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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF BICALUTAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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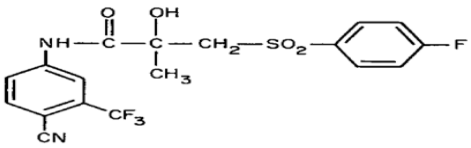
## ABSTRACT

A specific, accurate and precise Reverse Phase High Performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of bicalutamide in bulk and tablet dosage forms. A Symmetry $C_{18}$ (250 x 4.6 mm, 5µ) column with a moble phase of aetonitrile: water (90:10) was used. A flow rate of 0.5 ml/min was maintained. UV detection was performed at 270 nm. The retention time of bicalutamide was 6.15 min, and the total run time was 20 min. The method was validated as per ICH Q2 (R1) guidelines, for specificity, linearity, accuracy, precision, and robustness. The recovery of bicalutamide in tablets was found to be in the range 99-100.83%.

**KEYWORDS:** Bicalutamide, HPLC, Validation.

## INTRODUCTION

Bicalutamide is an oral non-steroidal anti-androgen, used in the treatment of prostate Cancer and hirsutism.[1] It competitively inhibits the action of androgens by binding to cytosol androgen receptors in the target tissue. It is chemically, N-[4- cyano-3(trifluoromethyl) phenyl]-3-[(4-fluorophenyl) sulfonyl)]-2-hydroxy-2-methyl propanamide. Literature survey reveals that various spectrophotometric[2] and HPLC methods[3-4] have been reported for the determination of bicalutamide in bulk and pharmaceutical dosage forms. In this study a simple, rapid, accurate, sensitive and precise HPLC method was developed for the estimation of bicalutamide in pharmaceutical dosage forms.



## EXPERIMENTAL

### Instrumentation

The separation was carried out on HPLC system (Waters) with Waters 1525 binary HPLC pump, UV absorbance detector, LC Solutions software and Enable C18H 250x4.6mm.

### Chemicals and Reagents

Bicalutamide was a gift sample by Hetero Labs Pvt. Ltd, Hyderabad. Acetonitrile of HPLC grade were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

### HPLC conditions

The mobile phase consisting Potassium dihydrogen orthophosphate and acetonitrile (HPLC grade) were filtered through 0.2 µm membrane filter before use, degassed mixture acetonitrile and HPLC water in the ratio of 90: 10 v/v was pumped into the column at a flow rate of 1.0ml/min. The detection was monitored at 270nm and the run time was 15min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

**Preparation of working standard solution:** Weighed and transfer about 10 mg o of bicalutamideinto a 10 ml clean, dry volumetric flask and made up to volume with mobile phase to get concentration 1 mg/ml (Stock). From stock solution 1ml was diluted with 10ml using mobile phase to get a concentration of100 µg/ml. From this solution 1ml of solution was transferred into 10ml volumetric flask and volume was adjusted with mobiles phase to get 10µg/ml.

### Preparation of sample drug solution for pharmaceutical formulations

Weighed contents of not less than 10 tablets. Crush pellets into uniform fine powder in suitable Powdering device. Weighed and transfer accuretly quantity of powder equivalent to about 10mg og Bicalutamide to a 10ml clea, dry grauated tube. Add about 10 ml of acetonitrile and Sonicate for about 20 min at room temparature with intermittent shaking.

Allow the solution to cool to room temperature and dilute volume with mobile phase and mix and filter through 0.2 micron filter.

**Assay**

Two commercial brands of tablets were chosen for testing suitability of the proposed method to estimate bicalutamide in pharmaceutical dosage forms. Twenty tablets were weighed accurately and powdered. A quantity equivalent to 50mg of bicalutamide was weighed accurately and transferred to 50ml volumetric flask. About 30ml of acetonitrile was added and kept in ultrasonic bath for 20min. This solution is filtered through a membrane filter and the volume was made up to the mark with mobile phase to get 1mg/ml concentration. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of bicalutamide present in tablet formulation was determined by comparing the peak area from the standard. The results are furnished in Table-6.

**Validation of proposed method**

Selectivity of the method was assessed on the basis of elution of bicalutamide using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters has been validated for the determination of bicalutamide.

