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# Method Development and Validation of Febuxostat in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Method

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**ABSTRACT**
Febuxostat is chemically 2-(3-cyano-4-isobutoxyphenyl) -4-methyl-1, 3-thiazole-5-carboxylic acid using for the treatment of gout and hyperuricemia. It achieves its therapeutic effect by decreasing serum uric acid, febuxostat is not expected to inhibit other enzymes involved in purine and pyrimidine synthesis and metabolism at therapeutic concentrations. Febuxostat inhibits xanthine oxidase by blocking its molybdo pterinium site to controls the conversion of hypoxanthine to xanthine leads to decreasing the uric acid production. A novel simple RP-HPLC method has been developed for the estimation of Febuxostat related substances in bulk and formulations. The chromatographic separation was achieved Inertsil C18 column using Acetonitrile and methanol (25:75) as mobile phase , flow rate was fixed at 1.0 ml/min because of good peak area and satisfactory retention time .The maximum absorbance was found to be at 315nm , injection volume was selected to be 20µl which gave a good peak area. Run time is to be 6 min because analyze gave peak around 2.922 and also reduce the total run time .The present recovery was found to be 98.0-102 was linear and precise over the same range .The analytical method was found linearity over the range of 20-70 ppm of the target concentration, the analytical passed both robustness and ruggedness tests on both cases, relative standard deviation was well satisfactory. The proposed method was optimized and validated as per the ICH guidelines.
**Key words:** Febuxostat, RP-HPLC, chromatographic separation, retention time.

## I. INTRODUCTION

Febuxostat was used to treat chronic gout and hyperuricemia. More effective than standard doses of allopurinol, but not more effective than higher doses of allopurinol. Febuxostat works by decreasing the amount of uric acid that is made in the body. It is used to prevent gout attacks. Febuxostat is a non-hygroscopic material, appearance was white crystalline powder that is freely soluble in dimethylformamide; soluble in dimethylsulfoxide; slightly soluble in methanol and acetonitrile; and practically insoluble in water. The melting range is 205°C to 208°C. Febuxostat is chemically designated as 2-[3-cyano4-(2-methylpropoxy) phenyl]-4-methyl-1, 3- thiazole-5-carboxylic acid. Its molecular formula is C16H16N2O3S, and its molecular weight is 316.375g/mol. [1-3]

## EXPERIMENTAL

**Equipments**:

The chromatographic technique performed on WATERS Model NO.2695 series Compact System Consisting of Inertsil-C18 ODS column, Electronic balance (SARTORIOUS), Digital pH meter(POLOMAN) and Sonicator ( FAST CLEAN).

**Materials**

Pharmaceutically pure sample of Febuxostat were obtained as gift samples from Fortune pharma training institute, Sri Sai Nagar, KPHB and Hyderabad, India. HPLC-grade Acetonitrile wasfrom qualigens reagents Pvt Ltd.

**METHOD DEVELOPMENT FOR HPLC[1-16]:**

**Trial: 1**

**Mobile Phase:** 100% pure degaussed methanol.

**Preparation of Standard Solution:**

10mg of Febuxostat drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from this and diluted to 10 ml with mobile phase.

**Chromatographic Conditions:**

Flow rate: 1.0ml/min
Column : Inertsil - C18 ODS column Detector wavelength:315nm
Column temperature: Ambient Injection volume: 20µl
Run time: 6min
Retention time: 3.261

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**Trail: 2**.
**Mobile Phase:** methanol and Acetonitrile were mixed in the ratio of 90:10V/V and sonicated to degas.
**Preparation of Standard Solution:**
10mg of Febuxostat drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

**Chromatographic Conditions:**
Flow rate: 1ml/min
Column : Inertsil -C18 ODS column
Detector wavelength: 315nm
Column temperature: Ambient
Injection volume: 20µl
Run time: 5min
Retention time:2.925

**Trail: 3**.
**Mobile Phase:** Methanol and Acetonitrile were mixed in the ratio of 80:20 V/V and sonicated to degas.
**Preparation of Standard Solution:**
10mg of Febuxostat drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

**Chromatographic Conditions:**
Flow rate: 1.0ml/min
Column : Inertsil - C18 ODS column
Detector wavelength: 315nm
Column temp: Ambient
Injection volume : 20µl
Run time: 6min
Retention time: 2.910

**OPTIMIZED METHOD**
**Mobile Phase:** Acetonitrile and Methanol were taken and sonicated to degas in the ratio of 25:75.
**Preparation of stock solution:**
10mg of Febuxostat drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 2 ml. was taken from this and diluted to 10ml.with mobile phase.

