A Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Salmeterol Xinafoate and Fluticasone Propionate in Bulk and Pharmaceutical Dosage Form.

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ABSTRACT
This is a simple, economic, sensitive stability indicating RP-HPLC method for the simultaneous estimation of Salmeterol Xinafoate and Fluticasone Propionate in bulk and pharmaceutical Formulation. The method was carried out on Octa-decyl C₁₈ column (5 μm, 25 cm x 4.6 mm, i.d) using methanol: water in the ratio of 70:30 and pH of the mobile phase up to 3 was adjusted with OPA at a flow rate of 0.8 ml/min. The wavelength for Salmeterol Xinafoate and Fluticasone Propionate at 232 nm was found to be appropriate. The linearity range was obtained in the concentration range of 20 to 100 μg/ml for Salmeterol Xinafoate (SLM) and 20 to 100 μg/ml for Fluticasone (FLT) respectively. The retention time of Salmeterol Xinafoate and Fluticasone Propionate were found to be 3.59 and 6.3 min, respectively. The regression equation for SLM and FLT were found to be as y = 0.009x - 0.003 and y = 0.009x - 0.031 with correlation coefficient (R²) 0.999 and 0.999, respectively. The developed method is found to be robust, accurate and sensitive which can be used for estimation of combination of Salmeterol Xinafoate and Fluticasone Propionate in pharmaceutical dosage forms. The method was applicable for the quality control of the mentioned drugs in raw material, bulk drug and pharmaceutical formulations.

KEYWORDS
Salmeterol Xinafoate, Fluticasone Propionate, RP-HPLC, Simultaneous estimation, Stability Study.
1. INTRODUCTION

Salmeterol Xinafoate (Figure 1) a long-acting β2-adrenergic agonist used in the management of moderate-to-severe persistent asthma and chronic obstructive pulmonary disease (COPD) [1]. Inhaled salmeterol works like other β2 agonists, causing bronchodilation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma. The long duration of action occurs by the molecules initially diffusing into the plasma membrane of the lung cells, and then slowly being released back outside the cell where they can come into contact with the β2-adrenoreceptors, with the long carbon chain forming an anchor in the membrane. Chemically it is 2-(hydroxymethyl)-4-[1-hydroxy-2-[6-(4-phenylbutoxy) hexylamino] ethyl] phenol; 1-hydroxynaphthalene-2-carboxylic acid [2]. Its molecular formula is C_{36}H_{45}NO_{7}, and its molecular weight is 603.7 g/mol with dose of 25 mcg and 50 mcg in Capsule form. Fluticasone propionate (Figure 2) is a highly selective agonist at the glucocorticoid receptor and used for prophylaxis and treatment of allergic rhinitis [3]. It mimics the naturally occurring hormone produced by the adrenal glands, cortisol or hydrocortisone used in the management of asthma and chronic obstructive pulmonary disease (COPD). Chemically it is [(6S,8S,9R,10S,11S,14S,16R,17R)-6,9-difluoro-17-(fluoromethylsulfanylcarbonyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydro cyclopenta [a] phenanthren-17-yl] propanoate [4]. Its molecular formula is C_{23}H_{31}F_{3}O_{5}S and its molecular weight is 500.6 g/mol with dose of 50 mcg, 100 mcg, 125 mcg and 250 mcg Capsule.

Figure 1. Chemical structure of Salmeterol Xinafoate

Figure 2. Chemical structure of Fluticasone Propionate

Literature survey reveals that, there are various methods reported for quantification of Salmeterol Xinafoate and Fluticasone Propionate by UV either single or in combination or with other drugs[5-7] HPLC [8-14] LC-MS method[15] in pharmaceutical dosage form, Previously mentioned methods lack sensitivity for spectroscopic methods while no Stability studies has been
reported so far for simultaneous estimation of Salmeterol Xinafoate and Fluticasone Propionate in combined dosage forms. Further LC-MS require a high volume of plasma and lengthy extraction procedure that leads to high cost. Hence, in the present study, a new, sensitive and suitable Stability indicating reversed-phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Salmeterol Xinafoate and Fluticasone Propionate in pharmaceutical dosage form. This work describes the validation parameters stated by the International Conference on Harmonization (ICH) guidelines which include specificity, precision, linearity, accuracy, range, stability of analytical solution, robustness and system suitability suitably parameter.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents
Both standards of Salmeterol Xinafoate and Fluticasone Propionate were obtained as gift sample from Glenmark Pharmaceuticals Ltd., while Pharmaceutical formulation was purchased from local market (Brand: Esiflo 250 Capsule labelled claim Salmeterol Xinafoate-50 mcg Fluticasone Propionate- 250 mcg make Lupin Pharmaceuticals Ltd). The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. HPLC grade methanol and ortho phosphoric acid (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

