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Assessment of suitability of saxagliptin hydrochloride for development of controlled release parenteral formulation by preformulation studies

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***Abstract*---**The main objective of pre-formulation study is to develop the stable, elegant, safe and effective drug delivery system by establishing drug kinetic profile, formulation compatibility with different excipients and physico-chemical parameters of new drug molecules. This could provide key evidence for implementing formulation design or requirement of the molecular alteration. So, in the present study preformulation studies were performed on Saxagliptin Hydrochloride (SXG) to assess its suitability for parenteral formulation. SXG is a potent and selective reversible inhibitor of dipeptidyl peptidase-4 used to treat type –II diabetes mellitus. The authenticity of SXG was established by differential scanning calorimetry (DSC) and fourier transform infrared spectroscopy (FTIR) spectra. An ultraviolet–visible (UV) spectrophotometric and high

performance liquid chromatography (HPLC) methods were employed for determination of SXG in bulk API (active pharmaceutical ingredient). The UV method was linear within the range of 1-40 µg/ml. The proposed methodology is robust which can be concluded from the lower percentage standard deviation percentage co efficient of variance (% CV) values of intraday and inter day variability. The retention time was observed 1.3 min of SXG in HPLC method. The higher regression coefficient value (0.999) indicates the methodology is robust. The outcome of the physico-chemical experiments of drug molecule indicates parenteral route is more suitable for SXG. Additionally, at different conditions like solid as well as liquid state, the drug molecule was observed stable.

***Keywords*---**diabetes mellitus, parenteral formulation, preformulation, saxagliptin HCl, stability.

Introduction

After drug discovery, with a background of physical, chemical and derived powered properties of the drug, the drug molecule has to be formulated in the form that can suitably be administered. Prior to initiation of formulation development, the first stage is physico-chemical data collection of drug molecule, identification of suitable potential drug salts and excipient, is known as pre-formulation. Preformulation is the bridging gap between new drug molecule and formulation-development. It also reveals scope for formulation development (Gopinath & Naidu, 2011).

Preformulation includes the application of bio-pharmaceutical principles for development of the suitable drug delivery system. Characterization of the drug molecule is the most important step in the pre-formulation study of product development. Hence, Preformulation studies are an important tool early in the development of both API and drug products. The interaction between the drug components and the excipient used in the formulation are generally included in the study, resulting in proper selection of excipients. The preliminary drug degradation profiles are incorporated in the study to get the knowledge of a stable formulation. A study of this subject aids the development of the monitoring process during the course of formulation development (Verma & Mishra, 2016).

The aim of pre-formulation study is to develop the sophisticated, stable, effective and safe dosage form by achieving kineticprofile, compatibility of drug entity with other formulation components and identify physico-chemical parameters. In general, preformulation study requires drug molecule characterization in solid as well as liquid state. Pre-formulation can assist to minimize product cost for effective the rapeutic formulation development. Diabetes mellitus is an illness in which one has a high blood sugar level, it is because of either the body doesn't make adequate insulin or body cells don't appropriately react to the insulin that is produced (Patra, 2012; Rother, 2007). In 2021, Approximately 537 million adults (20-79 years) are living with diabetes. The total figure of people living with

diabetes is anticipated to increase to 643 million by 2030 and 783 million by 2045 (Fralick et al., 2022).

Saxagliptin HCl is a potent and selective reversible inhibitor of dipeptidyl peptidase-4 used to treat type –II diabetes mellitus. Molecular formula of Saxagliptin HCl is C18H26ClN3O2 with a molecular mass of about 391.9g/mol (Prasad, Satyanaryana, & Krishnamohan, 2015). It belongs to class-III of Biopharmaceutical classification system. It is completely soluble in water, ethanol, methanol and slightly soluble in various organic solvents like ethyl acetate and dimethylene chloride (Rasul et al., 2021). The mechanism of action of Saxagliptin HCl an enzyme that deactivates and degrades incretin hormones, cytokines and other peptides. Released in response to meals, incretin hormones potentiate insulin release and decrease glucagon production, lowering serum glucose concentrations (Chandira, Palanisamy, Jaykar, Venkateswarlu, & Pasupathi, 2013).