**Specificity**

System suitability for specificity was carried out to determine whether there is any interference of any impurities at retention time of analytical peak. The study was performed by injecting blank. The chromatogram is shown in the figure 4.8-4.10.

**Linearity**

The linearity study was performed for the concentration of 100ng/ml to 3000ng/ml. Each dilution was injected into HPLC system. The area of each dilution was used to calculate the correlation coefficient. The chromatograms are shown in the figure and results are tabulated in table. Chromatograms are shown in figure no 4.22-4.28.

**Precision**

The standard solution was prepared as per the proposed assay method in six determinations and was injected into HPLC system. The retention time and peak area of six determinations was measured and %RSD was calculated.

**LOD and LOQ**

LOD and LOQ values are besend on the visual evaluation method, showing chromate gram figure no.4.31-4.39.

**Accuracy**

The accuracy study was performed for 80, 100, and 120 percentage of bicalutamide. Each dilution was injected in triplicate into HPLC system. The area of each level was used for calculation of %RSD. Chromatograms are shown in figure 4.35-4.44.

The present study reported in thesis was aimed to develop a new method to estimate bicalutamide in rat plasma and its application to pharmacokinetic study by RP-HPLC.

**RESULTS AND DISCUSSION**

The purity of bicalutamide is confirmed by melting point, TLC, UV, HPLC and IR spectroscopy studies. The characters of bicalutamide are as follows.

- Description: white crystalline powder, Melting point: 191-193$^0$C
- Solubility: practically insoluble in water, sparingly soluble in methanol soluble in acetonitrile.
- The TLC studies were performed on precoated silica gel plate and mobile phase consisted chloroform: Petroleum ether (2:1), toluene: ethyl acetate (1:1), toluene: methanol (4.3:5.7) indicated the presence of a single spot at $R_f$ 0.2, 0.5, 0.885 respectively. Detection was made in iodine and UV chamber.
- UV absorption is maximum at 270 nm.
- All characteristic peaks of bicalutamide IR spectrum were observed.

1689.53 (C=C stretch) 3100, (C-H bend) 3579.64 (N-H stretch), 1326.92 (N-H bend), 1515.94 (C=O stretch, amide group), 2229.56 (C=N stretch), 1141.78, 1326.93, 1180.35 (C-F stretch), 1053.06 (S-O stretch), 3336.62 (O-H stretch, alcoholic group)

- The purity of sample was also confirmed by RP-HPLC analysis using mobile phase acetonitrile: Water (90:10). The results indicated that a single prominent peak appeared

with a retention time 6.122 min and there were no minor impurities were observed, thus confirming the sample is pure.

- The retention time of bicalutamide was found to be 6.12min and the system suitability studies were done with 800ng concentartion of standard drug. The %RSD values are below 2%. The percentage purity of bicalutamide in pharmaceutical dosage form was found to be 109.312%. The chromatograms for sample, standard and blank injection showing in figure 4.2-4.4
- The %RSD of area of system precision was found to be 1.75. Precision results are within the limits (NMT 2). The % RSD for the area of all replicate injection found to be within the limits. Method precision should be performed to ***intraday*** and ***inter day.*** The linearity study was performed and the correlation coefficient of bicalutamide was found to be 0.999 (NLT 0.997)**.** Accuracy values was found to be within the limits (should not be more than 2)
- The assay method was established for estimation of bicalutamide in tablets and the % purity was found to be 101.312.