Both the drugs were characterized by physicochemical characteristics like solubility, Infrared Spectroscopy, M.P., $\lambda_{\text{max}}$ and these were considered as pure drug. The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. HPLC grade methanol and ortho phosphoric acid (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

2.2. Instrument and Chromatographic conditions
Binary Gradient HPLC System bearing model number HPLC 3000 Series, P-3000-M Reciprocating pump (40MPa) with UV detector, RP C\textsubscript{18} column (250×4.6 mm), particle size 5μ) was used and Sonicator: PCi Mumbai, Model No.3.5L 100H.

Various combinations of mobile phases were screened with respect to resolution, theoretical plate capacity factors and other system suitability parameters. Finally the separation was performed with freshly prepared mobile phase consist of methanol: water in the ration of 70:30 and pH up to 3 was adjusted with OPA with isocratic programming at a flow rate of 1.0 ml/min. 232 nm wavelength, injection volume of 20 μL and working temperature of 25°C was maintained during the entire process to obtain symmetric peaks of SLM and FLT.

2.3. Preparation of standard solution
Stock solutions were prepared by accurately weighed 10 mg Salmeterol and 10 mg Fluticasone which is transferred into two separate 100 ml volumetric flasks, about 75 ml of diluent was added to each flask and sonicated to dissolve, diluted up to mark with the diluent to obtain 100 μg/ml concentration of Salmeterol and 100 μg/ml concentration of Fluticasone separately.

2.4. Linearity study
From the prepared standard stock solutions of both, linearity was performed in the final concentration range of 20 to 100 μg/ml for Salmeterol and Fluticasone. Volume of 20 μL of each...
sample was injected with the help of Hamilton Syringe. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area Vs the drug concentration.

2.5. Validation of proposed method

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

2.5.1. Accuracy

It was done by recovery study using standard addition method at three different levels where known amount of standard SLM and FLT were added to pre-analyzed sample. Spiked known quantity of SLM and FLT Standard at 50%, 100% and 150% was added to a pre-quantified sample solution. Analyses of samples were done in triplicate for each level. From the results, % recovery was calculated.

2.5.2. Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

2.5.3. Intraday and Interday Precision

Intraday precision were determined by analyzing, the three different concentrations 40 μg/ml, 60μg/ml and 80μg/ml of SLM and FLT, for three times in the same day. Day to day variability was assessed using above mentioned method for three concentrations analyzed on three different days, over a period of one week.

2.5.4. Repeatability

It is measured by multiple injections of a homogenous sample of 60 μg/ml of SLM and FLT that indicate the performance of the HPLC instrument under chromatographic conditions.

2.5.5. Robustness

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of methanol using 60 μg/ml of SLM and FLT.

2.5.6. Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD= 3.3 SD/S and LOQ= 10 SD/S, where SD is the residual standard deviation and S is the slope of the line.

2.5.7. Specificity and selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

2.5.8. Ruggedness

From stock solutions, sample solutions of SLM and FLT both (60 μg/ml) were prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

2.6. System suitability test
System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

2.7. Analysis of Pharmaceutical formulation

To determine the contents of drugs in conventional capsule (Brand: Esiflo 250 Capsule labelled claim Salmeterol-50 mcg Fluticasone- 250 mcg make Lupin Pharmaceuticals Ltd), twenty open capsules were transferred into a 100 ml clean dry volumetric flask, add about 75 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. Solution was filter through 0.45μm membrane filter.

3. RESULTS AND DISCUSSION

3.1. Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method is to achieve the resolution between Salmeterol Xinafoate, Fluticasone Propionate and its degradation products. To achieve the separation of degradation products, octadecyl silane C₁₈ stationary phase and freshly prepared mobile phase consist of methanol: water in the ratio of 70:30 and pH up to 3 was adjusted with OPA with isocratic programming at a flow rate of 0.8 ml/min. 232 nm wavelength, injection volume of 10 μL loop and ambient temperature was maintained during the entire process to obtain symmetric peaks of SLM and FLT. The tailing factor obtained was less than two and retention time was about 3.59 and 6.73 min for SLM and FLT (Figure 3). This developed method was found to be specific and method was validated as per international guideline.

