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# Development of a New Green HPLC Method for Gatifloxacine Determination in Bulk Drug and Ophthalmic Formulations

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## Abstract:

**Background:** Control of the active pharmaceutical ingredient plays a crucial role in ensuring the required efficacy. Therefore, a new accurate, reproducible, and green RP-HPLC method was developed for determining Gatifloxacin in bulk and eye drops.

**Methods:** The chromatographic separation was carried out using (isopropanol: phosphate buffer, pH=3±0.1 ) (35:65) v/v as mobile phase, the column used was C8 5µ (4.6*150mm) Inert Sustain GL Sciences column at temperature 〖40〗^° C, the flow rate was 0.8 mL/min, the UV detector was set at 280 nm and the injection volume was 10 µL. The greenness of the method was evaluated using Eco-scale tool.

**Results:** ICH guidelines were applied to prove the validity of the developed method. The linearity was achieved in the range 10-120 mg/l for Gatifloxacin with an excellent correlation coefficient R2=1. The LOD and LOQ were found to be 0.026 and 0.08 mg/l respectively. The precision was studied as intra- and inter-day with relative standard deviations not more than 2%, and the accuracy by mean recoveries ranged from 99.36 to 102.25%. The developed method was successfully applied for to determine Gatifloxacin in marketed eye drops. Eventually, the Eco-scale tool revealed a high degree of greenness by using relatively more eco-friendly solvents.

**Conclusion:** A new, sensitive, green, and accurate reversed-phase liquid chromatographic method is developed and validated to determine gatifloxacin in bulk and ophthalmic formulations using a UV detector under isocratic conditions.

**Keywords:** RP- HPLC, Gatifloxacin, Eye Drops, Method Validation, Greenness Assessment.

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# تطوير طريقة كروماتوغرافية سائلة عالية الأداء جديدة خضراء لتحديد غاتيفلوكساسين كمادة أولية وفي الأشكال العينية

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## الملخّص:

**خلفية البحث وهدفه:** تلعب مراقبة المواد الفعالة صيدلانياً دوراً هاماً في ضمان الفعالية المنشودة، لذلك تم تطوير طريقة جديدة، ودقيقة ومضبوطة وخضراء لتحديد غاتيفلوكساسين كمادة أولية وفي الأشكال العينية.

مواد البحث وطرائقه: تم اجراء الفصل الكروماتوغرافي باستعمال إيزوبروبانول: وقاء فوسفاتي (35: (pH=3±0.1) (65حجم/ حجم، كطور متحرك، عمود C8 بأبعاد 5 InertSustain GL Sciences (4.6*150mm) µفي درجة الحرارة ⟦C40 °^ ⟧، كان معدل التدفق 0.8 مل / دقيقة، عند طول موجة 280 نانومتر، وحجم الحقنة10 ميكرولتر، تم تقييم خضرة الطريقة المطورة باستخدام أداة. Eco-Scale

**النتائج:** تم تطبيق إرشادات المؤتمر الدولي للتنسيق والمواءمة لإثبات مصدوقية الطّريقة المطوّرة، كانت الخطّية ضمن المجال 10-120 ملغ/ ل من أجل غاتيفلوكساسين ومعامل ارتباط ممتاز قدره 1 ، وكان حدا الكشف والمقايسة الكمية 0.026 ، 0.08 ملغ/ ل على الترتيب، تمت دراسة الدّقة خلال اليوم الواحد وبين أيام مختلفة فكانت الانحرافات المعيارية النسبية لا تزيد عن 2٪، وتراوحت المضبوطية بمتوسط استعادة من99.36% حتى 102.25% . تم تطبيق الطّريقة المطورة بنجاح لتحديد المركب في القطرات العينية التجارية. نهايةً، أظهرت نتيجة أداة Eco-Scale درجة عالية من الخضرة نظراً لاستخدام محلات أكثر صداقة للبيئة نسبياً.

**الاستنتاج:** تم تطوير طريقة كروماتوغرافيا سائلة بالطّور العكوس جديدة، حسّاسة، خضراء ودقيقة لتحديد غاتيفلوكساسين كمادة أولية وفي القطرات العينية، باستخدام التّحري بالأشعة فوق البنفسجية تحت شروط النظام المتساير.

**الكلمات المفتاحيّة:** الكروماتوغرافيا السّائلة عالية الأداء بالطّور العكوس، غاتيفلوكساسين، القطرات العينية، مصدوقيّة الطريقة، تقييم خضرة الطّريقة.

