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Original Article

SAXAGLIPTIN LEVELS AND ITS PHARMACOKINETIC APPLICATION IN PRESENCE OF SUCRALOSE IN ANIMALS SERUM BY HPLC METHOD

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ABSTRACT

Objective: It is to develop a simple, valid and rapid chromatographic method for quantification of saxagliptin in rat's serum in order to study saxagliptin pharmacokinetics parameters in sucralose fed rats simultaneously to detect any interaction possibility between saxagliptin and sucralose in rats.

Methods: In our developed method of analysis, mobile phase was consisted of phosphate buffer (pH =4) and methanol (70:30) v/v at flow rate of 1 ml/min with UV detection at 230 nm., C8 column of separation was used with the temperature of 40 °C using injection volume of 50 µl, samples run time was 10 min, and sildenafil citrate was used as internal standard. Saxagliptin was given to rats orally of (2g/kg) dose while sucralose was given with (11 mg/kg/day) dose.

Results: A successful HPLC method was validated and developed to determine saxagliptin in rats serum, overall intra-day precision and accuracy were reasonable with coefficient of variation percentage CV % values range (o.14-4.03) and accuracy % range (99.5-104), while inter-day precision and accuracy showed accepted precision with CV% range (0.15-2.81) and accuracy % range (99.9-116). The coefficient of correlation was 0.99949 with reasonable sensitivity and selectivity. Combination effect of saxagliptin with sucralose on saxagliptin serum profile was demonstrated as strong statistical effect according to Cohen's d and significant P values too.

Conclusion: A successful HPLC method was validated and developed to quantify saxagliptin in rats serum, combination effect of saxagliptin with sucralose over all time intervals of saxagliptin serum profile was demonstrated as strong statistical effect.

Keywords: HPLC, Saxagliptin, Sucralose, Pharmacokinetic, Interaction.

INTRODUCTION

Saxagliptin: ((±)-5-(4-(2-(5-ethyl-2-pyridinyl) ethoxy) phenyl) methyl)-2, 4-) thiazolidinedione monohydrochloride [1], the structural formula is as shown in fig. 1. It belongs to a different chemical class with different pharmacological action than the sulfonylureas, metformin, or the α-glucosidase inhibitors [2], it is a compound that belongs to a group named "thiazolidinediones" family, an oral antidiabetic agent that acts by decreasing insulin resistance [3]. Saxagliptin has the same mechanism of action by which all thiazolidinediones act inside the body. Its mechanism of action made it as one of the most effective drugs that is used in the management of type 2 diabetes mellitus [4, 5], saxagliptin can improve the sensitivity to insulin in muscle and adipose tissue [2, 7] which helps in hepatic gluconeogenesis inhibition [8] and improves glycemic control by reducing circulating insulin levels [9, 10].

Type 2 diabetes patients are usually treated with numerous pharmacological compounds which increases the susceptibility to be exposed to risky drug-drug interactions [7].

Saxagliptin was marketed at USA in 1999, now a days; it is marketed in more than 40 countries world wide [6].

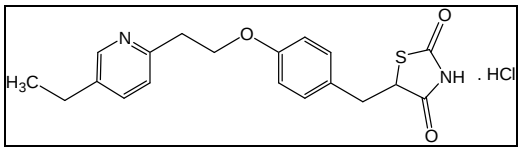

Fig. 1: Saxagliptin hydrochloride chemical structure

Saxagliptin is extensively metabolized by hydroxylation and oxidation via liver CYP450 enzymatic system [8], *in vitro* data emphasized that multiple CYP isoforms are involved in saxagliptin metabolism, mostly: CYP2C8 and CYP3A4 [10, 17].

Sucralose is a synthetic organochlorine sweetener, with chemical formula of Dichloro- 1, 6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy α-D-galactopyranoside [11], the chemical structure is shown in fig. 2. It is considered as one of the most common sweeteners in the world's food industries [12] and pharmaceutical manufacturing [13].

Sucralose is a derivative of the halogenated sucrose, mellow, with aromatic flavor and good stability, low in calories (around 3 calories per 1 gm), there are significant gaps in our current knowledge concerning pharmacokinetics of these sweeteners and their potential for sweetener–drug interactions. Recently, previous studies have approved that sucralose increases the expression of the P-glycoprotein intestinal transporter and induces CYP3A4 enzyme activity in intestine and liver [14, 15] at levels that have been associated with reduced bioavailability, pharmacokinetic and pharmacodynamic parameters of drug, it may occur if the drug is to be metabolized by CYP3A4 enzyme and concurrently taken with sucralose at doses that are approved by the FDA, these findings justify the need of further studies concerning potential sucralose-drug interactions.

