CHAPTER - 4

ESTIMATION OF GATIFLOXACIN
IN MARKETED TABLET DOSAGE
FORM BY HPTLC METHOD
4 Estimation of Gatifloxacin in Marketed Tablet Dosage Form by HPTLC Method

4.1 Introduction (106-109)

The most difficult condition that an analyst may be confronted with is to select most suitable procedure for a particular problem. With ever increasing number of analytical methods being reported, it is impossible to familiarize with all the available methods. We are mostly relying on knowledge, experience, scanning of literature and dependent on habits/fashions. One usually applies those methods which are well established or the analyst may have personal experience though the choice is often limited because of laboratory facilities in term of instruments/personnel. In view of variables in quantitative analysis such as in-homogeneity, sampling error and random error the selected method must reveal all these variables. First stage in selection of development of method is to establish what is to be measured and how accurately it should be measured. The selected method must have following parameters-

- Simple
- Specific
- Predictable, economical and convenient
- Accurate and Precise
- Avoid multiple source of key components (reagents, columns, TLC plates)
- To be fully automated before transfer for validation of its characteristics such as accuracy, precision, sensitivity.
ruggedness etc.

Modern pharmaceutical formulations are complex mixtures including, in addition to one or more medicinally active ingredients, a number of inert materials such as diluents, disintegrants, colors and flavors. In order to ensure quality and stability of final product, the analyst must be able to separate these mixtures into individual components prior to quantitative analysis. Moreover, comparison of the relative efficacy of different dosage forms of the same drug entity requires the analysis of the active ingredients in biological matrices, e.g. blood, urine and tissues.

Among the most powerful techniques available to analyst for the resolution of these mixtures is a group of highly efficient methods, collectively called chromatography.

A different chromatographic technique like TLC, GLC or HPLC is applicable for an analysis depends on various parameters. Solubility or volatility of the samples requires separation efficiency, concentration of the analyte, detection limit, cost of analysis, number of samples under analysis, sample preparation and other requirements of sample to be separated. TLC as practiced today is in two forms, some use it as a qualitative tool for separation of simple mixtures, other use it as a powerful separation tool for quantitative analysis with high sample throughput, the latter one referred to HPTLC.

TLC is an inexpensive simple method requiring little instrumentation which is used for separation of simple mixtures and for qualitative identification or semi-quantitative visual analysis of sample. By contrast, modern TLC is highly instrumental technique carried out on efficient fine particle layers.
HPTLC is capable of producing fast high resolutions and quantitative results with accuracy and precision rivaling those of gas chromatography (GC) and HPLC. It has many advantages relative to these popular methods.

HPTLC is an off-line process in which the various stages are carried out independently. The advantages of these arrangements using an open and disposable layer compared with an on-line column process such as LC are much higher sample throughput (lower analysis time) and lower cost per analysis than LC. The ability to process sample and standards simultaneously yet independently on a single plate under the same conditions leads to statistical improvements in data handling and better analytical precision and accuracy.

Many solvents can be used for preparation of TLC mobile phases because the phase is completely evaporated before detection and the plate is used only once. The availability of conventional linear, continuous, multiple, circular and two-dimensional development methods and the great variety of available mobile phases made this technique widely accepted.

A shortcoming of TLC is that although various steps have been automated and on-line coupling with other chromatographic and spectrometric techniques has been achieved, complete automation of TLC has not been realized. Also specificity of planar chromatography is limited by small migration distance in the non-forced flow planar techniques.
4.2 Experimental

4.2.1 Instruments

- Camag Linomat IV (semiautomatic spotting device)
- Camag glass twin-trough chamber (10 x 10) (20 x 10)
- Camag TLC Scanner 3
- Camag CATS 4 software (Camag Sonnenmattstr., Muttenz, Switzerland)
- 100-µl HPTLC Syringe (Hamilton Company, Reno, NV, USA)
- Shimadzu Libror AEG-220 weighing balance
- Sonicator (Frontline FS-4)
- Research centrifuge (Remi instruments)
- Cyclomixer (Remi instruments)

4.2.2 Materials and Reagents

- Analytical pure gatifloxacin (gift sample from Sun Pharma, Baroda)
- n-Butanol (SD fine chem. Ltd, Mumbai)
- Methanol A.R. (SD fine chem. Ltd, Mumbai)
- Ammonia 25% (SD fine chem. Ltd, Mumbai)
- Chloroform (Ranbaxy Laboratories, New Delhi)
- Sodium sulphate anhydrous (SD fine chem. Ltd, Mumbai)
- Potassium dihydrogen phosphate (SD fine chem. Ltd, Mumbai)
➤ Triple distilled water