3.2. Linearity study

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Salmeterol Xinafoate and Fluticasone Propionate were found to be as 20-100 µg/ml (Table 1). The regression equation for SLM and FLT were found to be as \( y = 0.009x - 0.003 \) and \( y = 0.009x - 0.031 \) with correlation coefficient (\( R^2 \)) 0.999 for both (Figure 4, 5).

3.3. Method Validation

3.3.1. Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 50%, 100%and 150% concentration levels. Known amounts of standard SLM and FLT were added to the pre-analyzed samples and were subjected to the proposed HPLC method. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.48 to 90.50 % for SLM and 99.57-100.37 % for FLT Results of recovery studies is shown in Table 2. Precision was evaluated by carrying out six independent sample preparations of a single sample by intra-day and inter-day precision. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise shown in Table 3.

3.3.2. Repeatability

It is measured by multiple injections of a homogenous sample of 60 µg/ml of SLM and 60 µg/ml of FLT and the % R. S. D. was found to be less than 2 (Table 4).
3.3.3. Robustness of the method
To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effects of change in flow rate, pH retention time, and in mobile phase ratio were studied. The method was found to be unaffected by small changes like +/- 10% in flow rate, +/- 0.2 change in pH, shown in Table 5.

3.3.4. Sensitivity
LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach and standard deviation of the response and slope. Limit of detection of SLM and FLT was determined 0.477 and 0.683 respectively. Limit of quantitation of SLM and FLT was determined 1.446 and 2.071, respectively.

3.3.5. Specificity and selectivity
The method is quite selective. There were no other interfering peak around the retention time of SLM and FLT; also the base line did not show any significant noise.

3.3.6. Ruggedness
Different analyst carried out precision studies in a similar manner carried out by first analyst. The % Assay was found to be 99.96 %, and 100.04% of SLM and FLT, respectively. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is rugged, shown in Table 6.

3.3.7. System suitability test
System suitability testing is essential for the assurance of the quality performance of the chromatographic system. The tailing factor, capacity factor, and theoretical plates for SLM and FLT were in the acceptance criteria as per the ICH guidelines (Table 7).

3.4. Analysis of Pharmaceutical formulation
The assay procedure was repeated for six times; the percentage content of SLM and FLT in the tablet formulation was determined as 99.96 %, and 100.04% respectively (Table 8).

3.4.1. Procedure for Forced Degradation Study
Forced degradation of each drug substances and the drug product was carried out under acidic, basic, oxidative stress, thermolytic and photolytic, conditions. Thermal degradation of drug was carried out in solid state. While remaining all studies were carried out in solution form. Solutions were prepared by dissolving drug with distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide, or aqueous hydrogen peroxide solution, which is further diluted with mobile phase to achieve a concentration of 150 µg/ml each of SLM and 30 µg/ml for FLT. These solutions were kept for 1 Hr. For thermal stress, samples of drug was placed in a controlled-temperature oven at 50°C for 1 hr. Solutions of drug substances and drug product were also kept at 80 °C for 48 h. For photolytic stress, samples of drug in solution state, was irradiated with UV radiation. The degradation studies (Figure 4-7) were tabulated in Table 9.

4. CONCLUSION
The present study was conducted to develop and validate a simple, sensitive and reproducible RP-HPLC method for quantitative determination of Salmeterol Xinafoate and Fluticasone Propionate with stressed stability studies under different conditions. The developed chromatographic assay fulfilled all the requirements to be identified as simple, specific, selective...
and reliable method, including accuracy, linearity, recovery and precision data. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Salmeterol Xinafoate and Fluticasone Propionate in pharmaceutical formulations without any interference from the Excipients and degraded peaks.

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6. CONFLICT OF INTEREST
There is no conflict of interest. The authors alone are responsible for content and writing of this article.

