Middle-East Journal of Scientific Research 25 (7): 1451-1457, 2017
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2017.1451.1457

Bioanalytical Method Development and Validation for Metformin and Canagliflozin Drugs in Human Plasma by RP-HPLC Method

[1,2]T. Deepan, [2]M.V. Basaveswara Rao and [1]M.D. Dhanaraju

[1]GIET School of Pharmacy andhra Pradesh, India
[2]Krishna University andhra Pradesh, India

Abstract: A simple, sensitive and selective HPLC method was developed for the simultaneous determination for metformin and canagliflozin in human plasma using a novel sample extraction procedure. Solid phase extraction of metformin, canagliflozin and pioglitazone (as internal standard) from plasma samples was performed with phosphate buffer: Acetonitrile (85:15, v/v) adjusting to pH 3.0 with sodium hydroxide using Inertsil ODS C_{18} (4.6 x 250mm, 5µm). The flow rate 1.0 ml/min and UV detection at 280 nm was employed. The retention time metformin, canagliflozin and internal standard (pioglitazone) was 4.62, 8.10 min and 10.64 min respectively. The linearity range for canagliflozin and metformin was found to be be 5-25µg/mL and 500-1250µg/mL respectively. The validation was successfully performed by means of accuracy and precision, selectivity and specificity, linearity, recovery under various conditions. This developed method can be successfully employed for the determination of metformin and canagliflozin in human plasma.

Key words: Metformin · Canagliflozin · Solid phase extraction · Human plasma · RP-HPLC

INTRODUCTION

Metformin HCl (1, 1-dimethylbiguanide HCl), is one of the most commonly used oral anti-hyperglycemic agents for the treatment of Type II diabetes mellitus. It is currently recommended as first-line therapy in overweight or obese patients. Metformin Hydrochloride is chemically known as 1, 1Dimethyl biguanide monohydrochloride [1] (Fig. 1).Canagliflozin is an oral selective Sodium-Glucose co-transporter 2 (SGLT2) inhibitor used for the management of type 2 Diabetes Mellitus. The chemical name (IUPAC) of Canagliflozin is (2S, 3R, 4R, 5S, 6R)-2-{3-[5-(4-fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl}-6 hydroxy methyl tetrahydropyran3, 4, 5-triol with molecular formula C24H25FO5S (Fig. 2). The combination of metformin and canagliflozin is available as tablet formulation for oral use in diabetes [2].

Literature survey reveals several methods such as liquid chromatography for HPLC in human plasma [3-5] LC-MS/MS [6] methods have been reported for the determination of MET individually and for canagliflozin bio analytical methods such as HPLC [7, 8] have been reported individually. For combinations such as metformin with other drugs such as HPLC [9-12] and LC-MS/MS [13-16] in biological matrices were reported. No methods were traced for simultaneous determination of metformin and canagliflozin in biological matrices by HPLC. In this work, we proposed a bio analytical method for simultaneous determination of metformin and canagliflozin in human plasma by HPLC.

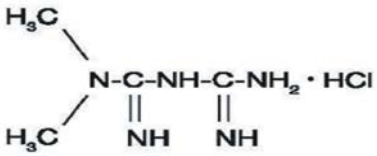

Fig. 1: Structure of metformin

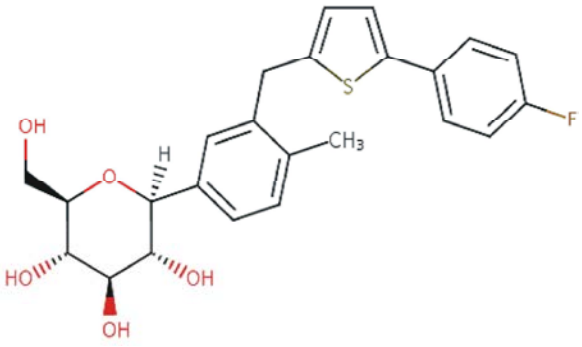

Fig. 2: Structure of canagliflozin

Corresponding Author: M.D. Dhanaraju, GIET School of Pharmacy Andhra Pradesh, India.