➤ Dosage forms were procured from local market

4.2.3 Chromatographic conditions

➤ Stationary phase: Silica gel 60 F_{254} precoated TLC plates
   (20x20 cm; layer thickness, 0.2 mm)

➤ Prewashing of plate with methanol and drying in oven (50 ±1°C, 5 min)

➤ Mobile phase: n-Butanol : methanol : 6 M ammonia (4 : 0.3 : 1 v/v/v)

➤ Chamber saturation time: 45 min

➤ Volume of mobile phase: 5 ml

➤ Temperature: 25 ± 2°C

➤ Migration distance: 60 mm

➤ Detection wavelength: 294 nm

Scanning parameters

➤ Scanning wavelength: 294 nm

➤ Lamp: Mercury

➤ Slit dimension: 3 x 0.45 mm

Spotting parameters

➤ Band width: 4 mm

➤ Space between two bands: 4 mm

➤ Spraying rate: 15 sec/μL
4.2.4 Preparation of standard solution of gatifloxacin

Gatifloxacin powder (10 mg) was weighed accurately and transferred to 10 mL volumetric flask. Methanol (6 mL) was added and the mixture was sonicated for 20 min. The volume was adjusted upto the mark with methanol (S1).

The solution (S1) (1 mL and 0.1 mL) were transferred in to two separate 10 mL volumetric flask and diluted to 10 mL with methanol. The final solution contained 100 μg/mL (S2) and 10 μg/mL (S3) of gatifloxacin respectively.

4.2.5 Preparation of sample solution

Twenty tablets of gatifloxacin were weighed and finely powered. The powder equivalent to gatifloxacin 10 mg was transferred to a 10 mL volumetric flask. It was suspended in methanol (6 mL) and sonicated for 20 min. The suspension was filtered through whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined and transferred to a 10 mL volumetric flask and volume was adjusted upto the mark with methanol. The solution (0.1 mL) was further diluted to 10 mL with methanol. The final solution contained an estimated 10 μg of Gatifloxacin per mL of the solution (T1).

4.2.6 Chromatographic method

Pretreatment to precoated plates

TLC plate was cut of required size and placed in twin trough glass chamber containing methanol as mobile phase. Methanol was allowed to travel upto upper edge of plate (ascending method). Plate was removed and allowed to dry in oven at 50 °C for 5 min. For the actual experiment the plate was allowed to
cool to room temperature and used immediately.

**Chromatographic separation**

With the help of microliter syringe, standard or sample solutions (40 μL) were applied on prewashed TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried and developed up to 60 mm at constant temperature using mixture of n-butanol : methanol : ammonia (4 : 0.3 : 1 v/v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 45 min.

The plate was removed from the chamber, dried and scanned in absorbance/reflectance mode of Camag TLC Scanner 3 at 294 nm. Peak area was recorded using Camag CATS 4 software.

**4.2.7 Calibration curve of standard Gatifloxacin**

Aliquots of 20, 30, 40, 50 and 60 μL of S1 were spotted on precoated TLC plate, using semiautomatic spotter under nitrogen stream. The TLC plate was developed, dried and scanned as described under section 4.2.6. The calibration curve was prepared by plotting peak area versus gatifloxacin concentration (ng/spot) corresponding to each spot.

**4.2.8 Quantification of gatifloxacin in tablet formulation**

The sample solution (T1) (40 μL) was applied on TLC plate, developed, dried and scanned as described under section 4.2.6. The amount of gatifloxacin present in sample was determined by fitting area values of peak corresponding to gatifloxacin into the equation of line representing calibration curve of gatifloxacin.
4.2.9 Validation of HPTLC method

4.2.9.1 Linearity

The linearity was determined by applying 20, 30, 40, 50 and 60 μL of standard solution of gatifloxacin (S1) on TLC plate and developed, dried and photometrically analyzed as described under 4.2.6.

4.2.9.2 Precision

4.2.9.2.1 Repeatability of measurement of peak area and peak height

(% CV < 1 % based on seven times measurement of same spot)

30 μL of standard solution of gatifloxacin (S1) was spotted on TLC plate and developed, dried and photometrically analyzed as described under 4.2.6.

The area and height of the spot was measured seven times without changing plate position and the % CV of the obtained data was computed.

4.2.9.2.2 Repeatability of sample application

(% CV < 3 % based on application of equal volume of seven spot)

30 μL of standard solution of gatifloxacin (10 μg/mL) was spotted on TLC plate and developed, dried and photometrically analyzed as described under 4.2.6.

