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Research Article

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Reversed-phase liquid chromatography with mass detection and characterization of saxagliptin degradation related impurities

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ABSTRACT

The current work on impurity profiling of Saxagliptin in its bulk drugs, included development of a stability-indicating reverse-phased liquid chromatographic method and its validation for the estimation of degradation related impurities (DRIs). Saxagliptin was subjected to acid and alkaline hydrolysis, H_2O_2 oxidation, thermal degradation and photolysis. Acid hydrolysis and base hydrolysis yielded significant degradants, which were isolated on a semi-preparative high performance liquid chromatography (HPLC) and characterized with the help of high-resolution mass spectroscopy, and the possible structures of the DRIs were revealed. The chromatographic separations were accomplished on Hypersil BDS C_{18} column (250 mm x 4.6 mm; 5 µm) using water and acetonitrile as a mobile phase with gradient elution at 1.0 ml/min flow rate, and eluents were detected using photo diode array detector at 213 nm wavelength. The method was validated with respect to accuracy, precision, linearity, robustness, and limits of detection and quantification as per International Conference on Harmonization (ICH) guidelines.

Key words: Saxagliptin, Degradation related impurities, Gradient elution, Mass-spectroscopy.

INTRODUCTION

Saxagliptin is a competitive inhibitor dipeptidyl peptidase-4 (DPP-4) used as an oral hypoglycemic agent for the management of type-II diabetes mellitus. The DPP-4 inhibitors increase the levels of glucagon like peptide-1 (GLP-1), which in turn enhances the insulin production. [1, 2]

A literature study about the analytical studies performed on Saxagliptin, including the spectrometry and high performance liquid chromatography (HPLC) reveals a few selected methods for the determination of Saxagliptin either single or in combination with some other drugs like, metformin, etc. [3-6] However, none of these methods have been able to address the impurity profiling of Saxagliptin, i.e. isolation and characterization of the impurities present. The current study is focused on the isolation and characterization of the degradation-related impurities (DRIs) developed as a result of the degradation studies.

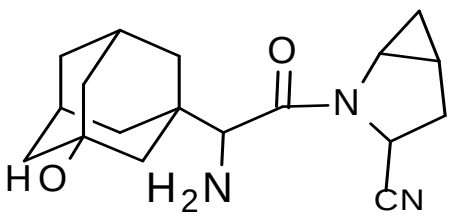


Fig. 1. Saxagliptin

EXPERIMENTAL SECTION

Chemicals:
Saxagliptin was obtained from AstraZeneca, Bengaluru, India. HPLC grade Acetonitrile was purchased from Merck chemicals (Darmstadt, Germany). AR-grade ammonium acetate, hydrochloric acid, sodium hydroxide, hydrogen peroxide, formic acid, were purchased from S.D. Fine Chemicals, India. HPLC grade water utilized from the Milli-Q water purification system, available in the laboratory.

Instrumentation:

HPLC Instrumentation:
Chromatographic work was carried on Prominence UFLC LC-20 system with a photo diode array (PDA) detector (Shimadzu, Japan), using LC-solution software (Shimadzu, Japan) for system control and data acquisition. Chromatographic separation was obtained using Hypersil BDS C_{18} column (250 mm X 4.6 mm; 5µm; Thermo Fischer Scientific). The mobile phase was composed of water (Solvent A) and acetonitrile (Solvent B) in a gradient elution, with a flow rate of 1.0 ml/min. The gradient elution was programmed as; time/percentage of Solvent B – 0/20, 3/20, 15/40, 28/10, with an equilibrium time of 2 min. The injection volume was 20µl and the eluents were monitored at 213 nm.

Semi Preparative HPLC instrumentation:
The isolation of the DRIs was carried out using Shimadzu semi-preparative HPLC system, (Shimadzu, Japan). The system consisted of two gradient LC-pumps, a PDA detector (Shimadzu, Japan) with a flow cell of 10 mm and a fraction collector. The system was controlled by Lab-solution software (Shimadzu, Japan) for the data acquisition and time programming. For the isolation of the DRIs, semi-preparative column Enable HPLC C_{18} (250 X 10 mm; 10µm) was used, with a simple gradient mobile phase of water (Solvent A) and acetonitrile (Solvent B). The separation was performed with gradient elution program: time/percentage of solvent B, 0/10, 5/40, 10/50, 17/10, with an equilibrium time of 3 min. The injection volume was 2ml, with a flow rate of 8 ml/min, and detection at wavelength 213 nm.

