Determination of Sitagliptin Levels in Rats Serum by HPLC and its Pharmacokinetic Investigation in Existence of Sucralose

Wael Abu Dayyih^{1*}, Mohammed Hamad²

¹ Dept of Pharmaceutical Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy and Medical Sciences, University of Petra, Queen Alia International Road, Amman - Jordan 11196, Jordan

² Dept of Basic Sciences, Collage of Sciences and Health Professions, King Saud Bin Abdulaziz University for Health Sciences, Jeddah 21423, Saudi Arabia

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*Corresponding author
Wael Abu Dayyih

Email:
wabudayyih@uop.edu.jo

ABSTRACT

It is an interest of the current work to develop a simple, valid and rapid chromatographic method for the quantification of sitagliptin in rats' serum. Accordingly, the pharmacokinetic parameters of sitagliptin was investigated for any possible interaction between sitagliptin and sucralose. The developed HPLC method was validated for sitagliptin determination in rat's serum. Results indicated an accomplished overall intra-day precision and accuracy values of 0.139-4.028 (CV%) and 99.48-107.12% respectively. On the other hand, inter-day precision and accuracy results recorded values of 0.150-2.810 (CV%) and 99.90-116.00% respectively. Moreover, the coefficient of correlation was determined at a value of 0.99950 with reasonable sensitivity and selectivity. Effect of sucralose showed strong significant correlation on sitagliptin serum profile. The precise, accurate, linear, sensitive and selective HPLC method will be elaborated in the context of the current work with emphasis on sucralose negative effect on sitagliptin level in rat's serum. Such finding is a guidance for physicians to reconsider the use of such sweeteners for diabetic patients

Key words: sitagliptin, sucralose, tadalafil, pharmacokinetic, interaction.

INTRODUCTION

Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor drug that has been introduced in 2006 for the treatment of type-2 diabetes. As an inhibitor, its main mechanism of action depends primarily on the suppression of glucagon like peptide-1 (GLP-1) and glucose dependent insolinotropic polypeptide (GIP) actives and thus leading to the increase of insulin secretion (Herma *et al.*, 2006; Abu Dayyih *et al.*, 2015; Dubla *et al.*, 2012; Pathade *et al.*, 2011; Khan *et al.*, 2011).

Sitagliptin, PO₄ (STGPN) has a typical gliptin structure (Figure 1) and is chemically known as 1,2,4-triazolo[4,3-a] pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-, phosphate (Herma *et al.*, 2006; El-Bagary *et al.*, 2011; Pulla *et al.*, 2011; Shyamala *et al.*, 2011).

On the other hand, non-caloric sugar substitutes have become common food and beverage products that are widely used by

type II diabetic patients. Amongst these sucrose derivative compounds, sucralose (SUCS) -a synthetic trichlorinated disaccharide- has been accepted as a safe sweetener by international regulatory bodies. However, sweeteners, in general, are not regarded as biological inert ingredients. Moreover, their pharmacokinetics and drug interaction have not been thoroughly investigated. There are, however, recent studies which proved that sucralose alters physiological parameters, most importantly the ones associated with increased expression of glucagon-like peptide-1 and with induced CYP3A4 enzyme activity in intestine and liver (Patil *et al.*, 2010; Nikolaou *et al.*, 2011; Zeng *et al.*, 2010; Zeng *et al.*, 2008; Niroji *et al.*, 2008). Such parameters were reported to cause reduction in the bioavailability, pharmacokinetic, and pharmacodynamic activities of certain drugs.

However, the impact of sucralose on the chemical integrity and thus bioavailability of sitagliptin has not been investigated so far.

Figure 1. Chemical structures of (a) sitagliptin (STGPN), (b) sucralose (SUCS), (c) tadalafil.

It is suggested that such delay in drug-sweetener studies is attributed to the lack of simple analytical method for the determination of sitagliptin in plasma and serum (El-Bagary *et al.*, 2011; Patil *et al.*, 2010; Vincent *et al.*, 2007; Beconi *et al.*, 2007).