The area and height of the seven spots were measured and the % CV of obtained data was calculated.
**Intra-day and inter-day precision:**

The Intra day precision (% CV) was determined for standard solution of gatifloxacin (200 - 600 ng/spot) for three times on the same day.

The Inter-day precision (% CV) was determined for standard solution of gatifloxacin (200-600 ng/spot) for five days.

### 4.2.9.3 Accuracy

The accuracy was determined by standard addition method. To a fixed amount (200 ng) of preanalyzed sample of gatifloxacin, increasing amount of gatifloxacin was added in the all levels of calibration curve (300, 400, 500 and 600 ng/spot) and analyzed as described under 4.2.6. The amount of gatifloxacin was calculated at each level.

### 4.2.9.4 Limit of Detection

Standard solution of gatifloxacin (1 μg/mL) (5, 10, 15, 20, 30 and 50 μL) were spotted on TLC plate, developed, dried and photometrically analysed as described under section 4.2.6 and the lowest concentration of analyte that can be detected was determined.

### 4.2.9.5 Limit of Quantitation

Lowest concentration of calibration curve for standard gatifloxacin was considered as limit of quantitation.

### 4.2.9.6 Specificity

Gatifloxacin solution (T1) (40 μL) was spotted on the TLC plate. The plate was developed, dried and photometrically analyzed as described under 4.2.6.
The purity of gatifloxacin peak was determined by comparing its spectra at three different levels i.e. peak start, peak apex and peak end.
4.3 Results and Discussion

Various methods like spectrophotometric, spectrofluorimetric and HPLC are reported for estimation of gatifloxacin. In present work HPTLC method was developed for estimation of gatifloxacin and its tablet formulation.

Gatifloxacin being soluble in methanol; methanol was selected for dissolution of the drug from tablet dosage form. To ensure complete release of drug from the formulation, the powder suspended in methanol was sonicated for 20 min.

Chromatographic conditions

- The plate was prewashed with methanol to remove any adsorbed impurities and dried at 50 °C for 5 min.
- Methanol was used for migration of the drug whereas n-butanol retards the migration of drug. Strong ammonia was used as modifier to obtain sharp peak.
- To obtain reproducible RF value, 45 min saturation time was necessary.
- The RF of gatifloxacin is 0.35 ± 0.03 (Fig. 4.1)

Selection of wavelength for scanning gatifloxacin

Gatifloxacin was determined by densitometric scanning after chromatographic separation. The light intensity remitted by chromatographic zones is usually lower than the sorbent layer around it. Therefore, absorption spectra of a compound determined directly on HPTLC plate is almost similar to the one recorded when the substance is in solution. The scanning at wavelength of maximum gives largest difference in absorbance between drug spot and blank area of sorbent layer.
The maxima of gatifloxacin was found to be at 294 nm and this wavelength was used for quantification of gatifloxacin (Fig. 4.2)

### 4.3.1 Method Validation

#### 4.3.1.1 Linearity

A representative calibration curve of gatifloxacin was obtained by plotting the mean peak area of gatifloxacin against the concentration over the range of 200-600 ng/spot. A correlation coefficient (r) was found to be 0.9967 and % CV was ranging from 1.38 - 2.76 %. The average linear regressed equation for the corresponding curve was $Y=14.31 \times + 3926.66$ (Table 4.1, Fig 4.3, 4.4).

#### 4.3.1.2 Precision

Repeatability of sample application seven times (Table 4.2) and repeatability of measurement of peak area based on seven repeat measurements (Table 4.3) of the same spot showed very low % CV i.e. 2.7 and 0.04 respectively according area which, in turn, ensured reproducible performance of the instrument.

The inter-day variation for determination of gatifloxacin was in the range of 1.71 - 3.27 %, while intra-day variation was ranging from 1.38 - 2.76 % (Table 4.4).

#### 4.3.1.3 Accuracy

The result indicates that the recovery of the added sample was between 96.28 - 103.04 %. This clearly indicates that the method is accurate and precise (Table 4.5).

#### 4.3.1.4 Limit of Detection and Limit of Quantitation

The minimum detectable quantity of gatifloxacin was found to be 10 ng/spot (Fig 4.5) while the limit of quantitation of
gatifloxacin was found to be 200 ng/spot (Fig. 4.3)

### 4.3.1.5 Specificity

Comparison of chromatogram of gatifloxacin in sample with standard gatifloxacin, showed no interference from the excipients (Fig. 4.6). Peak purity check showed a high degree of correlation between spectra scanned at peak start, peak apex and peak end positions ($r_{\text{start, apex}} = 0.9998$ and $r_{\text{apex, end}} = 0.9988$) of gatifloxacin peak which confirmed that peak represents a pure single component, i.e. gatifloxacin (Fig. 4.7). This was further supported by equally good correlation ($r=0.9991$) between the spectrum of standard gatifloxacin and the spectrum of gatifloxacin in sample (Fig. 4.8).