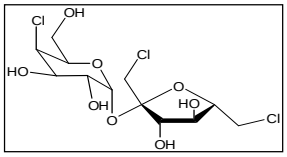

Fig. 2: Sucralose chemical structure

In our current study we aimed to design a new, simple, valid and rapid high performance liquid chromatography-ultraviolet method

according to EMEA guidelines for quantification of saxagliptin in rat serum in presence and absence of sucralose to determine any possible interaction between both substances through the monitoring of saxagliptin serum levels when sucralose is given concurrently.

MATERIALS AND METHODS

Chemicals

High performance liquid chromatography-ultraviolet HPLC/UV is a technique of analysis that could be used for determination of several compounds in plasma or serum [16, 18].

Methanol and phosphate buffer HPLC gradient were used for HPLC analysis (FULLTIME, USA), deionized water HPLC gradient was obtained from (TEDIA, USA), while saxagliptin hydrochloride raw material was from (JPM, Jordan).

Sildenafil citrate, the internal standard was purchased from (YASHICA pharmaceuticals, India), while sucralose was acquired from its finished product Splenda®.

Apparatus and chromatographic conditions

The HPLC system (HITACHI, Japan) consisted of S # L-2130 VWR-HITACHI pump model, S# L-2200 VWR-HITACHI auto sampler thermostat, S# L-2300 VWR-HITACHI column oven and S# L-2420 VWR-HITACHI UV detector.

Chromatographic separation was carried out using mobile phase consisted of (70:30) v/v phosphate buffer and methanol with pH of 4, column of separation was (ACE C8, 5 µm (250 x 4.6 mm i.d.) at temperature of 40 °C using injection volume of 50 µl, mobile phase flow rate was 1 ml/min and samples run time was 10 min, the signals were monitored and analyzed at λ= 230 nm and sildenafil citrate was used as internal standard.

Preparation of stock and working solutions

Saxagliptin oral dose of (2g/kg/day) was recommended for rats and prepared by dissolving the drug in methanol while sucralose oral dose of (11 mg/kg/day) was prepared as a working solution of (250 mg/ml) Splenda®, sucralose solution was prepared by dissolving Splenda®powder with distilled water at room temperature under sonication, both working solutions were prepared freshly and directly before experiment and kept away of heat and light.

Saxagliptin stock solution was prepared by dissolving (25 mg) in 50 ml methanol to get a stock solution of 500 µg/ml while saxagliptin working solutions were prepared from the stock solution by dilution with methanol directly before use and kept away of light and heat sources, sildenafil citrate was used as internal standard.

Calibration samples

Serial dilutions of samples were prepared by taking an appropriate volume of each working concentration that is previously prepared in methanol with sufficient volume of rats serum was added to reach 5 ml final volume with corresponding calibration concentration. Samples were vortexed, then used for analysis procedure, all final standard solutions were prepared freshly and directly before analysis. Six different calibration concentrations were analyzed under the same HPLC conditions for each calibration curve, peak area ratios were calculated and the correlation coefficient r^2 was accepted if it is more than 0.98.

Sample preparation (Extraction procedure)

Sample preparation was done by mixing 100 µl of serum with 75.0 µl of internal standard IS working solution, then sample was vortexed for 30 seconds and centrifuged at 12000 rpm for 5 min. Supernatant was transferred into rack, and 50 µl of supernatant was injected into HPLC unit.

Precision and accuracy

To examine and evaluate the precision and accuracy of our method of analysis, four different concentrations were prepared and used; lower limit of quantification, low, middle and high concentrations of saxagliptin by spiking with rats serum.

Intra-day precision and accuracy were evaluated by analyzing the four concentrations five times sequently in the same day while the same procedure was done at three different days for inter-day precision and accuracy evaluation.

Precision was assessed by comparing each concentration with the CV% (coefficient of variation) while the accuracy was assessed by comparing the mean calculated concentration with±20% of spiked concentration for lower limit of quantification while it was compared with±15% for other quality control samples.