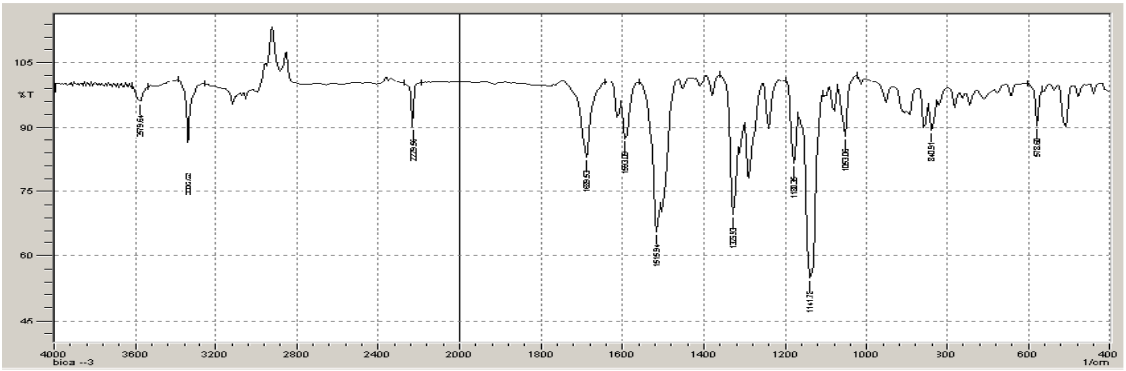



**Figure 1: IR spectrum of bicalutamide.**

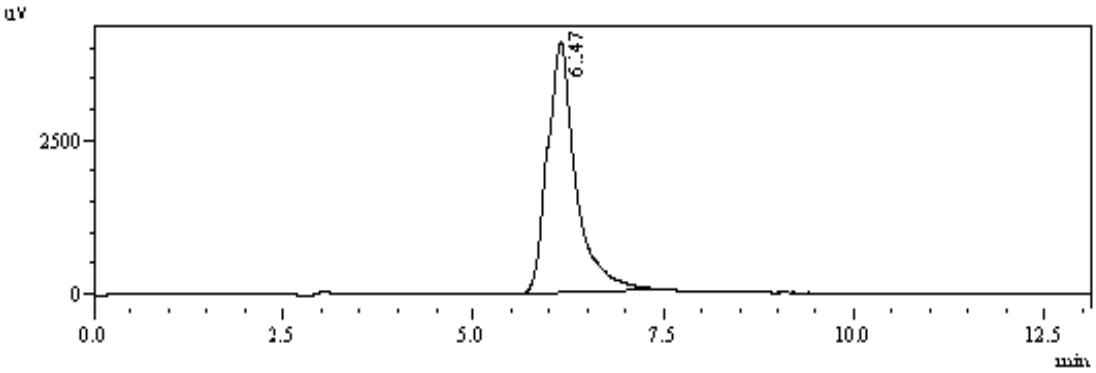



**Figure: 2 HPLC chromatogram of bicalutamide.**

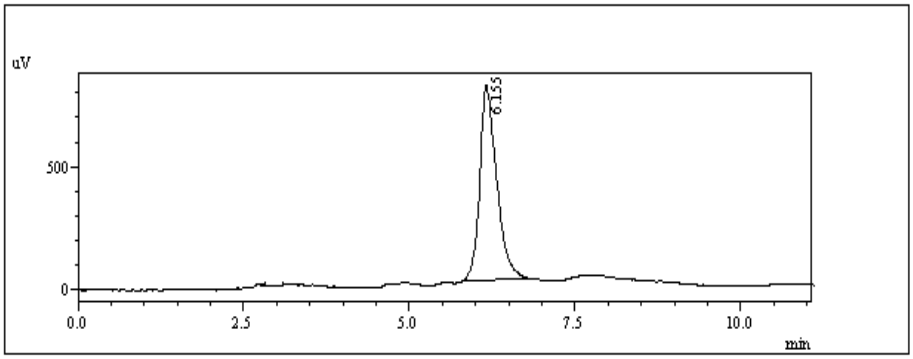



**Figure 3: chromatogram standard preparation.**

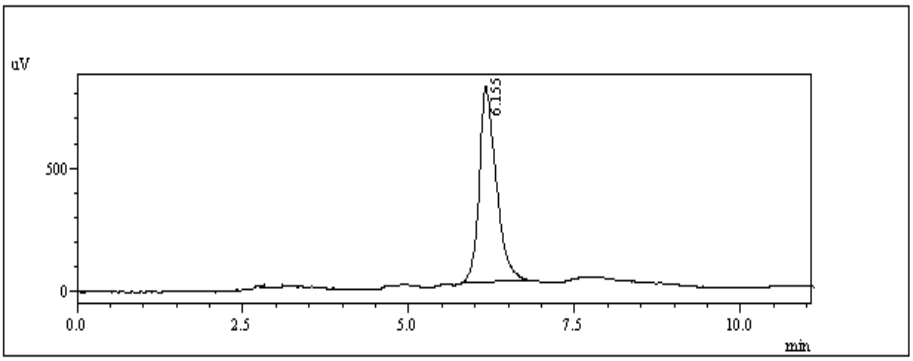



**Figure 4: chromatogram sample preparation.**

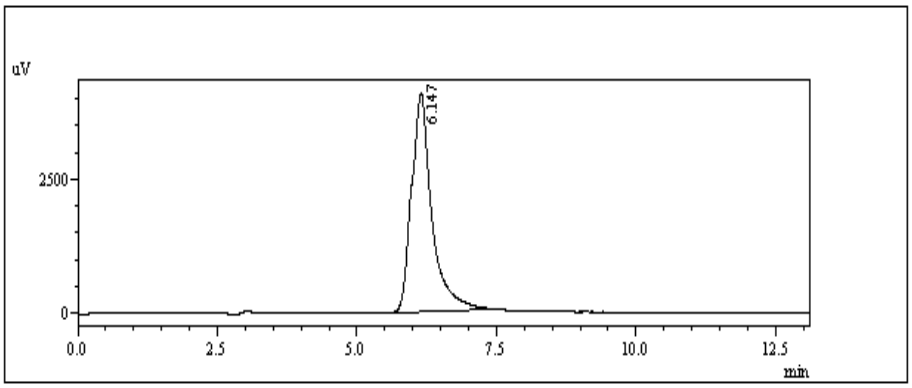



