www.pharmaerudition.org

ISSN: 2249-3875

International Journal of Pharmaceutical Erudition

Research for Present and Next Generation

MAY 2024

Vol: 14 Issue:01

(1-18)

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Research Paper

ELABORATE AND VALIDATION OF ANALYTICAL METHOD FOR COMBINATION OF ANTI- DIABETIC DRUGS IN PHARMACOKINETIC STUDIES

Dinesh Kumar Sharm*a, Mukesh Bansal, Gaurav Bhaduka, Dilip Agrawal

*Research Scholar, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur, Rajasthan

RP-HPLC analytical, and bio-analytical methods, developed for determination of SAXA and GLIM was found to be linear, accurate, precise, robust, suitable, sensitive, and specific, as per the regulatory guidelines. Both the methods involved simple and simultaneous estimation of SAXA and GLIM with gradient elution, and separations were performed at ambient temperature.The analytical method was used to determine the SAXA, and GLIM, simultaneously in tablet formulation, as well as in the dissolution study. All the ingredients of tablet formulation were not affecting the peaks of SAXA, and GLIM, which reflects specificity of developed method. The bio- analytical method was used to determine the SAXA and GLIM, simultaneously in rat plasma for during pharmacokinetic analysis. The plasma components did not interfere with the peaks of SAXA, and GLIM, and hence confirmed the specificity of the developed method.

Key Words: RP-HPLC, SAXA, GLIM, Bio-analytical method

INTRODUCTION

Diabetes

Diabetes is the fastest growing disease across the globe, and a substantial threat to human health. Diabetes mellitus (DM), often referred to as diabetes, is a group of metabolic diseases, which is characterized by a chronic elevation in blood glucose levels (Malecki, 2005). Undiagnosed or poorly controlled diabetes, can lead to a state of determined hyperglycemia, resulting in irreversible damage in different tissues, most significantly the retina, kidney glomeruli, neural tissue, and blood vessels (Wolfs, 2009). Owing to these complications, patients with diabetes often have a reduced life expectancy, and quality of life (Zimmet, 2001; Ahlqvist et al., 2018).

Antidiabetic drugs

DM is a chronic condition that typically cannot be cured. However, all the forms of diabetes have been treatable, since the development of readily available insulin, in 1921. The enhancement of insulin secretion, by pancreatic islet β-cells, has been a major goal for the treatment of type 2 diabetes mellitus (T2 DM) there are different classes of antidiabetic drugs, and their selection depends upon the various factors, *viz.*, type of the

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

diabetes, age, life style of the person, presence of other diseases, and numerous other factors. Antidiabetic drugs exert their useful effects *via* increasing insulin levels in the body, increasing the body' sensitivity to insulin or decreasing glucose absorption in the intestines. A list of antidiabetic drugs along with the molecular targets, mechanisms of action, and side-effects related to their use, are summarized in Table 1. Because of their adverse side effects, most of

Table1 : Currently available drugs and their targets for the treatment of diabetes

Drug Class	Molecular Target	Mechanismof Action	Adverseeffects	Generic Name
Insulin	Insulin receptor	Correctinsulin deficiency	Hypoglycemia, weight gain	Insulin glargin, Insulin lispro
Sulfonylurea	K_{ATP} Channel	Stimulate insulin secretion	Hypoglycemia, weight gain	GLIM, GLIP, GLYB
Meglitinides	K_{ATP} Channel	Stimulate insulin secretion	Hypoglycemia, weight gain	REPA, NATE
Biguanides	Unknown	Inhibition of hepaticglucose output	GI-disturbances, lactic acidosis	MET
TZD	PPARγ	Increaseinsulin sensitivity	Weight gain, edema,anemia	PIOG, ROSI
α-glucosidase inhibitors	α- glucosidase	Retard carbohydrate absorption	GIdisturbances	ACAR, MIGL
GLP -1 Analogues	GLP-1 Receptor	Stimulate insulin secretion	GIdisturbances, nausea, abdominal pain, weightloss	EXEN, LIRA
DPP-IV Inhibitors	DPP-IV	Increase blood level of the incretinGLP-1	Increasedriskfor infection and headache	VILD, SITA, SAXA
Amylin analogues	Calcitonin receptor & RAMP1,2,3	Slowgastric emptying &supress glucagon	Nausea	PRAM

these treatments are considered to be unsatisfactory, in terms of the prevention of complications, and preservation of quality of life. Hypoglycaemia and weight gain, are common side effects associated with most of the antidiabetic drugs.

Reagents and chemicals

The drugs SAXA, and GLIM, were procured *ex gratis* from Raks Pharma Pvt. Ltd., Vishakhapattanam, India. All other chemicals and solvents, like acetonitrile (ACN),methanol, sodium dihydrogen orthophosphate, orthophosphoric acid, and water, employed for the study were HPLC grade, supplied by Loba Chemie Pvt. Ltd., Mumbai, India. The excipients employed for making the tablets, like microcrystalline cellulose (MCC) pH 101, cornstarch, lactose monohydrate, magnesium stearate, and talc were also procured from Loba Chemie Pvt. Ltd., Mumbai, India. Water for HPLC was prepared by Micropore™ assembly installed at Shoolini University, Solan. All other chemicals were analytical grade, and glassware borosilicate.