Polymeric microspheres as a parenteral drug delivery system are primarily developed for sustained release of drugs for prolonged systemic therapeutic effects after subcutaneous (SC) or intramuscular (IM) administration. Polymers used for formulation of microspheres are biodegradable and biocompatible. In the present research PLGA is used as a polymer (Behera, Sahoo, Dhal, Barik, & Gupta, 2008; Mao, Guo, Shi, & Li, 2012). This polymer is usually used for biodegradable controlled release microparticles. In the present research solvent evaporation method is applied to formulate microparticles for injectable controlled release drug delivery system. Microspheres in the finished product are in a dry powder form. Prior to administration, a microsphere product is reconstituted in a liquid diluent which can be supplied in a separate container or in the liquid compartment of a dual-chamber prefilled syringe (Rosenstock et al., 2009; Tekkeli, Kızıltaş, & Dinçel, 2013). So, in the present study focus was given on preformulation studies of SXG. The main objective of the study was to assess SXG for its suitability to be formulated as SR microspheres for parenteral delivery.

Material and Method

Material

Biodegradable polymer, poly(lactide-co-glycolide), RESOMER® RG 502 H (lactide:glycolide = 50:50, Mw: 15000), RESOMER® RG 503 H (lactide:glycolide = 50:50, Mw: 35000), RESOMER® RG 504 H (lactide:glycolide = 50:50, Mw: 50000), RESOMER® RG 750 S (lactide:glycolide = 75:25, Mw: 1.25K dalton) were obtained as a gift sample from Evonik, and all of them were stored at -25℃ to 8℃ prior to use. Saxagliptin HCl API was obtained as a gift sample from Torrent Pharmaceuticals, India. Methanol, Ethanol, Ethyl Acetate, Methylene chloride (DCM) were obtained as a gift sample from Final Limited, India. Chemicals and solvents used were of high-performance liquid chromatography (HPLC) grade. Freshly prepared distilled water was used throughout the study.

Drug identification

The drug identification was performed by organoleptic properties, melting point,

UV, HPLC, FTIR and DSC.

Determination of thermodynamic solubility

Solubility study of drug was performed using different solvents such as methanol, ethanol, ethyl acetate and Dichloromethane (DCM). Samples were shaken on a rotary shaker at 37°C for 24 hours. The two phases are then separated by filtration (Prasad et al., 2015). Amount of solute in supernatant is then determined using UV spectrophotometric analysis at the corresponding λmax of each solvent.

Analytical preformulation

Analysis of SXG by UV spectrophotometry method(Madhavi & Rani, 2017). Standard stock solutions of SXG was prepared in water and scanned spectrophotometrically over the range of 200–400nm with double beam spectrophotometer (Shimadzu UV spectrophotometer, 240 j/PC, Japan), against the respective blank, to determine wave length of maximum absorbance (λmax). A stock solution containing 1000 µg/ml SXG was prepared by dissolving 25 mg SXG in 5 ml of water in a 25 ml of volumetric flask and volume was made upto 25 ml with the water. From these stock solutions, suitable aliquots were taken and diluted using appropriate solvent to get dilutions of 1-40 µg/ml. The determinations were conducted in triplicate and studied for three days to check intra and inter day variations. Calibration curve was constructed at concentrations range 1-40 µg/ml. Absorbance of each solution was measured at the wavelength of 213 nm. Calibration curve was constructed for SXG by plotting absorbance versus concentration at 213 nm wavelength. The determination was conducted in triplicate.

Analysis of SXG by HPLC method (Madhavi & Rani, 2017). Saxagliptin HCl was quantified using a Shimadzu prominence-iLC2010 high performance liquid chromatography (HPLC) system equipped with isocratic pump, auto sampler, and photodiode array detector. The mobile phase was Buffer: acetonitrile, 80:20 (v/v). The system was equipped with an all-time C18 column (50 x 4.6 mm, 3µ), temperature of column was 40℃ and the flow rate was set to 1 mL/min. The injection volume for drug loading samples was 100 µl. The chromatographs were analyzed with empower software at 213 nm. Linearity of the methodology was proved for concentration range of 4.67ppm to 37.40ppm.

Drug-polymer compatibility study

The physical stability and compatibility of Saxagliptin HCl with polymer was evaluated at 25℃ and 60% relative humidity (RH). Additionally, the samples were also closed in vials and stored in refrigerator (2–8℃). The samples were removed after 30 days. Fourier transform-infrared (FTIR) study (Ghule, Dhobale, Kedar, & Jadhav, 2019). The FTIR analysis was widely used for qualitative estimation and identification of functional group present in the compound. SXG was mixed with each of the components at an appropriate ratio; equivalent to that used in formulation process. Each mixture was stored in USP type-1 glass vial at

25°C±5°C, 60±5% RH (relative humidity) for one month. FTIRspectroscopy (Shimadzu; Model 8400, Japan) scanned from 4000 to 400 cm-1 by KBr pellet method, was used to identify the compatibility of drug molecule and other product components.