**Figure 5 Chromatogram specificity of standard**

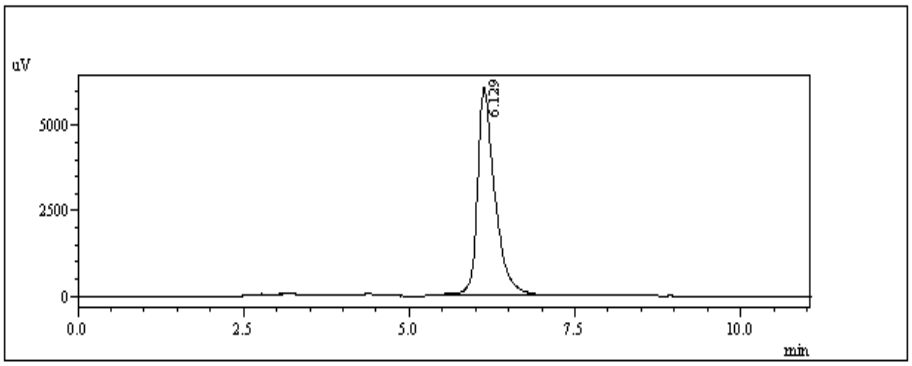



**Figure 6: Chromatogram specificity of sample.**

**Table 2: System precision.**

| System precision | | |
|---|---|---|
| **S.No** | **Retention time (Rt)** | **Area** |
| 1 | 6.120 | 20848 |
| 2 | 6.122 | 20811 |
| 3 | 6.119 | 20873 |
| 4 | 6.123 | 20805 |
| 5 | 6.123 | 21647 |
| 6 | 6.223 | 20578 |
| Avg | | 21460.33 |
| SD | | 368.157 |
| %RSD | | 1.75 |

