Annals of Pharmacy Practice and Pharmacotherapy
ISSN: 3062-4436
2021, Volume 1, Page No: 31-38

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Development of an RP-HPLC Method for Dapagliflozin and Metformin HCL Analysis

Khagga Bhavyasri[1]*, Thaneeru Surekha[1], Samreen Begum[1], Mogili Sumakanth[1]

[1]Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Affiliated to Osmania university, Barkatpura, Hyderabad, Telangana-500027, India.

***E-mail** ✉ bhavya.khagga@gmail.com

Received: 14 September 2021; **Revised:** 17 November 2021; **Accepted:** 17 November 2021

ABSTRACT

The present study aimed to investigate the RP-HPLC method for the determination of dapagliflozin and metformin HCl in bulk and combined formulation. For this purpose, the provided RP-HPLC approach was found to be straightforward, precise, specific, and cost-effective. The phase of stationary Chromatographic conditions was Phenomenex C18 250 x 4.6 mm. A 50:50 water: methanol ratio was employed as the fluid phase. The wavelength of observation was 230 nm, the diluent was water, and the flow rate was kept at 51.0 ml/min at a column temperature of 300 °C. Finally, the conditions were completed as an optimal process. Appropriate system variables were investigated by applying the standard six times, and the results were significantly below the approval standards: the linearity of metformin HCl was found to be 2-7 ppm, while the linearity of dapagliflozin was discovered to be 60-210 ppm with r^2 = 0.999; the precision values for them were 0.8214% and 0.6342%, respectively. The results showed that the LOD and LOQ values for metformin HCl were 263000 and 324000 ppb, respectively, while for dapagliflozin they were 345000 ppb and 415000 ppb. Using the foregoing procedure, the formulated market was assessed, and the percentages of metformin HCl and dapagliflozin were determined to be 99.85% and 99.73%, respectively.

Keywords: Dapagliflozin, Metformin, Liquid chromatography, Combined dosage forms, Simultaneous estimation

How to Cite This Article: Bhavyasri K, Surekha T, Begum S, Sumakanth M. Development of an RP-HPLC Method for Dapagliflozin and Metformin HCL Analysis. Ann Pharm Pract Pharmacother. 2021;1:31-8. https://doi.org/10.51847/4aAGsEQxu5

Introduction

Antidiabetic medications are pharmaceuticals that treat diabetes in a variety of ways. Each of these drugs reduces blood sugar to a safe level (normoglycemia) and alleviates the symptoms of diabetes, such as thirst, increased urination, and ketoacidosis. Additionally, antidiabetic drugs prevent or decrease the development of long-term effects of diabetes, including retinopathy (damage to the retina of the eye), neuropathy (injury to the nerves), and nephropathy (kidney disease). A disease known as type 1 diabetes results in the body creating insulin. Thus, for the efficient cure of type 1 diabetes, insulin is the sole drug. Because it functions similarly to natural insulin, injecting insulin lowers blood glucose levels. The condition known as insulin resistance occurs when the body's cells do not react to insulin as they would in those without diabetes [1-5].

Because SGLT-2 inhibitors stop glucose from being reabsorbed in the renal tubules, glucose disappears in the urine, resulting in glycosuria, which causes a small decrease in body weight and a reduction in blood sugar levels, increasing the risk of hypoglycemia. Oral preparations can be utilized either alone or in conjunction with other medications. In addition to GLP-1 agonists, they are recommended as a second or third medication for type 2 diabetics whose condition is managed with metformin hydrochloride [6-11]. Because they reduce the likelihood of hospitalization, they are regarded as first-line therapy for diabetic patients with cardiovascular disease, especially heart failure. Examples include dapagliflozin, empagliflozin, and canagliflozin.

In peripheral tissues like skeletal muscle, biguanides increase the uptake of glucose while decreasing the synthesis of glucose in the liver [12-15]. Although it should be taken cautiously in individuals with impaired liver or kidney function, metformin hydrochloride, a biguanide, is the most often prescribed drug for type 2 diabetes in adolescents and teens. Only metformin hydrochloride, which is frequently used for diabetes, did not result in weight increase [16-20].

Description of dapagliflozin

This medication helps individuals with type 2 diabetes manage their elevated blood sugar levels. Dapagliflozin helps avoid blindness, renal damage, and limb loss by controlling high blood sugar. Nerve issues and sexual function are also avoided. This drug is also used in patients with heart disease and type 2 diabetes to lower the danger of potentially failing hearts [21-26]. **Figure 1** depicts the dapagliflozin structure.