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# 1. Introduction:

Gatifloxacin (GTX) ((RS) 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3 methylpiperazin-l-yl)-4-oxo-3-quinolinecarboxylicacid) (Figure1) [1] has broad antimicrobial activity and is effective for the treatment of a wide variety of infectious diseases [1]

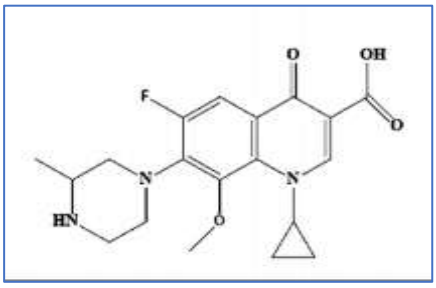


**Figure 1: structure of Gatifloxacin[1]**

Assay method for GTX has been cancelled in USP, however there is an official HPLC method in Indian Pharmacopoeia for Gatifloxacin determination as raw material and in ophthalmic solutions, infusions, and tablets [2].

A review of the literature on this drug had revealed chromatographic methods. Martin N Saad and his colleagues developed HPLC method for the simultaneous determination of Gatifloxacin and dexametasone in ophthalmic formulations using different column technologies including monolithic columns which showed the best results due to their hydrodynamic properties[3], another method was developed depending on ion pairing HPLC to determine GTX as bulk and in formulations[4], some HPLC methods have been conducted including reversed-phase HPLC method for drug determination in tablet dosage forms[5], stability-indicating HPLC method as bulk and in pharmaceutical preparations [6], HPLC method for the determination of Gatifloxacin stability in human plasma [7], on other hand ,spectrophotometric assays directly or after derivation[8,9] and spectrofluorimetric methods based on charge transfer complex formation [10] or metal ion interaction [1] have been developed.

Most HPLC methods even Pharmacopoeial ones use acetonitrile or consume large quantities of methanol in the mobile phase. Unfortunately, such solvents are considered harmful to both analysts and environment. Moreover, some methods as mentioned above, use special technology of column. Because of HPLC technique is considered a worldwide approach for drug determination [11,12], this work aims at developing a new reversed-phase HPLC for GTX determination as bulk and in eye drops in a relatively short retention time by using green mobile phase, and classical C8 column, which is available in all analytical laboratories. The developed method was validated according to ICH guidelines. Among various greenness assessment tools that depend basically on the twelve principles of green analytical chemistry (waste prevention, atom economy maximizing, less hazardous chemical synthesis, safer chemical and products designing, use of renewable sources, avoid chemical derivation, use catalysts, design for degradation, real time pollution prevention, accidents prevention) [13], Eco-Scale calculator was employed.

# 2. Materials and methods

## 2.1. Instrumentation

Chromatographic separation was carried out using a Shimadzu HPLC system LC 2030C 3D plus (Shimadzu Corporation, Japan), equipped with a PDA detector, an autosampler, column oven (Shimadzu Corporation, Japan). C8 column 5µm (4.6*250mm) InertSustain GL Sciences Inc.

Japan. Ultrasonic bath -BANDELIN SONOREX, Balance A and D Company Limited, Japan. pH meter WTW-inolab pH7310p, PTFE syring filter 0.45µm.

**2.2. Materials & standards**

Potassium dihydrogen phosphate ($KH_2PO_4$) (99% purity), ortho phosphoric acid (85% purity) were purchased from HIMEDIA-INDIA. Isopropanol, HPLC grade from Scharlou-SPAIN. Standard Gatifloxacin sesquihydrate (99.9% purity) was kindly provided by Miamed Pharmaceutical Industries (Evergreen chemical factory Co; Ltd, China). Eye drops (containing Gatifloxacin 0.3%) were purchased from Syrian market.

**2.3. Chromatographic conditions**

InterSustain C8 Column 5$\boldsymbol{\mu}$m (4.6 x250mm) (GL Sciences, Japan), temperature $40^{\circ}C$, and mobile phase consisted of (isopropanol: phosphate buffer, pH=3$\pm$0.1) (35 :65) v/v. Phosphate buffer consisted of 0.025M of $KH_2PO_4$, the pH was adjusted by ortho phosphoric acid. The mobile phase was degassed by sonication for 15 min and filtered through 0.45µm Millipore membrane filter before use. (Figure. 2) shows the chromatogram of GTX under optimal conditions (flow rate 0.8 mL/min, and UV detection at 280 nm, injection volume 10 µl).

