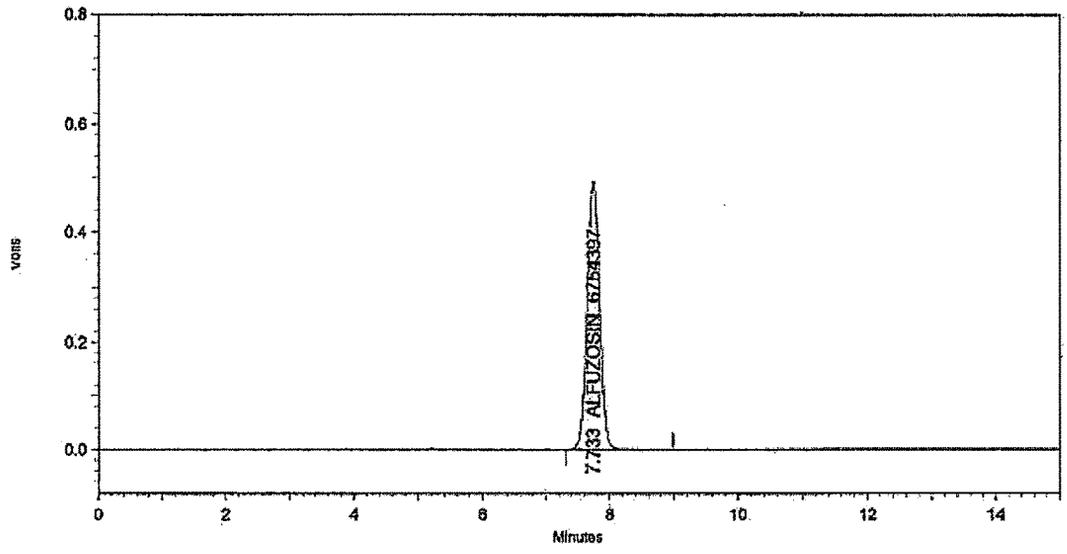


**Figure III. 2 Chromatogram of the standard sample for determination of assay of Alfuzosin Hydrochloride**

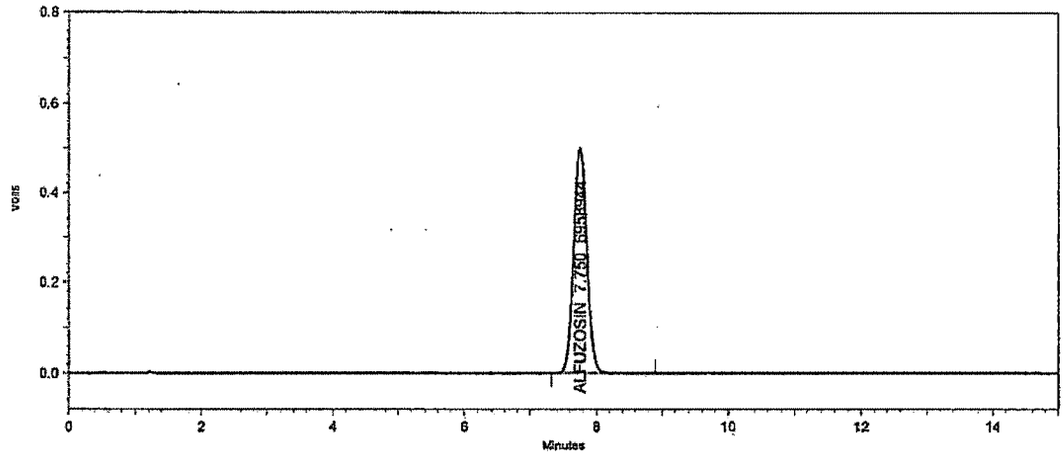


**Figure III. 3 Chromatogram of the test sample for determination of assay of Alfuzosin Hydrochloride**

## III.2 TEST PROCEDURE FOR CONTENT UNIFORMITY OF ALFUZOSIN HCl IN ALFUZOSIN ER TABLETS (10 mg) (BY HPLC)

**Chromatographic Conditions:**

| | | |
|---|---|---|
| Mobile phase | : | Buffer: Acetonitrile : Tetrahydrofuran<br>80: 20 : 1 |
| Buffer | : | 5ml of Perchloric acid was diluted in 900 ml water and pH was adjusted to 3.5 ± 0.05 with dilute sodium hydroxide solution and volume was made upto 1000 ml with water. |
| Column | : | Inertsil ODS 2, 150mm x 4.6mm, 5µm |
| Column temp. | : | 30°C |
| Flow rate | : | 1.5 ml/min |
| Wave length | : | 244 nm |
| Inj. Volume | : | 20 µl |
| Run Time | : | 15 minutes |
| Diluent | : | Water: Acetonitrile (70:30) |

**Blank Preparation:**

5 ml Methanol was transferred to a 25ml volumetric flask, diluted upto the mark with diluent and mixed.