Stability

Room temperature stability: three samples of quantification control low concentration (QCL) and quantification control high concentration)QCH) of concentrations (2 and 12.8 µg/ml) were prepared and analyzed with freshly prepared calibration at zero time and after 8 hs at room temperature of 28 °C. Area ratios % per QCL and QCH were calculated referring to related calibration.

Freeze and thaw stability: Three samples of QCL and QCH were prepared properly in serum with sufficient final volume covering all test cycles analysis.

At zero time: with corresponding calibration, samples were analyzed and concentrations were calculated, then, samples were stored and frozen at-20 °C.

After 12 hs: samples were thawed at room temperature 25 °C which was the samples processing temperature during analysis stages. After complete thawing, samples were analyzed again with corresponding calibration, and refrozen again for 24 h.

After 24 hs: Samples were thawed and analyzed under the same conditions. After each cycle, concentrations were calculated referring to related calibration.

Long term stability: three samples of (QCL and QCH) were analyzed with freshly prepared calibration at zero time. Samples then were frozen for 30 days at temperature of-20 °C. After long term freezing period was finished, samples were removed and thawed at room temperature.

Room temperature and long term stability test were also carried out for working solutions, stability test was assessed by comparing the calculated concentrations with the nominated concentrations as it should be within±15% for both QCL and QCH.

Preclinical study

The study protocol was approved by the Research Committee (October; 5/10/2013) at the Faculty of Pharmacy, University of Petra, Amman, Jordan.

Adult male Sprague Dawley laboratory rats were supplied at Petra University Animal House. Rat average weights were (0.230 kg±0.03). Rats were placed in air-conditioned environment with temperature of (20-25 °C) and exposed to a photoperiod cycle (12 h light/12 h dark) with humidity of 50% daily. Rats were under fasting for 24 hs, and weighed directly before the experiment. All used rats were in healthy conditions before and after experiment as rats were monitored for one month post analysis.

Saxagliptin and sucralose test solutions were freshly prepared directly in the laboratory before rats feeding in order to avoid any possible decomposition of either saxagliptin or sucralose.

A group consisting of total 80 healthy rats was used for the experiment. Rats were divided into groups of 8 rats, rats then were weighed and numbered orderly. Saxagliptin and sucralose oral doses were calculated according to each rat weight then given orally according to ordered numbers using gastric gavage. Trials analysis was performed among 3 days according to following arrangement:

At first day of trials: four groups of rats were used for analysis; two groups have received water at experiment zero time, then followed by saxagliptin after 1 h of water feeding, while the other

two group have received sucralose at zero time of experiment followed by saxagliptin after 1h (of sucralose feeding. Time intervals of blood samples pooling were: 0, 30 minutes, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 24 h.

At second day of trials: two groups have received water at zero time of experiment followed by saxagliptin after one h, while other two groups have received sucralose at zero time followed by saxagliptin after 1 h of sucralose feeding. Time intervals of blood samples pooling were: 0, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 24 h.

At third day of trials: two groups have received water at zero time of experiment followed by saxagliptin after one h, while the other two groups have received sucralose at zero time followed by saxagliptin after 1 h of sucralose feeding. Time intervals of blood samples pooling were: 0, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 24 h.

Tail tip of each rat was cut after weighing and numbering, approximately 200 µl of blood was pooled into eppendorf tube at each time interval under the same numbering order, after total time intervals of blood pooling is finished, samples were centrifuged for 10 minutes (12000 rpm) in order to obtain pure serum that is needed for analysis than frozen at-20 °C.

RESULTS AND DISCUSSION

Acceptable separation of saxagliptin and sildenafil citrate in serum was obtained as the saxagliptin peaks were sharp with no tailing or splitting, reasonable retention time and clear chromatograms, as mentioned in fig. 3, 4, 5 and 6 which represents an overlay chromatograms for blank rats serum, rats serum with IS, rats serum with LLOQ, and rats serum with IS and saxagliptin after 30 minutes of combination respectively.

Precision, accuracy and linearity were measured to estimate the method performance.

Linearity calibration curves were designed with six different trials, method calibration curves and correlation coefficient were collected after 6 replications, the correlation coefficient and calibrations equations were mentioned in table 1.