Instruments

The chromatographic system, to develop the analytical methods for the current investigation was performed on an Agilent Technologies 1200 series, HPLC system (Agilent Technologies, Waldbronn, Germany). It was equipped with a binary pump system (G1312A), an automatic injector (G1329A), and a photo diode array detector (G1315D). Data acquisition was performed using a chromatography software package (EZ Chrome Elite™). For sample preparation, ultrasonicator (Cleaner30A), filter assembly, syringe filters, and nylon membrane filters (Axiva, Delhi, India) were employed. The samples were accurately weighed using weighing balance (Citizon,Ambala, India). FT-IR spectra were recorded on Agilent technologies FT-IR (Agilent Technologies, Danbury, USA) (Cary 630). The melting point (°C) of the drugs, were recorded on melting point apparatus (Sentwin, Ambala, India). Hot air oven (INCO,Ambala, India), was used to dry the granules, during tablet preparation, while the granules were compressed using eight station rotary press compression machine (Kambert Machinery Co. Pvt. Ltd., Ahmedabad, India). Roche friabilator apparatus(INCO, Ambala, India) was used to check the friability of the tablets. Monsento hrardness apparatus (INCO, Ambala, India) was employed to determine the hardness.USP Dissolution apparatus, with temperature controller (Electrolab India Pvt.Ltd.),

Development and validation of RP-HPLCmethod

Optimization of chromatographic parameters

The chromatographic separation of the active drugs, i.e., SAXA, and GLIM, was performed using an Innoval C18 column (5 µm, 4.6 mm i.d × 250 mm) made of stainless steel. To get the

ideal chromatographic conditions for the simultaneous elution of SAXA and GLIM, a number of trials were performed. The method was optimized mainly w.r.t. mobile phase composition, elution type (isocratic or gradient), and flow rate. Two different mobile phase solvents for pumps were ACN for pump A,and buffer for pump B. Isocratic flow of the mobile phase was attempted for the simultaneous elution of the drugs. However, this approach was not successful in giving the separate, sharp, and symmetric peaks of SAXA and GLIM. Thus, gradient flow of mobile phase, by varying the ratio of ACN and buffer, over a period of total30 min, was followed. ACN and buffer solution were filtered through a 0.45 µm nylon-membrane filter, and ultra sonicated for about 20 mins. Samples were filtered through the syringe filter of 0.22 µm pore size, prior to the injection. The injection volume of the sample, to carry out the chromatography, was fixed as 10 µL. The column temperature was maintained at ambient temperature. Runtime ,and wavelength were selected by peak observed by chromatogram. These were optimized to obtain sharp and symmetric peaks.To select a common wavelength (λ), UV spectralanalysis, was employed at which both the drugs exhibited maximum absorption.

Stock and working solutions

Separate standard solutions of SAXA and GLIM, were prepared at a concentration of 500 µg/mL, and 250 µg/mL, respectively, dissolving the appropriated amount of bulk material in ACN, and ultra-sonicated for 20 mins. By adding an equal ratio of SAXA and GLIM solutions, a simultaneous stock solution was prepared, wherein, the final concentrations of SAXA, and GLIM, were 250 µg/mL, and 125 µg/mL, respectively. Further five levels in the range of, 15.63-250.00 µg/mL for SAXA, and 7.81-125.00µg/mL for GLIM, were prepared by diluting the simultaneous stock solution

Preparation of calibration graph

From the simultaneous stock solution, required five serial dilutions of SAXA (15.63,31.25, 62.50, 125.00, 250.00 µg/mL),and GLIM (7.81, 15.63, 31.25,62.50, 125.00 µg/mL), were prepared, and injected in HPLC at selected wavelength, in triplicate. A calibration curve was prepared between concentration, and average peak area, for both the drugs.

Validation study

Linearity and range

The linearity of the method used for the analysis of SAXA, and GLIM was evaluated from the calibration curves plotted between peak area and analyte concentration. Calibration curves, taking five points, in each case,were generated on the three consecutive days, with standard working solutions. Range is defined as the interval between the minimum and maximum concentration that is analysed by the given chromatographic conditions, within permissible

limit of precision and accuracy. Least square linear regression analysis, was applied on the obtained chromatographic data, employing MS-Excel 2013 spreadsheet software and the corresponding statistic study, was carried out. The regression analysis was performed by devising the regression equation in the form of $y = mx + c$ where, y = peak area, x = Concentration, m = slope and c= y-intercept.

Accuracy

Accuracy was determined by standard addition method (spiking) for both the drugs. Therein, a known amount of active (SAXA and GLIM), was added to a determined amount of placebo.The three concentrations used were 50,100 and 150% respectively, in previously analysed samples at 63.50 µg/mL for SAXA, and 31.35 µg/mL for GLIM. The study was carried out in triplicate for each concentration.

Precision

The precision study was carried out as per the ICH guidelines (Rao et al., 2013). The first one consisted of checking instrumental precision, wherein a sample corresponding to a concentration, within the linearity range, was injected six times, consecutively in to the chromatograph. Inter-day and intra-day precision were determined by analyzing three different concentrations for each drug in triplicate, selected from the linearity range,i.e.,31.25 µg/mL (LQC),62.50 µg/mL (MQC), and 125.00 µg/mL (HQC) for SAXA, and 15.63µg/mL (LQC), 31.25µg/mL (MQC), and 62.50µg/mL (HQC) for GLIM. Forinter-day precision, three replicates of each concentration were analysed on 1st and 2nd day, while for intra-day precision, same set of the concentrations were also analysed at different times, within a day. The concentrations for each drug were prepared from the same stock solution. The peak area was determined, and the precision was reported as % relative standard deviation (RSD).

Robustness

The study of robustness was carried out evaluate the influence of small, but deliberate variations, in the chromatographic conditions, for the simultaneous determination of SAXA, and GLIM, in the tablets. The chromatographic factors, chosen for this study, were as follows:

Detection wavelength: 225 nm and 235nm

Flow rate: 0.70mL/min and 0.80mL/min

Mobile phase: A:B :: 28:72 for 0-2 mins, A:B:: 48:52 for 2-25 mins, and A:B ::28:72 for 25-30mins.andA:B::32:68 for 0-2mins A:B::52:48 for 2-25mins, and A:B:: 32:68 for25-30 mins.