**Preparation of working standard solution:**
The stock solution equivalent to 20ppm to 80ppm were prepared, sonicated and filtered through 0.45µ membrane.

**TABLE: 1 Optimized chromatographic conditions:**

| Parameters | Method |
|---|---|
| Stationary phase (column) | Inertsil -ODS C18 (250 x 4.6 mm, packed with 5 micron) |
| Mobile Phase | Acetonitrile and Methanol (25:75) |
| Flow rate (ml/min) | 1.0 ml |
| Run time (minutes) | 6 |
| Column temperature (°C) | Ambient |
| l)µVolume of injection loop ( | 20 |
| Detection wavelength (nm) | 315nm |
| Drug RT (min) | 2.922 |

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**METHOD VALIDATION**

**SYSTEM SUITABILITY:**

A Standard solution was prepared by using Febuxostat working standard as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Febuxostat**,** retention times and peak areas.

**ACCEPTANCE CRITERIA:**

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %.
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Febuxostat peaks is NLT 3000.
4. The Tailing factor (T) for the Febuxostat peaks is NMT 2.0

**SPECIFICITY:**

**Febuxostat identification:**

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

**ACCEPTENCE CRITERIA:**

Chromatogram of standard and sample should be identical with near Retention time.

**PRECISION:**

**Repeatability:**

a. System precision: Standard solution prepared as per test method and injected five times.
b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

**ACCEPTANCE CRITERIA:** The % relative standard deviation of individual Febuxostat, from the six units should be not more than 2.0%. The assay of Febuxostat should be not less than 98% and not more than 102.0%.

**Intermediate precision (analyst to analyst variability):**

A study was conducted by two analysts as per test method

**ACCEPTENCE CRITERIA:**

The individual assays of Febuxostat should be not less than 98% and not more than 102% and %RSD of assay should be NMT2.0% by both analysts.

**ACCURACY (RECOVERY):**

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Febuxostat into each volumetric flask for each spike level to get the concentration of Febuxostat equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Febuxostat was calculated.

**ACCEPTANCE CRITERIA:**

The mean % recovery of the Febuxostat at each spike level should be not less than 98.0% and not more than 102.0%.

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

**LINEARITY OF TEST METHOD:**

A Series of solutions are prepared using Febuxostat working standard at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

**ACCEPTANCE CRITERIA:**

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

% of RSD for level 1 and Level 6 should be not more than 2.0%.

**RUGGEDNESS OF TEST METHOD:**

**System to system variability:**

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method.

Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

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**ACCEPTANCE CRITERIA:**
The % relative standard deviation of Febuxostat from the six sample preparations should be not more than 2.0%
The % assay of Febuxostat should be between 98.0%-102.0%.

**ROBUSTNESS:**
**Effect of variation of flow rate:**
A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.
Febuxostat was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