Table 1: Linearity calibration curves data for six calibration curves

Calibration no.	Calibration equation	Correlation coefficient (r^2)value
Calibration 1	y = 0.100898 x-0.00098911	0.999888
Calibration 2	y = 0.109219 x+0.00043395	0.999776
Calibration 3	y=0.099599 x-0.001155059	0.999166
Calibration 4	y = 0.101335 x-0.005413046	0.999990
Calibration 5	y = 0.098772 x+0.00257727	0.998991
Calibration 6	y = 0.096640 x-0.01064449	0.999111

High correlation coefficient which was over 0.99 for all calibrations indicated the reasonability of method linearity between the peak area ratio of saxagliptin in the serum and the quality control concentrations used in calibrations.

Inter-day and intra-day precision showed acceptable values as LLOQ the lower limit of quantification showed coefficient of variation (CV%) less than 20% while QCL, quantification control medium concentration QCM and QCH represented coefficient of variation values less than 15%, inter-day and intra-day results mentioned in table 2.

On the other hand, Inter-day and intra-day accuracy showed reasonable values as mean calculated concentration of LLOQ was within±20 % of nominated concentration while it was within±15 % for QCL, QCM and QCH.

Table 2: Inter-day over three days and intra-day precision and accuracy for QC samples

Inter-day: Day 1					
Sample ID	**Mean**	**SD**	**CV%**	**Error**	**Accuracy %**
ST(LLOQ)	0.26	0.0046	1.78	0.01	104
QCL	1.999	0.0038	0.191	0.01	99.95
QCM	7.10	0.0915	1.29	0.10	101.42
QCH	13.51	0.1229	0.91	0.15	105.54
Inter-day: Day 2					
ST(LLOQ)	0.25	0.0041	1.44	0.01	100
QCL	2.01	0.0030	0.15	0.02	100
QCM	7.31	0.0979	1.34	0.31	104
QCH	13.52	0.4502	3.33	0.60	105
Inter-day: Day 3					
ST(LLOQ)	0.25	0.0038	1.55	-0.004	100
QCL	2.14	0.0089	0.42	0.043	107
QCM	7.32	0.0856	1.17	0.42	104.57
QCH	13.71	0.3852	2.81	1.01	107.11
Intra-day					
ST(LLOQ)	0.26	0.0035	1.35	0.01	104
QCL	1.99	0.0027	0.14	-0.01	99.5
QCM	7.16	0.0959	1.34	0.16	102.2
QCH	13.31	0.5363	4.03	0.51	103.9

Three different stability tests were designed and carried out to assess the drug samples stability under all possible conditions that could be kept under among all experimental phases.

Freeze and thaw stability: data were obtained after each cycle of analysis for two quality control (QC) concentrations; QCL and QCH, samples were analyzed at zero time then after 12 h of freezing, samples were thawed for 3 hs then analyzed and finally after another 24 h of freezing and thawing, stability percentages were over 99% and accuracy percentages were over 98%. See fig. 9. All results were listed in table 3.

Table 3: Freeze and thaw stability data for QCL and QCH

QC Low (2 µg/ml)			
Time	**Mean**	**Accuracy%**	**Stability %**
0 h	2.01	103	
		102	
		99.8	
12 h	1.98	99.99	99
		99.98	
		99.97	
24 h	1.99	99.98	99.5
		99.98	
		99.87	
QC High (12.8 µg/ml)			
Time	Mean	Accuracy%	Stability %
0 h	12.7	99.68	
		99.89	
		99.79	
12 h	12.75	98.90	99.9
		98.79	
		98.99	
24 h	12.69	98.69	99.8
		98.87	
		98.89	