RESULTS AND DISCUSSION

RP-HPLC method development and validation SAXA and GLIM

Reference standard

SAXA (M/s Rax Pharmaceutical Ltd. Vishakhapattanam, A.P, India) and GLIM (M/s Chiros Pharmaceuticals, Solan, H.P, India), were procured *exgratis* from the respective

organizations, for the purpose of method development, and validation.

Optimization of the chromatographic parameters

The previously reported RP-HPLC methods for estimation of SAXA, and GLIM individually, or in combination with some other APIs, in pharmaceutical formulations have mostly used phosphate buffer, and ACN, as mobile phase in either isocratic mode or gradient mode (Mohammad et al., 2013; Aziz et al., 2015; Zhou et al., 2017;El-Zahar et al., 2017; Merey et al., 2017; Dai et al., 2018). However, no reports are traceable in the literature for the simultaneous estimation of SAXA and GLIM. Therefore, our aim was to develop, and validate a single analytical method, which could simultaneously estimate the SAXA, and GLIM, in pharmaceutical formulations, and in bulk drug mixture, for quality control purposes.

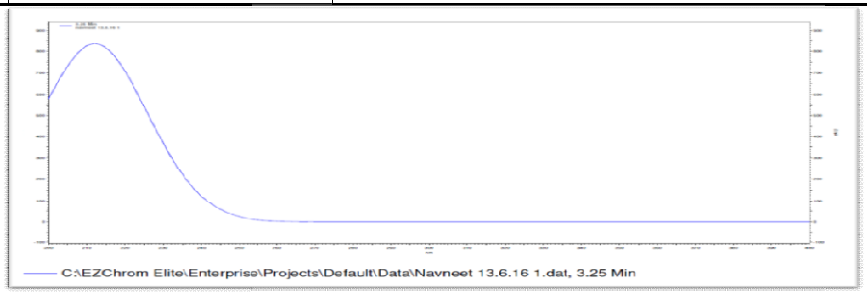
Chromatographic separation of the active drugs, i.e., SAXA and GLIM,was performed using an Innoval C18 column (5µm, 4.6 mm i.d × 250 mm) made of stainless steel. Two different mobile phases for pumps were :mobile phase A:acetonitrile (ACN) and mobile phase B: buffer. As, isocratic flow was not suitable for simultaneous estimation of SAXA, and GLIM, hence, gradient flow, of mobile phasesat flow rate of 0.75 mL/min from two different pumps was followed, and found to be most suitable. The gradient flow of solvents A and B, comprised of total time period of 30mins, and was according to the following order :A:B::30:70for0-2mins,A:B :: 50:50 for 2-25 mins, and A:B :: 30:70 for 25-30 mins. The composition of buffer was 0.1% sodiumdi-hydrogen ortho-phosphate, pH3.8 adjusted with ortho-phosphoric acid. Both, the ACN, and the buffer solution, were filtered through a 0.45µm nylon-membrane filter, and ultra-sonicated for 20 mins. Samples were filtered through the syringe filter of 0.22 µm pore size, prior to the injection. The injection volume of sample to carry out the chromatography was fixed as 10µL.The column temperature was maintained at ambient temperature. UV spectra were taken for SAXA, and GLIM employing optimized chromatographic parameters, and both drugs showed different wavelengths (Figure 4.1 and Figure 4.2). The wavelength at which both the drugs exhibited maximum absorbance was observed to be 230nm as obtained from PDA detector. Thus, chromatographic analysis was performed at 230nm. The optimized chromatographic parameters (Table 4.1), yielded well-resolved,sharp and symmetrical peaks for both the drugs with retention times of 3.28 mins forSAXAand 26.16 mins for GLIM.

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Table 2: Optimized chromatographic conditions for simultaneous analysis of SAXA and GLIM

S.No.	Parameters		Condition
1	Mobile phase	A	Acetonitrile
		B	0.1%sodium di-hydrogenortho-phosphate, pH 3.8 adjusted with ortho-phosphoric acid
2	Flow		Gradient flow of %B-70 for 0-2 mins, 50 for 2-25 mins, and 70 for 25-30 mins
3	Injection volume		10µL
4	Flow rate		0.75 mL/min
5	Detection wavelength		230nm
6	Retention time	SAXA	3.28 mins
		GLIM	26.16mins
7	Runtime		30mins
8	Temperature		Ambient temperature



