Ind. J. Pharm. Edu. Res., 2023; 57(4):1226-1231.
https://www.ijper.org

Original Article

Interaction Studies of Moxifloxacin with Anti-diabetic Drugs and Application of RP-HPLC in Analysis

Sania Bashir[1], Fatima Qamar[1,*], Safila Naveed[1], Syeda Zainab[2], Halima Sadia[1], Aisha Sana[1], Wajeeha Muzafar[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jinnah University for Women, Karachi, PAKISTAN.
[2]Department of Pharmaceutics, Jinnah University for Women, Karachi, PAKISTAN.
[3]Department of Chemistry, H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, PAKISTAN.

Correspondence:
Dr. Fatima Qamar
Faculty of Pharmacy, Jinnah University for Women, Karachi, PAKISTAN.
Email: fatimamudassar2009@hotmail.com

Received: 25-01-2023;
Revised: 03-04-2023;
Accepted: 14-08-2023.

ABSTRACT

Aim: Simple RP-HPLC method was developed and validated for the simultaneous estimation of Glimepiride, Glibenclamide and Moxifloxacin in pharmaceutical preparation. **Materials and Methods:** Mediterranean C_{18} column with dimensions of 150mm×4.6mm was used with HPLC Shimadzu_ LC-20 AT model, Version 1:62 is used for processing of chromatograms at room temperature (25± 2°C). 1 mL per minute was the flow rate and detector were set at 254nm with mobile Phase consisting of 85:10:5 v/v methanol: water: acetonitrile. **Results:** The developed method proved to be accurate, simple, effective precise and reproducible. One way ANOVA was also applied for further confirmation of the results. **Conclusion:** The method is applicable for the routine analysis of drugs in API and in formulation and for studying in vitro interaction studies.

Keywords: Moxifloxacin, Glimepiride, Glibenclamide, *in vitro* interaction studies.

INTRODUCTION

Moxifloxacin (MF) is a broad-spectrum anti-microbial agent chemically known as 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aS,7aS)-octahydro-6H-pyrrolo [3,4-b] pyridin-6-yl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid that belongs to a fluoroquinolone class.[1] It has great coverage against gram positive, gram negative bacteria, atypical and anaerobes.[2]

Glimepiride (GMP), third generation antidiabetic drug, belongs to the sulfonylurea class and is employed in the treatment of type II, non-dependent diabetes. This drug exerts its antidiabetic effects by elevating the insulin level through stimulation of pancreatic beta cells[3] and it also improves intracellular insulin receptor activity.[4] Glibenclamide (GLB) from diarylsulfonylurea class is an antidiabetic drug and is also used in the treatment of type 2 diabetes mellitus.[5] Simultaneous Determination of Cefepime and the Quinolones including Moxifloxacin in human urine has been reported by HPLC-UV,[6] Simultaneous Quantification of Linezolid, Levofloxacin, Rifampicin, and Moxifloxacin in Human Plasma has been reported using High-Performance Liquid Chromatography with UV detection.[7] Simultaneous estimated of pioglitazone hydrochloride, metformin hydrochloride, and glimepiride by RP-HPLC in tablet formulation has been estimated[8] for glimepiride. Venkatesh *et al.*, elaborated simultaneous estimation of glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone development using novel HPLC method,[9] Patil Sudarshan *et al.*, 2009[10] and Ranetti MC *et al.*, 2009,[11] quantified Glibenclamide using UV and HPLC methods.

One of the major problems associated with quinolone is dysglycemia, either hypoglycemia or hyperglycemia. Dysglycemia has been reported with the use of quinolones. However, moxifloxacin is relatively new member with many improved qualities. Literature survey revealed that all four generations of quinolones effect glucose homeostasis i.e., dysglycemia either hypoglycemia or hyperglycemia. In this perspective, severe association was found between fluoroquinolone and anti-diabetic drugs. The risk of dysglycemia increases with concomitant use of oral hypoglycemic agents and oral fluoroquinolones.[12] It is also reported that changes in pharmacokinetics of moxifloxacin occur when it is taken with high calorie or breakfast with high fat contents. Interaction of moxifloxacin is also reported with sucralfate and iron supplement and was evaluated for dysglycemia mostly by clinical data.