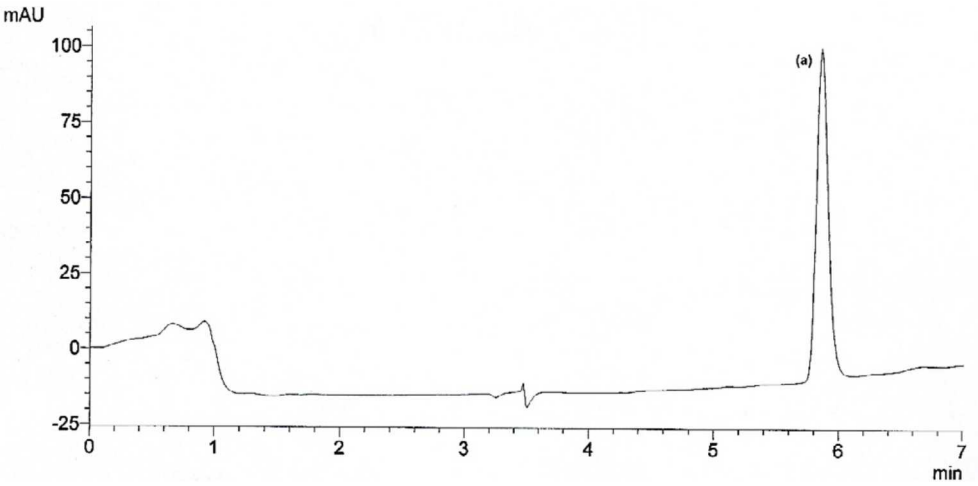

Fig. 3: Freeze and thaw stability chromatogram

Room temperature stability: serum samples of both QCL and QCH were analyzed at zero times and after 8 h of standing at room temperature of (28 °C±1), both stability and accuracy percentages were over 99%, see fig. 10, data was mentioned in table 4.

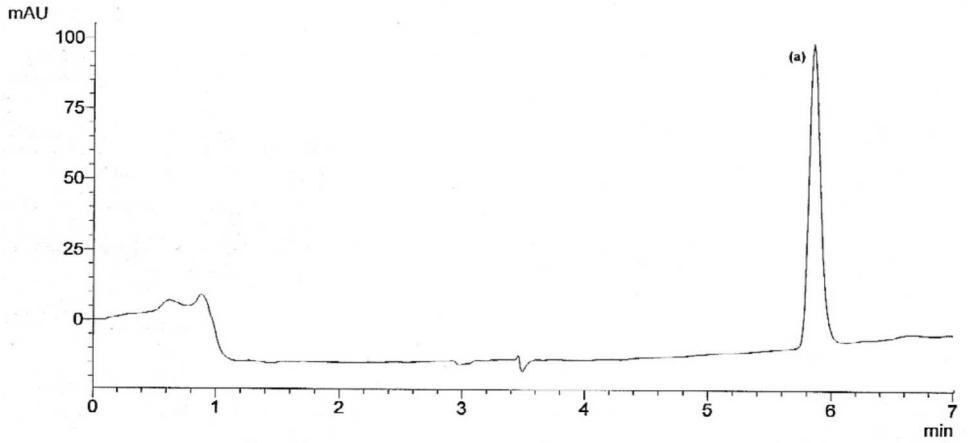

Fig. 4: Room temperature stability chromatograms

Table 4: Room temperature stability data for saxagliptin in serum for QCL and QCH

QC Low (2 µg/ml)			
Time (h)	**Mean**	**Accuracy%**	**Stability%**
0 h	2.1	110	99.8
		106	
		100	
8 h	2.08	105	
		99	
		99	
QC High (12.8 µg/ml)			
Time (h)	**Mean**	**Accuracy%**	**Stability%**
0 h	12.79	99.9	99.9
		100.1	
		99.9	
8 h	12.78	99.8	
		99.8	
		99.7	

Table 5: Long term stability data of saxagliptin in serum for QCL and QCH

QC Low (2 µg/ml)			
Time	**Mean**	**Accuracy%**	**Stability%**
0 h	1.98	99.72	99.90
		99.25	
		99.74	
30 days	1.99	100.90	
		99.91	
		99.50	
QC High (12.8 µg/ml)			
Time	**Mean**	**Accuracy%**	**Stability%**
0 h		98.89	99.88
		98.51	
		99.35	
30 days		99.95	
		99.27	
		98.81	

Table 6: First day trial serum data (number of groups) N=2 for both drug alone and drug with sweetener

Day-1-trials SX alone, N=2 SX+sucralose, N=2	**Serum average concentrations (µg/ml)**							
Group no.	**30 min**	**1 h**	**2 h**	**3 h**	**4 h**	**6 h**	**8 h**	**24 h**
Group 1+2 (SX alone)	5.516	10.992	15.139	13.003	10.939	8.941	6.203	0.511
Group 3+4 (SX+sucralose)	5.006	10.016	12.082	11.087	10.031	7.014	4.649	0.202

Long term freezing stability test was carried out as serum samples of both QCL and QCH were analyzed at zero time and after freezing for 30 days and thawing for 3 h, stability percentages were over 99% while accuracy % were over 98%,see fig. 11, data was collected then listed in table 5.