Fifure 1 : UV Spectra of SAXA at 3.28 mins

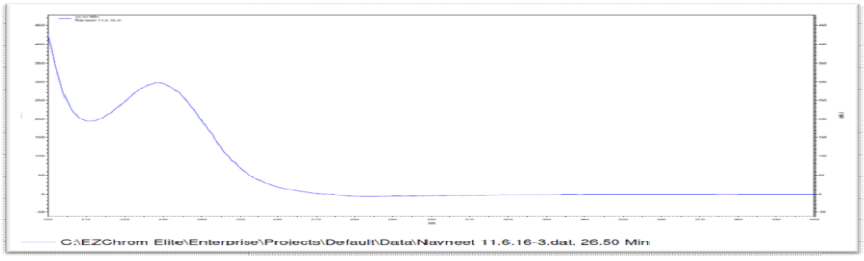


Figure2: UV spectra of GLIM at 26.16min

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

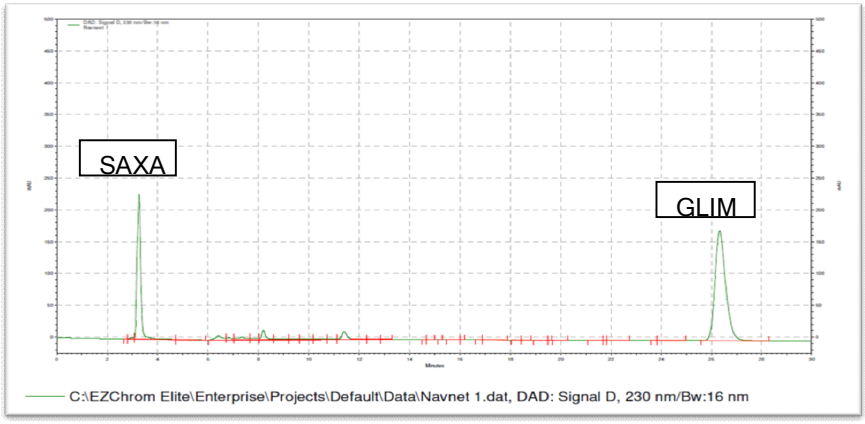


Figure3: HPLC chromatogram showing the peaks of SAXA, and GLIM at 3.28mins,and 26.16 mins

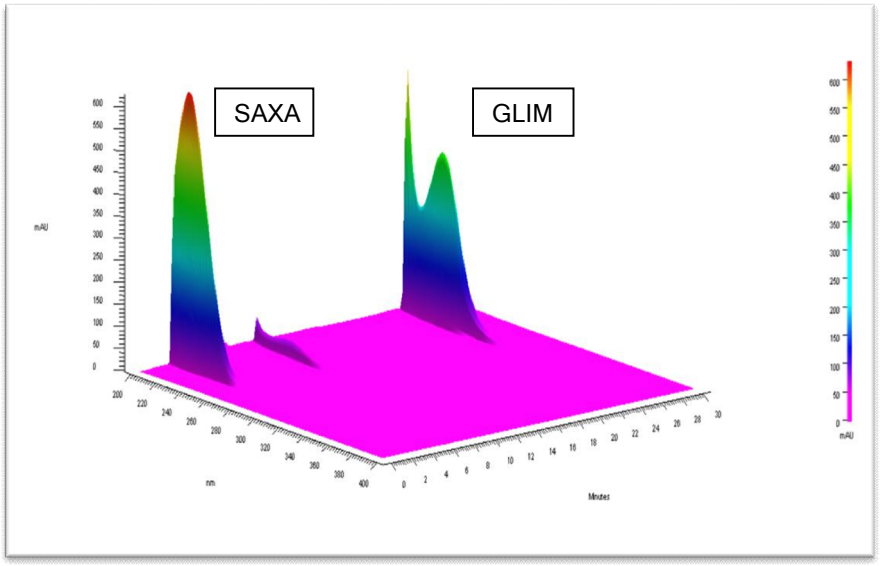


Figure4 3DHPLCchromatogramcombinationshowingthepeaksof SAXA,andGLIM at 3.28 mins,and 26.16 mins,

Construction of calibration curve

Five dilutions of SAXA, and GLIM were prepared from the simultaneous stock solution (SAXA:250µg/mL and GLIM:125µg/mL), by serial dilution. Each concentration was analyzed in triplicate and peak areas were recorded. % RSD was less than 2%, at each concentration, for both the drugs. The calibration graphs were also plotted between average peak area and concentration, for both SAXA, and GLIM.

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Table 2: Calibration curve data for analytical method of SAXA and GLIM

S.No.	Conc. (µg/mL)	Peak area			Avg. Area	SD	%RSD
SAXA							
1	15.63	483537	485793	493380	487570	5157	1.06
2	31.25	610970	610982	597819	606590	7596	1.25
3	62.50	1245882	1233108	1238495	1239162	6413	0.52
4	125.00	2494248	2401024	2452154	2449142	46685	1.91
5	250.00	5056333	5062130	5110675	5076379	29842	0.59
GLIM							
1	7.81	2084428	2070281	2021985	2058898	32741	1.59
2	15.63	2494944	2509772	2458197	2487638	26552	1.07
3	31.25	5347299	5238120	5283271	5289563	54861	1.04
4	62.50	9841902	9898158	9871476	9870512	28140	0.29
5	125.00	20066074	19865040	19949643	19960252	100936	0.51

Avg.=Average; Conc.= Concentration; RSD= Relative standard deviation; SD=Standard deviation

