CHAPTER II

DEVELOPMENT OF ANALYTICAL METHODS FOR GATIFLOXACIN
2.1 NEW ANALYTICAL METHODS FOR THE DETERMINATION OF
GATIFLOXACIN (GTF)

The structure of Gatifloxacin is as shown below

![Gatifloxacin Structure]

Gatifloxacin (GTF) is a synthetic broad-spectrum 8-methoxy fluoroquinolone antibacterial agent. Chemically it is 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid sesquihydrate. GTF is not official in any pharmacopoeia. Literature survey reveals the presence of some HPLC and spectrophotometric methods for the estimation of GTF. But the problem of assay of GTF in pharmaceutical formulations has been the subject of limited number of investigations.

Very few analytical methods have been reported for the estimation of GTF using visible spectrophotometry. The analytically important functional groups of GTF have not been exploited for designing sensitive, accurate and flexible visible spectrophotometric methods for the determination of GTF in pharmaceutical formulations. A total of ten visible spectrophotometric methods and one HPLC methods have been established for GTF in this chapter. The materials and methods used and the principle involved in these methods is also discussed in this chapter. These principles are also extended for the pharmaceutical formulations containing GTF.
2.2 MATERIALS AND METHODS:

This chapter deals with the details of the materials and instruments used for the present investigations. A summary of different reagents used for different methods are explained below.

2.2.1 INSTRUMENTATION:

All spectral and absorbance measurements were made on Shimadzu UV-150-02 (double beam) and Elico UV-VIS SL-150 spectrophotometers with 1 cm matched quartz cells. pH measurements were made on Systronics digital pH meter model 335. HPLC methods were developed on Shimadzu-LC-10 ATVP.

2.2.2 MATERIALS:

All the chemicals and reagents used were of analytical or pharmacopoeial grade and all solutions were freshly prepared in double distilled water. A brief account of the materials employed in the present investigations is furnished here.

Method M₁:

Ferric alum solution (Loba, 1% w/v in 1% HNO₃): prepared by dissolving 1 gm of Ferric alum in 100ml of 1% HNO₃.

0.1N HCl (Qualigens)

Method M₂:

Ferric Nitrate Solution (Loba 1% w/v in 1% HNO₃): prepared by dissolving 1 gm of Ferric Nitrate in 100ml of 1% HNO₃.

0.1 N HCl (Qualigens)

Method M₃:

Ferric Chloride solution (Loba 0.2% w/v in 0.1N HCl): prepared by dissolving 200mg FeCl₃ in 100ml of 0.1N HCl.

0.1N HCl (Qualigens)
Method M₄:

**Ferric Chloride solution** (Loba 0.003 M): prepared by dissolving 1.6 gm of FeCl₃ in 100ml of water, from this 1 ml was taken and diluted to 250ml with distilled water.

**1,10 – Phenanthroline solution** (Qualigens 0.01M): prepared by dissolving 198 mg in 100 ml of water.

**Ortho Phosphoric Acid** (Qualigens 0.02M): prepared by dissolving 0.2 ml of Orthophosphoric Acid in 100 ml of water.

0.1 N HCl (Qualigens)

Method M₅:

**Ceric ammonium sulphate (CAS)** (Loba 1% w/v): prepared by dissolving 1 gm of CAS in 100ml of 0.36 N H₂SO₄.

**Sodium Hydroxide** (Qualigens 0.1 N): prepared by dissolving 0.4 gm of NaOH in 100ml of water.

0.36 N H₂SO₄ (Qualigens)

0.1 N HCl (Qualigens)

Method M₆:

**Ceric ammonium sulphate (CAS)** (Loba 1% w/v): prepared by dissolving 1 gm of CAS in 100ml of 0.36 N H₂SO₄.

**3- Methyl – 2- benzothiazolinone hydrazone** (MBTH, Loba) (0.2 % w/v): prepared by dissolving 200mg of MBTH in 100 ml of water.