MATERIALS AND METHODS

Instrumentation: The analysis was performed by using waters Corporation (Milford USA) for method development and validation. This system comprised of a ternary gradient pump and auto sampler (2487 Separation module), column oven and a photo diode array detector. Inspire (4.6 x 250mm, 5µm) column was used. The instrumental settings were a flow rate of 1 mL/min, a column temperature at 25°C and a detector wavelength of 280 nm. The injection volume was 20 µL and the run time was 15 min respectively. Data acquisition was made with Waters empower software.

Materials and Reagents: Metformin hydrochloride and Canagliflozin reference standard were kindly supplied by Glenmarkpharma & Piramal healthcare respectively and tablets were purchased from a local market. Analytical-grade OPA (Orthophosphoric acid) and HPLC-grade, Acetonitrile and water were purchased from Merck (Darmstadt, Germany). A membrane filter of 0.45 µm porosity was used to filter and degassed the mobile phase. The tablets of metformin in combination with Canagliflozin were purchased from the Indian market. Double-distilled water was used throughout the experiment. Other chemicals were of analytical or HPLC grade.

Chromatographic Conditions: Metformin Hydrochloride and Canagliflozin was analyzed in intersilC_{18} column (250mm×4.6 mm, 5mm particle size) column for the chromatographic separation. The mobile phase was composed of phosphate buffer pH adjusted to 3.0 with Orthophosphoric acid and Acetonitrile (85:15v/v). Filtered through 0.45µm nylon membrane filter under vacuum filtration and pumped at ambient temperature, at a flow rate of 1 ml/min with UV detection wavelength at 280nm. Injection volume was 20µl. The run time was 15 min and the retention time of Metformin Hydrochloride and Canagliflozin was found to be 4.629 min and 8.108 min respectively.

Mobile Phase: The mobile phase consisted of buffer and Acetonitrile in the ratio of 85:15 (v/v). The pH of the mobile phase was adjusted to 3.0 with orthophosphoric acid. The buffer used in the mobile phase consisted of potassium dihydrogenortho phosphate in double-distilled water. The mobile phase was mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of 0.025M Phosphate Buffer: 3.4g of potassium dihydrogenortho phosphate was weighed and taken in a 1000ml volumetric flask and adjusted the P^H with orthophosphoric acid finally the solution was filtered by using 0.45 Micron membrane filter and sonicated it for 10 mins.

Preparation of Diluent: The diluents were prepared by accurately measured 850 ml (85%) of above buffer and 150 ml of Acetonitrile HPLC (15%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of Standard Solution of Metformin and Canagliflozin: Accurately weighed 250mg of Metformin, 25mg of Canagliflozin and 100mg of Pioglitazone working standard and transferred into a 5ml centrifuge tube then 2.5ml of plasma and 2.5ml of diluents was added. Then centrifuged it for 1hour at constant rpm. Then Supernant solution was collected from that centrifuge tube with the help of a clean and sterilize syringe and then it was transferred into a 10 ml clean dry volumetric flask and about 7 mL of diluent was added and sonicated to dissolve it completely and made up to the mark with the same solvent. (Stock solution)Further pipetted out 0.3l of above solution is transferred into a 10ml volumetric flask and dilute up to the mark with diluent. This mixed stock solution contains 750µg/ml of metformin and 75 µg/ml of canagliflozin.

Sample Preparation: A simple solid phase extraction (SPE) method was employed to extract both the analytes and IS from plasma. Accurately weighed equivalent weight of 250mg of Metformin, 25mg of Canagliflozin and 100mg of Pioglitazone tablet powder was transferred into a 5ml centrifuge tube then 2.5ml of plasma and 2.5ml of diluents were added. Then centrifuged it for 1hour at constant rpm. Then Supernant solution was collected from the centrifuge tube with the help of a clean and sterilize syringe and then it was transferred into a 10 ml clean dry volumetric flask, about 7 mL of diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further the supernatant liquids were collected in another Eppendorf tube and 20 µL supernatant was injected into the analytical column.