Differential Scanning Calorimetry (DSC) (Rasul et al., 2021). DSC is the thermal analysis method by which we can measure the interaction of drug with polymer. The thermal analysis of Drug, PLGA, physical mixture of Drug and PLGA was performed by using 3-5 mg of samples in a standard thermal aluminum pan with a comparable lid and heated from 0 to 300°C at a 10°C/min heating rate in METTLER TOLEDO DSC (METTLER TOLEDO, Switzerland).

Results and Discussion

Drug identification
Organoleptic properties and Melting Point

Saxagliptin HClis odourless and almost white powder which is sticky in nature. The melting-point of drug was in the range 96–102°C. Drug identification by UV. The maximum absorbance of SXG in water was found at 213nmas depicted in figure 1.

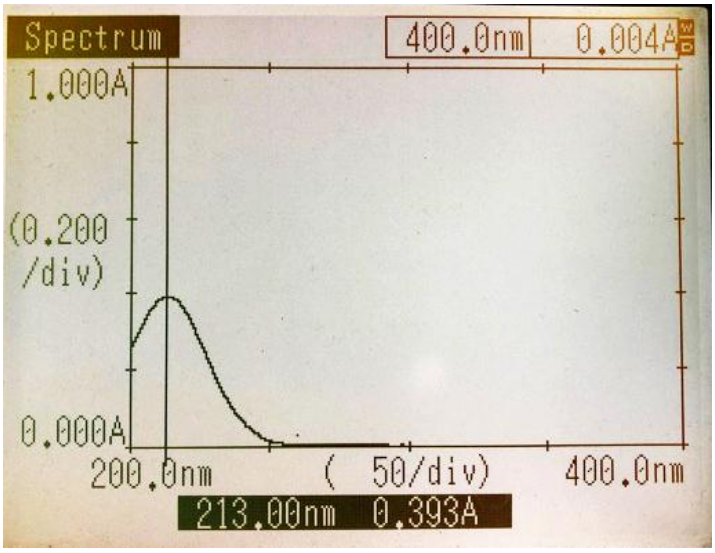

Figure 1. UV spectra of SXG in water at 213 λ-max

Drug identification by HPLC

The peak retention time of SXG was observed to be about 1.3 min as observed from figure 2.

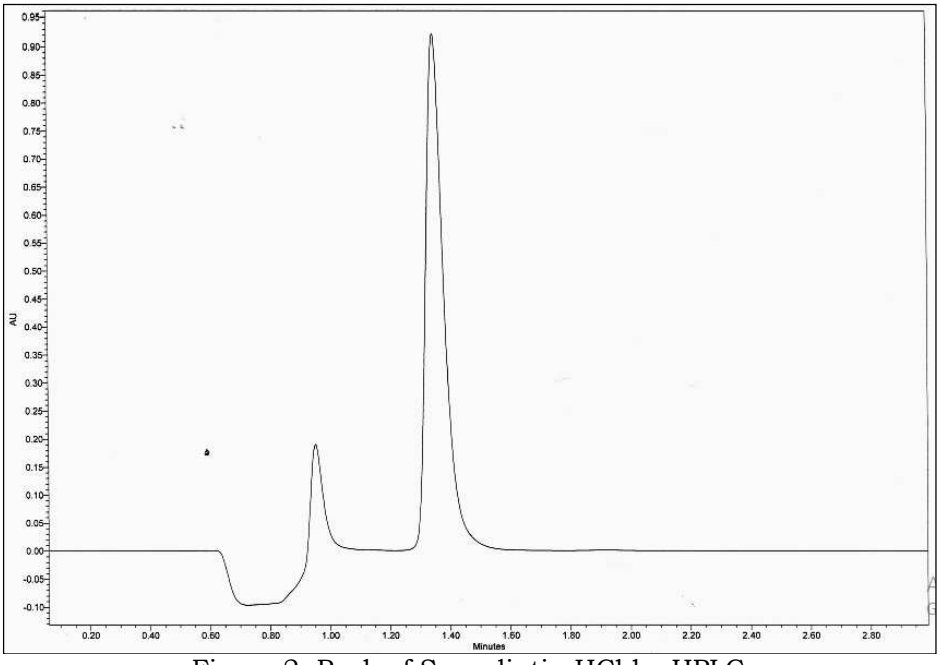

Figure 2. Peak of Saxagliptin HCl by HPLC

Drug identification by FTIR

The characteristic absorption spectra of SXG in FT-IR isillustrated in Figure 3and the functional groups responsible for characteristic peaks of SXG are mentioned in Table 1.