0.36 N H₂SO₄ (Qualigens)

0.1 N HCl (Qualigens)

Method M₇:

**Folin- Ciocalteu reagent** (Loba 2 N)

**Sodium Carbonate** (Loba 10 % w/v in water)

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0.1 N HCl

Method M₈:
Solochrome black – T (SBT) (Loba 0.5 % w/v): prepared by dissolving 500mg of SBT in 100ml water.
0.1 N HCl (Qualigens)
Chloroform (Qualigens)

Method M₉:
Methyl orange solution (MO) (Loba 0.1 % w/v): prepared by dissolving 100mg of MO in 100ml of water.
0.1 N HCl (Qualigens)
Chloroform (Qualigens)

Method M₁₀:
Bromothymol blue solution (BTB) (Loba 0.1 % w/v): prepared by dissolving 100mg of BTB in 100ml of water.
0.1N HCl (Qualigens)
Chloroform (Qualigens)

Method M₄₄:
Acetonitrile (HPLC grade, Qualigens)

2.3 EXPERIMENTAL

Method M₁:

Solution of Ferric Alum (1% w/v) in 1% w/v nitric acid was prepared in the usual way. Standard and sample solutions of GTF (1mg/ml) were prepared in 0.1N hydrochloric acid. Sample solution (from pharmaceutical formulation like tablet) was prepared by dissolving 100mg of the active ingredient in 50ml of 0.1 N hydrochloric acid and filtered. The filtrate was made up to 100ml with the same solvent.
Into a series of tubes, aliquots of GTF (1.0 – 4.0 ml, 100 μg/ml) were taken and then 1.0 ml of Ferric Alum reagent was added and kept aside for 10 min. The solution in each tube was then made up to 5.0 ml with distilled water and the absorption of the colored species was measured at 425 nm against reagent blank. The amount of GTF was calculated from its calibration graph. All the spectral characteristics are given in Table 2.1

For Pharmaceutical Preparations

Tablets

Four types of commercially available tablets were taken for analysis. Twenty tablets were accurately weighed and powdered separately. An accurately weighed quantity of tablet powder equivalent to 100mg of GTF was taken and the sample solution was made in the same manner described above and analyzed by the procedure described above. The results are given in Table 2.2

Chemistry of the colored species:

The enolic form of GTF gives a colored complex with Ferric Alum under acidic conditions. The color intensity and stability of the complex increases when the –COOH group is adjacent to phenolic group.

Method M2:

Solution of Ferric Nitrate (1% w/v) in 1% v/v nitric acid was prepared in the usual way. Standard and sample solutions of GTF (1mg/ml) were prepared in 0.1N hydrochloric acid. Sample solution (from pharmaceutical formulation like tablet) was prepared by dissolving 100mg of the active ingredient in 50ml of 0.1 N hydrochloric acid and filtered. The filtrate was made up to 100ml with the same solvent.

Into a series of tubes, aliquots of GTF (1.0 – 4.0 ml, 100 μg/ml) were taken and then 1.0 ml of Ferric Nitrate reagent was added and kept aside for 10 min. the
solution in each tube was then made up to 5.0 ml with distilled water and the absorption of the colored species was measured at 430 nm against reagent blank. The amount of GTF was calculated from its calibration graph. All the spectral characteristics are given in Table 2.1.

For Pharmaceutical Preparations:

Tablets

The procedure is the same that is discussed in method M1. The results are given in Table 2.2

Chemistry of the colored species:

The enolic form of GTF gives a colored complex with Ferric Nitrate under acidic conditions. The color intensity and stability of the complex increases when the –COOH group is adjacent to phenolic group.

Method M3:

Solution of Ferric Chloride (0.2% w/v) in 0.1 N hydrochloric acid was prepared in the usual way. Standard and sample solutions of GTF (1mg/ml) were prepared in 0.1N hydrochloric acid. Sample solution (from pharmaceutical formulation like tablet) was prepared by dissolving 100mg of the active ingredient in 50ml of 0.1 N hydrochloric acid and filtered. The filtrate was made up to 100ml with the same solvent.