Method Validation [17, 18]: Assay validation was performed in human plasma in accordance with international guidelines for bio analytical method

Table 1: Intraday precision for metformin and canagliflozin

Injection	Metformin	Canagliflozin	Pioglitazone
Injection-1	957498.0	158363.0	89485.0
Injection-2	958373.0	158376.0	89474.0
Injection-3	958377.0	158237.0	89648.0
Injection-4	958374.0	158373.0	89467.0
Injection-5	959484.0	158932.0	89364.0
Injection-6	954484.0	158383.0	89464.0
Average	957765.0	158444.0	89483.7
Standard Deviation	1726.6	245.3	91.7
%RSD	0.2	0.2	0.1

Table 2: Interday precision for metformin and canagliflozin

Injection	Metformin	Canagliflozin	Pioglitazone
Injection-1	959473.0	158387.0	87983.0
Injection-2	958474.0	158327.0	87838.0
Injection-3	958373.0	158363.0	87537.0
Injection-4	958363.0	158736.0	87538.0
Injection-5	959373.0	158373.0	87373.0
Injection-6	958363.0	157368.0	87293.0
Average	958736.5	158259.0	87593.7
Standard Deviation	534.3	461.8	267.1
%RSD	0.1	0.3	0.3

Table 3: Accuracy data for metformin in plasma

Concentration	% Recovery	Amount Recovered (mg)	Area	Average Recovery
50%	99.38	124.23	476290	99.98
100%	99.85	249.62	957024	
150%	100.72	377.68	1448027	

Table 4: Accuracy data for canagliflozin in plasma

Concentration	% Recovery	Amount Recovered (mg)	Area	Average Recovery
50%	100.25	12.53	77719	99.78
100%	99.57	24.89	154381	
150%	99.52	37.32	231466	

Table 5: Linearity range of metformin in plasma

S. No	Linearity Level	Concentration	Area
1	I	250	522088
2	II	500	734633
3	III	750	950658
4	IV	1000	1192066
5	V	1250	1430452
Correlation Coefficient		0.999	

Table 6: Linearity range of canagliflozin in plasma

S. No	Linearity Level	Concentration	Area
1	I	25	65477
2	II	50	110790
3	III	75	153097
4	IV	100	193120
5	V	125	239955
Correlation coefficient		0.99	

validation recommended by USFDA and EMEA [17, 18]. The validation parameters included selectivity and specificity, linearity and sensitivity, precision and accuracy, recovery, carry-over effects and stability.

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by pure drugs of metformin and canagliflozin. The solutions were suitably diluted at linearity level (750 µg/mL of metformin and 75 µg/mL of canagliflozin). Then each dilution was injected thrice (n=3). The percent recoveries of the drugs were determined. The results are shown in Tables 3 and 4 and Fig. 5.

Precision: To check the intra and inter-day variations of the method, solutions containing 750.0 µg/mL of metformin and 75 µg/mL of canagliflozin were subjected to the proposed HPLC method of analysis and results obtained were noted. The precision of the proposed method i.e., the intra and inter-day variations in the peak areas of the drugs solutions in plasma were calculated in terms of percent relative standard deviation (RSD) and the results are represented in Tables and . A statistical evaluation revealed that the percentage relative standard deviation of the drugs at linearity level for 6 injections was less than 2.0. Typical chromatogram of metformin and canagliflozin in plasma for intra and inter-day precision are shown in Fig. 4 and Tables 1 and 2.

Linearity: In order to find out the linearity range of the proposed HPLC method in plasma, curves were constructed by plotting peak areas obtained for the analyte against their concentrations. A good linear relationship (r^2=0.999) was observed between the concentrations of metformin and canagliflozin and their corresponding peak areas. The relevant regression equations were y=909.6.x+28373 for metformin (r^2=0.999) and y=1725x+23102for canagliflozin (r^2=0.999) (where y is the peak area and x is the concentration of metformin and canagliflozin (µg/ mL)). The slope, intercept and the correlation coefficient of the plots are shown in Tables 5 and 6. The linearity ranges for metformin and canagliflozin and their corresponding graphs are shown in Figures 7 and 8.