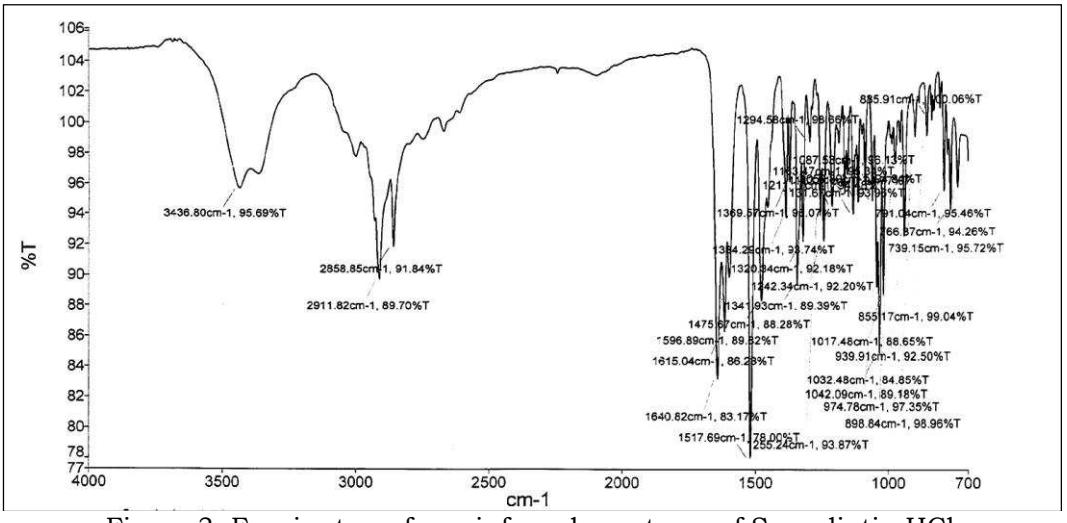

Figure 3. Fourier transform-infrared spectrum of Saxagliptin HCl

Table 1
Stretching bending of Saxagliptin HCl

Peak at wave number (cm-1)	Interpretation
3436.8	N-H stretch (Secondary amine)
2911.82	C-H stretch (aromatic)
1615.04	C-N stretch
1517.69	C-N stretch (aliphatic)

Drug identification by DSC
DSC thermogram of SXG is illustrated in Figure 4. Reported melting point value was found 96-102℃ and practically melting point value were found 96-98°C and 84.17°C by capillary method and DSC, respectively.

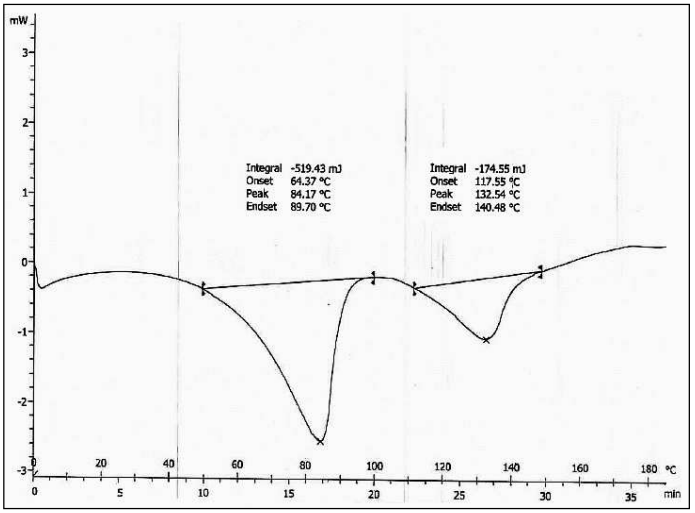

Figure 4. DSC (Differential Scanning Calorimetry) of Saxagliptin HCl

Determination of thermodynamic solubility

Saxagliptin HCl is a soluble in water, methanol and ethanol, sparingly soluble in dichloromethane, practically insoluble in ethyl acetate. The solubility of SXG in various solvents is shown in Table 2.