**2.4. Standard Solution**

Stock solution of GTX was prepared at concentration of 500 mg/L, using mobile phase as a solvent and stored in refrigerator at $4^{\circ}C$ temperature. Five working solutions were prepared by diluting the stock solution to obtain concentrations ranging from 10 to 120 mg/L..

**2.5. Method validation**

The developed method was validated according to the International Conference on Harmonization (ICH) guidelines, encompassing the following parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, selectivity and robustness[14].

**2.5.1. Linearity, limit of detection (LOD) and limit of quantification (LOQ)**

It was evaluated by plotting calibration curve using five concentrations: 10-30-60-90-120 mg/L of GTX against area under the curve, each concentration was injected in triplicate. Slope, intercept and correlation coefficient $R^2$ of the calibration curve were calculated.

LOD, LOQ were calculated based on LOD (3.3 SD/S) and LOQ (10 SD /S), where SD the standard deviation of the response and S the slope of the calibration curve.

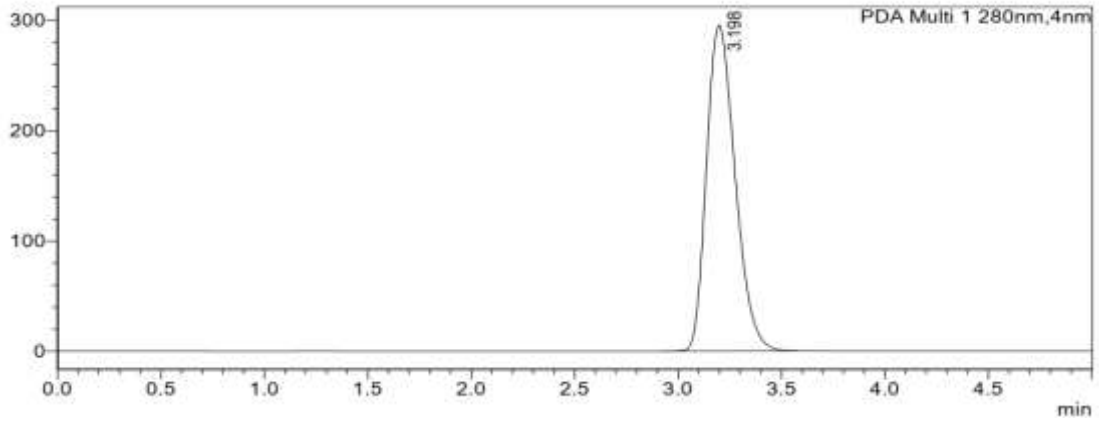


**Figure 2: standard chromatogram of GTX by developed method**

**2.5.2. Precision**

Two levels of precision were evaluated by applying the developed method on three concentrations on the same day (intra-day) and in three different days (inter-day), then relative standard deviation (RSD%) was calculated.

**2.5.3. Accuracy**

The accuracy of the developed method was evaluated using recovery and mean recovery at three different concentrations of GTX.

**2.5.4. Robustness**

Robustness was achieved by subjecting the method to a minor change in the conditions. Temperature was chosen as a parameter, then relative standard deviation (RSD %) was calculated.

**2.5.5. Selectivity**

The selectivity of the developed method was tested by applying the optimal chromatographic conditions to a mixture of common eye drop excipients (ethylenediaminetetraacetic acid EDTA, sodium chloride, benzalkonium chloride).

**2.5.6. Determination of Gatifloxacin in marketed eye drop (0.3% GTX)**

Equivalent to 0.6 g (2ml) of GTX was diluted to 100mL with mobile phase as a solvent, and after filtration, 10 µl was injected into the chromatographic system. Eventually, the practical content was calculated using a calibration curve equation.

**3. Results and discussion**

The first objective of this work is to replace acetonitrile with the minimum amount of safer solvent at the same time, using buffer solution as a maximum part of the mobile phase.

Since literature lacked examples of isopropanol as an organic modifier in mobile phases for Gatifloxacin analysis, this study investigated the optimal use of isopropanol alongside buffer to achieve accurate and reproducible Gatifloxacin determination.

**3.1. Mobile phase composition**

The optimal mobile phase composition, determined experimentally, consisted of 65% phosphate buffer and 35% isopropanol (Table 1). This composition effectively balanced rapid analyte elution with acceptable peak shapes.