Selectivity: An aqueous mixture of metformin and canagliflozin (750µg/ mL of metformin and 75µg/mL of canagliflozin) were prepared and injected into the column and the retention times were checked and any interference at the retention times was checked by comparing the

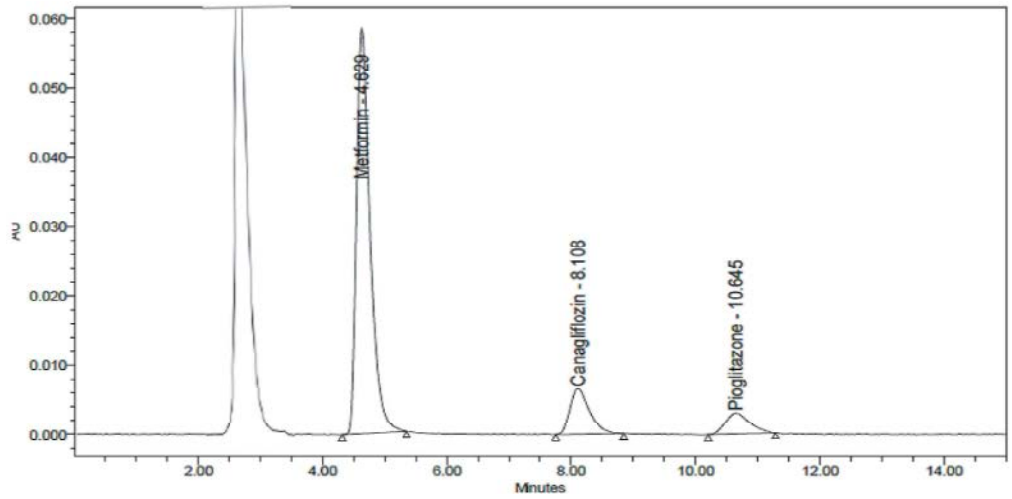

Fig. 3: Chromatogram of metformin and canagliflozin standard drug in plasma

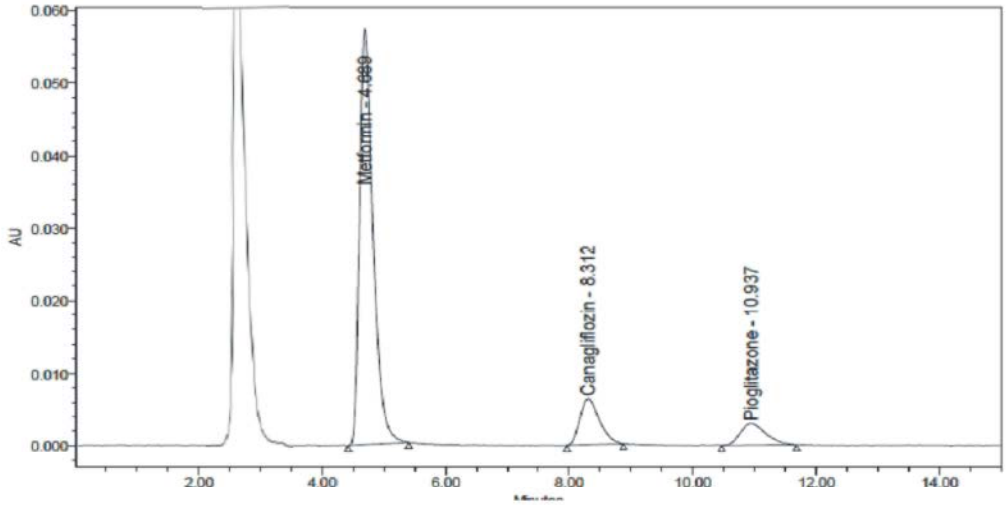

Fig. 4: Chromatogram of metformin and canagliflozin in plasma for precision

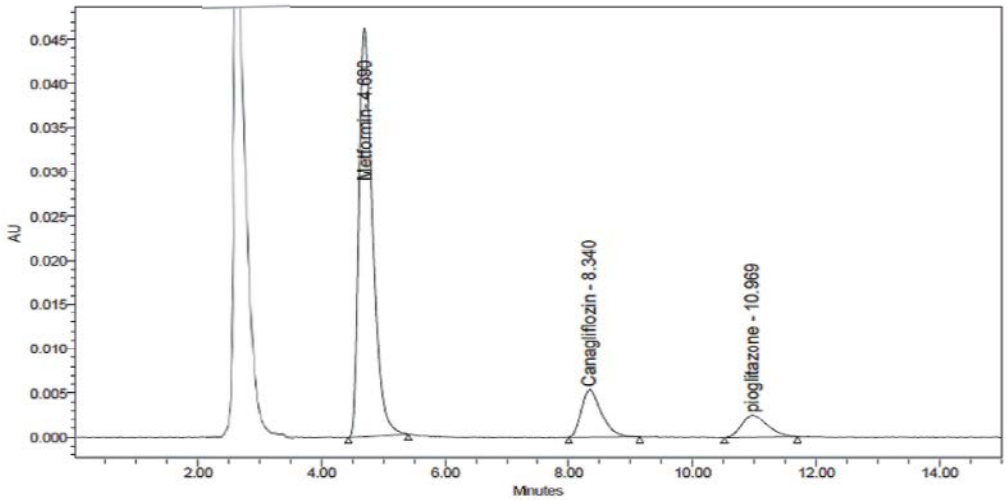

Fig. 5: Chromatogram of metformin and canagliflozin in plasma for Accuracy

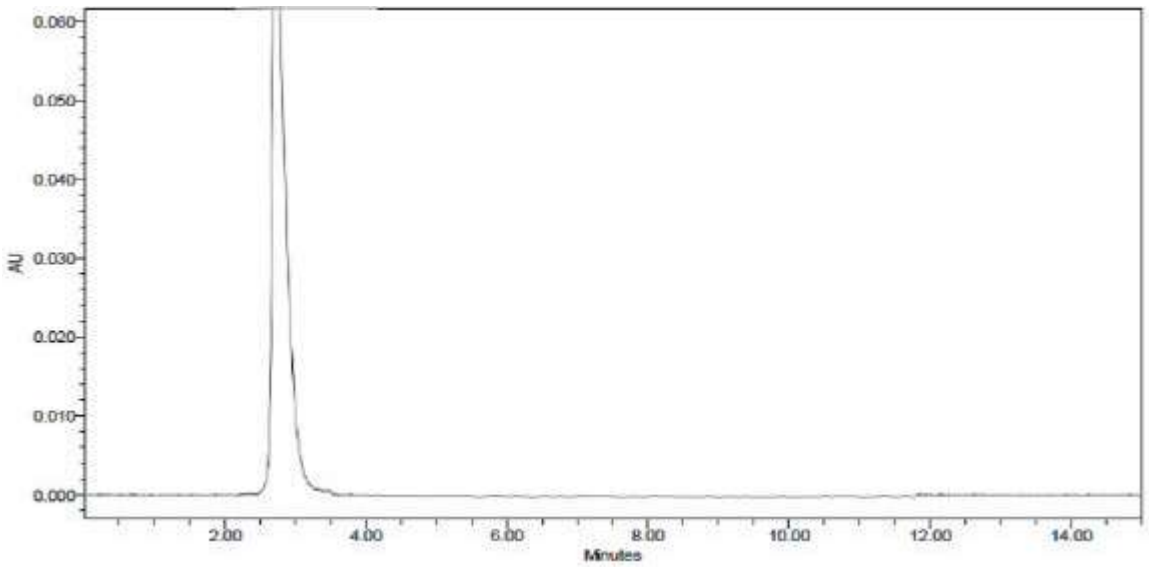

Fig. 6: Chromatogram of blank plasma sample

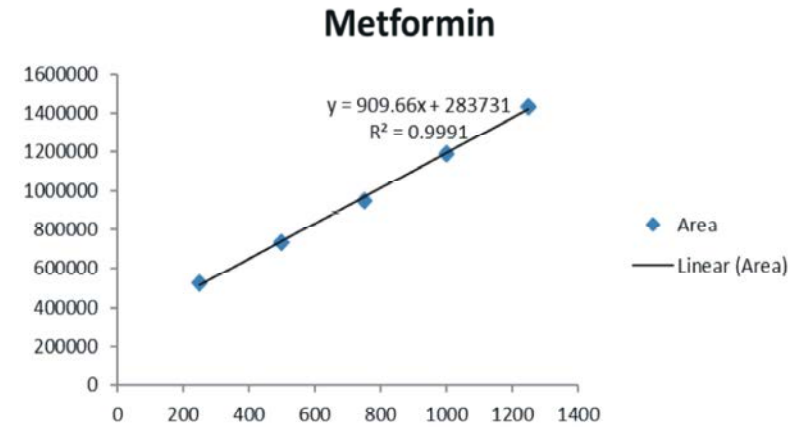

Fig. 7: Calibration curve for metformin in plasma

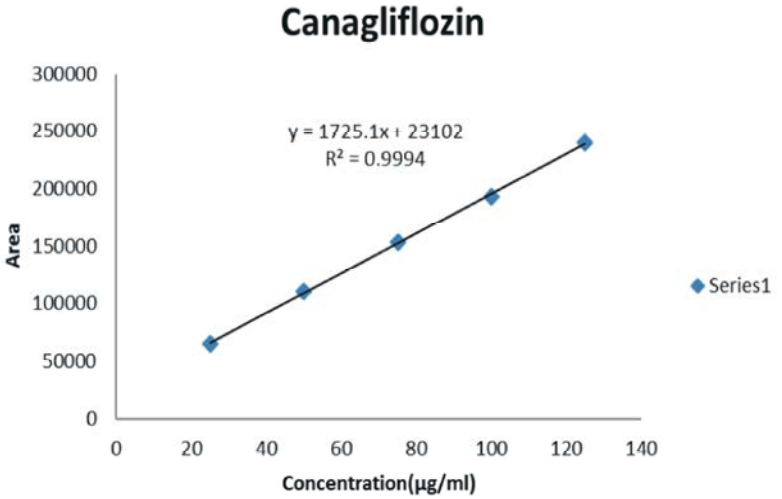

Fig. 8: Calibration curve for canagliflozin in plasma

response in the blank. No interference was observed at the retention times for metformin and canagliflozin extracted from plasma. The method was found to be precise and specific.