Table 2
Solubility parameters of different solvents

Solvents	Solubility* (mg/mL)
Water	202±10.1
Methanol	321±16.05
Ethanol	168±8.4
Ethyl Acetate	Insoluble
Dichloromethane (DCM)	0.15±0.008
*Result are represented as mean ± standard deviation	

(n=3)

Analytical preformulation
Analysis of SXG by UV spectrophotometry method

The spectrophotometry method developments for the identification of drug molecules has been increased considerably in now a day because of their key role in pharmaceutical analysis. The standard calibration curves were designed as per the experimental findings. The regression analysis indicates very good correlation (r2=0.9995) in water. These solutions followed Beer-Lambert's law and the linearity was found in the concentration range of 1-40 µg/ml in water. The standard curve of SXG is illustrated in Table 3 and Figure 5.

Table 3
Standard curve of Saxagliptin HCl in Water by UV

Conc. (ppm)	Absorbance at 213nm			Average	Std. Deviation	% RSD
0	0	0	0	0.000	0.000	0.000
1	0.026	0.026	0.026	0.026	0.000	0.000
5	0.103	0.102	0.103	0.103	0.001	0.562
10	0.196	0.195	0.197	0.196	0.001	0.510
15	0.311	0.309	0.312	0.311	0.002	0.492
20	0.391	0.39	0.392	0.391	0.001	0.256
24	0.472	0.47	0.473	0.472	0.002	0.324
30	0.584	0.582	0.586	0.584	0.002	0.342
40	0.785	0.783	0.787	0.785	0.002	0.255

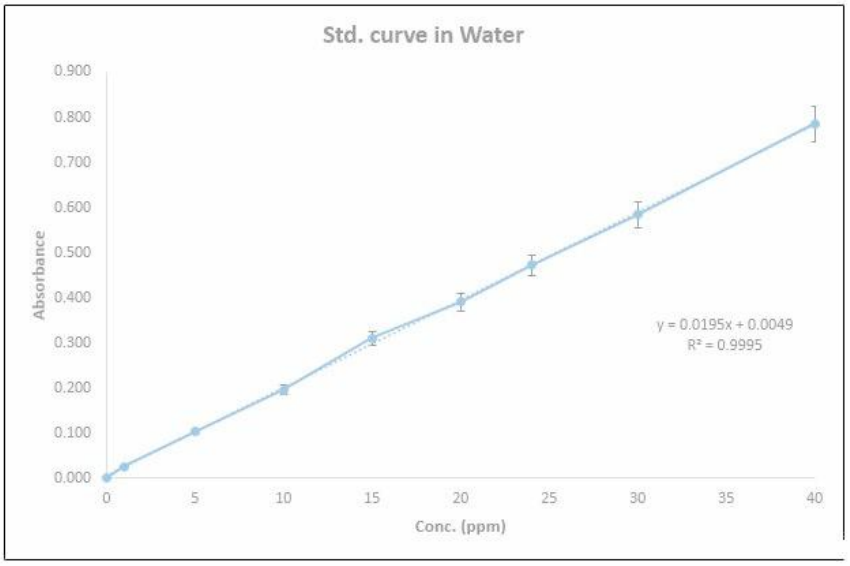

Figure 5. Standard curve of Saxagliptin HCl in Water

Analysis of SXG by HPLC method

The technique was developed to quantify Saxagliptin HCl for assay and in-vitro release. The chromatography was checked for its linearity and was showed to be linear from 4.67 ppm to 37.40 ppm which covers the linearity requirements for assay and in-vitro release, the chromatograph of the same is depicted in figure 6. The quantification of % Assay and/or % in-vitro release was done by estimating area of sample peak with area of standard peak of known concentration using formula Au/As*Cs/Cu*100 after conforming above mentioned system suitability requirements and the results are mentioned in Table 4. Method was observed to be appropriate for its intended uses.