Into a series of tubes, aliquots of GTF (1.0 – 4.0 ml, 200 μg/ml) were taken and then 1.0 ml of Ferric Chloride reagent was added and kept aside for 10 min. the solution in each tube was then made up to 5.0 ml with distilled water and the absorption of the colored species was measured at 425 nm against reagent blank. The amount of GTF was calculated from its calibration graph. All the spectral characteristics are given in Table 2.1
For Pharmaceutical Preparations

Tablets

The procedure is the same that is discussed in method M₁. The results are given in Table 2.2.

Chemistry of the colored species:

The enolic form of GTF gives a colored complex with Ferric Chloride under acidic conditions. The color intensity and stability of the complex increases when the –COOH group is adjacent to phenolic group.

Method M₄:

To a series of 10 ml graduated tubes, aliquots of GTF ranging from 0.5 to 2.0 ml (1 ml = 200 μg), 1.5 ml of Ferric Chloride (0.003 M) and 2.5 ml of 1,10-phenanthroline (0.01 M) were added successively to each tube. The tubes were then heated on a boiling water bath for 15 min, cooled to room temperature and 2 ml of O-phosphoric acid (0.02 M) was added to each tube and the total volume was brought to 10 ml with distilled water. The absorbance of the red colored species was measured at 530 nm against reagent blank within 60 min. The amount of GTF was computed from the calibration graph. All the spectral characteristics are given in Table 2.1.

For Pharmaceutical Preparations

Tablets

The procedure is the same that is discussed in method M₁. The results are given in Table 2.2.

Chemistry of the colored species:

The above method is based on the oxidation of GTF by Fe (III) to produce Fe (II), which subsequently reacts with 1,10-phenanthroline to form a red colored ferroin complex. O-phosphoric acid is used in this method to combine with residual iron to
form Fe (PO₄)₂⁻³ and to prevent photochemical reduction, thus facilitating the determination.

**Method M₅:**

Into a series of graduated test tubes 0.5 ml to 2.0 ml of working standard drug solution of GTF is pipetted and 1.2 ml of CAS is added and made up to the volume to 5.0 ml with 0.1 N NaOH. The absorbance of the colored species is measured against the reagent blank at the wavelength of maximum absorption 455 nm. The amount of the drug in the sample was computed from the calibration curve. All the spectral characteristics are given in Table 2.1

For Pharmaceutical Preparations

**Tablets**

The procedure is the same that is discussed in method M₁. The results are given in Table 2.2.

**Chemistry of the colored species:**

It is known that secondary amines undergo oxidation to give hydroxylamine which convert into stable nitroxides. Thus it may be stated that a nitroxide type of complex formed has yellow orange color.

**Method M₆:**

Aliquots of standard GTF (1.0-5.0 ml, 100μg/ml) were transferred into a series of 10 ml of volumetric flasks. To each of these flasks was added 1.0 ml of CAS solution mixed and kept aside for 2 min. then 1.0 ml of MBTH solution was added and kept aside for another 10 min. the solution was made up to the mark with distilled water and the absorbance was measured at 640 nm against its reagent blank within the stability period of 45 min after the color development. The amount of the drug was
computed from the respective calibration curve. All the spectral characteristics are given in Table 2.1

For Pharmaceutical Preparations

Tablets

The procedure is the same that is discussed in method M. The results are given in Table 2.2.

Chemistry of the colored species:

In the above method the reaction of MBTH with GTF in the presence of CAS proceeds via oxidative coupling, which is similar to that reported earlier for other fluoroquinolones. Under the reaction conditions, MBTH on oxidation loses two electrons and one proton, forming the electrophilic intermediate, which is the active coupling species. This intermediate reacts with GTF resulting in the formation of a colored chromogen.