Methods for sitagliptin assay and related compounds determination according to EMEA guidelines were not given a substantial attention when searching for a realistically feasible technique (Beconi *et al.*, 2007; EMA, 2011; Matsubara *et al.*, 2004; Tamimi *et al.*, 2014; Abu Dayyih *et al.* 2018). The foregoing was based on the fact that the only method most commonly discussed on sitagliptin analytical determination in plasma and urine of humans, rats and dogs was a high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS).

However, although MS gives a highly accurate determination of assay and related values of the drug, its high cost still represents a constraint in equipment availability on a broad range of applications. Further looking into less sophisticated techniques, Ultra-violet (UV) spectroscopic determination of sitagliptin was found to be more appropriately considered for assay determination of the drug. However, in addition to its incapability to test drugs in serum, UV methods cannot distinguish between the drug and its impurities and therefore this method was, and is generally, not considered for related compound determination. Consequently, an alternative technique became a must to develop herein taking a challenge to analytically quantify the drug and its related compounds within the serum (Pathade *et al.*, 2011; Pulla *et al.*, 2011; Zeng *et al.*, 2008; Dayyih *et al.*, 2018).

Many methods had been developed to detect and measure sitagliptin; RP-HPLC Method (Dubal *et al.*, 2012; Shyamala *et al.*, 2011; Patil *et al.*, 2010), Sensitive liquid chromatography tandem mass spectrometry method (Zeng *et al.*,

2010; Nirogi *et al.*, 2008), GC/MS method (Nikolaou *et al.*, 2011), Spectrofluorimetric and spectrophotometric methods (El-Bagary *et al.*, 2011) and UV Spectrophotometric Method (Pathade *et al.*, 2011).

In light of the above need for an alternative drug-quantification technique, the current research highlights the application of a new rapid high-performance liquid chromatography-ultraviolet method to monitor any sitagliptin-sucralose interactions. The new method will be investigated according to EMEA guidelines for the quantification of sitagliptin in rats' serum. Tadalafil (TDFL) -a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphor-diesterase type 5 (PDE5) will be used as an internal standard.

The current research is highlighting on developing of a new simple, sensitive, precise and accurate chromatographic method for measuring sitagliptin in rat's plasma, to be applied to investigate any possible sitagliptin-sucralose interactions in rat's plasma.

MATERIAL AND METHODS

Chemicals

HPLC grades of methanol and phosphate buffer were used as the mobile phase solvents for HPLC analysis (FULL-TIME, USA), deionized water HPLC grade was obtained from (TEDYA, USA), STGPN raw material ($\geq 98\%$) was thankfully donated from DAD company (Amman, Jordan). Tadalafil citrate ($\geq 98\%$) as the internal standard was purchased from TQ. Pharma. (Amman, Jordan), SUCS was acquired from its finished product Splenda® (Tate & Lyle, Newham, London, UK).

Apparatus and chromatographic

High performance liquid chromatography-ultraviolet HPLC/UV was the technique adopted in this study due to its

successful performance reported in the analysis of several compounds in plasma or serum (Abu Dayyih *et al.*, 2015; Zeng *et al.*, 2010; Zeng *et al.*, 2008; Nirogi *et al.*, 2008; Vincent *et al.*, 2007; Biconi *et al.*, 2007). The HPLC system (HITACHY, Japan) consisted of S # L-2130 VWR-HITACHY pump model, S# L-2200 VWR-HITACHY auto-sampler thermostat, S# L-2300 VWR-HITACHY column oven and S# L-2420 VWR-HITACHY- UV detector.

Chromatographic separation was carried out using a mobile phase which consisted of (65:35) v/v phosphate buffer and methanol of a pH value adjusted at 3.9. The method of HPLC assigned was as follows; the type of separation column used was ACE C18 (5 μ m, 250 x 4.6mm i.d.) operating at a temperature fixed at 40°C, an injection volume of 50 μ L, a mobile phase flow rate of 0.001L/min, and a sample run time of 9 min. Signals were monitored and analyzed at λ = 239nm. Tadalafil citrate was used as internal standard.