The summary of all validation parameters are shown in Table 4.6

### 4.3.1.6 Assay result for marketed gatifloxacin tablet dosage form

The developed HPTLC method was applied to determine the content of gatifloxacin in single component gatifloxacin tablet in nine different marketed formulations.

The gatifloxacin content of nine formulations was determined by comparing area from dosage unit with that of standard gatifloxacin.

The results shows all the formulations are in the range of 95 - 105 % of gatifloxacin.

The corresponding data along with % CV for the assay for gatifloxacin in each formulation is given in table 4.7
Conclusion

The results of the analysis of pure drug and tablet dosage forms indicate that the proposed method is simple, reproducible, specific, precise, accurate and reliable for estimation of gatifloxacin in its tablets. The results obtained are in good agreement with the label claim of gatifloxacin. It can be used for routine quality control of gatifloxacin tablet formulation in industry.
Fig. 4.1: Chromatogram showing the peak of gatifloxacin ($R_f = 0.35 \pm 0.03$).

Fig. 4.2: Spectra for standard gatifloxacin
Fig. 4.3: Chromatogram showing calibration curve for standard gatifloxacin
Table 4.1 Calibration data for gatifloxacin

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Peak area (n=5) (Mean ± SD)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>6834.81 ± 111.56</td>
<td>1.66</td>
</tr>
<tr>
<td>300</td>
<td>8475.33 ± 142.81</td>
<td>1.68</td>
</tr>
<tr>
<td>400</td>
<td>9701.76 ± 155.19</td>
<td>1.60</td>
</tr>
<tr>
<td>500</td>
<td>10903.22 ± 301.01</td>
<td>2.76</td>
</tr>
<tr>
<td>600</td>
<td>12755.10 ± 177.66</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Correlation: 0.9967
Slope: 14.31
 Intercept: 3926.66

\[ Y = 14.31 \times + 3926.66 \]

Fig. 4.4: Calibration curve for gatifloxacin
Table 4.2 Data for repeatability of sample application of gatifloxacin

<table>
<thead>
<tr>
<th>Track</th>
<th>SI</th>
<th>RF</th>
<th>height</th>
<th>X(calc)</th>
<th>area</th>
<th>X(calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s</td>
<td>0.37</td>
<td>391.0</td>
<td>390.98</td>
<td>8649.6</td>
<td>8648.55</td>
</tr>
<tr>
<td>2</td>
<td>s</td>
<td>0.37</td>
<td>403.6</td>
<td>403.62</td>
<td>9839.6</td>
<td>9830.69</td>
</tr>
<tr>
<td>3</td>
<td>s</td>
<td>0.37</td>
<td>410.1</td>
<td>410.06</td>
<td>8605.6</td>
<td>8605.57</td>
</tr>
<tr>
<td>4</td>
<td>s</td>
<td>0.37</td>
<td>409.8</td>
<td>409.77</td>
<td>8646.8</td>
<td>8646.84</td>
</tr>
<tr>
<td>5</td>
<td>s</td>
<td>0.38</td>
<td>402.7</td>
<td>402.68</td>
<td>8495.7</td>
<td>8495.71</td>
</tr>
<tr>
<td>6</td>
<td>s</td>
<td>0.38</td>
<td>390.2</td>
<td>390.18</td>
<td>8469.7</td>
<td>8469.73</td>
</tr>
<tr>
<td>7</td>
<td>s</td>
<td>0.38</td>
<td>392.2</td>
<td>392.17</td>
<td>8313.4</td>
<td>8313.42</td>
</tr>
</tbody>
</table>

Table 4.3 Data for repeatability of measurement of peak areas and height for gatifloxacin

<table>
<thead>
<tr>
<th>Track</th>
<th>SI</th>
<th>RF</th>
<th>height</th>
<th>X(calc)</th>
<th>area</th>
<th>X(calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>0.37</td>
<td>411.4</td>
<td>411.41</td>
<td>8534.2</td>
<td>8534.18</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>0.37</td>
<td>411.5</td>
<td>411.48</td>
<td>8537.3</td>
<td>8537.34</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>0.37</td>
<td>411.6</td>
<td>411.56</td>
<td>8540.5</td>
<td>8540.49</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>0.37</td>
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<td>411.52</td>
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<td>8542.93</td>
</tr>
<tr>
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<td>a</td>
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<td>411.66</td>
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<td>8541.18</td>
</tr>
<tr>
<td>6</td>
<td>a</td>
<td>0.37</td>
<td>411.5</td>
<td>411.51</td>
<td>8539.2</td>
<td>8539.24</td>
</tr>
<tr>
<td>7</td>
<td>a</td>
<td>0.37</td>
<td>411.5</td>
<td>411.47</td>
<td>8543.2</td>
<td>8543.18</td>
</tr>
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</table>