The buffer pH was carefully adjusted to 3 to achieve symmetrical peak shapes and minimize retention times, thereby improving the overall chromatographic performance.

**3.2. System suitability**

System suitability parameters were calculated to ensure that, the applied

chromatographic system is acceptable under optimal conditions

**Table 1: selection of mobile phase composition**

| Parameters | | | $t_R$ (min) | T | NTP |
|---|---|---|---|---|---|
| | Isopropanol% | Buffer % | | | |
| Mobile phase composition | 10 | 90 | 12.600 | 1.50 | 3600 |
| | 20 | 80 | 3.870 | - unknown peaks | 2100 |
| | **35** | **65** | **3.195** | **1.30** | **2500** |
| | 40 | 60 | 2.580 | overlapped | 859 |

**Table 2: System suitability**

| | |
|---|---|
| **Retention time ($t_R$)** | 3.195 |
| **Tailing factor (T)** | 1.30 |
| **Capacity factor (K)*** | 2.04 |
| **Number of theoretical plates (NTP)** | 2500 |

*** Dead time($t_m$) =1.05**

## 3.3 method validation

### 3.3.1. Linearity, LOD, LOQ

The calibration curve demonstrated an excellent linear relationship within the range of 10–120 mg/L. Triplicate measurements were performed for each concentration, and the average peak areas were plotted against the corresponding concentrations. The correlation coefficient was determined to be 1, as illustrated in Figure 3. Using the previously defined equations, the limits of detection (LOD) and quantification (LOQ) were calculated to be 0.026 mg/L and 0.08 mg/L, respectively

### 3.3.2. Accuracy and precision

To evaluate accuracy and precision, experiments were conducted at three different concentrations. The inter- and intra-day precision were assessed, yielding a relative standard deviation (RSD%) of less than 2. The detailed results are presented in Tables 3 and 4.

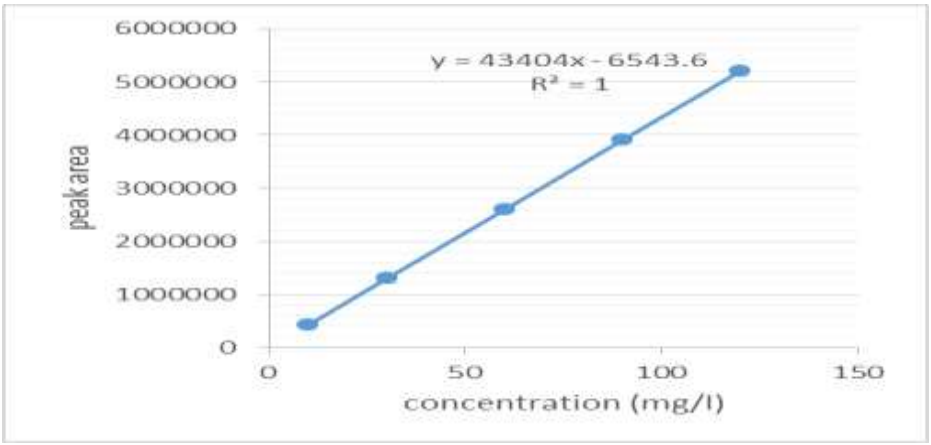


**Figure 3: Linearity of the developed method**

**Table 3: Accuracy of the developed method**

| Concentration (mg/l) | Found (mg/l)* | Recovery % |
|---|---|---|
| 20 | 20.45 | 102.25 |
| 60 | 59.62 | 99.36 |
| 120 | 120.66 | 100.55 |
| | | $Mean \mp SD = 100.72 \mp 1.45$ |

***Average of three separate determination**

**Table 4: Precision of the developed method**

| Concentration (mg/l) | intra − day* | | inter − day* | |
|---|---|---|---|---|
| | Recovery ± S.D* | Precision (RSD %) | Recovery%± S.D* | Precision (RSD %) |
| 20 | 100.55± 0.70 | 0.69 | 100.73±0.85 | 0.84 |
| 60 | 99.78± 0.23 | 0.23 | 101.02±0.26 | 0.25 |
| 120 | 100.56±0.92 | 0.91 | 99.33±0.77 | 0.78 |

***Average of three separate determination**

### 3.3.3. Robustness

To assess the robustness of the developed method, small variations in column temperature were introduced. These changes had an insignificant effect on both peak area and recovery, as demonstrated in Table 5.