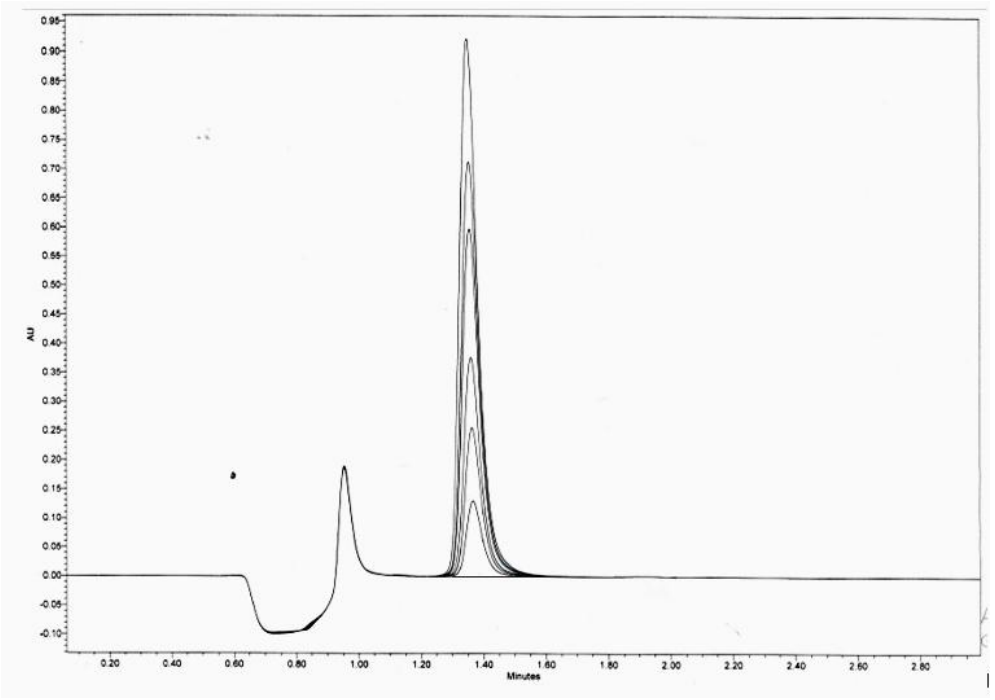

Figure 6. Linearity of Saxagliptin HCl by HPLC

Table 4
Linearity curve of Saxagliptin HCl

PPM with Potency	Set-1 Area	Set-2 Area	Set-3 Area	Average Area
4.67	495488	495576	495706	495590
9.35	989594	989709	989680	989661
14.02	1481183	1481134	1481006	1481108
23.37	2428195	2428342	2428229	2428255
28.05	2936024	2935721	2936022	2935922
37.40	3893286	3893145	3893378	3893270
		Slope		103709.611
		R^2		0.9999
		Intercept		17124.2057

Y Bias	0.7052

Drug-polymer compatibility study

The characteristic absorption peaks of SXG in FT-IR spectra as illustrated in Figure 3 confirms pure and stable drug profile. Moreover, stability of SXG has been also evaluated at different temperatures, light, moisture and oxidation condition. The results derived from stability study and pre-formulation study indicates stable characteristics of drug entity at various storage conditions which are illustrated in Table 5.

Table 5
At various conditions drug molecule stability under pre-formulation study

No.	Influencing factor	Packing material	Test Sample	Storage time (weeks)	Storage condition	Physical degradation	Drug content
1	Moisture	Open container	Pure drug	0	25℃/75 % RH	No	99.55
				1		No	99.73
2	Temperature	50 ml glass container with twist-off closure	Pure drug	0	70℃	No	99.49
				2		No	99.87
				4		No	99.93
3	Oxidation	25 mL glass flask with glass stopper	1%aqueous solution in 0.35 H_2O_2 Solution	0	50℃	No	99.65
				1		No	99.75
				3		No	99.49
4	Light	Open petridish	Pure drug substance	24 hrs	Xenon lamp	No	99.38
				48 hrs		No	99.65
		Amber colour petridish		24 hrs	Xenon lamp	No	99.59
				48 hrs		No	99.78

Fourier transform-infrared (FTIR) study

The FTIR spectral analysis showed that there is no appearance or disappearance of any characteristic peaks of pure drug Saxagliptin HCl and in the physical mixture which confirms the absence of chemical interaction between drug and polymers. The FT-IR spectra of physical mixture in initial condition and after 1 month study are shown in figure 7 and 8 respectively and the functional groups responsible for characteristic peaks are mentioned in Table 6.