Preparation of stock and working solutions

Oral STGPN was given at a recommended dose 1.90g/kg/day) for rats. It was prepared by dissolving the drug in methanol. On the other hand, SUCS, given at an oral dose of (10mg/kg/day), was prepared as a working solution of 0.25 g/mL by dissolving Splenda® at room temperature under sonication. Both working solutions were injected from fresh preparations that are kept away from heat and light (Abu dayyih *et al.*, 2015; Dubal *et al.*, 2012; Abu Dayyih *et al.*, 2018) (Abu Dayyih *et al.*, 2018; 2015; 2012).

STGPN stock solution was prepared at a concentration of 500 μ g/mL by dissolving 25mg in 50mL methanol. STGPN working solutions, preserved from light and heat, were prepared from the stock solution by dilution with methanol then injected directly for HPLC analysis. Tadalafil (TDLF) was used as internal standard.

Calibration samples

A series of dilutions of samples were prepared by completing an appropriate volume of each previous preparation with a sufficient volume of rat's serum to reach a final volume of 5mL at the corresponding calibration

concentration. Each sample was vortexed and directly injected for HPLC analysis. Six calibration curves were analyzed using the HPLC. In each calibration curve, peak area ratios were calculated and plotted against the concentrations with a correlation coefficient R^2 accepted not to exceed a value of 0.99.

Sample preparation (extraction procedure)

Sample preparation was carried out by mixing 100 μ L of serum with 75.0 μ L of internal standard (IS) working solution. The sample was then vortexed for 30s, centrifuged at 12000rpm for 5min and transferred into rack. 50 μ L of the supernatant was injected into HPLC unit.

Precision and accuracy

Precision and accuracy of the current method of analysis were evaluated on four different concentrations; lower limit of quantification, low, middle and high concentrations of STGPN by spiking with the rat's serum.

Intra-day precision and accuracy were evaluated by analyzing the four aforementioned concentrations. This was carried out five times in the same day and then repeated at three different days for the inter-day precision and accuracy evaluation. Furthermore, precision was assessed by comparing each concentration with the CV% (coefficient of variation) while accuracy was assessed by comparing the mean calculated concentration with $\pm$ 20% of spiked concentration for the lower limit of quantification while it was compared with $\pm$ 15% for the other quality control samples.

Stability

Different stability conditions were adopted in the current work. For the room temperature stability, three samples of quantification control low concentration (QCL) and quantification control high concentration (QCH) of concentrations 2 and 12.8 μ g/mL respectively, were prepared and analyzed with freshly prepared calibration at zero time and after 8 h at room temperature of 28°C. Percent area ratios per QCL and QCH were calculated and each corresponding calibration was constructed (Abu Dayyih *et al.*, 2015; Khan *et al.*, 2011; Beconi *et al.*, 2007; Dayyih *et al.*, 2018).

For freeze and thaw stability, three samples of QCL and QCH were properly prepared in serum with sufficient final volume covering all test cycles of analysis. For the zero-time stability, for each corresponding calibration, samples were analyzed, and concentrations were calculated, then, samples were stored and frozen at -20°C. For the 12h stability, samples were thawed at room temperature, i.e. 25°C, corresponding to the samples processing temperature during analysis stages. After complete thawing, samples were analyzed again for each corresponding calibration, then refrozen for 24h. For the 24h stability, samples were thawed and analyzed under the same conditions. After each cycle, concentrations were calculated relative to its corresponding calibration. For the long-term stability, three samples of (QCL and QCH) were analyzed with freshly prepared calibration at zero time. Samples were then frozen for 30 days at a temperature of -20°C. After the long term freezing period was complete, samples were removed and thawed at room temperature.

Room temperature and long-term stability test were also carried out for the working solutions. Stability test was assessed by comparing the calculated concentrations with the nominated (or **assigned**) concentrations as it should be within $\pm 15\%$ for both QCL and QCH.

Preclinical study

The preclinical study protocol was approved by the Research Committee (October 14/6/2016) at the Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan.

Adult male Sprague Dawley laboratory rats were supplied by Petra University Animal House. Average weight of rats was (0.230kg $\pm$ 0.03). Rats were placed in air-conditioned environment at a temperature of 20-25°C and exposed to a photoperiod cycle (12h light /12-h dark) at a humidity of 50% on daily basis. Rats were under fasting for 24h 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. STGPN and SUCS test solutions were freshly prepared directly in the laboratory

before rats feeding in order to avoid any possible decomposition of either STGPN or SUCS.