Sucralose–saxagliptin combination effect on saxagliptin serum levels: Rats serum levels of saxagliptin were calculated through serum samples analysis that obtained from rats groups per day of trials.

At first day of trials the samples were collected starting by zero time and ending by 24 h as last time interval,(fig. 3), samples were analyzed and data collected properly as in table 6.

At the second day of trials the samples were collected starting by zero time and ending by 24 h as last time interval, (fig. 4), samples were analyzed and data collected properly as in table 7**.**

At the third day of trials the samples were collected starting by zero time and ending by 8 hs as last time interval, (fig. 5), samples were analyzed and data collected properly as in table 8.

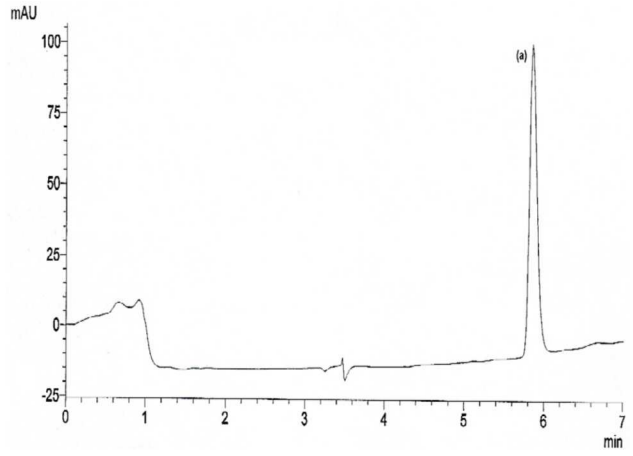

Fig. 5: Long term stability chromatograms

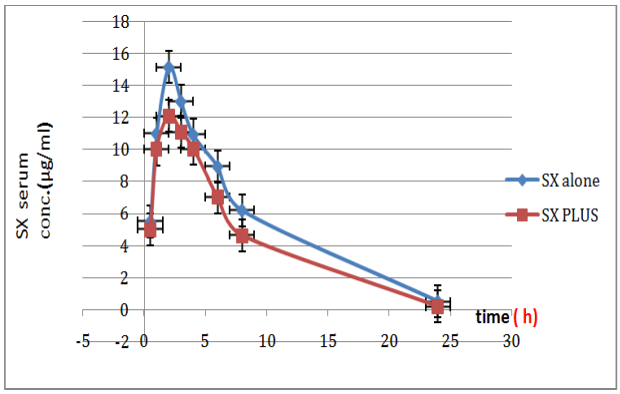

Fig. 6: First day trial serum–time profile curve

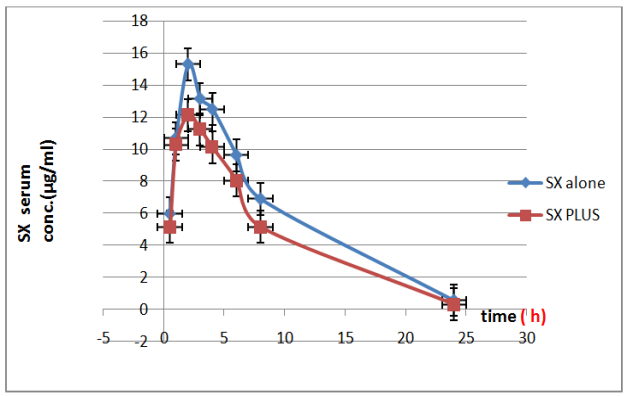

Fig. 7: Second day trial serum–time profile curve

Table 7: Second day trial data (number of groups) N=2 for both drug alone and drug with sweetener

Day-2-trials **SX alone, N=2** **SX+sucralose, N=2**	**Serum average concentrations (µg/ml)**							
Group no.	**30 min**	**1 h**	**2 h**	**3 h**	**4 h**	**6 h**	**8 h**	**24 h**
Group 1+2 (SX alone)	5.981	10.686	15.298	13.143	12.491	9.627	6.898	0.579
Group 3+4 (SX+sucralose)	5.146	10.274	12.130	11.241	10.125	8.035	5.145	0.313

Table 8: Third day trial data (number of groups) N=2 for both drug alone and drug with sweetener

Day-3-trials **SX alone, N=2** **SX+sucralose, N=2**	**Serum average concentrations (µg/ml)**							
Group no.	**30 min**	**1 h**	**2 h**	**3 h**	**4 h**	**6 h**	**8 h**	**24 h**
Group 1+2 (SX alone)	5.892	10.670	15.981	13.256	12.096	10.801	6.690	0.595
Group 3+4 (SX+sucralose)	5.682	10.400	12.023	11.025	10.713	8.707	5.011	0.302

Three days of trials results were collected with overall calculated serum concentrations profiles corresponding area under the curve (AUC) values, maximum concentration (Cmax) and maximum time (Tmax) were summarized in table 9, with statistical results, (fig. 6).