Structure

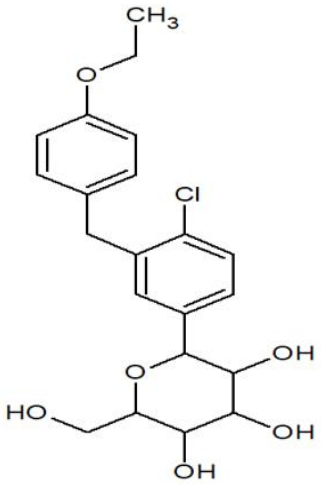

Figure 1. Dapagliflozin structure

Molecular weight: 408.873
Molecular formula: C21H25ClO6
Solubility: Methanol, water, acetonitrile [27-29]
The mechanism of action of dapagliflozin is the inhibition of sodium-glucose cotransporter 2 (SGLT2), which is mostly found in the proximal tubule of the blood vessel. Since 90% of glucose is reabsorbed by the kidneys with the aid of SGLT2, blocking it causes glucose to be excreted in the urine [30-32]. This excretion helps individuals with type 2 diabetes regulate their blood sugar levels better and may even help them lose weight.
pKa: 12.6
Applications: Regulating excessive blood sugar levels in patients with type 2 diabetes helps to minimize kidney damage and decreases the risk of heart attack or stroke.

Description of metformin hydrochloride

It is a medication used to treat type 2 diabetes. Metformin is a biguanide employed to cure hyperglycemia; it decreases the synthesis of glucose by the liver while enhancing the sensitivity of body tissues to insulin [33]. **Figure 2** shows the structure of metformin HCl.

Structure

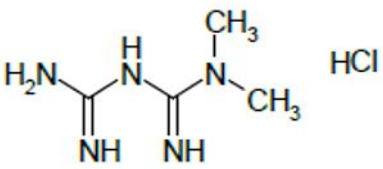

Figure 2. Metformin HCl structure

Molecular weight: 129.1639
Molecular formula: C4H11N5
Solubility: Water
Mechanism of action: Metformin decreases blood glucose levels by reducing hepatic glucose production, as well as lowering glucose absorption and utilization in the gut.
pKa: 12.4
Applications: It regulates blood sugar and guards against kidney damage, eyesight, and nerve issues.

Significance of combined dosage form
To enhance glycemic control in individuals with type 2 diabetes, a combination of medications is advised. These medications come in extended-release tablet format. Dapagliflozin lowers blood glucose levels by blocking the kidneys' ability to absorb glucose, while metformin hydrochloride lowers the absorption of sugar from the stomach and the ejection of sugar from the liver. Patients with type 2 diabetes and heart disease are prescribed this medication to lower their danger of heart failure [34]. Available brands are Xigduo and Oxramet.
The present study aimed to investigate the RP-HPLC method for the determination of dapagliflozin and metformin HCl in bulk and combined formulation. For this purpose, the provided RP-HPLC approach was found to be straightforward, precise, specific, and cost-effective.

Materials and Methods

Gland Pharma in Hyderabad, India, sent a complimentary sample of the pure medication dapagliflozin and metformin hydrochloride. The formulation of dapagliflozin and metformin HCl (OXRAMET) was acquired from a nearby pharmacy [11]. Methanol and water of HPLC quality. Hydrochloric acid (HCl), hydrogen peroxide (H2O2), and sodium hydroxide (NaOH) of analytical grade were used. **Figure 3** displays the commercial formulation of metformin HCl with dapagliflozin.
Diluent: Water was chosen based on drug solubility.

Figure 3. Formulations of dapagliflozin and metformin HCl

Dapagliflozin standard stock solution preparation
Weigh 10 mg of dapagliflozin accurately and transfer to 10 ml and volumetric flasks, then add 3/4th of the diluent and sonicate for 10 minutes. The flask was filled up to the mark with diluent and labeled as a standard stock solution (1000 g/ml of dapagliflozin).

Metformin standard stock solution preparation
Weigh 10 mg of dapagliflozin accurately and transfer to 10 ml and volumetric flasks, then add 3/4th of the diluent and sonicate for 10 minutes. The flask was filled up to the mark with diluent and labeled as a standard stock solution (1000 g/ml of metformin).

Standard working solution preparation (dapagliflozin)
Using a micropipette, 0.02 ml of dapagliflozin from the stock solution was extracted into a 10 ml volumetric flask and made up with a diluent (0.2 µg/ml of dapagliflozin).