Table 6
Compatibility of Saxagliptin HCl-Polymer mixture by FTIR

Saxagliptin HCl (API)	Saxagliptin HCl + Polymer mixture (Initial)	Saxagliptin HCl + Polymer mixture (1 M 25°C/60% RH)	Interpretation
X (cm^{-1})	X (cm^{-1})	X (cm^{-1})	
3436.8	3437.55	3437.61	N-H stretch (Secondary amine)
2911.82	2911.62	2911.55	C-H stretch (aromatic)
1615.04	1640.51	1640.51	C-N stretch
1517.69	1517.69	1517.73	C-N stretch (aliphatic)

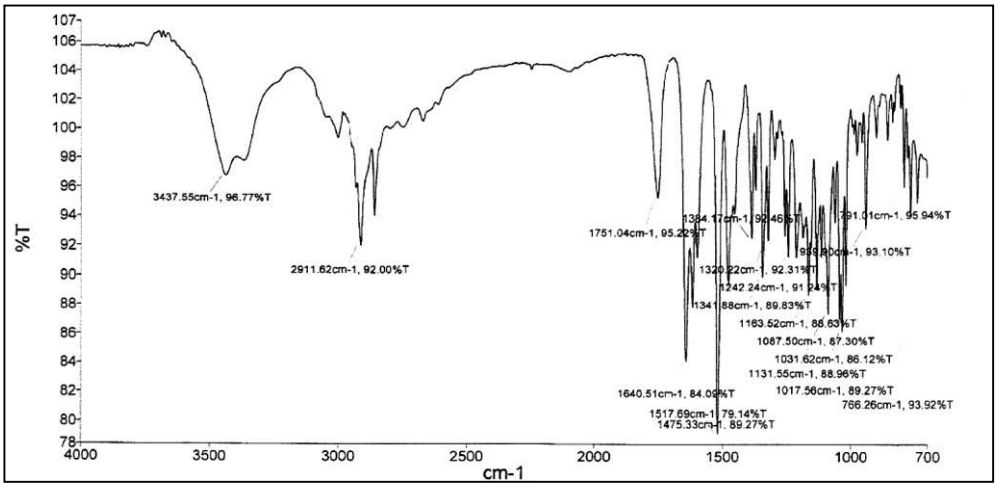

Figure 7. Fourier transform-infrared spectrum of Saxagliptin HCl-Polymer mixture (Initial)

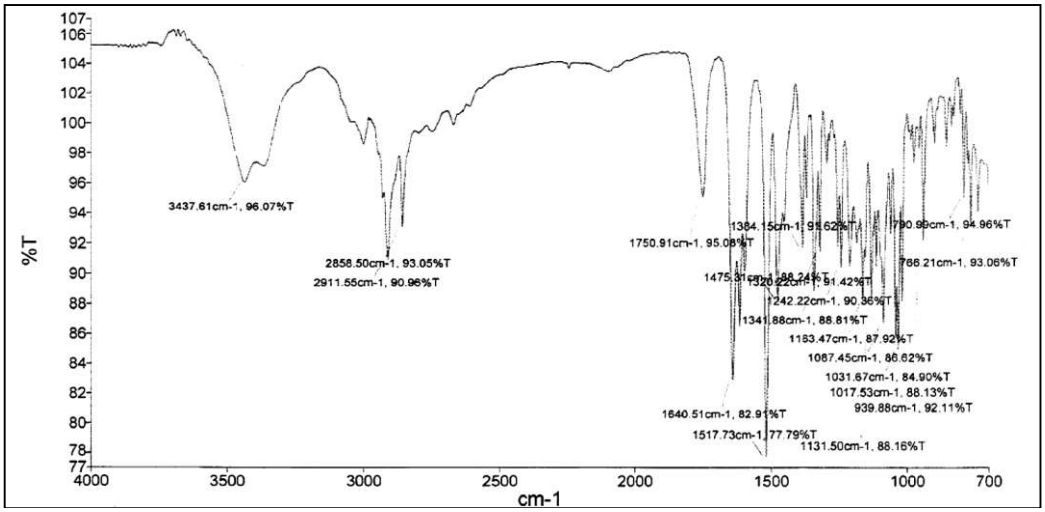

Figure 8. Fourier transform-infrared spectrum of Saxagliptin HCl-Polymer mixture (1 Month, 25℃/60%RH)

Differential Scanning Calorimetry (DSC)

DSC thermogram of SXG and polymer mixture showed that there is no change observed in the endothermic peak of drug and polymer in physical mixture at initial condition and after 1 month, which indicates there is no any chemical interaction between drug entity and polymers as shown in figure 9 and 10 respectively.