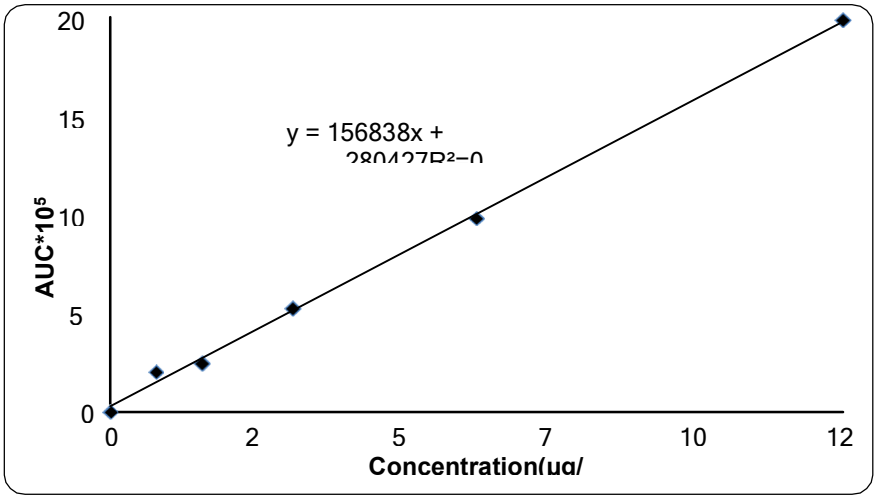


Figure3: Calibration curve for GLIM

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

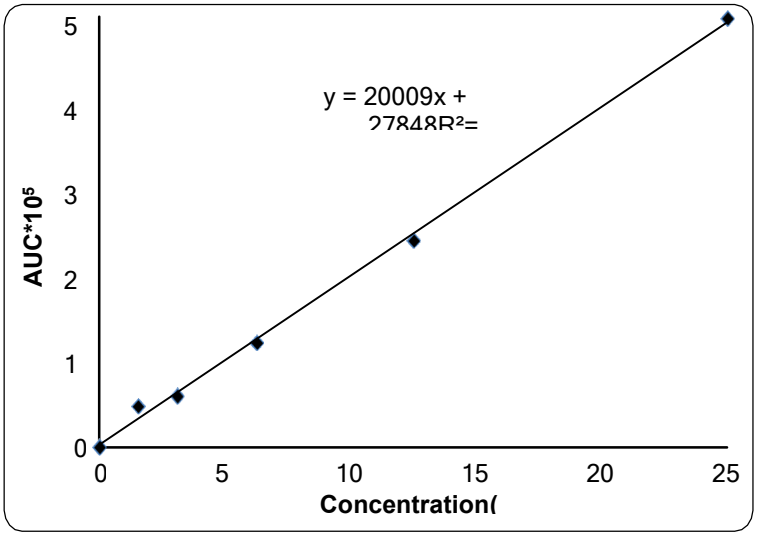


Figure4: Calibration curve for SAXA

All drug samples were protected from the light by employing amber coloured glass vials.

Preparation of calibration graph

From the simultaneous stock solution, required five serial dilutions of SAXA (15.63, 31.25, 62.50, 125.00, 250.00 µg/mL), and GLIM (7.81, 15.63, 31.25, 62.50, 125.00 µg/mL), were prepared, and injected in HPLC at selected wavelength, in triplicate. A calibration curve was prepared between concentration, and average peak area, for both the drugs.

Validation study

Linearity and range

The linearity of the method used for the analysis of SAXA, and GLIM was evaluated from the calibration curves plotted between peak area and analyte concentration. Calibration curves ,taking five points, in each case, were generated on the three consecutive days, with standard working solutions. Range is defined as the interval between the minimum and maximum concentration that is analysed by the given chromatographic conditions, within permissible limit of precision and accuracy. Least square linear regression analysis, was applied on the obtained chromatographic data, employing MS-Excel 2013 spreadsheet software and the corresponding statistic study,was carried out. The regression analysis was performed by devising the regressionequation in the form of $y = mx + c$ where, y = peak area, x = Concentration, m = slope and c= y-intercept.

Accuracy

Accuracy was determined by standard addition

method (spiking) for both the drugs.Therein, a known amount of active (SAXA and GLIM), was added to a determined amount of placebo. The three concentrations used were 50, 100 and 150% respectively, in previously analysed samples at 63.50 µg/mL for SAXA, and 31.35 µg/mL for GLIM. The study was carried out in triplicate for each concentration level. The accuracy, of the developed method was calculated based on % recovery.

Precision

The precision study was carried out as per the ICH guidelines (Rao et al., 2013). The first one consisted of checking instrumental precision, where in a sample corresponding to a concentration, within the linearity range, was injected six times, consecutively into the chromatograph. Inter-day and intra-day precision were determined by analyzing three different concentrations for each drug in triplicate, selected from the linearity range,i.e.,31.25 µg/mL (LQC), 62.50 µg/mL(MQC), and 125.00 µg/mL (HQC) for SAXA, and 15.63 µg/mL (LQC), 31.25 µg/mL (MQC), and 62.50 µg/mL (HQC) for GLIM. Forinter-day precision, three replicates of each concentration were analysed on 1st and 2nd day, while for intra-day precision, same set off the concentrations were also analysed at different times, within a day. The concentrations for each drug were prepared from the same stock solution. The peak area was determined, and the precision was reported as % relative standard deviation (RSD).

Robustness

The study of robustness was carried out to evaluate the influence of small, but deliberate variations, in the chromatographic conditions, for the simultaneous determination of SAXA, and GLIM,in the tablets. The chromatographic factors, chosen for this study, were as follows:

Detection wavelength: 225 nm and 235nm

Flow rate: 0.70mL/min and 0.80mL/min

Mobile phase: A:B :: 28:72 for 0-2 mins, A:B:: 48:52 for 2-25 mins,and A:B ::28:72 for25-30mins. and A:B::32:68 for 0-mins A:B::52:48 for 2-25mins, and A:B:: 32:68 for25-30 mins. Where mobile phase A: acetonitrile (ACN), and mobile phase B: buffer (was 0.1%sodiumdi-hydrogen ortho-phosphate, pH3.8 adjusted with Orthophosphoricacid) The peak area was determined, and the robustness was reportedas % RSD.

Detection limit and quantification limit

LOD, is the smallest concentration, which the analytical method,on a given instrument and chromatographic conditions, is able to differentiate the compound from the background noise, While, the LOQ, is the smallest concentration which is quantifiable, with defined precision, and accuracy (ICH, 2005). To determine LOD, and LOQ separately for SAXA, and GLIM, a sample containing

selected minimum concentration for each drug, was injected repeatedly, and analysed to calculate the standard deviation. Finally, LOD, and LOQ were calculated as per the equations 3.3and 3.4, wherein, "s" is the slope of the curve(s), and σ is standard deviation of y-intercept of regression line. Systems uitability testing (FDA, 2015;ICH,2005) The system suitability of the developed analytical method was studied for the different chromatographic parameters *viz.,* tailing factor (Tf), number of theoretical plates (N), retention time (Rt), asymmetric factor (As) of the peaks, HETP, area, resolution(Rs), and selectivity(α)

System suitability was evaluated on the following parameters, by analyzing six replicates of the analyte concentration i.e., SAXA,and GLIM.

a. Injection repeatability: Peak areas and retention times of six replicates separately for SAXA, and GLIM were recorded, and calculated the % RSD which should be less than one.

b. Tailing factor : Tailing factor was calculated

Preparation and evaluation of tablet formulation

Preformulation study

Melting point determination

The melting point (°C) of the SAXA and GLIM drugs, were recorded on melting point apparatus. All samples were run at a heating rate of 20°C/min over a temperature range 40-430 °C using Sentwin, melting point apparatus.