**ACCEPTANCE CRITERIA:**
The Tailing Factor of Febuxostat standards should be NMT 2.0 for Variation in Flow.

**LIMIT OF DETECTION AND QUANTITATION (LOD and LOQ):**
From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

## II. RESULTS AND DISCUSSION

**Method development:**

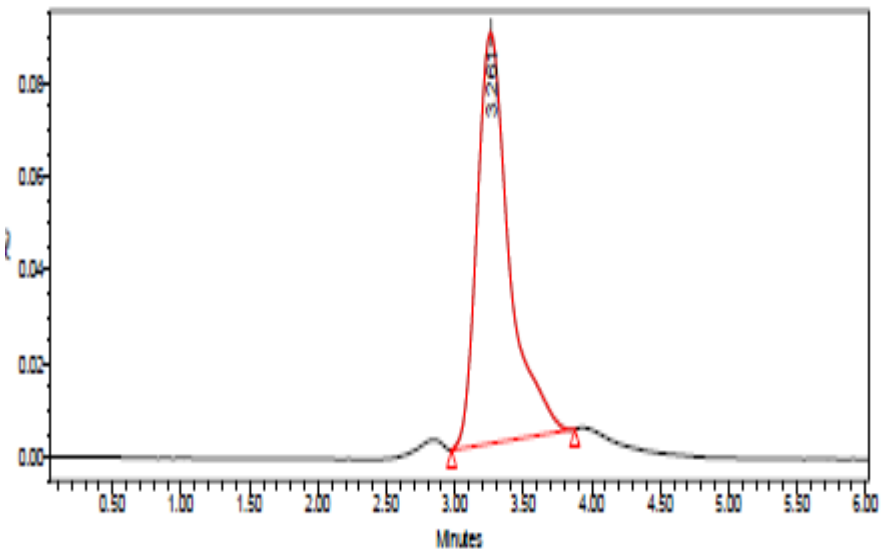


**Fig 1: Chromatogram of Trial 1**

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**TABLE : 2 Inference : Got noise base line and peak tailing.**

| S.NO | Name of the peak | Retention time(min) |
|---|---|---|
| 1. | FEBUXOSTAT | 3.261 |

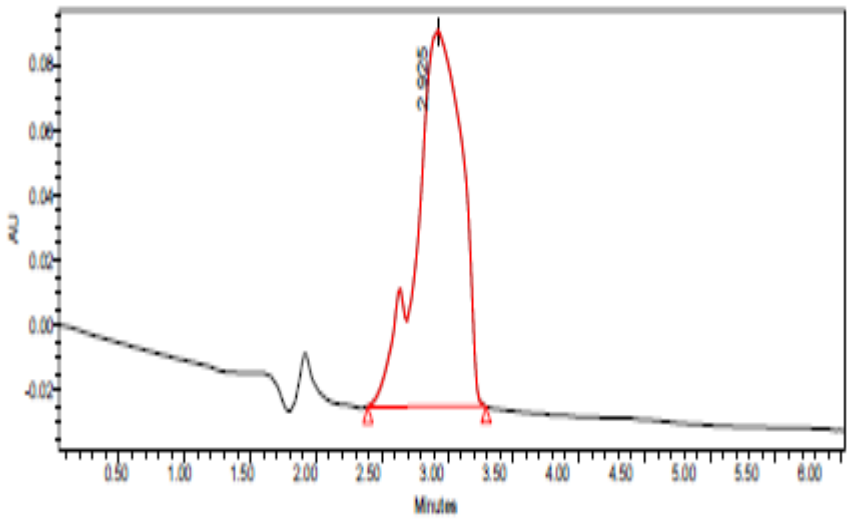


**Fig 2 : Chromatogram of Trial 2**

**TABLE : 3 Inference: Got more asymmetry**

| S.NO | Name of the peak | Retention time(min) |
|---|---|---|
| 1 | FEBUXOSTAT | 2.925 |

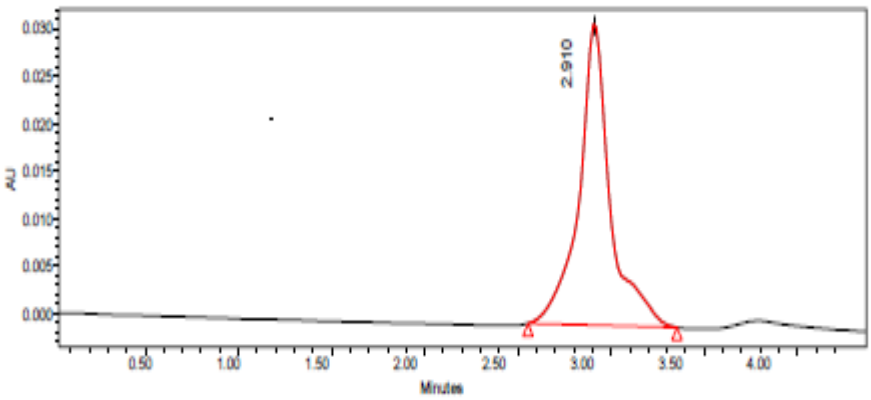


**Fig 2: Chromatogram of Trial 3**

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**Table No. 4 Inference: Got Bad Peak.**

| S.NO | Name of the peak | Retention time(min) |
|---|---|---|
| 1. | **FEBUXOSTAT** | 2.910 |