The aim of the present study is to develop and validate an HPLC method for the simultaneous identification and quantification of the drugs that could be utilized in routine for analysis and to apply the method to study for their interaction.

DOI: 10.5530/ijper.57.4.146

MATERIALS AND METHODS

Instrumentation and analytical conditions

UV-vis spectrophotometer model 1601, shimadzu (Japan) with shimadzu UV probeversion 4.2 software attached to core 13 computer containing path length of 10mm with an advanced LC 20AT shimadzu HPLC system attached to UV-vis detector (SPD-10A(v)), equipped with CBM102 communication BUS module Shimadzu with core 13 was employed for the present investigation.

Column used for the HPLC system was mediterranea C_{18} with dimensions of 150mm×4.6mm having internal diameter of 5µm. In addition, weighing balance, GAST, pump for mobile phase filtration, Ultrasonic Sonicator (BRANSON 5210), pH meter (C9330), Class A volumetric flasks (Germany) were used. Flow rate was set at 1 mL/min with wavelength 254nm. Column temperature was maintained at room temperature.

Materials and Reagents

Moxifloxacin (brand name Avelox strength: 400 mg), Glibenclamide (brand name: Euglucon strength: 5 mg) and Glimepiride (brand name: Getryl Strength: 4 mg) were purchased from local pharmacy store. HPLC grade methanol was used. All the chemicals are of high purity grade.

Stock and working solutions

Stock solution having strength of 400 µg $mL^{-1}$of Moxifloxacin (MX), Glibenclamide (GLB) and Glimepiride (GLP) were prepared individually. 40mg of moxifloxacin is dissolved in 100 mL volumetric flask and 40mg of glibenclamide and glimepiride were dissolved in 100 mL volumetric flask individually using mobile phase as a diluent. Obtained solutions were further diluted up to 25 – 400 µg/mL^{-1}. All the performance was carried out at room temperature i.e., 25°C.

Sample preparation

For the preparation of sample solution, 20 tablets of all three drugs were individually powdered (60 mesh) and amount equivalent to 40 mg was taken. Calculated amount was transferred in 100 mL volumetric flask and volume was made up with the mobile phase up to desired concentration. Final solution was then filtered through whatmann filter paper having pore size of 0.45 µm and then analyzed for the drug content.

Validation procedure

The ICH guidelines were followed for each step of the validation process.[13] Technique validation proves that the performance characteristics of the method are appropriate for the intended usage. System appropriateness, selectivity, specificity, linearity (concentration-detector response relationship), accuracy, precision, sensitivity, detection, and quantification limit recovery from the matrix were only a few of the various criteria that were evaluated.[14] The system suitability was assessed by five replicate analyses of the drug. By examining the repeatability, peak symmetry (symmetry factor), theoretical plates of the column, resolution between the peaks of statins and moxifloxacin, mass distribution ratio (capacity factor), and relative retention, the system applicability of the approach was assessed. Specificity is a method's capacity to distinguish between the target analyte and other elements present in the sample.[15] The recovery assay performed on the formulation sample data was used to evaluate the method's accuracy. As a result, known dosages of each drug were made in triplicate and spiked into the appropriate formulation at three levels (80%, 100%, and 120%). The average recovery was then determined as the mean value achieved. Two distinct, non-consecutive days were used for analysis in order to test the method's accuracy. In accordance with the ICH recommendations, the LOD and LOQ were determined. The robustness of this procedure was assessed using two separate instruments in two different labs. Lab 1 was in the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy Jinnah university for women, while Lab 2 was in the Department of Pharmacy, Jinnah University for Women, Karachi.

Interaction studies

Direct method used for the study of MX interaction with GLP and GLM. Stock solutions of 400µg/mL was prepared of each drug in methanol separately. Then MX was mixed separately with GLP and GLB in 1:1 manner so that final concentration of each solution was 200µg/mL.

It was kept in water bath maintained at 37°C for 3 hr. Aliquots of the sample were drawn periodically and injected three times in the system after filtration through 0.45 µm filter paper. The % recovery of each drug was calculated and one Way ANOVA was applied by using SPSS.