Method M:

Aliquots of GTF solution (1-4 ml, 500 µg/ml) were transferred to a series of 20 ml graduated tubes. Then 2.5 ml of FC reagent and 9 ml of sodium carbonate solution were added simultaneously and kept aside for 10 min at room temperature. The solution was made up to volume with distilled water. The absorbance was measured at 780 nm against reagent blank. The colored species was stable for 3 hrs. The amount of drug in the sample was computed from Beer's Plot. All the spectral characteristics are given in Table 2.1

For Pharmaceutical Preparations

Tablets

The procedure is the same that is discussed in method M. The results are given in Table 2.2
Chemistry of the colored species:

The color formation by FC reagent with GTF under alkaline conditions may be explained in the following manner based on the analogy with the reports of the earlier workers, the mixed acids in the FC reagent involve the following chemical species.

\[ 3 \text{H}_2\text{O}. \text{P}_2\text{O}_5. 13\text{WO}_3. 10 \text{H}_2\text{O} \text{ and } 3\text{H}_2\text{O}. \text{P}_2\text{O}_5. 14 \text{WO}_3. 4 \text{MoO}_3. 10 \text{H}_2\text{O}. \]

GTF probably effects a reduction of 1,2 or 3 oxygen atoms of tungstate and / or molybdate in FC reagent thereby producing one or more of the possible reduced species, which have a characteristic intense blue color.

Method M₈:

Aliquots of working standard solutions of GTF ranging from 1- 4 ml (100μg/ml) were transferred to a series of 125 ml separating funnels. To these, 0.5 ml of 0.1 N HCl and 1 ml of SBT dye solution (0.5 %) were added. The total volume of the aqueous phase was adjusted to 10 ml with distilled water. Chloroform (10 ml) was added to each separating funnel. The contents were shaken and allowed to stand for clear separation of the layers. The chloroform layers were separated and their absorbance was measured at 520 nm against reagent blank. The amount of drug present in the sample solution was calculated from its calibration curve. All the spectral characteristics are given in Table 2.1.

For Pharmaceutical Preparations

Tablets

The procedure is the same that is discussed in method M₁. The results are given in Table 2.2.
Chemistry of the colored species:

GTF possessing secondary amine involves in association complex formation with an acidic dye (SBT), which is extractable from the aqueous phase. The protonated nitrogen (positive charge) of GTF molecule in acid medium is expected to attract the oppositely charged portion (negative charge) of the dye and behaves as a single unit being held together by electrostatic attraction.

Method M₉:

Aliquots of working standard solutions of GTF ranging from 0.5 - 3 ml (500µg/ml) were transferred to a series of 125 ml separating funnels. To these, 6 ml of 0.1 N HCl and 2 ml of MO dye solution (0.1 % w/v) were added. The total volume of the aqueous phase was adjusted to 10 ml with distilled water. Chloroform (10 ml) was added to each separating funnel. The contents were shaken and allowed to stand for clear separation of the layers. The chloroform layers were separated and their absorbance was measured at 425 nm against reagent blank. The amount of drug present in the sample solution was calculated from its calibration curve. All the spectral characteristics are given in Table 2.1

For Pharmaceutical Preparations

Tablets

The procedure is the same that is discussed in method M₁. The results are given in Table 2.2.

Chemistry of the colored species:

GTF possessing secondary amine involves in association complex formation with an acidic dye (MO), which is extractable from the aqueous phase. The protonated nitrogen (positive charge) of GTF molecule in acid medium is expected to
attract the oppositely charge portion (negative charge) of the dye and behaves as a single unit being held together by electrostatic attraction.

**Method M10:**

Aliquots of working standard solutions of GTF ranging from 0.5 – 2.5 ml (100μg/ml) were transferred to a series of 125 ml separating funnels. To these, 6 ml of 0.1 N HCl and 2 ml of BTB dye solution (0.1 % w/v) were added. The total volume of the aqueous phase was adjusted to 10 ml with distilled water. Chloroform (10 ml) was added to each separating funnel. The contents were shaken and allowed to stand for clear separation of the layers. The chloroform layers were separated and their absorbance was measured at 420 nm against reagent blank. The amount of drug present in the sample solution was calculated from its calibration curve. All the spectral characteristics are given in Table 2.1.

**For Pharmaceutical Preparations**

**Tablets**

The procedure is the same that is discussed in method M1. The results are given in Table 2.2.

**Chemistry of the colored species:**

GTF possessing secondary amine involves in association complex formation with an acidic dye (BTB), which is extractable from the aqueous phase. The protonated nitrogen (positive charge) of GTF molecule in acid medium is expected to attract the oppositely charged portion (negative charge) of the dye and behaves as a single unit being held together by electrostatic attraction.