FTIR spectroscopy

Infrared spectroscopy was performed using Attenuated total reflection Fourier transform infrared spectrometer (ATR-FTIR) to determined interaction between excipients, and the active drugs (SAXA and GLIM). All the excipients, drugs, and optimized formulation, were determine against FTIR light wave number ranging from 4000 cm^{-1} to 650 cm^{-1}. All FTIR spectra were recorded using an ATR stage

Preparation of tablets

Wet granulation method was employed to prepare the tablet containing the active drugs, i.e., SAXA and GLIM (Patel et al., 2016). The process flow diagram of tablet preparationis depicted in Figure.

The active drugs and excipients were passed through different mesh screens. SAXA, GLIM, MCC, lactose monohydrate, and cornstarch, were passed through 30 mesh, while magnesium stearate and talc, were passed through 60 mesh screen.

A 10 % w/v aqueous solution of starch was employed as a binder. Drugs (SAXA and GLIM), MCC, and lactose monohydrate were mixed thoroughly in a required amount,and sufficient volume of binder solution was added, to obtain a damp mass. Afterwards, the mass was sieved through 20 mesh screen to obtain

granules, and were subsequently dried at 60 °C for 1 h. Finally, magnesium stearate, and talc were added to the granules, and mixed uniformly. Lastly, the tablets were compressed by using eight station tablet punching machine.

Bioanalytical RP-HPLC method development and validation for SAXA and GLIM

Optimization of chromatographic parameters

For the development of simultaneous bio-analytical method of SAXA and GLIM, the chromatographic column employed was C-18 (5 µm, 4.5 mm X 250 mm). The same chromatographic conditions, *viz.* ,mobile phase composition, elution type and flow rate were considered for the optimization of bioanalytical method. The details are already explained.

Stock solutions

Standard solutions of SAXA, and GLIM, separately were prepared at a concentration of 1000 µg/mL for each, dissolving the appropriated amount of bulk material in ACN.

Based on these solutions, and by means of an adequate dilution, a simultaneous stock solution was prepared by above mentioned SAXA, and GLIM, solutions in required ratio to make the concentration of SAXA as 500 µg/mL, and GLIM as 250 µg/mL.

Working solutions with plasma treatment

Working solution was prepared by protein precipitation of plasma using ACN as a precipitation solvent. In 200 µL of rat plasma taken in 1.5 mL of micro centrifuge tubes, 200 µL of stock solution containing SAXA, and

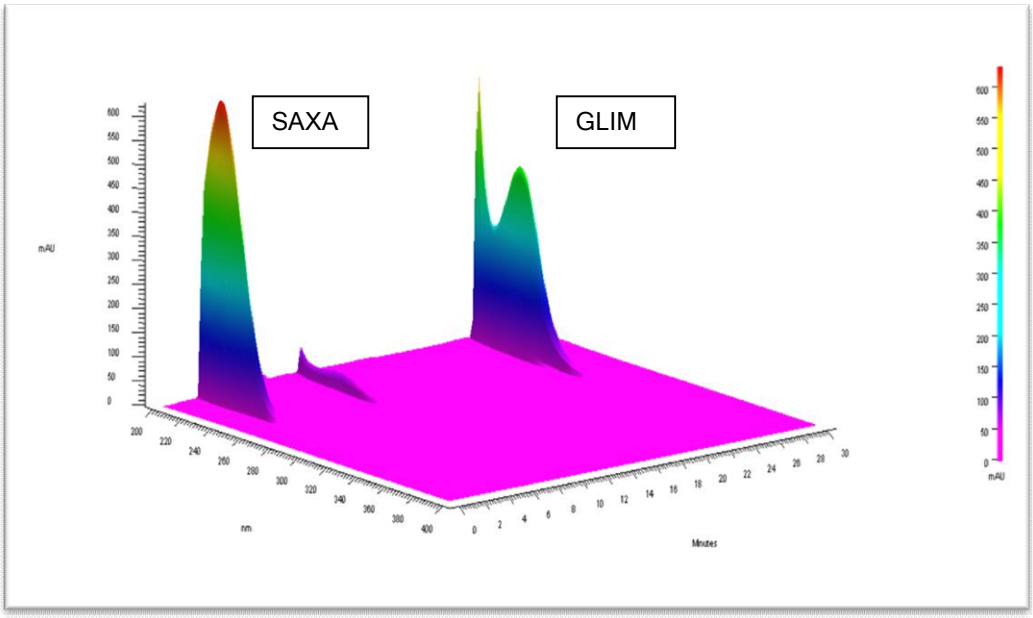


Figure 5 : 3D HPLC chromatogram combination showing the peaks of SAXA, and GLIM at 3.28 mins, and 26.16 mins, respectively

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Table3: Calibration curve data for analytical method of SAXA and GLIM