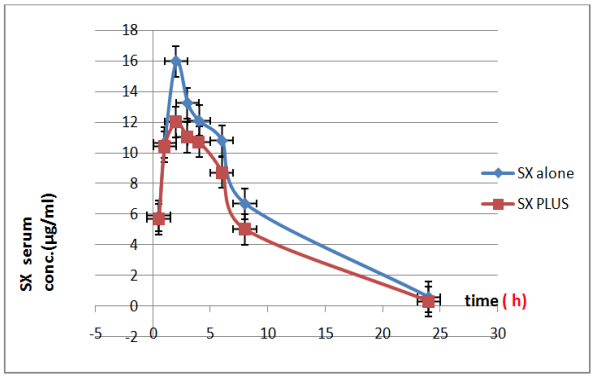

Fig. 8: Third day trial serum–time profile curve

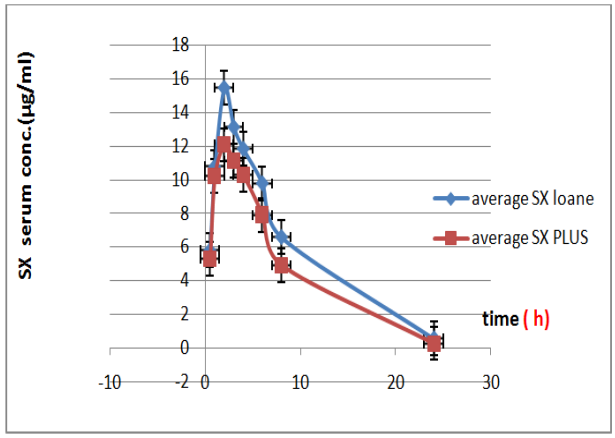

Fig. 9: Serum concentration–time profile graph (0-24) h of oral administration of drug, (SX = saxagliptin alone, SX Plus = saxagliptin with sucralose)

Table 9: Serum concentration–time profile kinetic parameters

Drug	**C max (µg/ml)**	**T max (h)**	**T ½ (h)**	**AUC (µg/mlh)**
SX	15.237±0.03	2	2	130.6±2.1
SX plus sucralose	12.078±0.1	2	2	100±2.0
P value	0.009**	**P < 0.01		0.001**

Concentrations values were treated statistically to evaluate the effect size and significance of combination between saxagliptin and sucralose, Cohen's d value was used for effect size evaluation of combination while P value was used for estimation of combination significance, Combination effect size values were exceeding 0.8 for all intervals except at 30 minutes as it was smaller, combination effect significance was strong as all P values were less than 0.01, Two examples of analysis chromatograms during HPLC procedure run were mentioned below (figure7 and fig. 8).

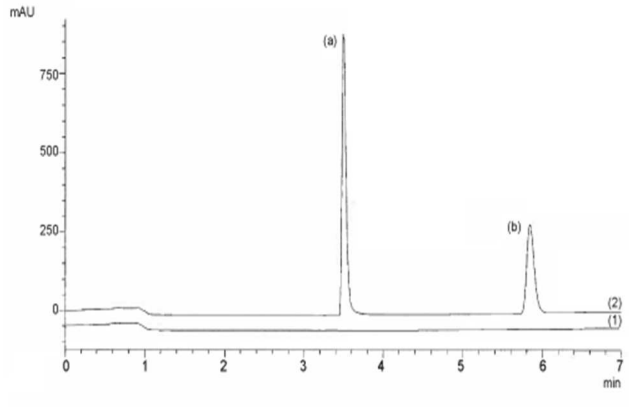

Fig. 10: Blank chromatogram (1) and separation chromatogram (2): Saxagliptin (b peak) and internal standrad slidenafil citrate (a peak) chromatogram