RESULTS

Method validation

The proposed method was validated according to the guidelines given by ICH (International Conference on the Harmonization) and was validated for the for all the parameters like system suitability, specificity, linearity, selectivity, Trueness, linearity, precision, limit of detection and quantification (Figure 1).

System suitability

System suitability parameters are summarized in Table 1; all the values were within the limits.

Selectivity and specificity

The selectivity and specificity of the method shows good resolution factor as the peaks of moxifloxacin, glimepiride and

Table 1: System suitability for Moxifloxacin, Glimepiride and Glibenclamide.

Parameters	Moxifloxacin	Glimepiride	Glibenclamide
Ret. Time	2.10	3.29	4.30
Capacity factor	1.10	1.06	1.40
Theoretical Plates	2869.00	2367.00	2400.00
Tailing factor	1.94	1.29	1.58
Resolution	1.65	2.82	2.33

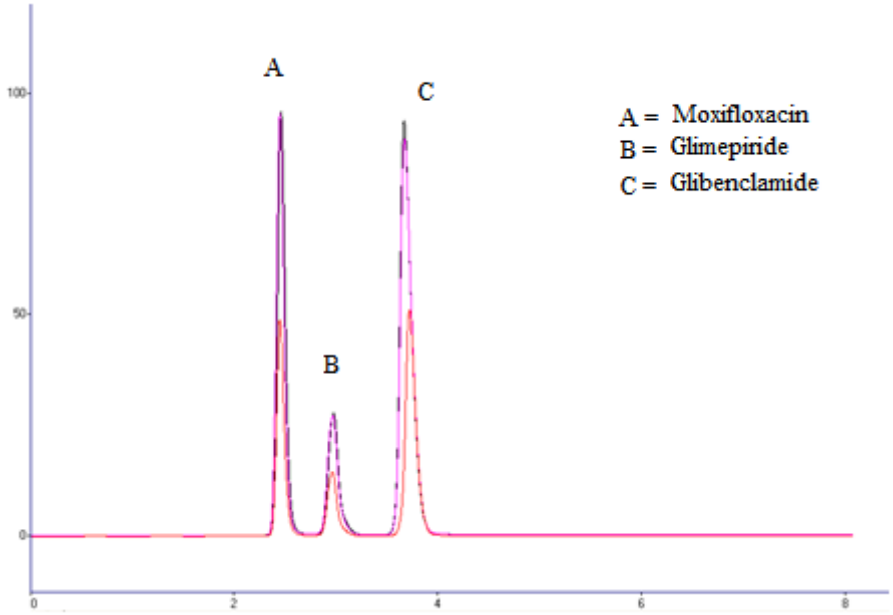

Figure 1: Accuracy of Moxifloxacin, Glimepiride and Glibenclamide.

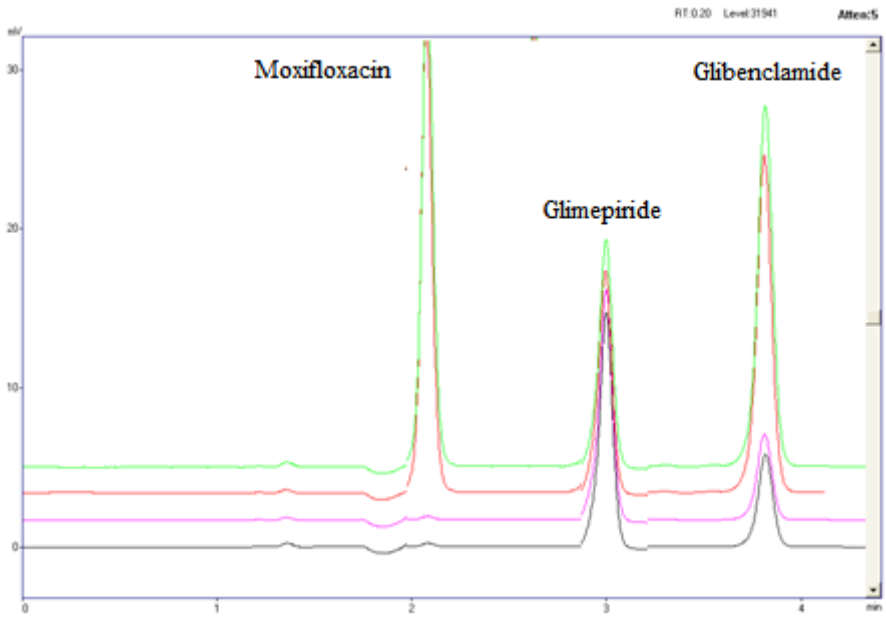

Figure 2: Linearity of Moxifloxacin, Glimepiride and Glibenclamide.