Standard working solutions preparation (metformin)
Pipette out 0.1 ml of metformin from the stock solution into a 10 ml volumetric flask and fill with diluent (10 µg/ml of metformin).

Preparation of combined standard working solutions
1 ml of dapagliflozin 0.2 ppm and 1 ml of metformin HCl 10 ppm were pipetted into a 10 ml volumetric flask and three-quarters of diluent was added and sonicated for 10 minutes and made up to the mark with diluent to get a mixed standard solution containing 0.2 ppm of dapagliflozin and 10 ppm of metformin so that the drugs dapagliflozin and metformin were in the ratio equal to that of the marketed formulation.

Preparations of serial dilutions for calibration curve
For metformin HCl: Pipette 0.1 ml from the stock into a 100 ml volumetric flask, add the diluent, shake vigorously, and make up the solution with water to reach a concentration of 100 ppm. Pipette out 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 ml of the 100 ppm solution and transfer to separate 10 ml volumetric flasks, making up the final volume to 10 ml with diluent to obtain 2, 3, 4, 5, 6, and 7 ppm solutions respectively.
For Dapagliflozin: Pipette 0.6, 0.9, 1.0, 1.2, 1.5, 1.8, 1.8, and 2.1 ml from 1000 ppm solution into separate 10 ml volumetric flasks and dilute to 10 ml with diluent to give 60, 90, 120, 150, 180, and 210 ppm solutions, respectively.

Preparations of serial dilutions for accuracy
For metformin HCl: To the sample solution of 0.2 + 10 ppm of dapagliflozin and metformin HCl, 2 ppm of metformin standard is spiked at 50% accuracy level. 4 ppm of metformin standard is spiked at 100% accuracy level. 6 ppm of metformin standard is spiked at 150% accuracy level.
For Dapagliflozin: To the sample solution of 0.2 + 10 ppm of dapagliflozin and metformin HCl, 60 ppm of dapagliflozin standard is spiked at 50% accuracy level. 120 ppm of dapagliflozin standard is spiked at a 100% accuracy level. 180 ppm of dapagliflozin standard is spiked at a 150% accuracy level.

Results and Discussion

Validation of the method
The technique has been approved based on the following criteria: system specificity, accuracy, precision, limit of detection (LOD), quantitation, suitability, and linearity.

System suitability
Operational criteria were generated as per protocol from a dapagliflozin and metformin standard solution and injected five times into the HPLC apparatus. Standard chromatograms, which were obtained by calculating the % RSD of retention time, theoretical plates, tailing factor, and peak areas from five duplicate injections, were used to determine the system appropriateness factors. Both the relative standard deviation (% RSD) and the standard deviation were within the acceptable range. The Rt values for metformin HCL and dapagliflozin were 2.178 and 3.338, respectively.

Specificity
There were no peaks seen after injecting a blank.

Precision
Repeatability: After injecting six mixed working standard solutions of dapagliflozin 0.2 ppm and metformin 10 ppm, the percentage amount was calculated, and the results showed that the % RSD for dapagliflozin and metformin HCl were, respectively, 0.8214 and 0.6342.

Linearity
Six reference solutions containing 2 ppm to 7 ppm of metformin and 60 ppm to 210 ppm of dapagliflozin are injected to show the test method's linearity. **Figure 3** displays the graph for metformin HCl, which was created by plotting concentrations versus peak area. **Figure 3** displays the graph for metformin HCl, which was created by plotting concentrations versus peak area. At 25%, 50%, 75%, 100%, 125%, and 150%, the linearity for metformin HCl was 2.172, 2.174, 2.160, 2.170, 2.171, and 2.173, respectively. Linearity was determined to be 3.336, 3.338, 3.311, 3.330, 3.329, and 3.331 for dapagliflozin at 25%, 50%, 75%, 100%, 125%, and 150%, respectively.

Accuracy

Three injection concentrations of 50%, 100%, and 150% are administered in triplicate. The mean percentage recovery for metformin was 99.83%, 99.79%, and 99.75% for 50%, 100%, and 150% each, while the mean percentage recovery for dapagliflozin was 99.45%, 99.79%, and 99.84% for 50%, 100%, and 150% each.

Limit of detection (LOD)

Dapagliflozin and metformin's LODs were determined to be 345000 ppb and 263000 ppb, respectively, using this approach.