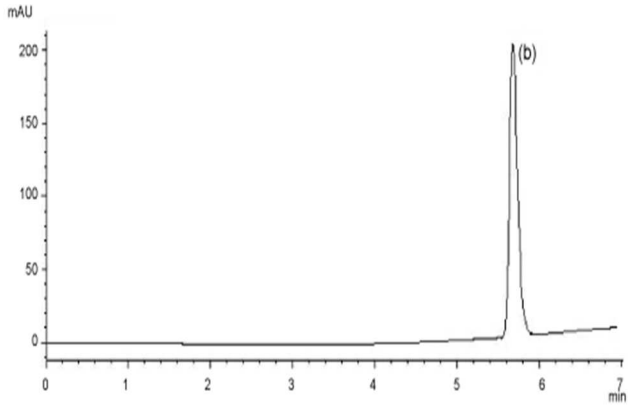

Fig. 11: Saxagliptin chromatogram

Method validation was evaluated referring to EMEA guidelines

Precision and accuracy results were reasonable as CV% values and mean calculated concentrations were within range of acceptance while method linearity performance was accepted as all correlation coefficients were over 0.98.

For method stability: three stability tests showed reasonable results, which confirm method stability as accuracy percentages and stability percentages were accepted with reasonable values.

Method of analysis was designed with a novel mobile phase consistence and low cost comparing with previously used mobile phases in other methods.

Sucralose–saxagliptin combination effect was first demonstrated in current research.

The statistical analysis of the results showed a significant combination interaction between saxagliptin and sucralose when both compounds are given concurrently, as P values represented strong combination effects, which were less than 0.01.

This combination interaction could be justified by sucralose induction effect on CYP 450 enzymes, specifically 3A4 subtype by which saxagliptin is extensively metabolized by it.

Saxagliptin is basically absorbed in stomach where CYP3A4 isoenzymes are located profusely in parietal cells endoplasmic reticulum, this enzyme will exert its metabolic biotransformational effect over saxagliptin once absorbed, induction effect of sucralose over the CYP3A4 metabolic enzyme will also activate the metabolism of saxagliptin in liver too, which results in saxagliptin plasma/serum levels reduction and over production of saxagliptin active and inactive metabolites.

In rats, CYP3A1, 3A2, 3A9, 3A18, 3A23 and 3A62 have been reported as CYP3A forms. CYP3A23 was classified as identical to CYP3A1 by the analysis of its gene.

CYP3A62 form has been identified as a new rat CYP3A isoenzyme with expression profile similar to human CYP3A4 and rat CYP3A9. CYP3A62 is a predominant form in the intestinal tract, where CYP3A1 and-3A2 were found only in liver [17].

As recent studies illustrated a distinctive binding affinity variance of saxagliptin to reactive sites of CYP3A enzymes during metabolic reactions which justifies its variable bioavailability between humans and rats, this variance impresses a possible perceptible clinical differences in saxagliptin human plasma levels when combined with sucralose, which strongly recommends further clinical research in human.

CONCLUSION

A successful HPLC method was validated and developed to quantify saxagliptin in rat's serum, the method was precise and accurate with rational linearity performance and reasonable sensitivity and selectivity.

Concerning stability and recovery tests, all obtained results were reasonable and accepted according to EMEA guidelines.

Combination effect of saxagliptin with sucralose over all time intervals of saxagliptin serum profile was demonstrated as strong statistical effect according to Cohen's d and significant P values too.

Cmax showed a significant change between presence and absence of sucralose while Tmax didn't show any change, which suggests the possibility of interaction between saxagliptin and sucralose during combination.

Advanced clinical research on human volunteers to make more precise results concerning saxagliptin–sucralose combination interaction is suggested through the detection and quantification of saxagliptin and its active metabolites as these metabolites are also pharmacologically active in the human body of the diabetic patient.

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ABBREVIATION

HPLC: high performance liquid chromatography, QC: quality control, EMEA: European medicines agency, LLOQ: lower limit of quantification, QCL: quantification control low concentration, QCM: quantification control medium concentration, QCH: quantification control high concentration, CYP: cytochrome p, CV%: coefficient of variation, AUC: area under the curve, Cmax: maximum concentration, Tmax: maximum time at Cmax, r^2: correlation coefficient, IS: internal standard, SX: saxagliptin, HR: h, ST: standard, NA: not available.

CONFLICT OF INTERESTS

Declared None

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