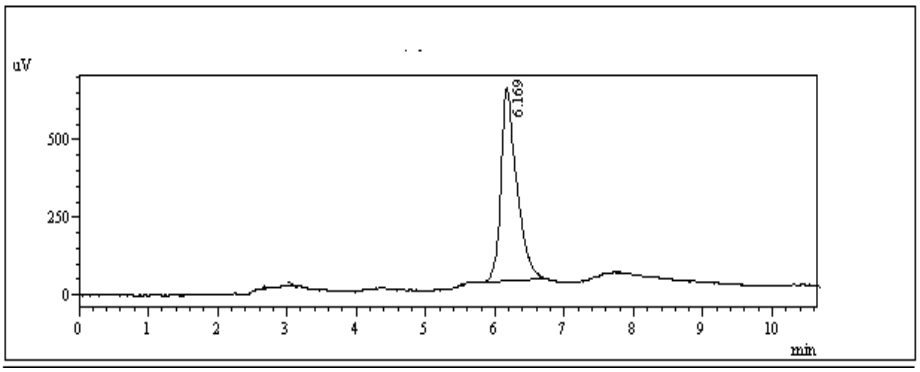



**Figure 7: Chromatogram system precision.**

**Table 3: Intra-day precision data.**

| S.No | Concentration (ng/mL) | Morning Mean Peak area* | Afternoon Mean peak area* | Evening Mean Peak area* | SD | % RSD |
|---|---|---|---|---|---|---|
| 1 | 600 | 10559 | 10563 | 10571 | 42 | 0.39 |
| 2 | 1000 | 12765 | 12769 | 12762 | 54 | 0.42 |
| 3 | 1200 | 14521 | 14536 | 14541 | 48 | 0.33 |

*** Average of 3 determinations.**

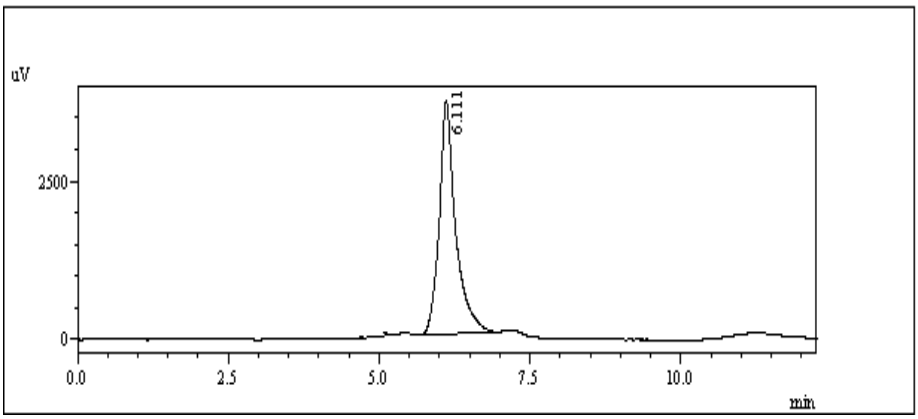



**Figure 8: Chromatogram method precision 100%**

**Table 4: Inter-day precision data.**

| S.No | Concentration (ng/mL) | Morning Mean Peak area* | Afternoon Mean peak area* | Evening Mean Peak area* | SD | %RSD |
|---|---|---|---|---|---|---|
| 1 | 600 | 10559 | 10563 | 10531 | 46.5 | 0.64 |
| 2 | 1000 | 12760 | 12775 | 12762 | 39 | 0.30 |
| 3 | 1200 | 14702 | 14735 | 14741 | 44 | 0.29 |