Limit of quantitation (LOQ)

The LOQs for metformin and dapagliflozin using this approach were 324000 ppb and 415000 ppb, respectively.

Robustness

%RSD OF DAPA and MET for Flow rate, -0.1 ml/min, were found to be 0.6214 and 0.7369, respectively, whereas %RSD OF DAPA and MET for Flow rate, +0.1 ml/min, were found to be 0.8624 and 0.5159. These two tiny, intentional adjustments to the procedure include flow rates of -0.1 ml and +0.1 ml.

Assay of marketed formulation

The apparatus was injected with the sample and reference solutions separately, making it easier to record chromatograms and determine the amount of drug contained in the sample. **Figure 4** displays the standard's assay chromatogram. **Figure 5** shows the sample's assay chromatogram. The percentage assay findings for metformin HCl and dapagliflozin are displayed in **Table 1**. **Table 2** shows the HPLC validation parameter summary.

Calculation

$$ASSAY = \frac{Sample\ peak\ area}{Standard\ peak\ area} \times \frac{Standard\ dilution\ factor}{Sample\ dilution\ factor} \times \frac{Average\ weight\ of\ tablets}{Label\ claim} \times Potency\ of\ standard \quad (1)$$

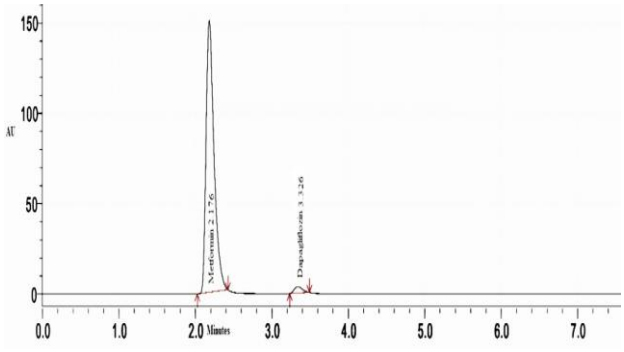

Figure 4. Assay chromatogram of standard

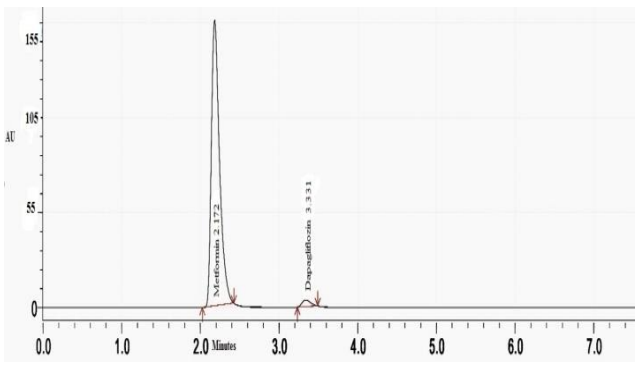

Figure 5. Assay chromatogram of sample

Table 1. Assay results

Name of the Drug	Label claim	Assay (%)
Dapagliflozin	10 mg	98.41%
Metformin	500 mg	99.58%

Table 2. Summary of validation parameters (HPLC)

Parameters	Dapagliflozin	Metformin
Calibration range (µg/ml)	60-210 ppm	2-7 ppm
Optimized wavelength	230 nm	230 nm
Retention time	2.178 min	3.338 min
Correlation coefficient (r^2)	0.999	0.999
Precision (%RSD)	0.8214	0.6342
Recovery (%)	99.79%	99.78 %
Limit of detection (ppb)	345000	263000
Limit of quantitation (ppb)	415000	324000

Conclusion

The provided RP-HPLC method was determined to be precise, cost-effective, and unambiguous for estimating the levels of metformin HCl and dapagliflozin in tablet and bulk dose forms. Phase Phenomenex C18, stationary, 250 x 4.6 mm, 5 m. Chromatographic conditions were that the mobile phase was made up of 50:50 water and methanol, the flow rate was kept at 1.0 ml/min, the detection wavelength was 230 nm, and the temperature was kept at 30 °C. Using water as a diluent, the conditions were finalized as part of an optimized procedure. System suitability parameters were examined by injecting the standard six times, and the results were significantly below the acceptance criteria. The linearity study for metformin HCl revealed that it was 2-7 ppm, and for dapagliflozin, it was 60-210 ppm, with $r^2 = 0.999$. The accuracy values for dapagliflozin and metformin HCl were determined to be 0.8214% and 0.6342%, respectively. Dapagliflozin has a LOD of 345000 ppb and a LOQ of 415000 ppb. Metformin has a LOD of 263000 and a LOQ of 324000 ppb. The aforementioned approach was used to assess the formulated market, and the percentages of dapagliflozin and metformin HCl were discovered to be 99.73% and 99.85%, respectively.