**Standard Preparation:**

About 50 mg of Alfuzosin HCl working standard was accurately weighed into a 250ml volumetric flask, 10 ml of methanol was added to it and sonicated to dissolve the standard. The volume was made up to 250 ml with diluent and mixed. 5 ml of this solution was diluted to 25 ml with diluent and mixed.

**Sample Preparation:**

1 tablet was accurately weighed and transferred into a 250 ml volumetric flask. About 50 ml of methanol was added and was allowed to stand for about 10 minutes with intermittent shaking to disperse the tablet. It was then sonicated for about 15 minutes with intermittent shaking. Further 100 ml of diluent was added and sonicated for 30 minutes with intermittent shaking. It was then allowed to cool to room temperature and the volume was made up to 250ml with diluent, further mixed and filtered through 0.45µ filter. Filtrate was used after discarding 3ml of the filtrate.

**System Suitability Test:**

*To meet the system suitability criteria, following parameters were fulfilled:*

- Number of theoretical plates for the analyte peak in standard preparation were kept not less than 3000.
- Tailing factor for the analyte peak in standard preparation was kept not more than 2.0.
- Relative standard deviation for five replicate injections of standard preparation was kept not more than 2.0%.

**Procedure:**

- Blank preparation was injected.
- Standard preparation was injected five times and compliance for system suitability test was checked.
- Sample preparation was injected in duplicate.

**Calculation:** Assay was calculated by using the following formula and MS excel programme

$$\% \text{ Assay} = \frac{\text{Area of Sample}}{\text{Av. Area of Std.}} \times \frac{\text{Std. concentration}}{\text{Sample concentration}} \times \text{Potency of std}$$

## III.3 TEST PROCEDURE FOR DISSOLUTION OF ALFUZOSIN HCl IN ALFUZOSIN ER TABLETS (10 mg) (BY HPLC)

**Chromatographic Conditions:**

| | | |
|---|---|---|
| Mobile phase | : | Buffer: Acetonitrile<br>75: 25 |
| Buffer | : | 5ml of Perchloric acid was diluted in 900 ml water and pH was adjusted to 3.5 ± 0.05 with dilute sodium hydroxide solution and volume was made upto 1000 ml with water. |
| Column | : | C8, Inertsil 150mm X 4.6mm, 5µm |
| Column temp. | : | 30°C |
| Flow rate | : | 1.2 ml/min |
| Wave length | : | 244nm |
| Inj. Volum | : | 20 µl |
| Run Time | : | 10 min. |
| Diluent | : | Dissolution media. |

**Dissolution Parameters:**

| | | | |
|---|---|---|---|
| Apparatus | : USP Type-II, Paddle | Medium | : 0.01N HCl |
| RPM | : 100 | Volume | : 500 ml |
| Temp | : 37.0°C ±0.5°c | Volume withdrawn | : 10 ml |

**Blank Preparation:**

Dissolution Medium was used as blank.

**Standard Preparation:**

About 50mg of Alfuzosin HCl working standard was accurately weighed into a 250ml volumetric flask, 5 ml of methanol was added and sonicated to dissolve the standard. The volume was made up to 250 ml with diluent and mixed. 5 ml of this solution was diluted to 50 ml with dissolution medium and mixed.

**Sample Preparation:**

One tablet was placed into each of six bowls and the apparatus was operated for 24 hour. 10ml of the sample was withdrawn at the end of specified time interval and same quantity of fresh medium was added after each withdrawal and filtered through 30µ filter.

**System Suitability Test:**

*To pass the system suitability test, following conditions were fulfilled for standard preparation:*

- Number of theoretical plates for the analyte peak in standard preparation were kept not less than 3000.
- Tailing factor was kept not more than 2.0.
- Relative standard deviation for five replicate injections of standard preparation was kept not more than 2.0%.

**Procedure:**

- Blank was injected (dissolution medium).
- Standard preparation was injected five times and and compliance for system suitability test was checked.
- Sample preparation was then injected.