S.No.	Conc. (µg/mL)	Peak area			Avg. Area	SD	%RSD
SAXA							
1	15.63	483537	485793	493380	487570	5157	1.06
2	31.25	610970	610982	597819	606590	7596	1.25
3	62.50	1245882	1233108	1238495	1239162	6413	0.52
4	125.00	2494248	2401024	2452154	2449142	46685	1.91
5	250.00	5056333	5062130	5110675	5076379	29842	0.59
GLIM							
1	7.81	2084428	2070281	2021985	2058898	32741	1.59
2	15.63	2494944	2509772	2458197	2487638	26552	1.07
3	31.25	5347299	5238120	5283271	5289563	54861	1.04
4	62.50	9841902	9898158	9871476	9870512	28140	0.29
5	125.00	20066074	19865040	19949643	19960252	100936	0.51

Avg.=Average; Conc.= Concentration; RSD= Relativestandard deviation;SD=Standard deviation

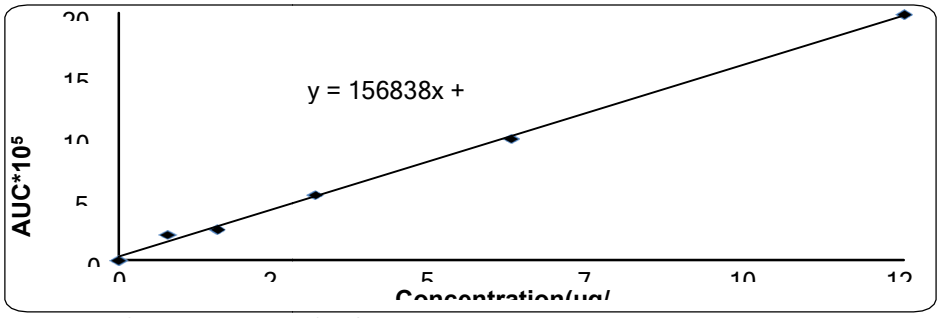
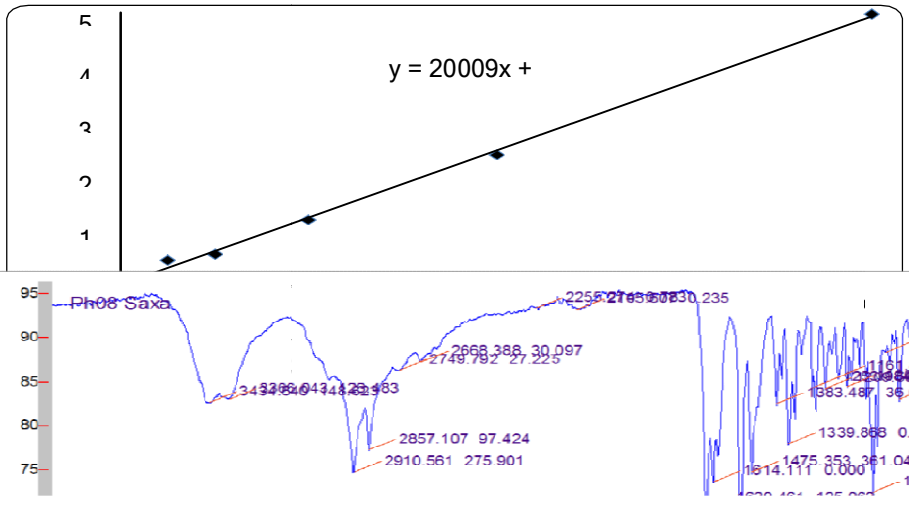


Figure 6: Calibration curve for GLIM



ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Figure8: FTIR spectrum of SAXA

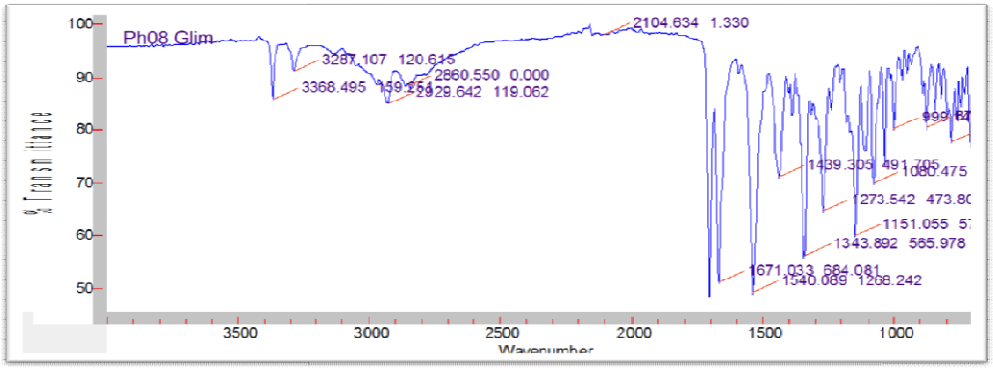


Figure 9: FTIR spectrum of GLIM

Table 4: Interpretation data of SAXA

Peaks at wavenumber (cm^{-1})	Interpretation
3434.64	OH stretching
2910.56	C-H stretching
2914	NH stretching
1644	Amide C=O stretch
1516.04	C-N stretching
1032.22	C-O stretching

Table 5: Interpretation data of GLIM

Peaks atwavenumber(cm^{-1})	Interpretation
3368.495	N-H stretch(Secondaryamine)(Dueto SO_2NH-)
3287.107	N-H stretch (Secondaryamine)(DuetoCO-NH-)
3135.24	C-H stretch (aromatic)
2929.648	C-H stretch(aliphatic)
1708.12	C=O stretch
1671.033	N-C=O stretch

GLIM, was added. Afterward 600 µL of ACN was added and vortexed for 20 sec. Now, the resulting solution was centrifuged at 5000 rpm for 10 min. The supernatant was taken, and filtered through nylon syringe filter paper (0.22 µm), transferred to HPLC vials. The working solution so prepared contained 100 µg/Ml of SAXA, and 50 µg/mL of GLIM.