**Method M44:**

A stock solution (1000 μg/ml) of pure drug was prepared by dissolving 100mg of GTF and 100 mg of ciprofloxacin (internal standard) separately in 100ml
volumetric flasks containing 70 ml of triple distilled water. Daily working standard solutions of GTF and internal standard were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Initially a mobile phase consisting of acetonitrile: buffer in the ratio of 85: 15 was tried. Symmetry RP- C 18 columns 250 mm were used. Early elution with tailing of peaks was observed in the above condition. Then the composition of mobile phase was changed to 70: 30. Under these conditions broad peaks shape and pronounced tailing was observed. For the same mobile phase, if the ratio was changed to 20: 80, GTF was eluted at around 6.85 min with symmetric peak shape. All the spectral characteristics are given in Table 2.3.

For Pharmaceutical Preparations

Tablets

Four types of commercially available tablets were taken for analysis. Twenty tablets were accurately weighed and powdered separately. A quantity equivalent to 100mg of GTF was taken and the sample was analysed as discussed above. The results are given in Table 2.4.
FIG 2.1

ABSORPTION SPECTRA OF GTF IN FERRIC ALUM M1

WAVELENGTH (nm)

CONCENTRATION (mcg/ml)

BEER'S LAW PLOT OF GTF IN FERRIC ALUM M1

CONCENTRATION (mcg/ml)
FIG 2.2

**ABSORPTION SPECTRA OF GTF IN FERRIC NITRATE M₂**

![Absorption Spectra Graph]

**BEER'S LAW PLOT OF GTF IN FERRIC NITRATE M₂**

![Beer's Law Plot Graph]
FIG 2.3

**ABSORPTION SPECTRA OF GTF IN FERRIC CHLORIDE REAGENT M₃**

![Absorption Spectra Graph]

**BEER'S LAW PLOT OF GTF IN FERRIC CHLORIDE REAGENT M₃**

![Beer's Law Plot Graph]
FIG2.4

ABSORPTION SPECTRA OF GTF IN FERRIC CHLORIDE/OPA REAGENT M₄

WAVELENGTH (nm)

ABSORBANCE

BEER'S LAW PLOT OF GTF IN FERRIC CHLORIDE/OPA REAGENT M₄

ABSORBANCE

CONCENTRATION (mcg/ml)
FIG 2.5

ABSORPTION SPECTRA OF GTF IN CAS REAGENT M₅

WAVELENGTH (nm)

ABSORBANCE

BEER’S LAW PLOT OF GTF IN CAS REAGENT M₅

CONCENTRATION (mcg/ml)

ABSORBANCE
FIG 2.6

ABSORPTION SPECTRA OF GTF IN CAS/MBTH $M_6$

BEER'S LAW PLOT OF GTF IN CAS/MBTH $M_6$

59
FIG 2.7

**ABSORPTION SPECTRA OF GTF IN F.C. REAGENT M₇**

![Graph showing absorbance vs. wavelength (nm)]

**BEER'S LAW PLOT OF GTF IN F.C. REAGENT M₇**

![Graph showing absorbance vs. concentration (mcg/ml)]
FIG 2.8

ABSORPTION SPECTRA OF GTF IN SBT
REAGENT Mg

WAVELENGTH (nm)

ABSORBANCE

0.29
0.3
0.31
0.32
0.33
0.34
0.35

460 480 500 520 540 560

BEER'S LAW PLOT OF GTF IN SBT Mg

ABSORBANCE

0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

0 200 400 600

CONCENTRATION (mcg/ml)
FIG 2.9

ABSORPTION SPECTRA OF GTF IN MD REAGENT M₃

WAVELENGTH (nm)

ABSORBANCE

BEER'S LAW PLOT OF GTF IN MO REAGENT M₉

ABSORBANCE

CONCENTRATION (mcg/ml)
FIG 2.10

**ABSORBANCE SPECTRA OF GTF IN BTB M₁₀**

![Graph showing absorbance spectra with wavelength (nm) on the x-axis and absorbance on the y-axis.]