Preparation of Sample Solution:
Stock solutions of Saxagliptin and its DRIs were prepared by dissolving known amounts of components in acetonitrile. The specification concentration of Saxagliptin was 1000 µg/ml for DRIs and 100 µg/ml for the assay determination. For the sample loading in semi-preparative HPLC the concentration taken was, 30,000 µg/ml.

Forced Degradation Studies:
Forced degradation studies were performed on Saxagliptin, for which 1.0 mg/ml concentration of sample was used. Samples were withdrawn at particular intervals, and injected to monitor the degradation process.

Acid Degradation studies: The sample solutions for acid degradation were prepared in 1N hydrochloric acid (HCl) and kept at 60˚C, for 6 hours. Samples were withdrawn at regular intervals for monitoring the degradations.

Alkaline Degradation studies: The sample solutions for alkaline degradation were prepared in 1N sodium hydroxide (NaOH) and kept at 60˚C, for 6 hours. Samples were withdrawn at regular intervals for monitoring the degradations.

Oxidation Degradation studies: The sample solutions for oxidative degradation were prepared in 30% hydrogen peroxide (H_2O_2) and kept at room temperature for 10 days. Samples were withdrawn at regular intervals for monitoring the degradations.

Thermal Degradation studies: The samples for thermal degradation were kept in oven at 80˚C for 10 days. Samples were withdrawn at regular intervals for monitoring the degradations.

Photo Stability studies: The samples were exposed to light using a petri-dish for 10 days. Samples were taken at regular intervals to monitor degradations.

RESULTS AND DISCUSSION

Method Development and Optimization:
Hypersil BDS C_{18} column (250 X 4.6 mm; 5µm) was finalized as the column for this study, after trial and error runs with other columns like, C_8, phenyl and cyano. For the optimization of the column different mobile phase

combinations involving methanol, acetonitrile, buffer solution (Ammonium acetate) were tried, in isocratic and gradient modes.
Finally, good separation and peak shape where observed with the use of water and acetonitrile, in a gradient mode of elution. The DRIs were optimized on the same developed mobile phase, and a chromatogram of Saxagliptin along with its DRIs was observed as given in Fig. 2.

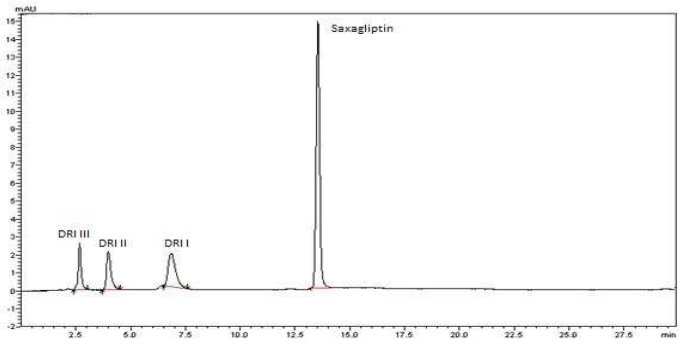


Fig. 2. Chromatogram of Saxagliptin with 1 % Spiked DRIs

Forced Degradation Studies: The forced degradation studies were performed on Saxagliptin under different stress conditions, yielding three important degradation related impurities (DRIs) of Saxagliptin. The results of the degradation studies have been tabulated below, in Table. 1.

Table. 1. Forced Degradation studies results for Saxagliptin and its DRIs

Stress Condition	Time	Saxagliptin Assay (%w/w)	DRI (%w/w)	Remark
Acid Hydrolysis, HCl 1N, 60˚C	6 hours	82.64	7.27 (DRI-I) 5.06 (DRI-II)	DRI-I and DRI-II
Base Hydrolysis, NaOH 1N, 60˚C	6 hours	80.13	15.27 (DRI-III)	DRI-III
Oxidation, 30% H_2O_2	10 days	99.27	-	No DRI
Thermal, 80˚C	10 days	99.01	-	No DRI
Photo-Stability	10 days	99.97	-	No DRI

Structural Elucidation:
DRI-I: The MS data of DRI-I showed $[M+H]^+$ ion at *m/z* 316 ($C_{18}H_{26}N_3O_2$) indicating the presence of the keto-iminopiperazine ring. The spectral data also showed ion at *m/z* 271 for the loss of ammonia from *m/z* 288, thereby confirming the presence of amine group. Thus, with the help of the mass spectral data and literature study the DRI-I was confirmed as, 4-(3-hydroxytricyclo[3.3.1.1^{3,7}]dec-1-yl)-6-imino octahydro-3H-cyclopropa[4,5]pyrrolo[1,2-a]pyrazine-3-one.