**Table 5: Robustness of the developed method**

| $(c)^0$ | 38 | 40 | 42 | Average% + SD | RSD % |
|---|---|---|---|---|---|
| recovery% of GTX | 99.64 | 100.33 | 99.20 | 99.72% ± 0.56 | 0.57 |

### 3.3.3. Selectivity

The method demonstrated excellent selectivity, as it was successfully applied in the presence of excipients without any interference with the drug peak, as shown in the chromatogram in Figure 4.

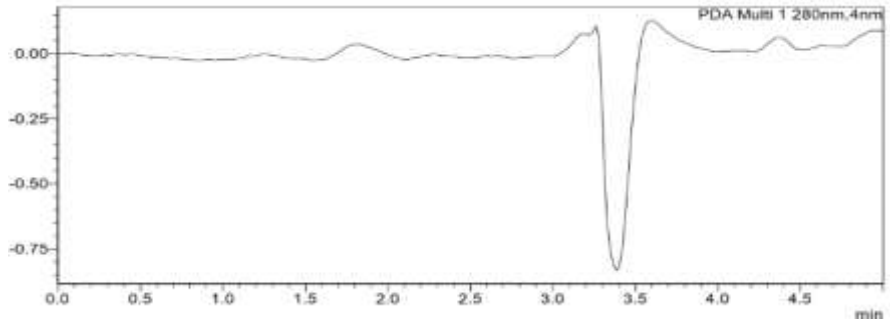


**Figure 4: Selectivity of the developed method**

### 3.4. Determination of Gatifloxacin in ophthalmic formulations

The results of determination of GTX in marketed eye drops (0.3%) are summarized in table 6. The mean recovery was found to be 100.74%, based on five replicate measurements. Figure 5 presents the chromatogram of GTX in the marketed ophthalmic solution.

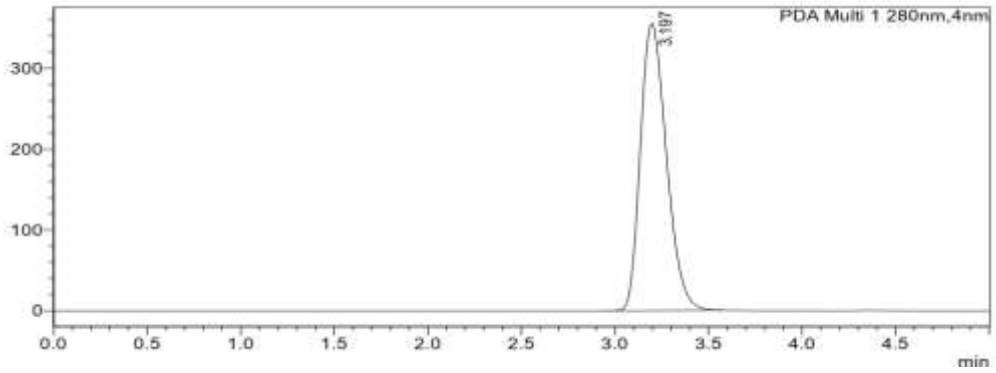


**Figure 5: Chromatogram of GTX in marketed eye drops**

**Table 6: Determination of GTX in eye drops**

| Number of experiment | Recovery % | Average recovery % ± SD |
|---|---|---|
| **1** | 100.52 | 100.74 ± 0.65 |
| **2** | 101.15 | |
| **3** | 99.98 | |
| **4** | 100.42 | |
| **5** | 101.64 | |

## 4.Greenness assessment using analytical Eco-Scale tool

The Analytical Eco-Scale is based on the principle that an ideal green analysis has a value of 100. For each parameter of the analytical procedure—such as reagent amounts, hazards, energy consumption, and waste generation—penalty points are assigned. The total penalty points for the procedure are used to calculate the Eco-Scale score using the following formula:

Analytical Eco-Scale = 100 - Total Penalty Points

The Eco-Scale score reflects the greenness of the method as follows:

75 indicates excellent green analysis.
50 indicates acceptable green analysis.
50> indicates inadequate green analysis [15].

As shown in Table 7, the developed method achieves an excellent level of greenness, reflecting its environmentally friendly attributes.