**OPTIMIZED METHOD**

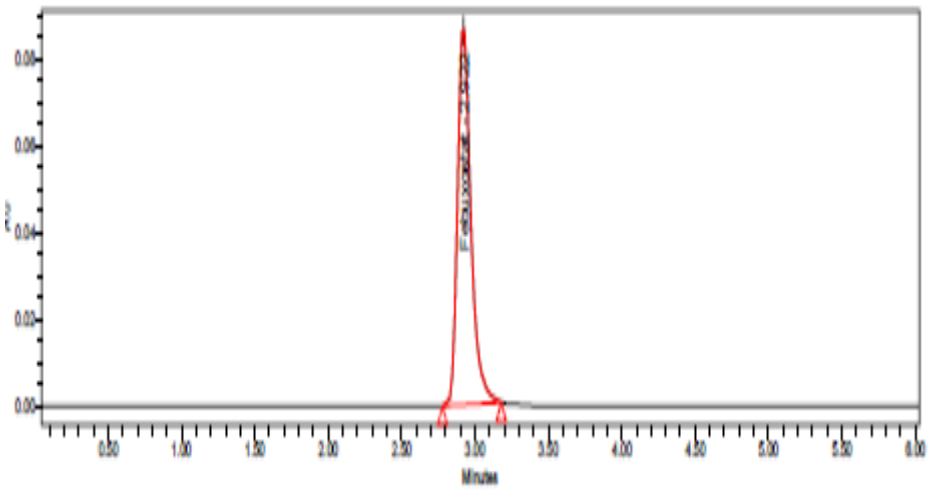

**Fig 3: Chromatogram of standard**

**TABLE: 4 Inference: Got chromatogram at an Rt of 2.922for standard**

| S.NO | Name of the peak | Retention time(min) |
|---|---|---|
| 1 | **FEBUXOSTAT** | 2.922 |

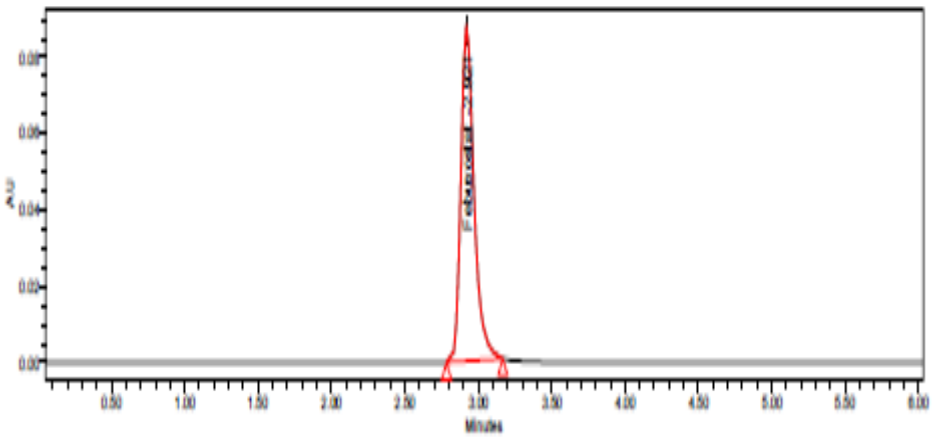

**Fig 4: Chromatogram of sample**

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**TABLE: 5 Inference: Got same peak with same Rt2.921as of standard.**

| S.NO | Name of the peak | Retention time(min) |
|---|---|---|
| 1. | **FEBUXOSTAT** | 2.921 |

**SYSTEM SUITABILITY:**

**TABLE-6: Data of System Suitability**

| Injection | RT | Peak Area | USP Plate count | USP Tailing |
|---|---|---|---|---|
| 1 | 2.921 | 566245 | 9005 | 1.116 |
| 2 | 2.920 | 566022 | 9046 | 1.119 |
| 3 | 2.922 | 566378 | 9014 | 1.120 |
| 4 | 2.923 | 566624 | 9094 | 1.117 |
| 5 | 2.921 | 566099 | 9055 | 1.118 |
| Mean | 2.921415 | 566273 | 9042 | 1.118 |
| SD | 0.001095 | 238.896 | ------- | ------- |
| % RSD | 0.024156 | 0.042 | ------- | ------- |

**System precision:**

**TABLE-7 Data of Repeatability (System precision)**

| | Injection | Peak Areas of Febuxostat | %Assay |
|---|---|---|---|
| **Concentration 40ppm** | 1 | 566123 | 100.12 |
| | 2 | 566540 | 100.05 |
| | 3 | 566219 | 100.14 |
| | 4 | 566360 | 100.16 |
| | 5 | 566354 | 100.16 |
| **Statistical Analysis** | **Mean** | 566319 | 100.12 |
| | **SD** | 158.2425 | 0.046 |
| | **% RSD** | 0.027 | 0.046 |