**BEER'S LAW PLOT OF GTF IN BTB M₁₀**

![Graph showing absorbance vs concentration (mcg/ml) with concentration on the x-axis and absorbance on the y-axis.]

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FIG 2.3a
RINGBOM PLOT OF GTF IN FERRIC CHLORIDE
REAGENT M₃

% Transmission

Log Concentration in mcg

FIG 2.4a
RINGBOM PLOT OF GTF IN FERRIC
CHLORIDE/OPA REAGENT M₄

% Transmission

Log Concentration in mcg
FIG 2.7a
RINGBOM PLOT OF GTF IN F.C. REAGENT M₇

Log Concentration In mcg
% Transmission

FIG 2.8a
RINGBOM PLOT OF GTF IN SBT REAGENT M₈

Log Concentration In mcg
% Transmission

67
**Chromatogram of Gatifloxacin M**

**Chromatographic conditions**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase (column)</td>
<td>Bondapak C-18 (250x4.6 mm, packed with 5 micron)</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>acetonitrile:buffer (20:80)(pH3.0)</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>1.0</td>
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<tr>
<td>Column back Pressure (psi)</td>
<td>1540</td>
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<tr>
<td>Run time (minutes)</td>
<td>14</td>
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<tr>
<td>Column temperature (°C)</td>
<td>Ambient</td>
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<tr>
<td>Volume of injection loop (u.l)</td>
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<tr>
<td>Detection wavelength (nm)</td>
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<tr>
<td>Internal standard</td>
<td>Ciprofloxacin</td>
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<tr>
<td>Drug RT (min)</td>
<td>3.643</td>
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<tr>
<td>Internal standard RT (min)</td>
<td>3.501</td>
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TABLE 2.1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

<table>
<thead>
<tr>
<th>DATA</th>
<th>Proposed Methods</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>M₁</td>
</tr>
<tr>
<td>λ max</td>
<td>425</td>
</tr>
<tr>
<td>Beer’s law limits (ug/ml)</td>
<td>10-40</td>
</tr>
<tr>
<td>Molar absorptivity (lit.mole⁻¹.cm⁻¹)</td>
<td>3.801x10³</td>
</tr>
<tr>
<td>Sandells Sensitivity (ug/cm²/0.01 abs.unit)</td>
<td>0.105</td>
</tr>
<tr>
<td>Regression Equation (Y=a+b+c) slope (b)</td>
<td>0.0018</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.029</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9999</td>
</tr>
<tr>
<td>% Relative Standard Deviation</td>
<td>1.12</td>
</tr>
<tr>
<td>% Range of error 0.05 level</td>
<td>0.9364</td>
</tr>
<tr>
<td>0.01 level</td>
<td>1.3364</td>
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</table>
### TABLE 2.2
ASSAY AND RECOVERY OF THE DRUG IN DOSAGE FORMS

<table>
<thead>
<tr>
<th>DATA</th>
<th>LESSED AMOUNT</th>
<th>Amount found (mg)</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet 1</td>
<td>400</td>
<td>339±0.15, 329±0.11, 349±0.13, 369±0.19, 359±0.15, 359±0.18, 359±0.18, 359±0.56</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T = 1.05, F = 0.98, T = 1.06, F = 1.12, T = 1.09, F = 1.06, T = 1.08, F = 1.05</td>
<td></td>
</tr>
<tr>
<td>Tablet 2</td>
<td>400</td>
<td>359±0.15, 329±0.11, 349±0.13, 359±0.19, 369±0.18, 369±0.18, 369±0.18, 369±0.56</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T = 1.13, F = 0.98, T = 1.06, F = 1.08, T = 1.09, F = 1.06, T = 1.08, F = 1.05</td>
<td></td>
</tr>
<tr>
<td>Tablet 3</td>
<td>400</td>
<td>369±0.17, 329±0.11, 349±0.13, 359±0.19, 369±0.18, 369±0.18, 369±0.18, 369±0.56</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T = 1.11, F = 1.07, T = 1.06, F = 1.08, T = 1.09, F = 1.06, T = 1.08, F = 1.05</td>
<td></td>
</tr>
<tr>
<td>Tablet 4</td>
<td>400</td>
<td>339±0.23, 329±0.19, 349±0.23, 359±0.19, 369±0.18, 369±0.18, 369±0.18, 369±0.56</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T = 1.07, F = 1.04, T = 1.06, F = 1.08, T = 1.09, F = 1.06, T = 1.08, F = 1.05</td>
<td></td>
</tr>
</tbody>
</table>