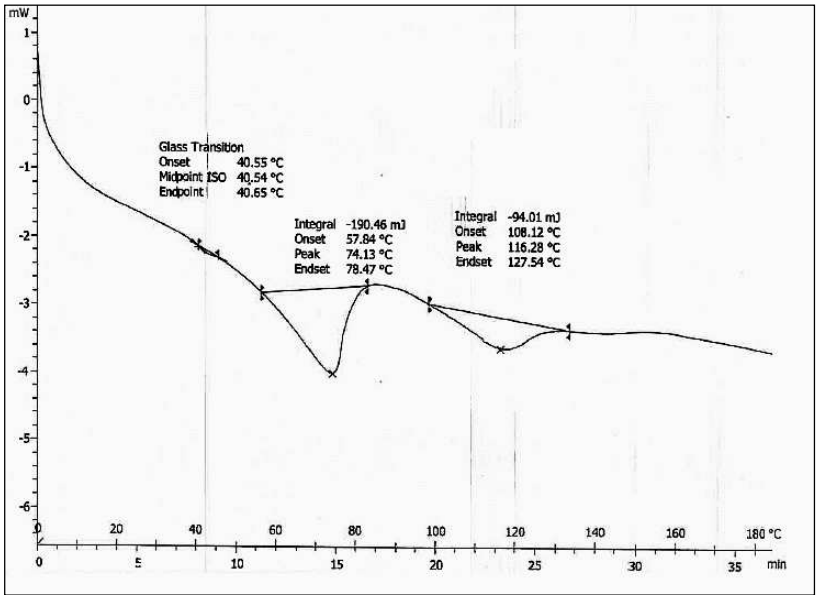

Figure 9. DSC (Differential Scanning Calorimetry) of Saxagliptin HCl-Polymer mixture (Initial)

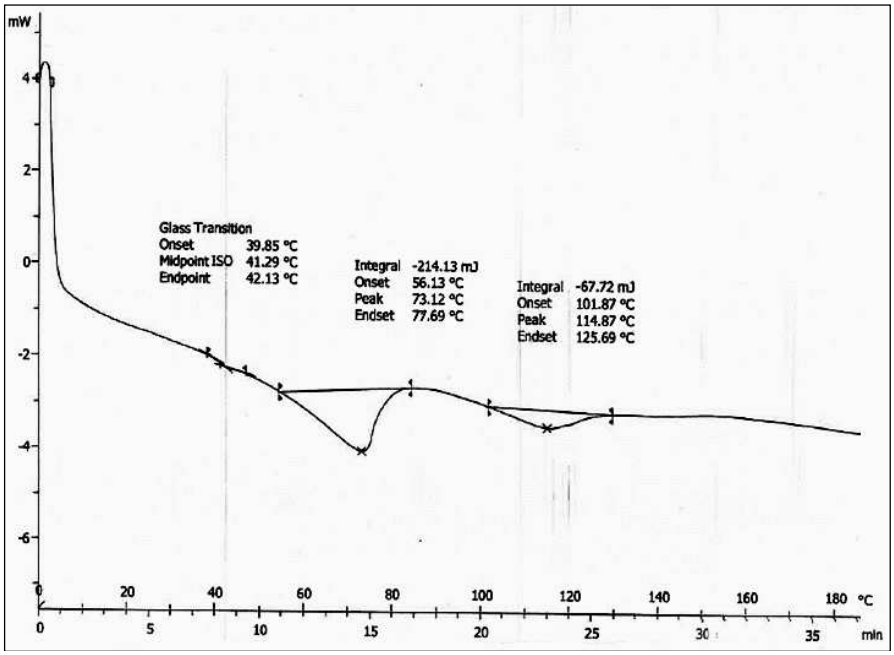

Figure 10. DSC (Differential Scanning Calorimetry) of Saxagliptin HCl-Polymer mixture (1 Month, 25℃/60%RH)

Conclusion

From the outcomes of the various preformulation studies, it can be summarized that SXG is suitable for sustained release microsphere parenteral formulation. The results of UV, HPLC, FT-IR and DSC suggested the drug is authentic. The UV method and HPLC method showed good correlation indicating they can be used for quantification of drug cargo in bulk and in vitro studies. The solubility study of drug suggested that it is soluble in organic media suggesting its suitability for sustained release formulation. Stability study under pre-formulation studies indicates stable characteristics of drug entity ratifying final stability of formulation.

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