**Calculation:** % drug release was calculated by using following formula

$$\% \text{ Drug release} = \frac{\text{Sample area}}{\text{Av. Std. Area}} \times \frac{\text{Std. Conc.}}{\text{Sample Conc.}} \times \% \text{ Potency of Std}$$

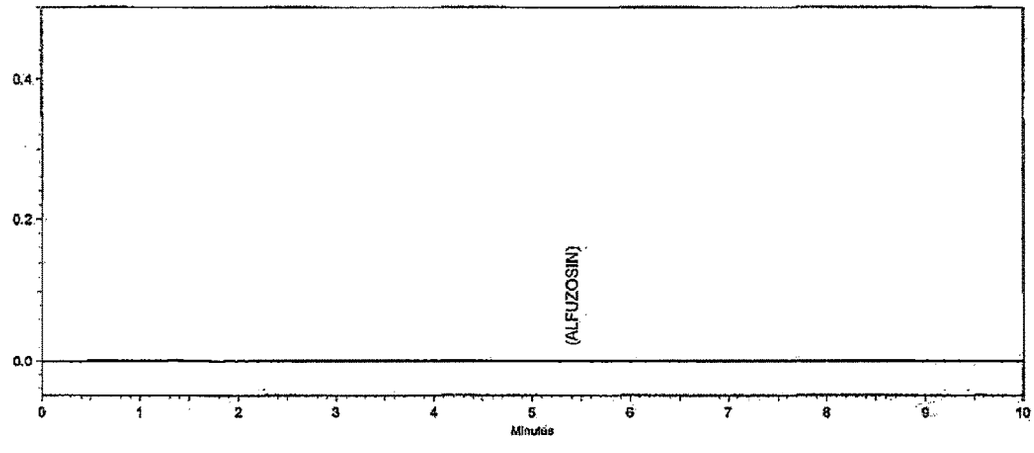


**Figure III. 4 Chromatogram of the blank sample for determination of dissolution of Alfuzosin Hydrochloride**

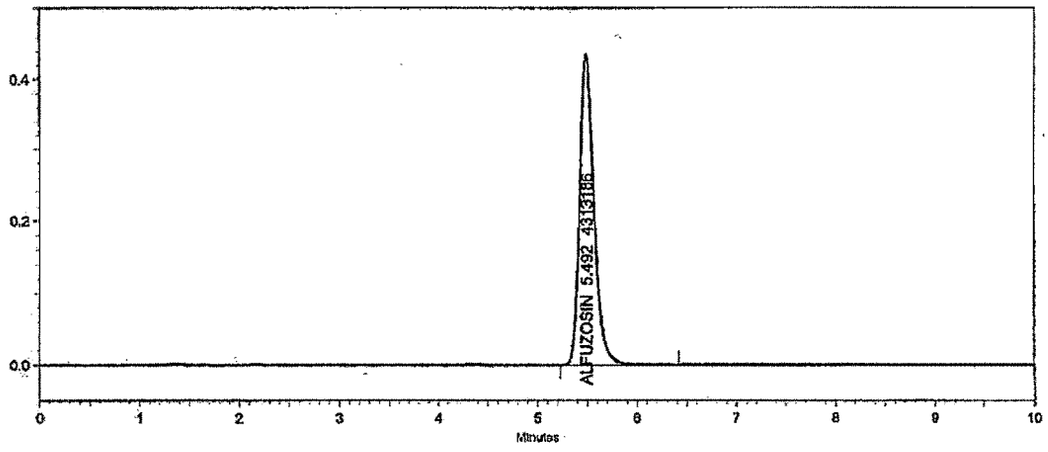


**Figure III. 5 Chromatogram of the standard sample for determination of dissolution of Alfuzosin Hydrochloride**

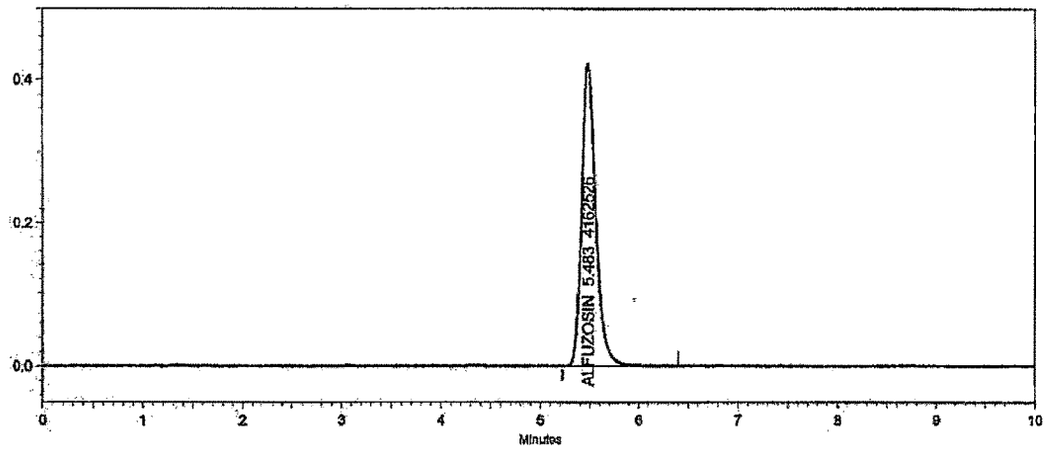


**Figure III. 6 Chromatogram of the test sample for determination of dissolution of Alfuzosin Hydrochloride**

## III.4 TEST PROCEDURE FOR CONTENT RELATED SUBSTANCES OF ALFUZOSIN HCl IN ALFUZOSIN ER TABLETS (10 mg) (BY HPLC)

**Chromatographic Conditions:**

| | | |
|---|---|---|
| Mobile phase | : | Buffer: Acetonitrile : Tetrahydrofuran<br>80: 20 : 1 |
| Buffer | : | 5ml of Perchloric acid was diluted in 900ml water and pH was adjusted to 3.5 ± 0.05 with dilute sodium hydroxide solution and volume was made upto 1000ml with water. |
| Column | : | Inertsil ODS 2; 150mm x 4.6mm, 5µm |
| Column temp. | : | Ambient |
| Flow rate | : | 1.5 ml/min |
| Wave length | : | 244nm |
| Inj. Volume | : | 50 µl |
| Diluent | : | Water: Acetonitrile (70:30) |

**Blank Preparation:**

10 ml Methanol was transferred to a 25ml volumetric flask, diluted upto the mark with diluent and mixed.

**Resolution Solution Preparation:**

*Alfuzosin Impurity A stock standard preparation:*

Accurately about 1mg each of Alfuzosin HCl working standard and Alfuzosin impurity A working standard was weighed into a 50 ml volumetric flask. About 5 ml of methanol was added and sonicated to dissolve. The volume was made to 50ml with diluent and mixed.