A total of 80 healthy rats were used for the experiment. Rats were divided into groups of 8 rats, then weighed and numbered orderly. STGPN and SUCS oral doses were calculated based upon each rat's weight then administered according to their numbers in order using gastric gavage. Trial analysis was performed in 3 days according to the following arrangement:

In the first day of trials: Four groups of rats were used for analysis; two groups received water at zero-time experiment, then followed by STGPN after 1h of water feeding, while the other two group received SUCS at zero time of experiment followed by STGPN after 1h of SUCS feeding. Time intervals of blood samples pooling were: 0, 30min, 1h, 2h, 3h, 4h, 6h, 8h and 24h.

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

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

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

RESULTS AND DISCUSSIONS

Separation between chromatogram peaks between STGPN and Tadalafil in serum was satisfactorily attained. Individual peaks of STGPN were sharp, clear of tailing or splitting, of reasonable retention time and high resolution. An overlay chromatogram of all the aforementioned peaks in samples representing blank rat's serum, rats serum with IS, rats serum

Figure 2. (A) Blank chromatogram (1) and separation chromatogram (2): STGPN (peak b) and internal standard Tadalafil citrate (peak a) chromatogram (B) STGPN chromatogram.

Table I. Linearity of six calibration curves.

Calibration no.	Calibration equation	Correlation coefficient (R^2)
Calibration 1	$y=0.1008981 x - 0.00098912$	0.999890
Calibration 2	$y=0.1092190 x + 0.00043396$	0.999777
Calibration 3	$y=0.0995990 x - 0.001155060$	0.999168
Calibration 4	$y=0.1013354 x - 0.005413047$	0.999991
Calibration 5	$y=0.0987726 x + 0.00257729$	0.998992
Calibration 6	$y=0.0966405 x - 0.01064450$	0.999113

Table II. Inter- day precision and accuracy 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)	0.264	0.0046	1.782	0.011	104.011
QCL	1.998	0.0038	0.193	0.013	99.961
QCM	7.103	0.0915	1.301	0.104	101.431
QCH	13.51	0.1229	0.910	0.15	105.541
Inter-day: Day 2					
ST(LLOQ)	0.254	0.0042	1.45	0.011	100.10
QCL	2.017	0.0031	0.16	0.020	100.01
QCM	7.314	0.0980	1.35	0.311	104.2
QCH	13.522	0.4503	3.34	0.601	105.1
Inter-day: Day 3					
ST(LLOQ)	0.256	0.0039	1.56	-0.0039	100.03
QCL	2.144	0.0090	0.43	0.04321	107.0
QCM	7.328	0.0857	1.18	0.4222	104.58
QCH	13.715	0.3853	2.82	1.0116	107.12
Intra-day					
ST(LLOQ)	0.260	0.0036	1.36	0.011	104.02
QCL	1.991	0.0028	0.15	-0.012	99.51
QCM	7.164	0.0960	1.35	0.164	102.21
QCH	13.312	0.5364	4.04	0.516	103.94

with Low LOQ, and rat's serum with IS and STGPN after 30min of combination, respectively (Figures 2).

Method performance attributes including precision, accuracy, and linearity were carried out and statistically tested over six replications. Calibration curves were constructed over six different trials (Table I) resulting in a correlation coefficient greater than 0.999 thus confirming the good linearity that was established between the peak area of STGPN in serum and its concentrations. Precision of inter- and intra- day tests demonstrated acceptable lower limit of quantification (LLOQ) values with coefficient of variation (CV%) determined to be less than 20%. For the quantification control concentrations representing low (QCL), medium (QCM) and high (QCH) values for the six runs, a coefficient of variation less than 15% was recorded (Table II). On the other hand, inter-day and intra-day tests showed accuracy values with lower limit of quantification (LLOQ) lying within a CV% of $\pm 20\%$ for defined sample concentrations, whilst within $\pm 15\%$ for the QCL, QCM and QCH.