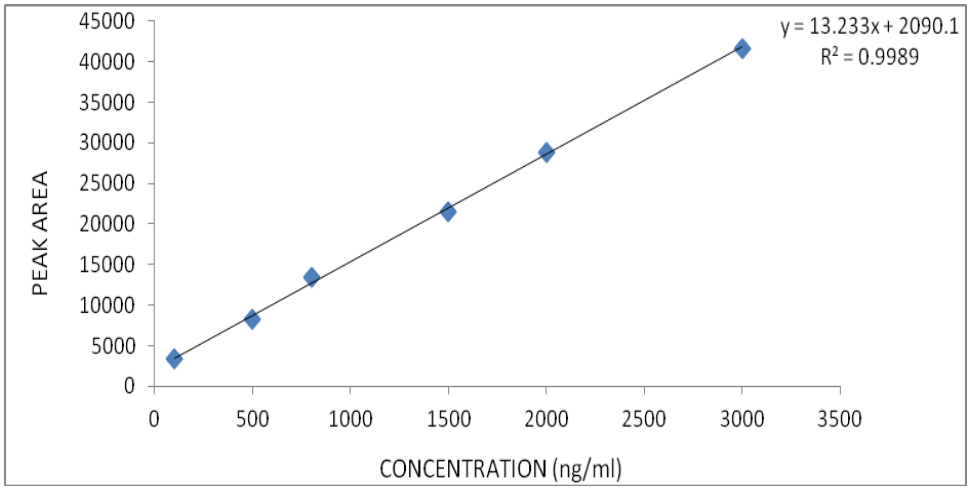



**Fig 9: Standard calibration curve for bicalutamide.**

**Table 5 accuracy data.**

| Concentration level | Peak area | Mean | Standard deviation | % RSD |
|---|---|---|---|---|
| 80 % | 10637 | 10643 | 38 | 0.35 |
| | 10643 | | | |
| | 10650 | | | |
| 100% | 12801 | 12806 | 32 | 0.30 |
| | 12803 | | | |
| | 12814 | | | |
| 120% | 14770 | 14776 | 36 | 0.24 |
| | 14779 | | | |
| | 14881 | | | |

**Table 6: Percentage purity of bicalutamide.**

| Bicalutamide | Bicalutamide |
|---|---|
| Brand name | TABI ( DR.Reddy's) |
| Active ingredient | Bicalutamide |
| Label claim | 50 mg |
| Average weight | 127.66 mg |
| Sample weight | 10 mg |
| Equivalent weight | 25.532 |
| Average weight in ml | 600ng |
| Area of sample | 10765 |
| Pecentage of assay | 101.32 |

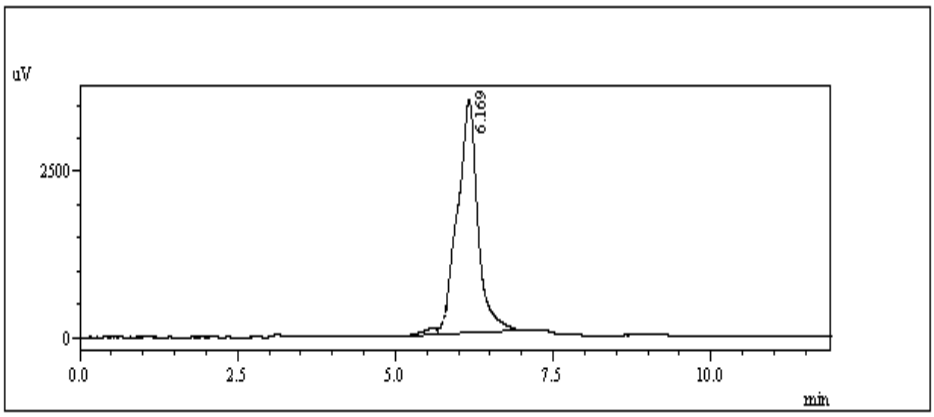



**Figure 10: Chromatogram showing assay of bicalutamide tablets**

## CONCLUSION

- A new method has been established for estimation of bicalutamide by RP-HPLC using shimadzu LC-10AT and – detector.
- The chromatographic conditions are Phenomenex-Luna 5µ, $C_{18}$ column, mobile phase of acetonitrile:water (90:10 v/v).
- The assay method was established for estimation of bicalutamide in tablets. And the %purity was found to be 101.312.
- The proposed RP-HPLC method is a suitable technique for the determination of bicalutamide in pharmaceutical dosage form without any interference.
- All the validation parameters were carried out as per ICH Q2(R1) guidelines. This RP-HPLC method may be considered more specific and sensitive.
- The developed method can be used for routine and quality control analysis of the investigated drug to provide simple, accurate and reproducible quantitative analysis for determination of bicalutamide.

## SUMMARY OF THE METHOD

| Validation parameter | Result |
|---|---|
| Mobile phase | Acetonitrile |
| Flow rate | 0.5 ml/min |
| Detection wavelength | 270 nm |
| Retention time | 6.12 min |
| Run time | 20 min |
| LOD ( based on the visualevaluation method) | 20ng/ml |
| LOQ (based on the visual evaluation method) | 50ng/ml |
| Linearity | 0.9999 |
| Precision | Within the limits |
| Accuracy | Within the limits |
| Recovery | 101.32% |

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