**Diluted Standard Preparation:**

Accurately about 20 mg of Alfuzosin HCl working standard was weighed into a 250 ml volumetric flask. About 10 ml of methanol was added and sonicated to dissolve. The volume was made upto 250 ml with diluent and mixed. Further 2 ml of this solution was diluted to 100 ml with diluent and mixed.

**Sample Preparation:**

10 tablets were accurately weighed and transferred into a 500ml volumetric flask. About 350ml of methanol was added and allowed to stand for about 10 min with intermittent shaking to disperse the tablets. It was further sonicated with intermittent shaking for about 30 min and allowed to cool to room temperature and the volume was made up with methanol and mixed. It was filtered through 0.45µ Nylon filter after discarding first 3 ml of filtrate. 10 ml of the filtrate was diluted to 25 ml with diluent and mixed.

**System Suitability Test:**

*To meet the system suitability criteria, following parameters were fulfilled:*

- Resolution between peak due to Alfuzosin and Alfuzosin impurity A in resolution solution preparation was kept less than 1.2.
- Number of theoretical plates for the analyte peak in standard preparation were kept not less than 3000.
- Tailing factor for the analyte peak in standard preparation was kept not more than 2.0.
- Relative standard deviation for five replicate injections of standard preparation was kept not more than 2.0%.

**Procedure:**

- Blank was injected.
- Resolution solution preparation was injected once and standard preparation six times and compliance for system suitability test was checked.
- Sample preparation was injected in single and the chromatogram was recorded.

*Disregard Limit:*

In the sample chromatogram, any peak due to blank and peak with an area less than 0.1 times the area obtained in the chromatogram of diluted standard preparation (0.02%) was disregarded.