Table 2: Trueness of Moxifloxacin, Glimepiride and Glibenclamide.

Drugs	Conc. *µg/mL	RSD (%)	Recovery (%)
Glimepiride	80%	0.01	99.90
	100%	0.00	100.80
	120%	0.01	100.80
Moxifloxacin	80%	0.03	100.20
	100%	0.02	100.00
	120%	0.06	99.90
Glibenclamide	80%	0.01	99.90
	100%	0.01	100.00
	120%	0.01	100.80

*Conc. = concentration

Table 3: Interday and Intraday Precision.

Drugs	Conc. Injected µg/ml	Inter day		Intra day	
		RSD (%)	Recovery (%)	RSD (%)	Recovery (%)
Moxifloxacin	450.00	0.0002	99.67	0.0004	98.90
	225.00	0.0004	99.80	0.0007	99.05
	112.50	0.0001	100.01	0.0004	100.54
	56.25	0.0004	100.32	0.0009	100.53
	28.12	0.0007	99.80	0.0004	100.01
Glimepiride	450.00	0.0002	100.20	0.0002	101.34
	225.00	0.0001	100.02	0.0004	100.23
	112.50	0.0003	100.58	0.0003	100.52
	56.25	0.0003	99.80	0.0002	99.96
	28.12	0.0004	100.05	0.0003	100.05
Glibenclamide	400.00	0.0012	100.43	0.0012	99.99
	200.00	0.0012	99.99	0.0001	100.45
	100.00	0.0007	100.01	0.0007	99.99
	50.00	0.0004	99.99	0.0004	100.01
	25.00	0.0002	100.53	0.0002	99.99

*Conc- concentration

Table 4: Regression characteristics of Moxifloxacin, Glimepiride and Glibenclamide.

Drugs	R^2	Regression Equation	LOD (ng/mL)	LOQ (ng/mL)
Moxifloxacin	0.99	Y = 10347x + 30977	4.33	5.31
Glibenclamide	0.99	Y = 6528x + 63472	2.30	6.98
Glimepiride	0.99	Y = 6928x + 65672	2.22	5.65

glibenclamide separated well from the excipients. The proposed method shows good resolution.

Linearity

Moxifloxacin, Glimepiride and Glibenclamide showed good linear relationship (Figure 2) and having regression equation Y = 10347x + 30977, Y = 6528x + 63472 and Y = 6928x + 65672 of all three drugs simultaneously.

Trueness

The Trueness of the proposed method was validated and the results obtained from the experiments are shown in Table 2.

Table 5: *In vitro* Interaction studies.

Time	% Recovery				% Recovery			
min	MOX	GLP	F-value	p-value	MOX	GLP	F-value	p-value
0	79.96	88.65	3395618.00	0	85.78	99.75	6741003.00	0
30	104.02	82.89	40233649.00		101.20	101.76	1009.38	
60	105.07	76.10	75516100.00		104.65	103.87	2784.80	
90	107.14	76.50	16887544.00		104.87	99.75	44782.23	
120	110.02	75.65	53158361.00		105.98	101.30	22763.30	
150	111.67	72.08	70555321.00		107.31	100.00	53144.10	
180	112.06	67.62	88871112.00		107.88	102.84	25247.20	

Percentage recovery of MX, GLB and GLP was in the range of 100.03%, 100.08%and 100.08% respectively.

Precision

For our method to be precise that is producing reproducible results, five different concentrations of both the drugs were individually injected into system and tested for repeatability and reproducibility. The results obtained were analyzed for inter and intraday variations as shown in Table 3 and it was found that there was no significant difference among the values when tested within a day and between the days as shown in Table 3.

Robustness

Slight deliberate changes were done to check the robustness of the method in some parameter like pH variation, mobile phase ratio and in total volume variation.

LOD and LOQ

Limit of detection and limit of quantification, results are shown in Table 4.