Acknowledgments: I want to acknowledge our Principal Professor. M. Sumakanth of RBVRR Women's College of Pharmacy for allowing me to perform research work.

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Dezani TM, Dezani AB, Serra CH. Development and validation of RP-HPLC method for simultaneous determination of lamivudine, stavudine, and zidovudine in perfusate samples: application to the single-pass intestinal perfusion (SPIP) studies. Braz J Pharm Sci. 2021;57:e19073.
2. Chaowuttikul C, Palanuvej C, Ruangrungsi N. Quantification of chlorogenic acid, rosmarinic acid, and caffeic acid contents in selected Thai medicinal plants using RP-HPLC-DAD. Braz J Pharm Sci. 2020;56(2):e17547.
3. Lee MG, Choi YH, Lee I. Effects of diabetes mellitus induced by alloxan on the pharmacokinetics of metformin in rats: restoration of pharmacokinetic parameters to the control state by insulin treatment. J Pharm Pharm Sci. 2008;11(1):88-103.

4. Martínez-Flórez A, Galizzi M, Izquierdo L, Bustamante JM, Rodriguez A, Rodriguez F, et al. Repurposing bioenergetic modulators against protozoan parasites responsible for tropical diseases. Int J Parasitol Drugs Drug Resist. 2020;14:17-27.
5. Brocks D, Gabr RQ, Padwal RS. Determination of metformin in human plasma and urine by high-performance liquid chromatography using small sample volume and conventional octadecyl silane column. J Pharm Pharm Sci. 2010;13(4):486-94.
6. Salem HF, Nafady MM, Ali AA, Khalil NM, Elsisi AA. Evaluation of metformin hydrochloride tailoring bilosomes as an effective transdermal Nanocarrier. Int J Nanomed. 2022;17:1185-201.
7. Urooj A, Sundar PS, Vasanthi R, Raja MA, Dutt KR, Rao KN, et al. Development and validation of RP-HPLC method for simultaneous estimation of dapagliflozin and metformin in bulk and in synthetic mixture. World J Pharm Pharm Sci. 2017;6(7):2139-50.
8. Patel KJ, Chaudhary AB, Bhadani SM, Raval RJ. Stability indicating RP-HPLC method development and validation for estimation of dapagliflozin and metformin HCL. World J Pharm Pharm Sci. 2017;6(9):796-809.
9. Deepan T, Rao MB, Dhanaraju MD. Development of validated stability indicating assay method for simultaneous estimation of metformin and dapagliflozin by RP-HPLC. Eur J Appl Sci. 2017;9(4):189-99.
10. Olaya MD, Vergel NE, López JL, Viña D, Guerrero MF. Quantification of chlorogenic acid, rosmarinic acid, and caffeic acid contents in selected Thai medicinal plants using RP-HPLC-DAD. Braz J Pharm Sci. 2020;56:e17609.
11. Jani BR, Shah KV, Kapupara PP. Development and validation of UV spectroscopic first derivative method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture. J Bioequiv. 2015;1(1):102.
12. Parmar SH, Luhar SV, Narkhede SB. Development and validation of UV-spectroscopic first derivative and high performance thin layer chromatography analytical methods for simultaneous estimation of dapagliflozin propanediol monohydrate and saxagliptin hydrochloride in synthetic mixture. Eur J Biomed Pharm Sci. 2018;5:668-84.
13. Sanagapati M, Lakshmi DK, Reddy NG, Sreenivasa S. Development and validation of stability-indicating RP-HPLC method for determination of dapagliflozin. J Adv Pharm Educ Res. 2014;4(3).
14. Mishra K, Soni H, Nayak G, Patel SS, Singhai AK. Method development and validation of metformin hydrochloride in tablet dosage form. J Chem. 2011;8(3):1309-13.
15. Lambers Heerspink HJ, De Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab. 2013;15(9):853-62.
16. Venkataraman S, Manasa M. Forced degradation studies: regulatory guidance, characterization of drugs, and their degradation products-a review. Drug Invent Today. 2018;10(2):137-46.
17. Naveed S, Shafiq A, Khan M, Jamal M, Zafar H, Hashim H, et al. Degradation study of available brands of metformin in Karachi using UV spectrophotometer. J Diabetes Metab. 2014;5(328):2.
18. Aubry AF, Gu H, Magnier R, Morgan L, Xu X, Tirmenstein M, et al. Validated LC-MS/MS methods for the determination of dapagliflozin, a sodium-glucose co-transporter 2 inhibitor in normal and ZDF rat plasma. Bioanalysis. 2010;2(12):2001-9.
19. Bhoomaiah B, Shree AJ. Development and validation of RP-HPLC method for simultaneous determination of metformin and miglitol in bulk and pharmaceutical formulation. Int J Pharm Pharm Sci. 2014;6(6):135-41.
20. Debata J, Kumar S, Jha SK, Khan A. A new RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. Int J Drug Dev Res. 2017;9(2):48-51.
21. Maher HM, Abdelrahman AE, Alzoman NZ, Aljohar HI. Stability-indicating capillary electrophoresis method for the simultaneous determination of metformin hydrochloride, saxagliptin hydrochloride, and dapagliflozin in pharmaceutical tablets. J Liq Chromatogr Relat Technol. 2019;42(5-6):161-71.
22. Hassib ST, Taha EA, Elkady EF, Barakat GH. Validated liquid chromatographic method for the determination of (canagliflozin, dapagliflozin or empagliflozin) and metformin in the presence of (1-cyanoguanidine). J Chromatogr Sci. 2019;57(8):697-707.
23. Manoharan G, Ismaiel AM, Ahmed ZM. Stability-indicating RP-HPLC method development for simultaneous determination and estimation of dapagliflozin in raw and tablet formulation. Chem Res J. 2018;3(2):159-64.