**Calculation** : % Known impurity and unknown impurity was calculated using following formula

$$\% \text{ Kown imp.} = \frac{\text{Impurity area}}{\text{Av. Area of diluted std}} \times \frac{\text{Std dilutions}}{\text{Test dilutions}} \times \frac{\text{Standard potency}}{100} \times \frac{1}{\text{rrf}} \times 100$$

$$\% \text{ Unkown imp.} = \frac{\text{Impurity area}}{\text{Av. Area of diluted std}} \times \frac{\text{Std dilutions}}{\text{Test dilutions}} \times \frac{\text{Standard potency}}{100} \times 100$$

% Total impurities = % Known impurities + % Unknown impurities

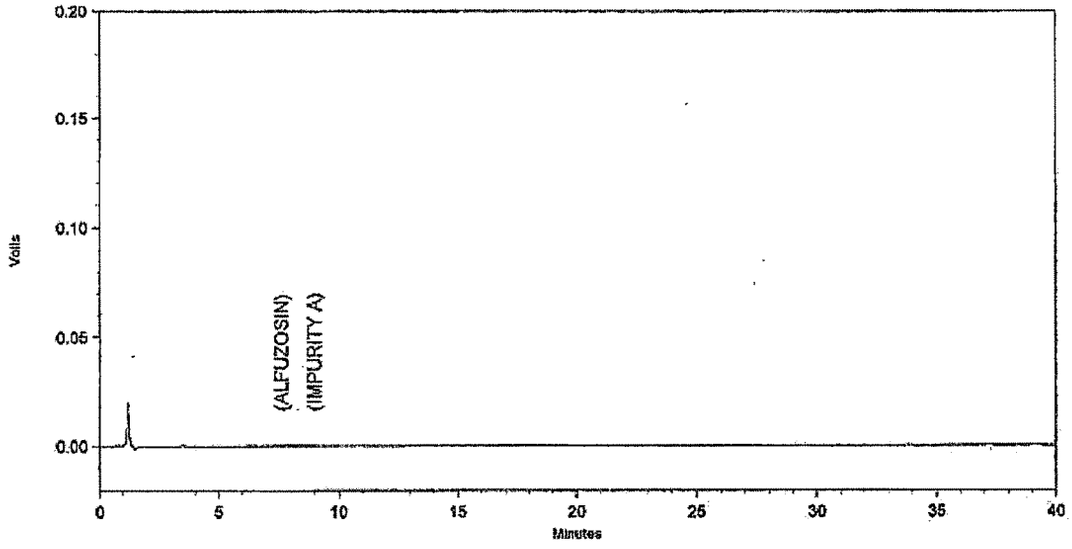