Substance: GATIFLOXACIN

85
Table 4.4 Precision of HPTLC method for estimation of standard gatifloxacin

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Intra-day Precision</th>
<th>Inter-day Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=3)</td>
<td>(n=5)</td>
</tr>
<tr>
<td></td>
<td>Peak area (mean ± SD)</td>
<td>% CV</td>
</tr>
<tr>
<td>200</td>
<td>6834.35 ± 111.56</td>
<td>1.66</td>
</tr>
<tr>
<td>300</td>
<td>8475.33 ± 142.81</td>
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<tr>
<td>400</td>
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</tr>
<tr>
<td>500</td>
<td>10903.22 ± 301.01</td>
<td>2.76</td>
</tr>
<tr>
<td>600</td>
<td>12755.10 ± 177.66</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Table 4.5 Accuracy data of gatifloxacin

<table>
<thead>
<tr>
<th>Taken Concentration of gatifloxacin (n = 3)</th>
<th>Added Concentration of gatifloxacin found (n = 3)</th>
<th>% Recovery ± % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0</td>
<td>206.08 ± 02.47</td>
</tr>
<tr>
<td>200</td>
<td>100</td>
<td>291.06 ± 06.61</td>
</tr>
<tr>
<td>200</td>
<td>200</td>
<td>385.10 ± 15.12</td>
</tr>
<tr>
<td>200</td>
<td>300</td>
<td>486.31 ± 09.51</td>
</tr>
<tr>
<td>200</td>
<td>400</td>
<td>581.67 ± 15.28</td>
</tr>
</tbody>
</table>
Table 4.6 Summary of validation parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity range</td>
<td>200 - 600 ng/spot</td>
</tr>
<tr>
<td>2</td>
<td>Limit of detection</td>
<td>10 ng/spot</td>
</tr>
<tr>
<td>3</td>
<td>Limit of quantitation</td>
<td>200 ng/spot</td>
</tr>
<tr>
<td>4</td>
<td>Precision (% CV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Repeatability of measurement (area)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>- Repeatability of sample application</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>(area)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Intra-day</td>
<td>1.38 - 2.76</td>
</tr>
<tr>
<td></td>
<td>- Inter-day</td>
<td>1.71 - 3.27</td>
</tr>
<tr>
<td>5</td>
<td>Accuracy (%)</td>
<td>96.28 - 103.04</td>
</tr>
<tr>
<td>6</td>
<td>Specificity</td>
<td>Specific</td>
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</table>
**Fig. 4.5 : Detection limit of HPTLC method for estimation of gatifloxacin**

**Fig. 4.6 : Chromatogram showing peak of gatifloxacin solution from tablet formulation**
**HPTLC method for tablet Dosage forms**

**Fig : 4.7** Peak purity spectra for gatifloxacin from tablet formulation at the peak start, peak apex and peak end position of the spot (Correlation = 0.9998).
Fig. 4.8: Comparison of spectra of a drug from the tablet formulation & the spectra of pure drug (gatifloxacin).
Table 4.7 Determination of gatifloxacin in marketed tablet dosage forms

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Form</th>
<th>Labeled amount of drug (mg)</th>
<th>Average amount of drug found (n=5)</th>
<th>% of gatifloxacin ± CV (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>400</td>
<td>411.16</td>
<td>102.79 ± 2.99</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>400</td>
<td>391.08</td>
<td>97.77 ± 6.42</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>400</td>
<td>386.48</td>
<td>96.87 ± 5.70</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>400</td>
<td>405.63</td>
<td>101.41 ± 5.86</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>400</td>
<td>403.62</td>
<td>100.91 ± 4.64</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>400</td>
<td>383.65</td>
<td>95.91 ± 6.96</td>
</tr>
<tr>
<td>7</td>
<td>G</td>
<td>400</td>
<td>385.45</td>
<td>96.36 ± 4.24</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>400</td>
<td>382.32</td>
<td>95.58 ± 5.57</td>
</tr>
<tr>
<td>9</td>
<td>I</td>
<td>400</td>
<td>416.21</td>
<td>104.53 ± 6.24</td>
</tr>
</tbody>
</table>