Three different conditions were adopted for testing drug stability; Freeze and thaw stability, Room temperature, and Long-term freezing. Stability data of the freeze-dried, samples were obtained when the samples representing QCL and QCH concentrations were analyzed at zero time, after 12 and 24h of freezing. All samples were thawed for 3h then analyzed. Assay data indicated 99% of the drug with accuracy values exceeding 98% (Figure 3, Table III).

For stability studies at room temperature, serum samples representing QCL and QCH concentrations analyzed at zero time and after 12h at room temperature ($28^{\circ}\text{C} \pm 1$) had stability and accuracy values exceeding 99% (Table IV). Finally, for the long term freezing condition, serum samples representing QCL and QCH concentrations analyzed at zero time and after 30 days and thawing for 3h had stability and accuracy values exceeding 99% and 98% respectively (Figure 3, Table IV).

SUCS –STGPN combination effect on STGPN serum levels:

Assay of STGPN samples in rat's serum was determined at each day of trials. Samples

representing the first day of trials were collected at zero time and at 24h. Assay results (Figure 4A), while samples representing the last time interval (Table V). Samples collected on the second day of trials (Figure 4B) were analyzed at zero time and 24h (Table V). Samples collected on the third day of trials had an overall serum concentration profiles represented by area under the curve (AUC), maximum concentration (C-max), and maximum time (T-max) (Table VI).

Statistical treatment of the samples was carried out to evaluate size and significance in the combination of STGPN and SUCS in the oral dose (is this correct). Effect size was expressed by Cohen's d value while the *P* value was used to indicate significance. Cohen's d values for the combination effect size did not exceed 0.8 for all intervals except at 30min where lower d values were recorded. With regard to *P* values, combination effect significance was strong as all *P* values were less than 0.01. Finally, two examples on the designed procedure for HPLC analysis are illustrated in the chromatograms (Figure 3 and 4).

Method validation was evaluated for quantitative purposes according to EMEA guidelines (Herma *et al.*, 2006; Dayyih *et al.*, 2018). Concerning precision and accuracy, values determined were reasonably acceptable as indicated by the low CV% values obtained. In addition, Method linearity established an accepted correlation coefficient confirmed above 0.999.

Accordingly, the three aforementioned stability tests verified the reliability of the HPLC procedure to analyze the drug with or without the presence of SUCS with reasonable accuracy and precision data obtained. In consequence, such a validated method gives more emphasis to the use of the current method as a replacement to most widely used HPLC procedures which are highly expensive and more complex to operate.

Having a SUCS –STGPN combination being investigated for the first time in the current research, the initial statistical analysis of the data presented herein suggests a significant interaction between the two compounds when administered concurrently. Such interaction was supported by a *P* value test outcome <0.01 for the combination effect which indicates

Table III. Freeze and thaw stability for QCL and QCH.

Time	QC Low (2µg/mL)			QC High (12.8µg/mL)		
	Mean	Accuracy%	Stability %	Mean	Accuracy%	Stability %
0h	2.012± 0.112	103.10	100.60	12.711± 2.322	99.69	99.30
		102.01			99.90	
		99.84			99.80	
		99.98			98.91	
12h	1.981± 0.210	99.98	98.46	12.753± 3.112	98.80	100.33
		99.99			99.00	
		99.97			98.70	
		99.99			98.88	
24h	1.990± 0.096	99.89	99.50	12.692± 1.996	98.90	99.85

Table IV. Room temperature and Long-term stability of STGPN in serum for QCL and QCH.

Room temperature						
QC Low (2 µg/mL)				QC High (12.8 µg/mL)		
Time	Mean	Accuracy%	Stability%	Mean	Accuracy%	Stability%
0h	2.11±0.068	110.1	105.50	12.82±3.126	99.91	100.15
		106.1			100.12	
		100.2			99.91	
8h	2.29±1.001	105.3	108.53	12.78±1.735	99.84	99.69
		99.5			99.82	
		99.6			99.77	
Long-term stability						
QC Low (2 µg/mL)				QC Low (2 µg/mL)		
Time	Mean	Accuracy%	Stability%	Mean	Accuracy%	Stability%
0h	1.98±1.002	99.72	99.00	12.76±1.886	98.89	99.88
		99.25			98.51	
		99.74			99.35	
30days	1.99±0.196	100.90	100.50	12.68±2.093	99.95	
		99.91			99.27	
		99.50			98.81	

Table V. Three days data for both drug alone and drug with SUCS (n=2).