**Figure III. 7 Chromatogram of the blank sample for determination of related substance of Alfuzosin Hydrochloride**

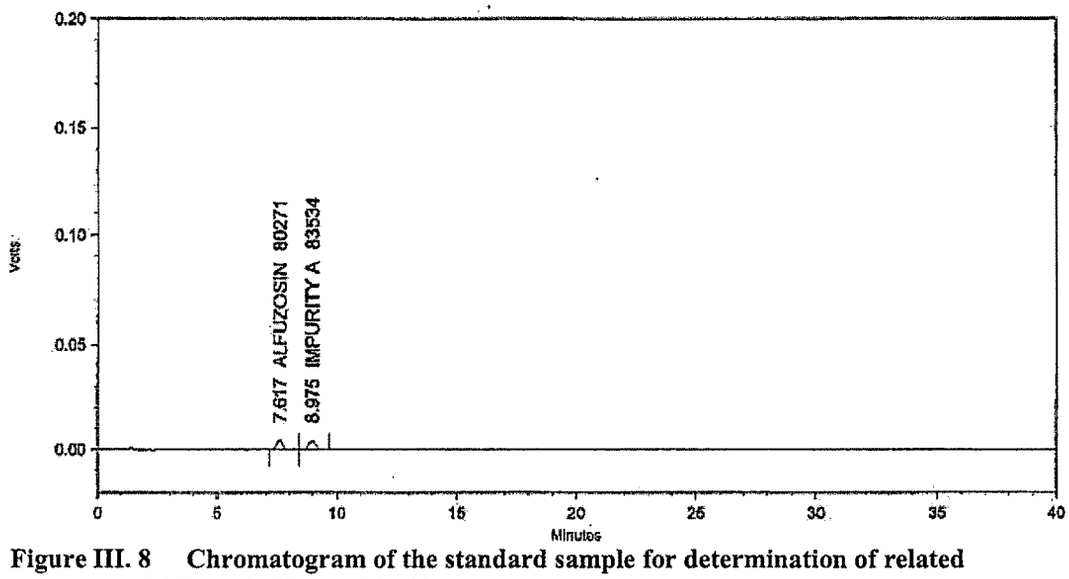


**Figure III. 8 Chromatogram of the standard sample for determination of related substance of Alfuzosin Hydrochloride**

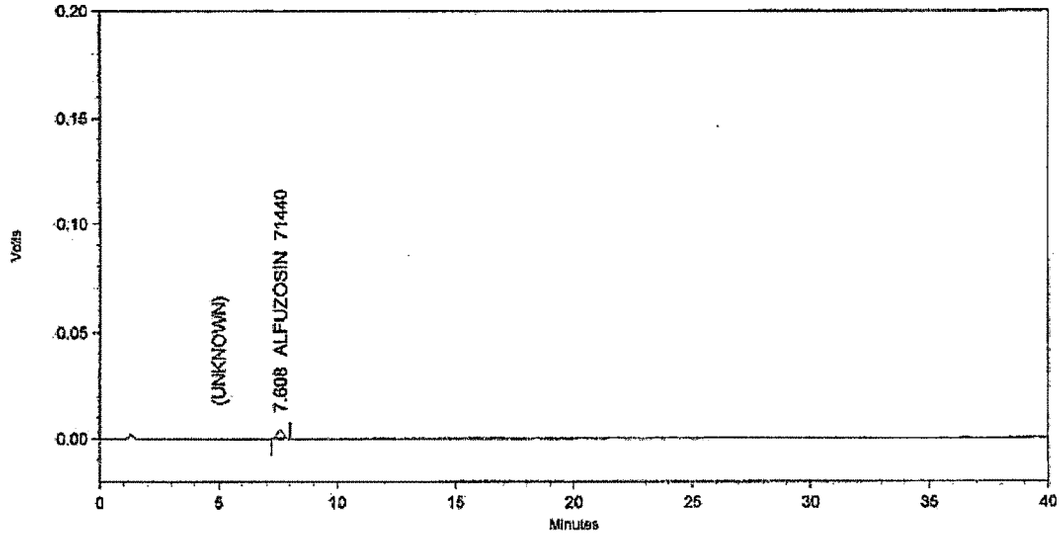


**Figure III. 9 Chromatogram of the test sample for determination of related substance of Alfuzosin Hydrochloride**

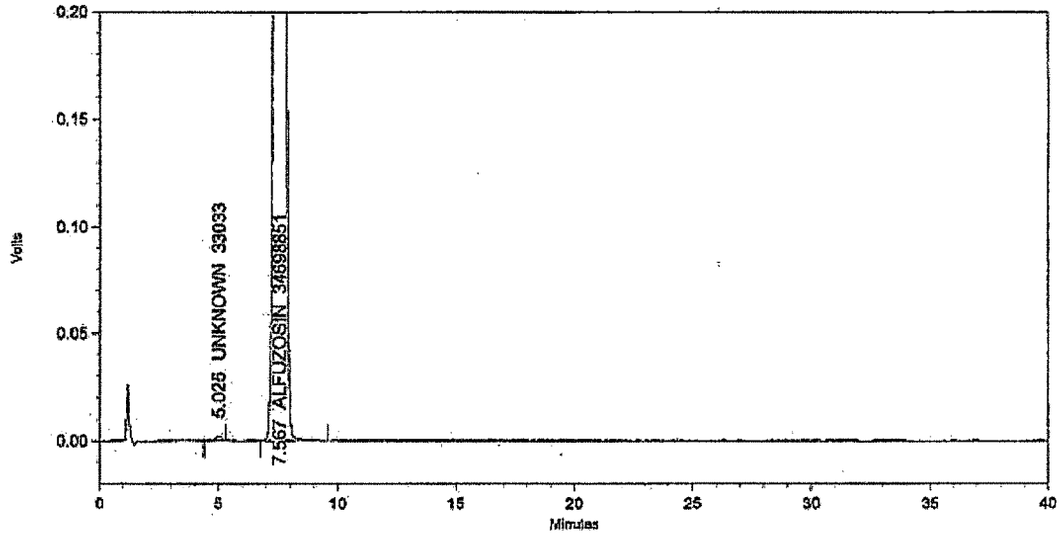


**Figure III. 10 Chromatogram of the test sample for determination of related substance of Alfuzosin Hydrochloride**