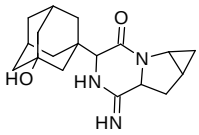


Fig. 3. DRI-I

DRI-II: The MS data of DRI-II showed $[M+H]^+$ ion at *m/z* 335 ($C_{18}H_{27}N_2O_4$). The spectra indicates that there was no substitution on the adamantly group, due to the presence of major product ion at *m/z* 180. Based on the further spectral data the DRI-II was confirmed as, (1S, 5S)-2-[amino(3-hydroxytricyclo[3.3.1.1^{3,7}]dec-1-yl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carboxylic acid.

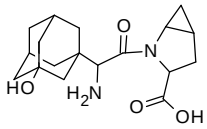


Fig. 4. DRI-II

DRI-III: The MS data of DRI-III showed $[M+H]^+$ ion at *m/z* 335 ($C_{18}H_{27}N_2O_4$). The DRI-III is isomeric to DRI-II. The spectra shows two major ions at *m/z* 317 and *m/z* 289. The other product ions confirm presence of carboxylic group in the DRI-III structure, which was confirmed as, N-[1-(3-hydroxytricyclo[3.3.1.1^{3,7}]dec-1-yl)ethyl]-2-azabicyclo[3.1.0]hexane-3-carboxamide.

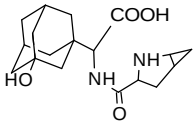
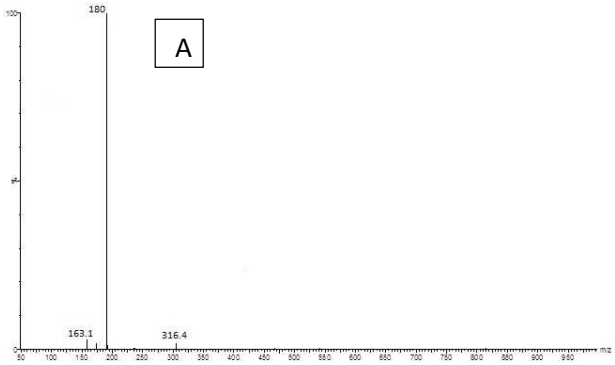
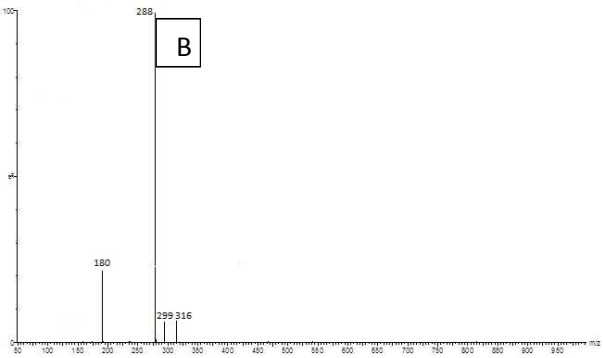
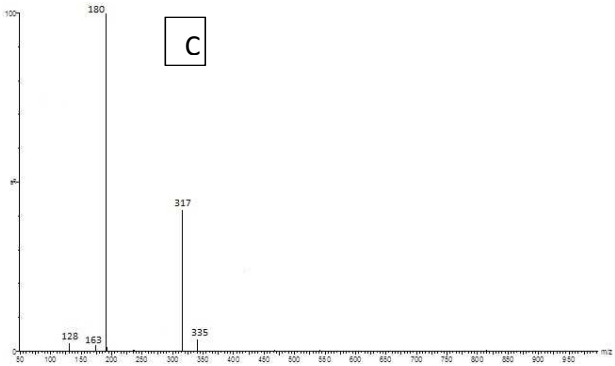