Preparation of calibration curve

From the simultaneous stock solution, required serial dilutions were prepared to have different concentration of SAXA (0.50,1, 2,4,6,8,10,20, 30, 40, 50, 60 µg/mL) and GLIM (0.25, 0.50, 1,2,3,4,5,10,15,20,25,30 µg/mL).10µL of each Concentration was injected in HPLC at selected wavelength in triplicate. A calibration curve was prepared between concentration, and average peak area.

Validation study

Validation study, was performed according to the similar methodology, as described under section however, the samples were performed by spiking with rat plasma(Shahet al., 2000; Burhenne2012).Mumbai, India) was used to perform the dissolution test. Disintegration apparatus (INCO, Ambala, India) was employing to perform the disintegration test .

FTIR spectroscopy study

FTIR spectra of pure SAXA and GLIM are depicted in the Figure, While the ir interpretations, are included in the Table. Overlay FTIR spectra, of drugs and the excipients, and the optimized formulation are portrayed in Figure. The FTIR spectrum of both SAXA, and GLIM matched with the reported literature (Rani andAbbulu 2014; Jyothi et al., 2014; Kataria et al., 2016). It is vivid from figures that all the peaks pertaining to the excipients are intense, and clear in the FTIR spectrum of the tablet formulation Thus it can be inferred that there wash no interaction, between the drugs and the excipients, employed in the formulation of tablets.

CONCLUSION:

RP-HPLC analytical, and bio-analytical methods, developed for determination of SAXA and GLIM was found to be linear, accurate, precise, robust, suitable, sensitive, and specific, as per the regulatory guidelines. Both the methods involved simple and simultaneous estimation of SAXA and GLIM with gradient elution, and separations were performed at ambient temperature.

REFERENCE

1. Abu Ruza S, Millership J, McElnay J. 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 . 2005; 817:277-86.

2. Acharya S, Patra S, Pani NR.

Optimization of HPMC and carbopol concentrations in non-effervescent floating tablet through factorial design. Carbohydrate Polymers .2014; 102:360-8.

3. Ahlqvist E, Stormle P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol. 2018;6:361-9.

4. Ahren B. Emerging dipeptidyl peptidase-4 inhibitors for the treatment of diabetes. Expert Opinion on Emerging Drugs. 2008; 13:593-607.

5. Bhardwaj SK, Dwivedi K, Agarwal KK. A Review: HPLC Method Development and Validation. International Journal of Analytical and Bioanalytical Chemistry.2015;5(4):76-81.

6. Bhargavi S, Suryasagar G, Sowmya DK, Ashok K, Nama S. UV Spectrophotometric Method for Determination of Glimepiride in Pharmaceutical Dosage Forms. International Journal of Pharmaceutical Sciences Review and Research. 2013; 21:131-3.

7. Bondu AK, Chipirishetti R, Pillutla B, Bodapati P, ChP,Talari S. Method Development and Validation of Metformin Hydrochloride and Saxagliptin by RP-HPLC.World Journal of Pharmaceutical Research. 2014;3(4):1549-57.

8. Brubaker PL, Drucker DJ .Min ireview: Glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. Endocrinology.2004; 145(6):2653-9.

9. Burhenne J .Bioanalytical Method Validation. Journal of Analytical and Bioanalytical Techniques. 2012; 3(7):1000.

10. Caglar S, Alp AR. A Validated High Performance Liquid Chromatography Method for the Determination of Saxagliptin and Metformin in Bulk, a Stability Indicating Study. Journal of Analytical and Bioanalytical Techniques. 2014; 12:1-5.

11. Chacra AR, Tan GH, Apanovitch A, Ravichandran S, List J, Chen R. Saxagliptin added to a submaximal dose of sulphonylurea improves glycaemic control Compared with up titration of sulphonylurea in patients with type 2 diabetes: a randomized controlled trial. International Journal of Clinical Practice.2009; 63(9):1395-406.

12. Chacra AR, Tan GH, Ravichandran S, List J, Chen R. Safety and efficacy of saxagliptinin combination with submaximal sulphonylurea versus up-titrated sulphonylurea over 76 weeks. Diabetes and Vascular Disease Research. 2011;8(2):150-9.

13. Deeb SE, Schepers U, Tzig HW. Fast HPLC method for the determination of glimepiride, glibenclamide, and related substances using monolithic column and flow program. Journal of Separation Science.2006; 29(11):1571-7.

14. De Fronzo RA, Hissa M, Garber AJ, Gross JL, Duan RY, Ravichandran S, etal. The efficacy and safety of saxagliptin when added to metformin therapy in patients with inadequately
controlled type 2 diabetes on metformin alone. Diabetes Care.2009; 32:1649-55.

15. Derosa G, Cicero AF, Fogari E, D'Angelo A, Bianchi L, Maffioli P. Pioglitazone compared to glibenclamide on lipid profile and inflammation markers in type 2diabetic patients during an oral fat load. Hormone and Metabolic Research. 2011; 43:505-12.

16. Derosa G, Maffioli P, Ferrari I, Mereu R, Ragonesi PD, Querci F, et al. Effects ofone year treatment of vildagliptin added to pioglitazone or glimepiride in poorly controlled type2 diabetic patients. Hormone and Metabolic Research. 2010;42:663–9.

17. Devi R, Mohammad H.Stability Indicating RP-HPLC Method for the Simultaneous Determination of Atorvastatin Calcium, Metformin Hydrochloride, and Glimepiride in Bulk and Combined Tablet Dosage Form. International Scholarly Research Notices. 2014; 1-