DISCUSSION

In vitro interaction studies

The interaction method had been used to study the interactions of moxifloxacin with anti-diabetic agents (Glimepiride and Glibenclamide). The percent recovery was calculated by AUC (area under the curve) of moxifloxacin with interacting drugs (Glimepiride and Glibenclamide). It was observed that glimepiride was available up to 59.5-115.7% at 37°C. One way ANOVA was applied to evaluate the interaction results. Individual test verified that significant interaction was observed as shown in Table 4. These results indicated that MX and antidiabetic GLP and GLB may affect the efficacy of each other. One way ANOVA test was applied to study the *in vitro* interaction studies and level of significance was evaluated using p test values. ANOVA p value was observed to be less than 0.05 in case of MX and GLP. From the p value it was evident that there is a significant difference between the percent availability of both drugs thereby, indicating that these drugs could possibly effect the efficacy of each other. Likewise, in case of MX and GLB, p value was also found to be less than 0.05 for MX and GLB. Hence forth, it was apparent that there was significant difference between the percent availability of these drugs. On the basis of the results depicted in Table 5, it can be concluded that MX when comes in combination with GLP and GLB may vary their availabilities. Furthermore, it has also been reported also that raised level of glibenclamide was observed in presence of moxifloxacin at different pH levels using UV spectrophotometer.[12] In another study it was reported that moxifloxacin lowered blood glucose level in non-diabetic and diabetic animals and has effect on the hypoglycemic action of oral anti-diabetics in the alloxan diabetic rabbits.[16-20]

CONCLUSION

The developed method showed accurate, precise, results for the simultaneous determination of Moxifloxacin, Glimepiride and Glibenclamide in bulk and pharmaceutical preparation. Advantage of HPLC method is that it analyzed drugs in combination in short time, less retention time, less complex composition of mobile phase with flow rate of 1 mL per min. Therefore, this method is applicable for the routine analysis of drugs in API and in formulation and for studying *in vitro* interaction studies. *In vitro* interaction studies and results analyzed via SPSS employing One Way ANOVA test that illustrates our results are significant $p< 0.005$ i.e., there is difference in percent availability between both drugs (MX and GLP, MX and GLB). Hence, they may affect the availability of each other. However further *in vivo* interaction studies required for confirmation of these interactions.

ACKNOWLEDGEMENT

We would like to thank the Management of University for support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of Variance; **RP-HPLC:** Reverse phase high pressure liquid chromatography; **HPLC-UV:** High pressure liquid chromatography-Ultraviolet spectroscopy; **MF:** Moxifloxacin; **GMP:** Glimepiride; **GLB:** Glibenclamide; **ICH:** International Conference on the Harmonization; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **RSD:** Relative standard deviation; **API:** Active Pharmaceutical Ingredien; **SPSS:** Statistical Package for the Social Sciences; **µg/mL:** Microgram per milliliter**; ng/mL:** Nanogram per milliliter.

SUMMARY

- The aim of the present study is to develop and validate an HPLC method for the simultaneous identification and quantification of the drugs: Moxifloxacin, Glimepiride and Glibenclamide.
- Advantage of HPLC method is that it analyzed drugs in combination in short time and less complex composition of mobile phase.
- *In vitro* interaction studies and results analyzed via SPSS employing One Way ANOVA test.