	First day data							
	Serum average concentrations (µg/mL)							
	30min	1h	2h	3h	4h	6h	8h	24 h
Group 1+2 (STGPN alone)	5.515	10.991	15.140	13.004	10.940	8.942	6.204	0.512
Group 3+4 (STGPN+ SUCS)	5.007	10.017	12.081	11.088	10.030	7.013	4.650	0.201
Second day data								
Group 1+2 (STGPN alone)	5.982	10.687	15.299	13.144	12.492	9.628	6.899	0.580
Group 3+4 (STGPN+ SUCS)	5.147	10.275	12.131	11.242	10.126	8.036	5.146	0.314
Third day data								
Group 1+2 (STGPN alone)	5.893	10.671	15.982	13.257	12.097	10.802	6.691	0.596
Group 3+4 (STGPN+ SUCS)	5.683	10.401	12.024	11.026	10.714	8.708	5.012	0.303

Figure 3. a Freeze and thaw stability chromatogram b Long-term stability chromatogram.

Figure 4 a. First day; b. Second day; c. Third day trial serum–time profile curves.

Table VI. Serum concentration – time profile kinetic parameters.

Drug	C max (µg/mL)	T max (h)	T $\frac{1}{2}$ (h)	AUC (µg/mL*h)
STGPN	15.238 $\pm$ 0.029	2	2	130.7 $\pm$ 2.11
STGPN + SUCS	12.079 $\pm$ 0.099	2	2	101 $\pm$ 2.02
Pvalue	0.009**	< 0.01**		0.001**

** p<0.01 significance

a significant interaction to dominate the drop of drug level in serum and increase in drug metabolites.

Referring back to the mechanism of action of SUCS, it is thought that this combination interaction is more likely attributed to CYP 450 enzyme inhibition by the synthetic sweetener, specifically inhibition of the 3A4 subtype by which STGPN is extensively metabolized. Once STGPN is absorbed in the stomach, CYP3A4 iso-enzymes, located profusely in parietal cells endoplasmic reticulum, will induce metabolic biotransformation of STGPN. Moreover, induction effect of SUCS over the CYP3A4 metabolic enzyme will also activate the metabolism of STGPN in the liver. All of the aforementioned inhibitions of enzyme activities will accordingly result in STGPN plasma/serum levels reduction and over production of STGPN active and inactive metabolites.

The CYP3A forms in rats, i.e. the CYP3A1, 3A2, 3A9, 3A18, 3A23 and 3A62 have the CYP3A23 being identical to the CYP3A1 by the analysis of its gene. The CYP3A62 form has been identified as an iso-enzyme with expression profile similar to the human CYP3A4 and rat CYP3A9. In connecting the dots, the CYP3A62 is a known to be the predominant form in the intestinal tract, whereas the CYP3A1 and -3A2 are found only in liver (Matsubara *et al.*, 2004; Tamimi *et al.*, 2014; Dayyih *et al.*, 2018).

Moreover, a recent study has illustrated the presence of a distinctive binding affinity of STGPN to reactive sites of the CYP3A enzymes during metabolic reactions (Vincent *et al.*, 2007; Matsubara *et al.*, 2004; Dayyih *et al.*, 2018). This may further confirm a predicted variable bioavailability in humans as justified by the current observed *in-vivo* reduction of STGPN in rats. Such finding may lead to a possible perceptible clinical difference in STGPN human plasma levels when combined with SUCS, which strongly recommends further clinical research in humans.

CONCLUSIONS

A successful HPLC method was validated and developed to quantify STGPN in rats' 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 STGPN with SUCS over all time intervals of STGPN serum profile demonstrated as strong effect on STGPN pharmacokinetic according to Cohen's d and significant P values. C-max showed a significant change between presence and absence of SUCS while T-max did not show any change. This suggests the possibility of interaction between STGPN and SUCS during combination.

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