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**LINEARITY:**

**TABLE 8: Data of Linearity**

| Concentrati on (ppm) | Average Area | Statistical Analysis | |
|---|---|---|---|
| 0 | 0 | Slope | 14156 |
| 20 | 283120 | y-Intercept | -818.1 |
| 30 | 424680 | Correlation Coefficient | 0.999 |
| 40 | 566240 | | |
| 50 | 702073 | | |
| 60 | 849360 | | |
| 70 | 990920 | | |

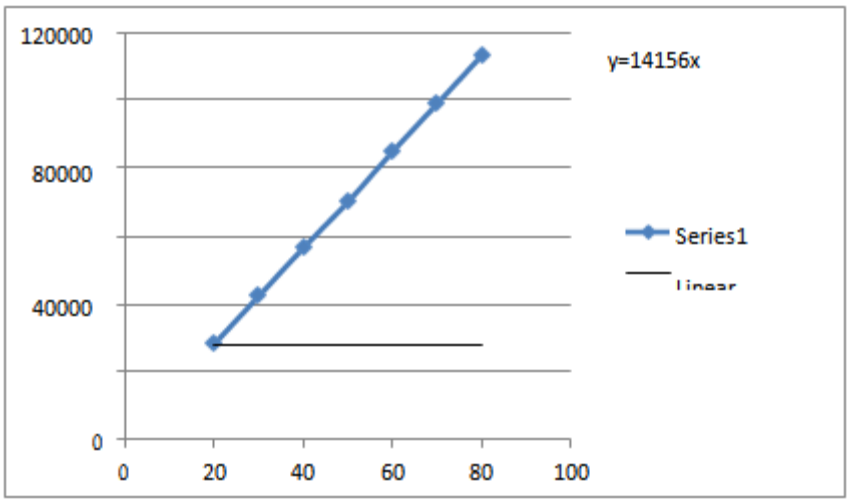


**Fig: 5 Linearity Plot** (Concentration Vs Response)

**System to System variability:**

**TABLE: 09 Data of system to system variability (sample)**

| S.NO: | Peak area | Assay % of Febuxostat |
|---|---|---|
| 1 | 566078 | 100.11 |
| 2 | 566534 | 100.19 |

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| | | |
|---|---|---|
| 3 | 566782 | 100.24 |
| 4 | 566196 | 100.13 |
| 5 | 566987 | 100.27 |
| 6 | 566420 | 100.03 |
| Mean | 566499 | 100.16 |
| %RSD | 0.0608 | 0.089 |

**Robustness:**

**TABLE: 10 Data for Effect of variation in flow rate:**

| Flow 0.8 ml | Std Area | Tailing factor | Flow 1.0 ml | Std Area | Tailing factor | Flow 1.2 ml | Std Area | Tailing factor |
|---|---|---|---|---|---|---|---|---|
| | 560215 | 1.116 | | 566602 | 1.120 | | 567045 | 1.133 |
| | 560481 | 1.120 | | 566315 | 1.122 | | 567354 | 1.135 |
| | 560398 | 1.122 | | 566812 | 1.120 | | 567205 | 1.134 |
| | 560124 | 1.128 | | 566094 | 1.121 | | 567700 | 1.134 |
| | 560820 | 1.127 | | 566384 | 1.122 | | 567403 | 1.133 |
| Avg | 560407 | 1.122 | Avg | 566441 | 1.121 | Avg | 567341 | 1.133 |
| SD | 270.683 | 0.0049 | SD | 170.2416 | 0.00089 | SD | 244.44 | 0.0008 |
| %RSD | 0.048 | 0.44 | %RSD | 0.0202 | 0.0804 | %RSD | 0.043 | 0.073 |

**LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ):**

From the linearity plot the LOD and LOQ are calculated:

$$\text{LOD} = \frac{3.3\sigma}{S}$$

$$= \frac{3.3 \times 238.896}{14156}$$

$$= 0.055$$

$$\text{LOQ} = \frac{10\sigma}{S}$$

$$= \frac{10 \times 238.896}{14156}$$

$$= 0.168$$

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## III. SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 315nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C18 chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of Acetonitrile and Methanol (25:75)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonocation time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyze gave peak around2.922and also to reduce the total run time.

The present recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.055. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-70ppm of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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