**Table 7: Eco-Scale score of the developed method**

| Reagents | Penalty points (PPs) |
|---|---|
| Amount | 1 |
| Hazardous | 2 |
| **1×2× 2=4** | |
| **Instrumentation** | |
| Energy | 0 |
| Occupational Hazard | 3 |
| Waste (amount) | 3 |
| Waste (management) | 3 |
| **9** | |
| **100 - (4+9) = 87** | |

## 5.Conclusion:

Developing new analytical methods is a complex task, requiring the optimization of numerous parameters and conditions. In light of growing concerns about the future of our planet and its resources, the need for green analytical methods has become increasingly important. This study successfully developed and validated a sensitive, fast, precise, and environmentally friendly HPLC method for the determination of Gatifloxacin in bulk and ophthalmic solutions.

The main objective was to replace harmful solvents, such as acetonitrile, with greener alternatives in the mobile phase and to incorporate a relatively high percentage of the aqueous component to minimize waste. The greenness of the method was assessed using the Analytical Eco-Scale tool, which demonstrated a high score. Based on these findings, the method is environmentally friendly and suitable for application in quality control laboratories.

## 6. Acknowledgment :

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## References:


1. Mostafa M M, Abd El-Wahab Z H , Salman A A , Abdelbaset W M. The use of complex formation manner for spectrophotometric analysis of Gatifloxacin drug based on Co(II), Ni(II) and La(III) ions . *Heliyon*,7 (2021),e06051.
2. Indian Pharmacopoeia, vol 2, 2018.
3. Saad M N, Essam H M, Elzanfaly E S, and M. Amer S M.A Two-Step Optimization Approach: Validated RP-HPLC Method for Determination of Gatifloxacin and Dexamethasone in Ophthalmic Formulation .*Journal of Chromatographic Science*, (58) 6, 2020,504–510.
4. Venugopal K, Snehalatha M, Bende G, and Saha R. Development and Validation of an Ion-Pairing RP-HPLC Method for the Estimation of Gatifloxacin in Bulk and Formulations. *Journal of Chromatographic Science*, Vol. 45, April 2007.
5. Bera A K, Kumar De A, and Pal B .Simple isocratic RP-HPLC method development and validation for estimation of gatifloxacin in tablet dosage form. *International Journal of Pharmaceutical Sciences and Research.*5(9), 2014, 3741-3745.
6. Aljuffali I A , Abul Kalam M, Sultana Y, Imran A , Alshamsan A. Development and validation of stability-indicating high performance liquid chromatography method to analyze gatifloxacin in bulk drug and pharmaceutical preparations. *Saudi Pharmaceutical Journal* ,23, 2015, 85–94.
7. Al-Dgither S, Naseeruddin Alvi S, M. Hammami M M. Development and validation of an HPLC method for the determination of gatifloxacin stability in human plasma. *Journal of Pharmaceutical and Biomedical Analysis*, 41, 2006, 251–255.
8. Amin A S, Abou El-Fetouh Gouda A , R El Sheikh , Faten Zahran F. Spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical formulation. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, (67),5, 2007, 1306-1312.
9. K. Venugopal, Ranendra N. Saha. New, simple and validated UV-spectrophotometric methods for the estimation of gatifloxacin in bulk and formulations. *Il Farmaco*, (60), 11–12, November–December 2005, 906-912.
10. Jasmin Shah F, Rasul Jan M, Ullah I, Shah S. Sensitive Spectrofluorimetric Method for Determination of Fluoroquinolones through Charge-Transfer Complex. American Journal of Analytical Chemistry ,(4),10, October 2013.
11. Alzeer S. Extraction of caffeine and its metabolite paraxanthine from urine and measuring both of them by high-performance liquid chromatography. *Damascus University Journal for Medical Sciences*, 39(3), 2023. (in Arabic)
12. Ghazal L, Almardini M A, Allous I. Development and validation of HPLC method for the assay of Etoricoxib in pharmaceutical dosage form. *Damascus University Journal for Medical Sciences*, 39(2),2023. (in Arabic)
13. Al Shatti B J, Alsairafi Z, Al Tannak NF. Green chemistry and its implementation in pharmaceutical analysis. *Reviews in Analytical Chemistry*, 42(1), 2023, 20230069.
14. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2(R1), 2005,1-13.
15. Gałuszka A, Konieczka P, Migaszewski Z M, Namies´nik J. Analytical Eco-Scale for assessing the greenness of analytical procedures. *Trends in Analytical Chemistry*, vol. 37, 2012.


**Statements and Declarations**

**Competing Interests** :

- The corresponding author states that there is no conflict of interest.