Fig. 5. DRI-III







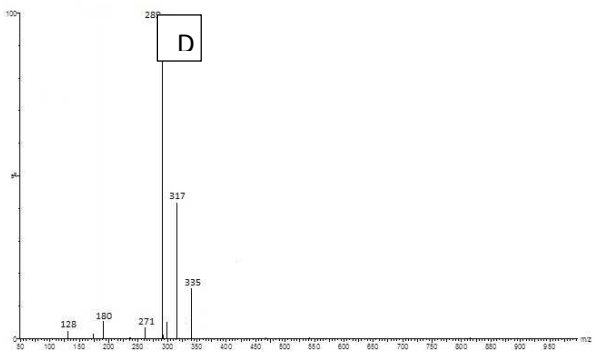


Fig. 6. Mass spectra of (A) Saxagliptin, (B) DRI-I, (C) DRI-II, (D) DRI-III

Method Validation:

To make sure that the developed method is reproducible and reliable, it was validated as per the ICH guidelines. [7-9]

Accuracy: For accuracy studies, recovery experiments were performed in triplicate at three concentrations, i.e., 50%, 100% and 150% of impurities spiked to Saxagliptin (1000 µg/ml). For assay method, accuracy was studied at three concentration levels of 50 µg, 100 µg and 150 µg, respectively. The results for the accuracy are given in the Table. 3., below.

Table. 3. Accuracy Results

Parameters	Saxagliptin	DRI-I	DRI-II	DRI-III
Accuracy (50%)				
Amount added (µg/ml)	25	0.75	0.75	0.75
Amount recovered (µg/ml)	24.68	0.759	0.737	0.771
Percentage Recovery	98.72	101.10	98.26	102.80
% RSD	0.064	1.171	0.068	0.486
Accuracy (100%)				
Amount added (µg/ml)	50	1.5	1.5	1.5
Amount recovered (µg/ml)	50.429	1.535	1.482	1.497
Percentage Recovery	100.84	102.00	98.66	99.33
% RSD	0.372	1.193	0.076	1.597
Accuracy (150%)				
Amount added (µg/ml)	75	2.25	2.25	2.25
Amount recovered (µg/ml)	75.18	2.23	2.27	2.22
Percentage Recovery	100.24	99.11	100.83	98.69
% RSD	0.086	0.632	0.325	0.477

Precision: The precision studies were carried out by injecting six replicates of standard solution for Saxagliptin (100 µg/ml), and for the DRIs by spiking 0.15% of each DRI with Saxagliptin (1000 µg/ml). The % RSD of each DRI were calculated and tabulated in Table. 4.

Table. 4. Precision Results

Parameters	Saxagliptin	DRI-I	DRI-II	DRI-III
System Precision (%RSD)	0.984	1.604	1.183	1.678
Method Precision (%RSD)	0.315	0.978	0.635	1.327

LOD and LOQ: The LOD and LOQ of Saxagliptin and the three DRIs were estimated at signal to noise ratio (S/N) of 3:1 and 10:1, respectively, by injecting a series triplicate injections with known concentrations. The results of which are given in Table. 5.

Table. 5. LOD and LOQ Results

Parameters	Saxagliptin	DRI-I	DRI-II	DRI-III
LOD (µg/ml)	0.03	0.08	0.05	0.05
LOQ (µg/ml)	0.10	0.25	0.15	0.15

Linearity: The linearity test solutions of Saxagliptin and its DRIs were prepared at ten different concentration levels ranging from LOQ to 250% of the specification level (Saxagliptin 1000 µg/ml). The peak area vs concentration was studied and co-efficient of regression (r^2) values were ≥ 0.9940, there by showing good linearity of the method. The data for linearity is summarized in Table. 6.

Table. 6. Linearity Results

Parameters	Saxagliptin	DRI-I	DRI-II	DRI-III
Range (µg/ml)	10 - 100	0.2 - 2.5	0.2 - 2.5	0.1 - 2.0
Co-efficient of Regression (r^2)	0.9998	0.9993	0.9986	0.9940

Robustness: Robustness of the developed method was evaluated by changing flow rate by 10% (1.0 ml ± 0.1 ml), mobile phase composition and column temperature. By evaluating all these chromatographic conditions, the resolution between the analytes was found to be good, while the tailing factor of each analyte was below 1.2; indicating the robustness of the method.

CONCLUSION

Three major Degradation related impurities of Saxagliptin were successfully isolated by semi-preparative HPLC and characterized with the help of Mass spectroscopy. Furthermore, a simple gradient RP-HPLC method was developed and validated for the determination of Saxagliptin and its degradation related impurities, as per the ICH guidelines.

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