Sensitivity: To determine the sensitivity in terms of LLOQ, 'Lower Limit of Quantification' where the response of LLOQ must be at least five times greater than the response of interference in blank matrix at the retention time of the analyte(s). The LLOQ obtained by the proposed method were. 2.98µg/ml and 9.98 µg/ml for metformin and canagliflozin respectively.

RESULTS AND DISCUSSION

The developed HPLC method was optimized for the analysis of metformin hydrochloride and canagliflozin in human plasma. Different mobile phases were tested to find the best condition to quantify metformin and canagliflozin in plasma. Different ratios of methanol, acetonitrile and potassium hydrogen phosphate were tried and the optimum mobile phase was finalized. Then the method was validated for selectivity, linearity, limit of quantification, accuracy, precision and recovery as per the international guidelines

The LLOQ obtained for metformin and canagliflozin by the proposed method in plasma was 2.98 and 9.98µg/mL respectively. The retention times obtained for metformin, canagliflozin and internal standard (pioglitazone) in plasma were 4.62, 8.10 and 10.64 min respectively. Quantitative linearity of drugs in plasma was obeyed in the concentration range of 250-1250µg/mL for metformin and 5-25µg/mL for canagliflozin respectively. The relevant regression

equations were y=909.6x+28373 for metformin (r^2=0.999) and y=1725x+23102 for canagliflozin (r^2=0.999) (where y is the peak area and x is the concentration of metformin and canagliflozin (µg/mL)). The intra-day and inter-day drugs variations in plasma by the proposed method showed percentage relative standard deviation were less than 2%, indicating that the method is precise. The corresponding mean recoveries of the drugs in plasma were 99.30-100.72%. This reveals that the method is quite accurate. The percentage relative standard deviation obtained for the drugs spiked in plasma for stability studies were less than 2%.

CONCLUSION

The optimized HPLC method is selective, accurate, precise and repeatable. The method is linear over a wide range and utilizes a mobile phase which can be easily prepared. The column used is a widely available reversed phase C-18. Even for an injection volume of 20 µl the method is quite sensitive. The method was validated as per ICH guidelines and all the parameters met within the acceptance criteria. It can be concluded that the method is suitable for the routine quantification of metformin and canagliflozin in human plasma