- Average±standard deviation of six determinations the t- and f- values refer to comparison of the proposed methods with the reference method theoretical values. Reference method: Theoretical values at 95% confidence limits \( t = \)...

- UV method (λmax 292 nm in 0.1 n 1 M)
- Recovery of 10 mg added to the pharmaceutical formulations (Average of 3 determinations)
### TABLE 2.3

OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED HPLC METHODS FOR GTF M₄₄

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection wavelength (nm)</td>
<td>240</td>
</tr>
<tr>
<td>Linearity range (ng/ml)</td>
<td>0.25 - 500</td>
</tr>
<tr>
<td>Detection limits (ng/ml)</td>
<td>0.00128</td>
</tr>
<tr>
<td>Regression equation (Y = a + b \cdot C)</td>
<td></td>
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<tr>
<td>Slope (b)</td>
<td>0.7560</td>
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<tr>
<td>Standard deviation of slope (S_b)</td>
<td>0.00196</td>
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<tr>
<td>Intercept (a)</td>
<td>0.00084</td>
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<tr>
<td>Standard deviation of intercept (S_a)</td>
<td>0.00324</td>
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<tr>
<td>Standard error of estimation (S_e)</td>
<td>0.00309</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9999</td>
</tr>
<tr>
<td>Relative standard deviation (%)*</td>
<td>0.0571</td>
</tr>
<tr>
<td>% Range of error (Confidence limits)*</td>
<td>0.0571</td>
</tr>
<tr>
<td>0.05 level</td>
<td>0.0477</td>
</tr>
<tr>
<td>0.01 level</td>
<td>0.0706</td>
</tr>
<tr>
<td>% Error in bulk sample*</td>
<td>0.0691</td>
</tr>
</tbody>
</table>

*Average of eight determinations.

**Average of three determinations.
### TABLE 2.4
INTER-AND INTRA-DAY PRECISION FOR GATIFLOXACIN ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED HPLC METHODS. M44

<table>
<thead>
<tr>
<th>Concentration of GTF (µg/ml)</th>
<th>Observed concentration of GTF (µg/ml)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (n=5)</td>
<td>% CV</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>4.97</td>
<td>0.55</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>10.01</td>
<td>0.19</td>
</tr>
</tbody>
</table>

### ESTIMATION OF GTF IN TABLETS M44

<table>
<thead>
<tr>
<th>Pharmaceutical formulation</th>
<th>Labeled amount (mg)</th>
<th>Amount obtained by proposed method</th>
<th>% Recovery of proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>200</td>
<td>200.60</td>
<td>100.31</td>
</tr>
<tr>
<td>Tablets</td>
<td>400</td>
<td>398.12</td>
<td>99.94</td>
</tr>
</tbody>
</table>
2.4 CONCLUSION

Since Gatifloxacin is a relatively new drug and the analytical methods available for its assay are very limited, it is worthwhile to develop some methods for its assay. As part of the present investigations, ten methods have been developed for the purpose of assay of GTF and a simple HPLC method was also developed. It can be seen from the results presented above that the proposed methods have reasonable sensitivity. Statistical analysis of the results shows that the proposed procedures have good precision and accuracy. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives present in them.

The order of sensitivity among the ten proposed methods is $M_6 > M_3 > M_8 > M_{10} > M_2 > M_1 > M_7 > M_4 > M_5 > M_9$. Beer's law limits ($\mu g/ml$) of the proposed methods are better than many of the reported spectrophotometric methods. All the proposed methods are simple sensitive and reliable with good precision and accuracy. These methods can be used for the routine determination of GTF in bulk samples and in pharmaceutical formulations.