7. REFERENCES
1. https://en.wikipedia.org/wiki/Salmeterol
2. https://pubchem.ncbi.nlm.nih.gov/compound/salmeterol_xinafoate#section=3D-Status
3. https://en.wikipedia.org/wiki/Fluticasone_propionate
4. https://pubchem.ncbi.nlm.nih.gov/compound/fluticasone_propionate
5. M.S. Kondawar, R.R. Shah, J.J. Waghmare, M.K. Malusare, N.D. Shah (2011). UV Spectrophotometric Method for Simultaneous estimation of Salmeterol Xinafoate and Fluticasone propionate in Bulk and Dosage form. International Journal of PharmTech Research. 3, 1801-1806.
6. S. Ahmed, S. Hesham, A. Mohammad (2012). New developed spectrophotometric method for simultaneous determination of salmeterol Xinafoate and fluticasone propionate in bulk powder and Seritide diskus inhalation. Bulletin of Faculty of Pharmacy. 50, 121–126.
7. P.U. Patel, U.B. Patel (2016). Development and Validation of Spectrophotometric Method for Simultaneous Determination of Roflumilast and Salmeterol in Synthetic Mixture. International Journal of Pharmacy and Pharmaceutical Research. 5, 2, 68-77.
8. S. Ramanjaneyulu S, G. Vijaykumar, K.B. Chandrasekhar (2013). Development and validation of RP-HPLC Method for the Simultaneous Estimation of Salmeterol and Fluticasone in Powder for Inhalation Capsules. An International Journal of Advances in Pharmaceutical Sciences. 4, 5, 960-969.
9. P.S. Jain, A.P. Gorle, S.S. Patil, R.S. Chavan, P.R. Bari, S.J. Surana (2015). Stability-indicating RP-HPLC Method for Estimation of Salmeterol xinafoate in Bulk and in Pharmaceutical Formulation. International Journal of Pharmaceutical Chemistry and Analysis. 2, 1, 28-33.
10. D. Murnane, G.P. Martin, C. Marriott (2005). Validation of a Reverse-Phase High Performance Liquid Chromatographic method for Concurrent Assay of a Weak Base (Salmeterol Xinafoate) and a pharmacologically active steroid (fluticasone propionate). Journal of Pharmaceutical and Biomedical Analysis. 2, 3, 1149–1154.
11. K. Srinivasaro, V. Gorule, K.A. Venkata (2012). Development and Validation for Simultaneous Estimation of Budesonide and Salmeterol Xinafoate in Metered Dose Inhalation Form by RP-HPLC. *International Journal of Pharmaceutical and Phytopharmacological Research*. 1, 5, 271-275.

12. G.M. Ashlesha, N.D. Hiral, N.S. Bhanubhai (2013). Validated Reversed Phase High Performance Liquid Chromatographic Method for Determination of Three Novel Steroids in Bulk and Pressurized Metered Dose Commercial Preparations Using a Common Mobile Phase. *International Journal of Applied Science and Engineering*. 11, 2, 125-135.

13. E. Paczkowska, D. Smukowska, E. Tratkiewicz, P. Bialasiewicz (2015). HPLC Method for Simultaneous Determination of Salmeterol Xinafoate and Fluticasone Propionate for the Quality Control of Dry Powder Inhalation Products. *Acta Chromatographica*. 27, 2, 309-320.

14. P.R. Priyank, J.B. Dave, P.J. Mittal, K.S. Bhargav (2014). Estimation of Fluticasone Propionate in Nasal Preparation and In Combination With Formoterol Fumarate In Inhalation Preparation. *World Journal of Pharmacy and Pharmaceutical Sciences*. 3, 6, 1148-1160.

15. N.L. Yuan, B. Tattam, F.B. Kenneth, J.S. Paul (2001). Quantification of epimeric budesonide and fluticasone propionate in human plasma by liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry. *Journal of Chromatography B*. 761, 177-185.

**Figure 3.** Linearity plot of Salmeterol Xinafoate
Figure 4. Linearity plot Fluticasone Propionate

Figure 5. Typical Chromatogram of Standard Salmeterol Xinafoate and Fluticasone Propionate At 232 Nm With Retention Time 3.59 and 6.3 Minutes Respectively.

Figure 6. Acidic Degradation.
Figure 7. Alkaline Degradation.

Figure 8. Peroxide Degradation.

Figure 9. Heat Degradation.
Figure 10. Photolytic Degradation.

Table 1. Linearity Study of SLM and FLT.

| Sr. No. | SLM | FLT |
|---------|-----|-----|
|         | Concentration [µg/ml] | Mean peak area ± SD [n=5] | %RSD | Concentration of [µg/ml] | Mean peak area ± SD [n=5] | %RSD |
| 01      | 20  | 173.6±3.05 | 1.76 | 20  | 208.8±3.70 | 1.77 |
| 02      | 40  | 311.4±5.98 | 1.92 | 40  | 412.2±6.87 | 1.67 |
| 03      | 60  | 453.0±7.28 | 1.61 | 60  | 619.4±5.81 | 0.94 |
| 04      | 80  | 571.6±4.83 | 0.84 | 80  | 823.2±7.12 | 0.86 |
| 05      | 100 | 729.6±5.90 | 0.81 | 100 | 1055.4±9.34 | 0.89 |

Table 2. Results of Recovery Studies of SLM and FLT.