REFERENCES

1. Ahmad I, Bano R, Musharraf SG, Ahmed S, Sheraz MA, ul Arfeen Q, *et al.* Photodegradation of moxifloxacin in aqueous and organic solvents: a kinetic study. AAPS PharmSciTech. 2014;15(6):1588-97. doi: 10.1208/s12249-014-0184-x, PMID 25139764.
2. Sweetman SC. Dose adjustment in renal impairment: response from Martindale: the Complete Drug Reference. BMJ. 2005;331(7511):292-3. doi: 10.1136/bmj.331.7511.292-a, PMID 16052024.
3. Appelbaum PC, Hunter PA. The fluoroquinolone antibacterials: past, present and future perspectives. Int J Antimicrob Agents. 2000;16(1):5-15. doi: 10.1016/s0924-8579(00)00192-8, PMID 11185413.
4. Kang F, Singh J. *In vitro* release of insulin and biocompatibility of *in situ* forming gel systems. Int J Pharm. 2005;304(1-2):83-90. doi: 10.1016/j.ijpharm.2005.07.024, PMID 16181752.
5. Bähr M, Von Holtey M, Müller G, Eckel J. Direct stimulation of myocardial glucose transport and Glucose Transporter-1 (GLUT1) and GLUT4 protein expression by the sulfonylurea glimepiride. Endocrinology. 1995;136(6):2547-53. doi: 10.1210/endo.136.6.7750476, PMID 7750476.
6. Eurich DT, Simpson SH, Majumdar SR, Johnson JA. Secondary Failure Rates Associated with Metformin and Sulfonylurea Therapy for Type 2 Diabetes Mellitus. Pharmacotherapy. 2005;25(6):810-6. doi: 10.1592/phco.2005.25.6.810.
7. Ocaña González JA, Callejón Mochón M, Barragán de la Rosa FJ. Simultaneous Determination of Cefepime and the Quinolones Garenoxacin, Moxifloxacin and Levofloxacin in Human Urine by HPLC-UV. Microchim Acta. 2005;151(1-2):39-45. doi: 10.1007/s00604-005-0391-y.
8. Baietto L, D'Avolio A, De Rosa FG, Garazzino S, Patanella S, Siccardi M, *et al.* Simultaneous quantification of linezolid, rifampicin, levofloxacin, and moxifloxacin in human plasma using high-performance liquid chromatography with UV. Ther Drug Monit. 2009;31(1):104-9. doi: 10.1097/FTD.0b013e31819476fa, PMID 19077929.
9. Liang H, Kays MB, Sowinski KM. Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma. J Chromatogr B. 2002;772(1):53-63. doi: 10.1016/S1570-0232(02)00046-6.
10. Laban-Djurdjević A, Jelikić-Stankov M, Djurdjević P. Optimization and validation of the direct HPLC method for the determination of moxifloxacin in plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;844(1):104-11. doi: 10.1016/j.jchromb.2006.07.001, PMID 16890030.
11. Jain D, Jain S, Jain D, Amin M. Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulation. J Chromatogr Sci. 2008;46(6):501-4. doi: 10.1093/chromsci/46.6.501, PMID 18647470.
12. Lakshmi K, Rajesh T, Sharma S, Lakshmi S. dLad, N. Pharm Chem. 2009;1(1):238-46. Bhoir S, Bhoir I, Sundaresan M. Indian J Pharm Sci. 2003;65(6):650.
13. Patil Sudarshan S, Bonde C. Int J ChemTech Res. 2009;1(4):905-9.
14. Ranetti M-C, Ionescu M, Hinescu L, Ionica E, Anuta V, Ranetti AE, *et al*. Farmacia. 2009;57(6):728-35.
15. Akhtar F, Gul S, Ashfaq S, Rehman I, Mirza AZ. UV Spectroscopic Method for Optimization and Determination of Glibenclamide in Bulk, Pharmaceutical Dosage Form and its Application for *in vitro* Interaction Studies. J Anal Test. 2020;4(4):281-90. doi: 10.1007/s41664-020-00146-9.
16. Venkatesh P, Harisudhan T, Choudhury H, Mullangi R, Srinivas NR. Simultaneous estimation of six anti-diabetic drugs–glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay. Biomed Chromatogr. 2006;20(10):1043-8. doi: 10.1002/bmc.635, PMID 16506282.
17. Rostom AH, Al-Sultan AI. Hypoglycemic Effect of Some Fluoroquinolones on Normal and Diabetic Experimental Animals. Egypt J Hosp Med. 2007;28(1):404-17. doi: 10.21608/ejhm.2007.17670.
18. Guideline, I. Fed Regist. 1997;62.
19. Mehta AC. Quality Management in Drug Analysis. Analyst. 1997;122(7):83R-8R. doi: 10.1039/a700563f.
20. Hernandez EC, University of Kentucky: 1997.

Cite this article: Bashir S, Qamar F, Naveed S, Sana A, Sadia H, Zainab S, et al. Interaction Studies of Moxifloxacin with Antidiabetic Drugs and Application of RP-HPLC in Analysis. Indian J of Pharmaceutical Education and Research. 2023;57(4):1226-31.