24. International conference on harmonization guidance for industry in: Q2B, text on validation of analytical methods, Switzerland, IFPMIA; 1996. p. 1-8.
25. Kommineni V, Chowdary KP, Prasad SV. Development of a new stability indicating RP-HPLC method for simultaneous estimation of saxagliptine and dapagliflozin and its validation as per ICH guidelines. Indo Am J Pharm Sci. 2017;4(09):2920-32.
26. Araujo ED, Rosa AM, Amaral MS, Sversut RA, Baroni AC, Oliveira LC, et al. Stability-indicating HPLC-DAD method for the simultaneous determination of fluoroquinolone in combination with a non-steroidal anti-inflammatory drug in pharmaceutical formulation. Braz J Pharm Sci. 2020;56(4):e17758.
27. Goring S, Hawkins N, Wygant G, Roudaut M, Townsend R, Wood I, et al. Dapagliflozin compared with other oral anti-diabetes treatments when added to metformin monotherapy: a systematic review and network meta-analysis. Diabetes Obes Metab. 2014;16(5):433-42.
28. Zhang M, Zhang L, Wu B, Song H, An Z, Li S. Dapagliflozin treatment for type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. Diabetes/Metab Res Rev. 2014;30(3):204-21.
29. Rosenstock J, Hansen L, Zee P, Li Y, Cook W, Hirshberg B, et al. Dual add-on therapy in type 2 diabetes poorly controlled with metformin monotherapy: a randomized double-blind trial of saxagliptin plus dapagliflozin addition versus single addition of saxagliptin or dapagliflozin to metformin. Diabetes Care. 2015;38(3):376-83.
30. Khalil GA, Salama I, Gomaa MS, Helal MA. Validated RP-HPLC method for Simultaneous determination of Canagliflozin, Dapagliflozin, Empagliflozin and Metformin. Int J Pharm Chem Biol Sci. 2018;8(1):1-13.
31. Patel ND, Shah DB, Maheshwari DG. A review on chromatographic and spectrophotometric methods for estimation of gliflozin in bulk and in different dosage forms. J Glob Trends Pharm Sci. 2015;6(4).
32. Harnaskar VR, Datar PA, Shete RV, Gire S. A review on analysis of dapagliflozin. Curr Pharma Res. 2018;8(2):2321-35.
33. Ziaei E, Emami J, Kazemi M, Rezazadeh M. Simultaneous determination of docetaxel and celecoxib in porous microparticles and rat plasma by liquid-liquid extraction and HPLC with UV detection: in vitro and in vivo validation and application. J Pharm Pharm Sci. 2020;23(1):289-303.
34. Mubeen G, Noor K. Spectrophotometric method for analysis of Metformin. Indian J Pharm Sci. 2009;71(1):100.