| Drug | Initial amount [µg/ml] | Amount added [µg/ml] | Amount recovered ± S.D. [µg/ml, n =3] | % Recovery | % RSD |
|------|------------------------|---------------------|--------------------------------------|------------|-------|
| SLM  | 60                     | 0                   | 60.29 ±0.67                          | 100.48     | 0.89  |
|      | 60                     | 30                  | 89.89 ±0.89                          | 98.88      | 1.49  |
Table 3. Results of Precision Studies of SLM and FLT (Intra-Day and Inter-Day)

| Drug | Concentration [µg/ml] | Intraday Amount Found [µg/ml] | Inter day Amount Found [µg/ml] |
|------|----------------------|-------------------------------|-------------------------------|
|      |                      | Mean±S.D.                     | Mean±S.D.                     |
|      |                      | [% RSD]                       | [% RSD]                       |
|      |                      | [n= 3]                        | [n= 3]                        |
| SLM  | 40                   | 40.13 ± 2.00                  | 0.28                          |
|      |                      |                               | 40.77 ± 2.00                  | 0.29                          |
|      | 60                   | 60.09 ± 5.57                  | 0.50                          |
|      |                      |                               | 60.25 ± 3.06                  | 0.27                          |
|      | 80                   | 80.65 ± 5.03                  | 0.34                          |
|      |                      |                               | 80.39 ± 5.51                  | 0.35                          |
|      | 40                   | 40.51 ± 8.50                  | 0.69                          |
|      |                      |                               | 40.87 ± 4.16                  | 0.34                          |
| FLT  | 60                   | 60.77 ± 7.64                  | 0.41                          |
|      |                      |                               | 60.37 ± 10.21                 | 0.55                          |
|      | 80                   | 81.05 ± 9.45                  | 0.38                          |
|      |                      |                               | 80.50 ± 6.66                  | 0.27                          |

Table 4. Results of Repeatability Study of SLM and FLT.

| Drug | Concentration [µg/ml] [n=6] | Peak Area | Mean[µg/ml] ± % RSD |
|------|----------------------------|-----------|---------------------|
| SLM  | 60                         | 622.833   | 60.15 ± 0.94        |
| FLT  | 60                         | 377.677   | 60.25 ± 0.255       |

Table 5: Robustness Evaluation of the HPLC Method for SLM and FLT.

| Chromatographic conditions | SLM | FLT |
|----------------------------|-----|-----|
| Tailing (T')               | Capacity Factor (K') | Theoretical Plate (N) | Tailing (T') | Capacity Factor (K') | Theoretical Plate (N) |
| A: Mobile phase pH          |     |     |

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### Table 6. System Suitability Test for SLM and FLT.

|                | SLM                      | FLT                      |
|----------------|--------------------------|--------------------------|
| **Proposed method** | **System suitability parameters** | **Proposed method** |
| **Retention time (Rt)** | 3.59 | Retention time (Rt) | 6.30 |
| **Capacity factor (K')**  | 1.18 | Capacity factor (K') | 0.98 |
| **Theoretical plate (N)** | 2838.7 | Theoretical plate (N) | 7465.8 |
| **Tailing factor (T)**    | 1.16 | Tailing factor (T)  | 0.95 |
Table 7. Analysis of Tablet Formulation.

| Drug | Label claim [mg] | Amount found [mg] | Amount found [%] | SD | %RSD |
|------|------------------|-------------------|------------------|----|------|
| SLM  | 250              | 249.67            | 99.86 %          | 0.46 |      |
| FLT  | 50               | 50.06             | 100.12%          | 1.13 |      |

Table 8. Forced Degradation of SLM and FLT.

| Sample Exposure condition | Total Number of products with their Rt | SLM Degradation remained (60 µg/ml) | Recovery (%) | FLT Degradation remained (60 µg/ml) | Recovery (%) |
|--------------------------|---------------------------------------|------------------------------------|---------------|------------------------------------|---------------|
| Acidic,                 | 1 (5.1)                               | 58.86                              | 90.81         | 28.25                              | 56.50         |
| Basic,                  | 3 (2.9, 3.4, 6.2)                     | 48.88                              | 81.48         | 38.57                              | 64.29         |
| Peroxide                | 8 (3.8, 4.2, 5.2, 5.4, 5.9, 6.8, 8.4, 8.9) | 51.40                              | 85.67         | 41.83                              | 69.73         |
| Heat                    | 0                                     | 58.68                              | 97.80         | 56.40                              | 94.01         |
| Photolytic              | 0                                     | 59.26                              | 98.78         | 58.32                              | 97.20         |