REFERENCES

1. Edward C., Chao, 2011. Canagliflozin. Drugs of the Future. 36(5): 351-357. doi:10.1358/dof.2011.36.5.1590789
2. Dunn, C.J. and D.H. Peters, 1995. Metformin. A review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus. Drugs., 49(5): 721-49.
3. Valentina Porta, Simone Grigoleto Schramm, Eunice Kazue Kano, Eunice Emiko Koono, Yara Popst Armando and Kazuo Fukuda, 2008. Cristina Helena dos Reis Serra, HPLC-UV determination of metformin in human plasma for application in pharmacokinetics and bioequivalence studies, Journal of Pharmaceutical and Biomedical Analysis, 46: 143-147.
4. Himal Paudel Chhetri, Panna Thapa and Ann Van Schepdael, 2013. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation, Saudi Pharmaceutical Journal, http://dx.doi.org/10.1016/ j.jsps. 12.011.
5. Hossein Amini and Abolhassan Ahmadiani, 2005. Parisa Gazerani Determination of metformin in human plasma byhigh-performance liquid chromatography, Journal of Chromatography B, 824: 319-322
6. Sanjay Gurule, Dipanjan Goswami, Arabinda Saha, Yogesh Modhave, Arshad Khuroo and Tausif Monif, 2012. Bio analytical method development and validation using incurred samples: Quantitative estimation of metformin inhuman K3EDTA plasma by LC–MS/MS, Clinical Research and Regulatory Affairs, 29(1): 23-34.
7. Ishpreet Kaur, Sharad Wakode, Harsharan Pal Singh and Satish Manachanda, 2016. Development and Validation of a Stability-Indicating Reverse Phase HPLC-PDA Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Form, Pharm Methods, 7(1): 54-62.

8. Muzaffar Iqbal, Nasr Y. Khalil, Amer M. Alanazi, Khalid A. Al-Rashood, 2015. A simple and sensitive high performance liquid chromatography assay with a fluorescence detector for determination of canagliflozin inhuman plasma, DOI: 10.1039/c5ay00074b, Analytical methods.
9. Deepan, T., M.V. Basaveswara Rao and M.D. Dhanaraju, 2017. Development of Validated Stability Indicating Assay Method for Simultaneous Estimation of Metformin and Dapagliflozin by RP-HPLC, European Journal of Applied Sciences, 9(4): 189-199, Idosi Publications.
10. Shanmugakumar, S.P. Nakasthra, K. Mamatha, P. Yamuna and J. Swamy, 2015. Development and Validation of Glimepiride and Metformin in Human Plasma by HPLC: An application study, International Journal of Advances in Pharmaceutical Analysis, IJAPA, 5(3): 51-57.
11. Abu Ruz, S., J. Millership and J. McElnay, 2005. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. Journal of Chromatography B; 817: 277-286.
12. Ashutosh, K.S., D. Manidipa, Seshagiri and S.D. Gowri, 2015. New Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Metformin and Alogliptin in Human Plasma, J. Chromatograph Sep. Tech., 6(6).
13. PavanKumar, P., T.E.G.K. Murthy and M.V. Basaveswara Rao, 2015. Development, validation of liquid chromatography?tandem mass spectrometry method for simultaneous determination of rosuvastatin and metformin in human plasma and its application to a pharmacokinetic study, Journal of Advanced Pharmaceutical Technology & Research, 6(3).
14. Zhong, G., H. Bi, S. Zhou, X. Chen and M. Huang, 2005. Simultaneous determination of metformin and gliclazide in human plasma by liquid chromatography-tandem mass spectrometry: application to a bioequivalence study of two formulations in healthy volunteers. Journal of Mass Spectrometry, 40: 1462-1471.
15. Bonde, S.L., R.P. Bhadane, A. Gaikwad, D. Katale, S. Gavali and A.S. Narendiran, 2013. A simple and sensitive method for determination of metformin and sitagliptin in human plasma using liquid chromatography and tandem mass spectrometry. International Journal of Pharmacy and Pharmaceutical Sciences, 5: 463-470.
16. Mistri, H.N., A.G. Jangid and P.S. Shrivastav, 2007. Liquid chromatography tandem mass spectrometry method for simultaneous determination of antidiabetic drugs metformin and glyburide in human plasma. Journal of Pharmaceutical and Biomedical Analysis, 45, 97-106.
17. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM368107.pdf.
18. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf.