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Development and Validation of Stability Indicating Analytical method For Estimation of Dapaglifliozin and Saxagliptin and characterization of some degradant Impurities using LC-MS/MS

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ABSTRACT

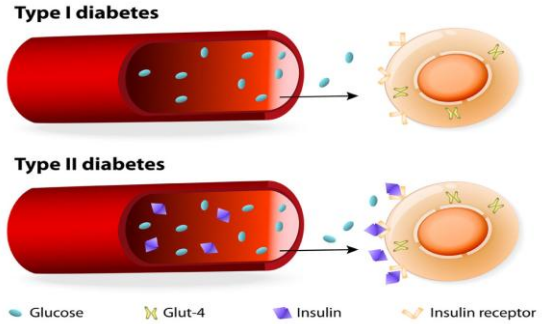
The goal of this study was to see how Dapagliflozin and Saxagliptin degraded under acidic, basic, oxidative, photolytic, and thermal stress conditions, as recommended by the International Conference on Harmonization (ICH). Under basic and acidic stress circumstances, dapagliflozin was found to be liable, whereas it was stable under oxidative, photolytic, and thermal stress conditions. Similarly, Saxagliptin was found to be unstable under oxidative, photolytic, and thermal stress conditions, but liable to acidic and basic stress conditions. Two degradation products (DPs) for dapagliflozin and two degradation products (DPs) for saxagliptin were identified, and their chromatographic separation was performed on a Hypersil, BDS, C18, (150mm x 4.6mm, 5m) column with a mobile phase consisting of buffer (pH-5): isocratic acetonitrile. The ion transitions were measured in positive mode, with Dapagliflozin's MRM transition being 409.300245.200 Da and Saxagliptin's MRM transition being 316.300299.700 Da. All of the stressed samples were analyzed using ESI-MS/MS and LC-MS/MS. MRM scan mode was used to describe Dapagliflozin and Saxagliptin, as well as their degradation products, and ESI-MS/MS spectra were used to derive fragmentation patterns. By comparing fragmentation patterns of Dapagliflozin and Saxagliptin with those of Dapagliflozin and Saxagliptin, structural elucidation of DPs of Dapagliflozin and Saxagliptin was obtained. As per the ICH guidelines, the developed method has been validated for specificity, linearity, accuracy, precision, and robustness.

Key Words: Dapagliflozin, Saxagliptin, LLC-MS/MS method. Validation, ICH Q2 (R1) guidelines.

INTRODUCTION[1-18]

Definition of Diabetes Mellitus [1-3]

Diabetes mellitus, commonly known as just diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. Diabetes affects approximately 200 million people worldwide, including more than a quarter of elderly living in developed countries. Diet and exercise are first line treatments along with oral hypoglycemic drugs to achieve the goal of improving glycogenic control and preventing both micro vascular and macro vascular complications.



Types of Diabetes Mellitus[2]

Antidiabetic Drugs[4-5]

Antidiabetic are a class of drugs that are used to treat high sugar level in blood. Antidiabetic therapy seeks to prevent the complications such as stroke and myocardial infarction. Evidence suggests that reduction of diabetes level can decrease the risk of stroke by 34%, of ischemical heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease. There are many classes of antidiabetic, which lower blood sugar by different means. Among the most important and most widely used medications are Biguanides, Sulphonylureas, Non-sulphonylurea, Thiazolidinediones, Alpha-glucosidase inhibitors, Dipeptidyl peptidase IV (DPP-IV) inhibitors and Glucagon-like peptide-1 (GLP-1) analogues.

INTRODUCTION TO MASS SPECTROMETRY[11-12]

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio (*m/z)* of charged particles (ions). Although there are many different kinds of mass spectrometers, all of them make use of electric or magnetic fields to manipulate the motion of ions produced from an analyte of interest and determine their *m/z*. The basic components of a mass spectrometer are the ion source, the mass analyzer, the detector, and the data and vacuum systems. The ion source is where the components of a sample introduced in a MS system are ionized by means of electron beams, photon beams (UV lights), laser beams or corona discharge. In the case of electrospray ionization, the ion source moves ions that exist in liquid solution into the gas phase. The ion source converts and fragments the neutral sample molecules into gas-phase ions that are sent to the mass analyzer. While the mass analyzer applies the electric and magnetic fields to sort the ions by their masses, the detector measures and amplifies the ion current to calculate the abundances of each mass-resolved ion. In order to generate a mass spectrum that a human eye can easily recognize, the data system records, processes, stores, and displays data in a computer.The interface between a liquid phase techniques (HPLC) with a continuously flowing elute, and a gas phase technique carried out in a vacuum was difficult for a long time. The advent of electrospray ionization changed this. Currently, the most common LC-MS interfaces are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo-ionization (APPI).

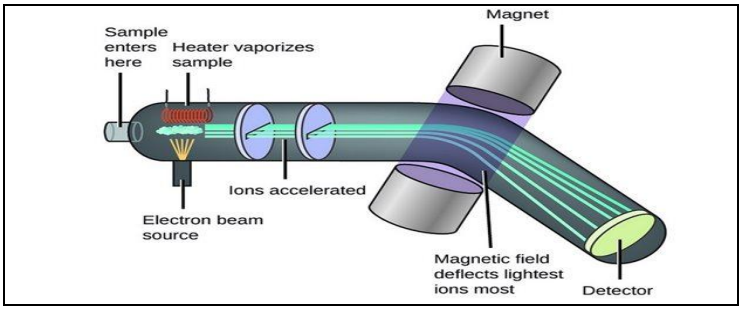


Fig.1.10: Diagram of Mass Spectrometry

INTRODUCTION TOLC/MS/MS (Triple Quadrupole)[13-14]

A linear series of three quadrupoles can be used; known as a triple quadrupole mass spectrometer. The first (Q1) and third (Q3) quadrupoles act as mass filters, and the middle (q2) quadrupole is employed as a collision cell. This collision cell is an RF only quadrupole (nonmassfiltering) using argon, helium or nitrogen gas (~10-3 Torr, ~30 eV) to induce collision induced dissociation of selected parent ion(s) from Q1. Subsequent fragments are passed through to Q3 where they may be filtered or scanned fully.

This process allows for the study of fragments (daughter ions) which are crucial in structural elucidation. For example, the Q1 may be set to "filter" for a drug ion of a known mass, which is fragmented in q2. The third quadrupole (Q3) can then be set to scan the entire *m/z* range, giving information on the sizes of the fragments made. Thus, the structure of the original ion can be deduced.

MATERIAL AND METHOD

Melting Point of Drugs

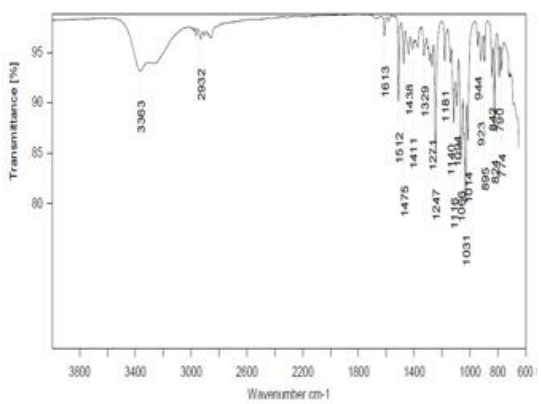
Drug Name	Reported (°C)	Observed (°C)
Dapagliflozin	63-66^{o}C	64^{o}C
Saxagliptin	217-220^{o}C	219^{o}C

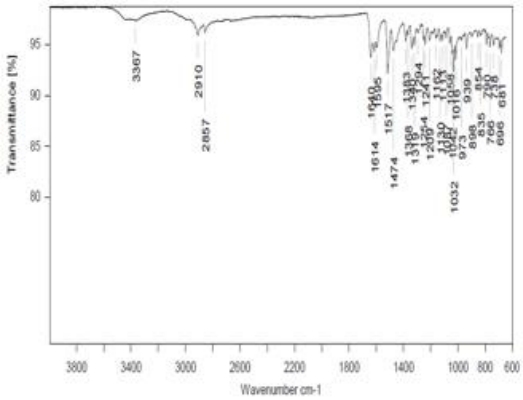
Solubility Study

Solubility Data of Dapagliflozin and Saxagliptin

Solvent	Solubility of Dapagliflozin	Solubility of saxagliptin
Water	Insoluble	Very slightly soluble
Acetonitrile	Soluble	Slightly Soluble
Methanol	Freely soluble	Soluble

Identification by IR Spectroscopy





IR Spectra of Dapagliflozin IR Spectra of Saxagliptin

IR Interpretation of Dapagliflozin

Functional Group	Frequency (cm^{-1})
C-Cl stretching	600-800
O-H stretching	3363
C=Cstretching	1411-1512

IR Interpretation of Saxagliptin

Functional Group	Frequency (cm^{-1})
N-H stretching	3367
C=O stretching	1640
C=N stretching	2857-2910

METHOD DEVELOPMENT

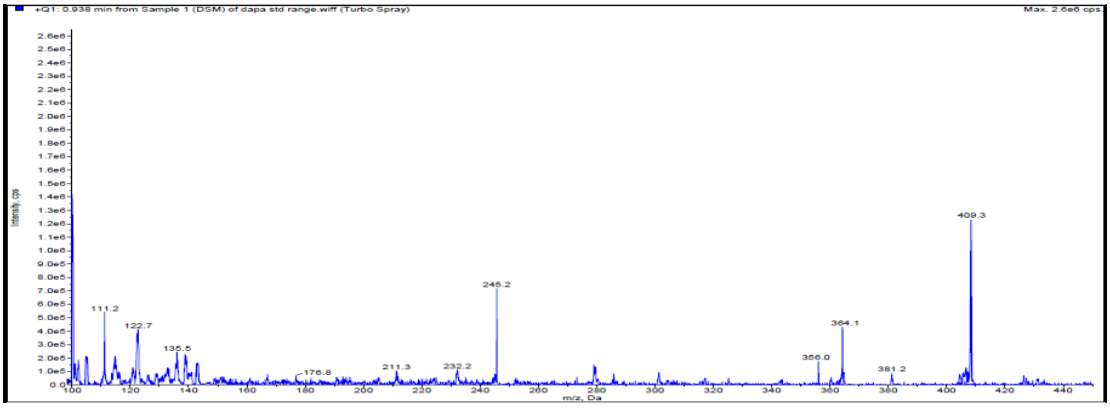
Mass Spectrometric conditions

Instrument	Liquid chromatography Mass spectro Dapaer (API-2000) equipped with auto sample, auto injector, column oven, ion source ESI electron spray ionizer with Q1 and collision energy.		
Ion Source setting		***Scan setting***	
Ion source	ESI	Polarity	Positive ion
Curtain Gas	20psi	Scan type	MRM
Ion Spray Voltage	5500	Scan time	1-10 min
Temperature	400°C	Declustering Potential	80
Ion Source Gas(GS1)	50psi	Focusing Potential	350
Ion Source Gas(GS2)	60psi	Entrance Potential	10
Scan type	Dapagliflozin	MRM:(Q1)409.300 Da and (Q3) 245.200 Da	
	Saxagliptin	MRM:(Q1)316.300 Da and (Q3) 299.700 Da	

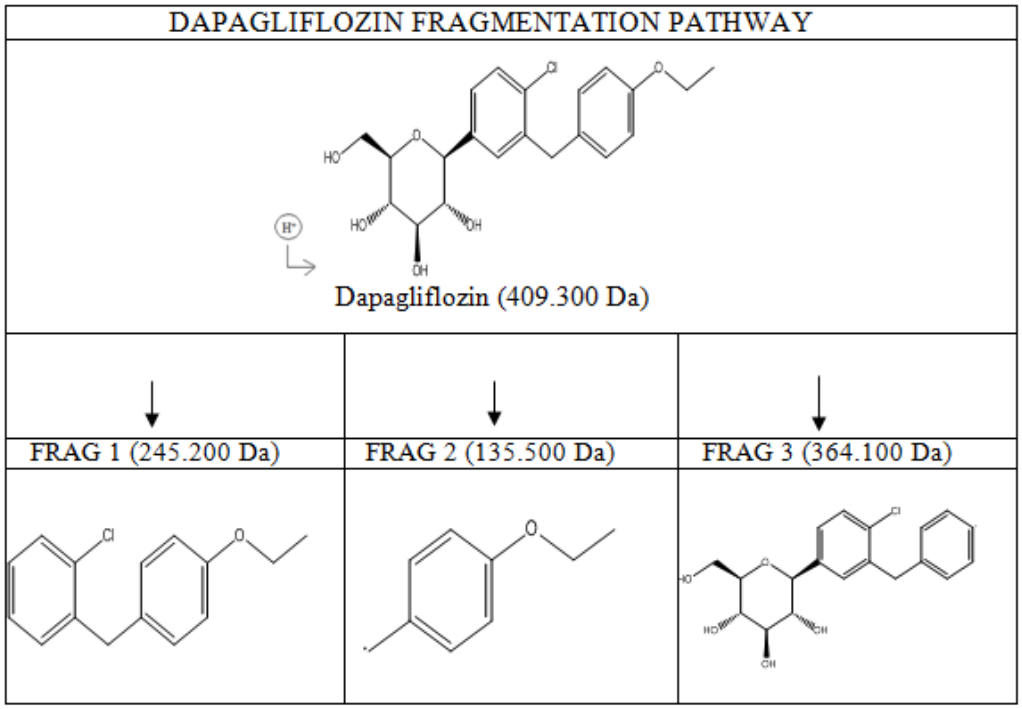
- **Chromatographic condition:**

Column	:	Agilent, Zorbax, C18, (150mm x 4.6mm), 5µm			
Flow rate	:	1.0 mL/min	Injection volume	:	20 µL
Column oven temperature	:	35 °C	Run time	:	10 min
Column oven compartment	:	Ambient	Mode	:	Isocratic
Dapagliflozin R.T	:	About 1.6 min			
SaxagliptinR.T	:	About 5.2 min			

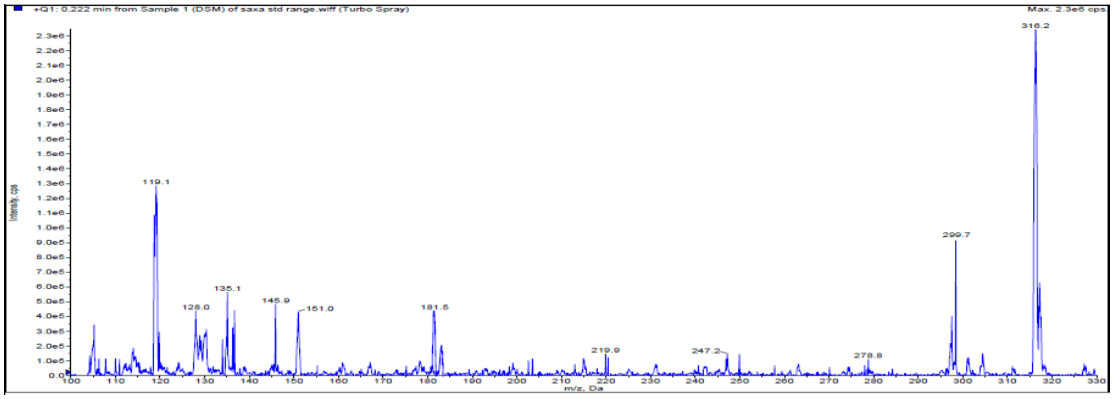
Mass Determination



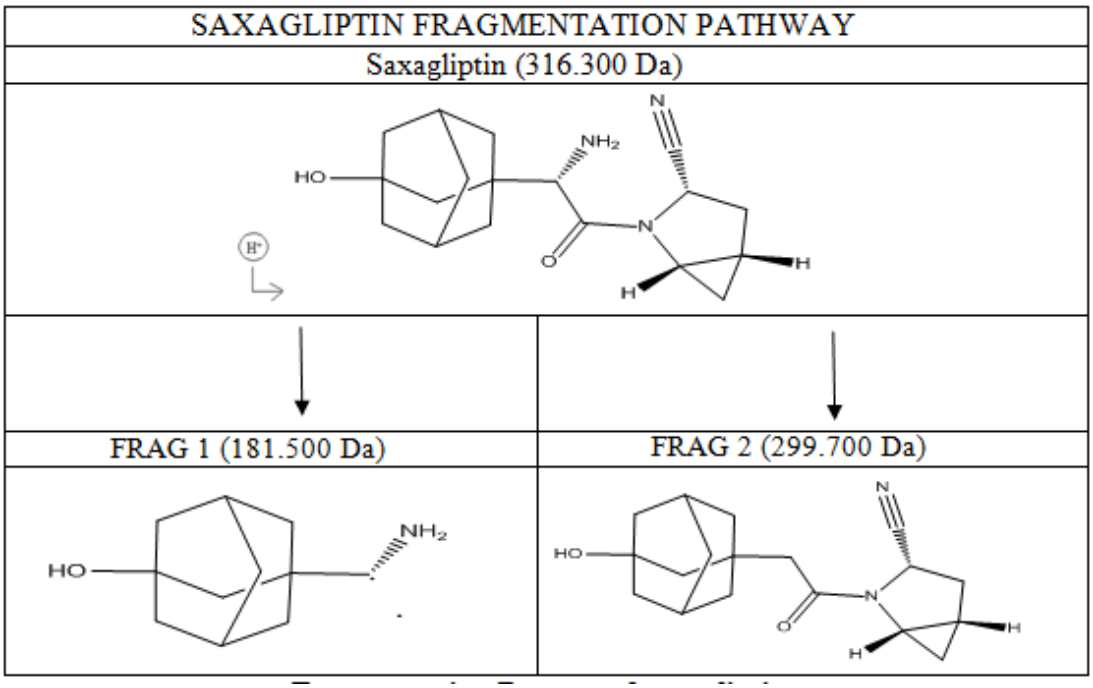
Mass spectra of Dapagliflozin



Fragmentation Pattern of Dapagliflozin

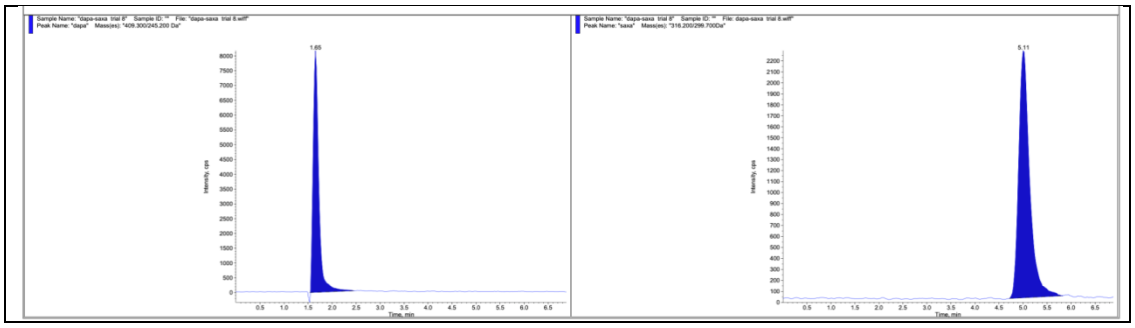


Mass spectra of Saxagliptin



Fragmentation Pattern of saxagliptin

Chromatographic Trials



Chromatogram of Dapagliflozin and Saxagliptininbuffer (pH-5): acetonitrile (70:30v/v) (FINAL)

FORCED DEGRADATION STUDY

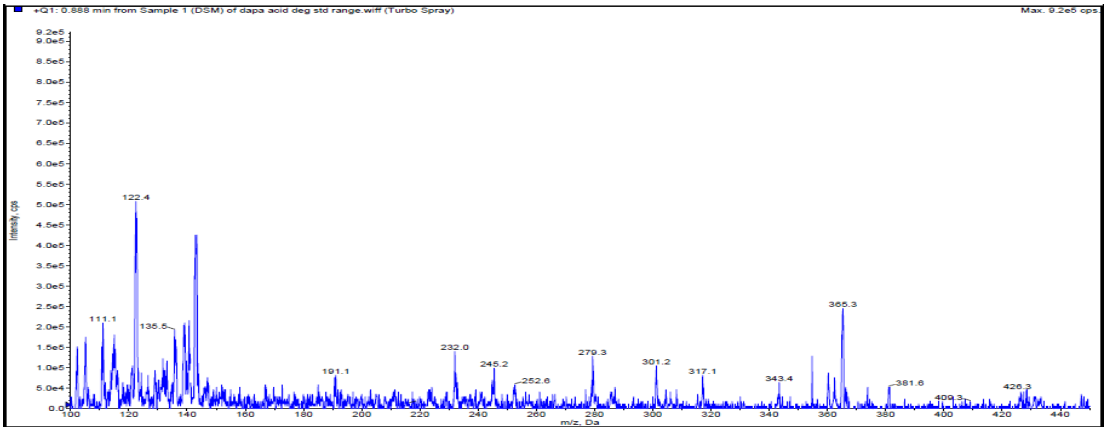
Different Degradation Conditions for Dapagliflozin

Sr. No.	Stress Type	Stress Condition
1	Acid Degradation	1 N HCl at 60^{o}C for3 hr.
2	Base Degradation	1 N NaOH at 60^{o}C for 5 hr.
3	Oxidative Degradation	30.0 % H_2O_2at 60^{o}Cfor 4 hrs.
4	Thermal Degradation	105^{o}C for 5 days
5	Photolytic Degradation	UV for 5 days

Different Degradation Conditions for Saxagliptin

Sr. No.	Stress Type	Stress Condition
1	Acid Degradation	1 N HCl at 60°C for 6 hr.
2	Base Degradation	1 N NaOH at 60°C for 8 hr.
3	Oxidative Degradation	30.0 % H_2O_2 at 60°C for 5 hrs.
4	Thermal Degradation	105 °C for 5 days
5	Photolytic Degradation	UV for 5 days

ESI-MS/MS Spectra and Fragmentation Pattern of acidic Degradation Solution of Dapagliflozin



Mass Spectra of acidic degradation solution of Dapagliflozin

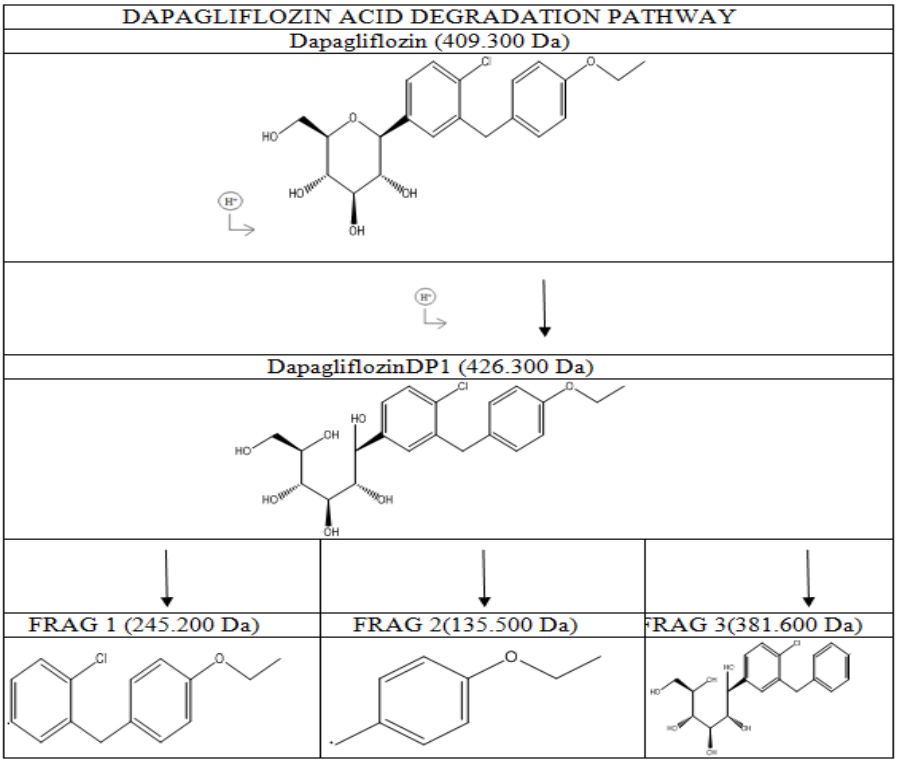
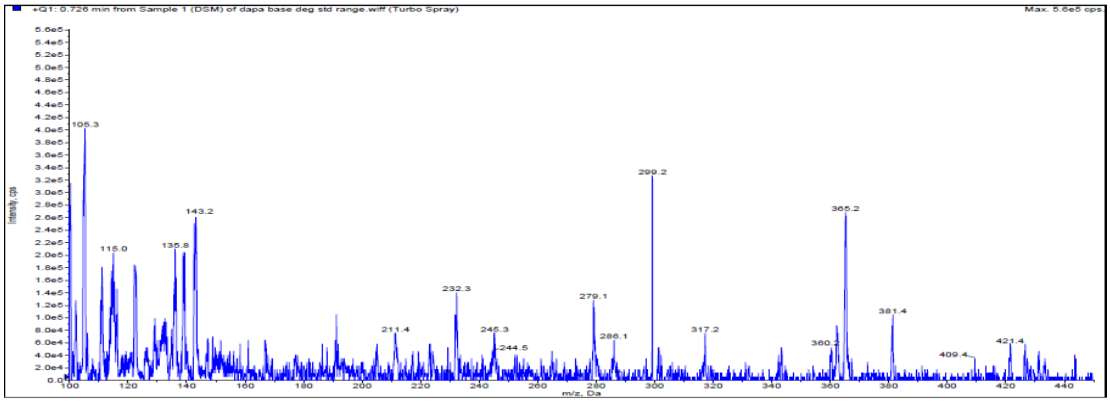
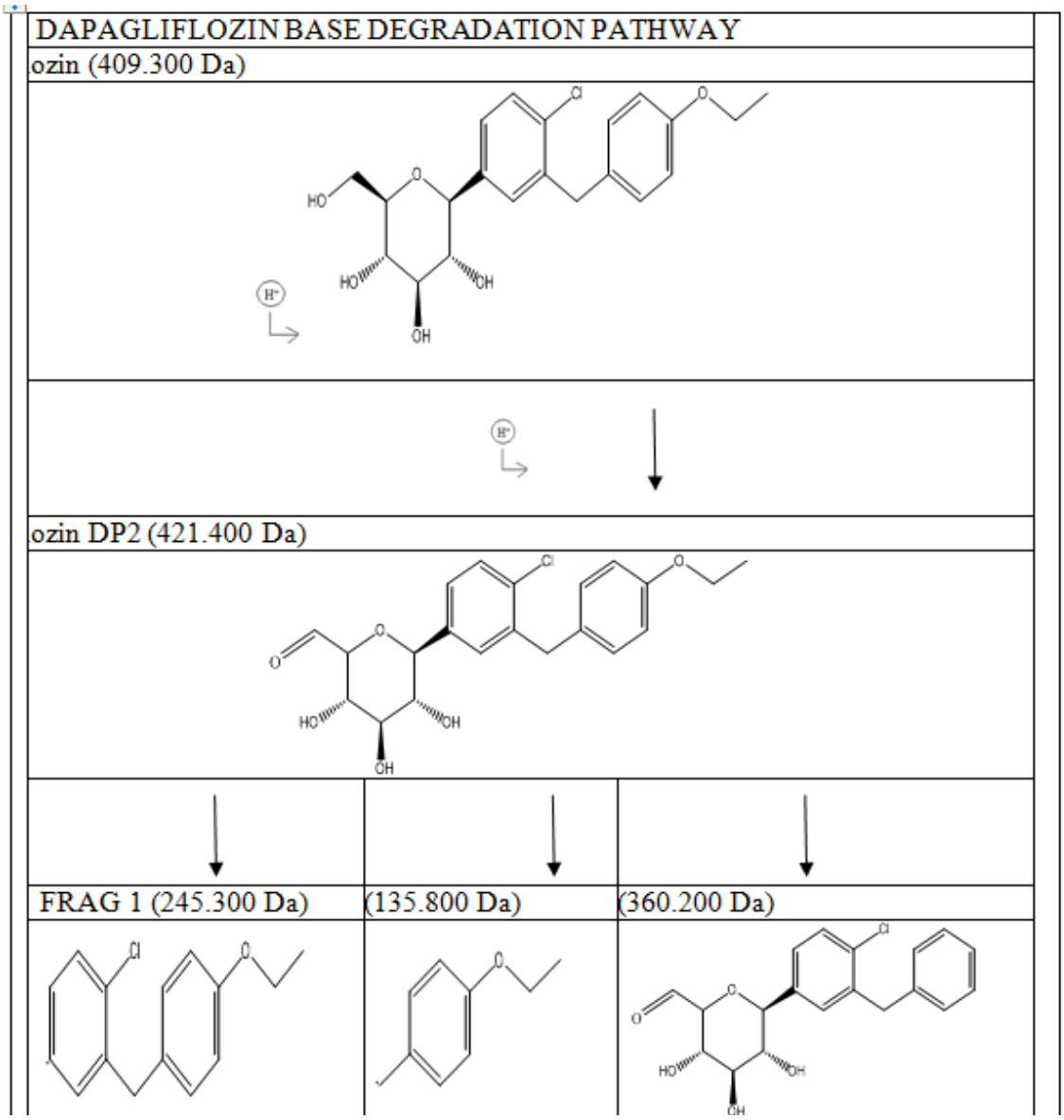


Fig 6.17: Dapagliflozin acidic Degradation Pathway

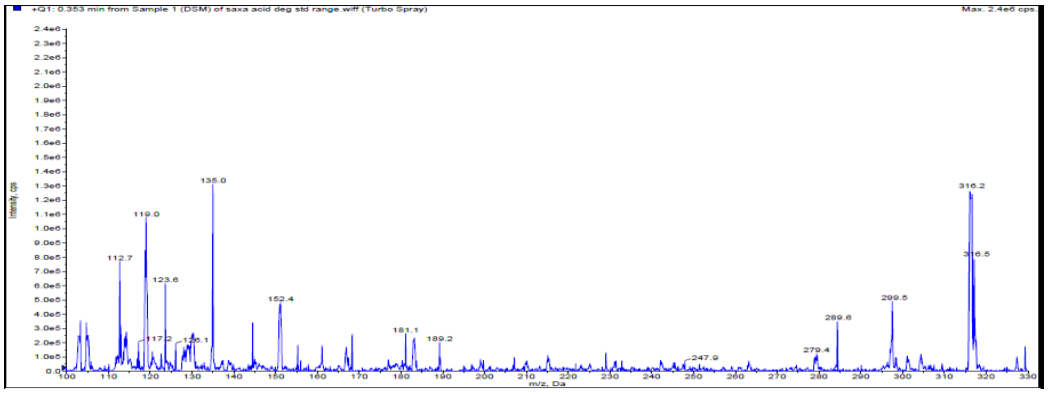
ESI-MS/MS Spectra and Fragmentation Pattern of basic Degradation Solution of Dapagliflozin



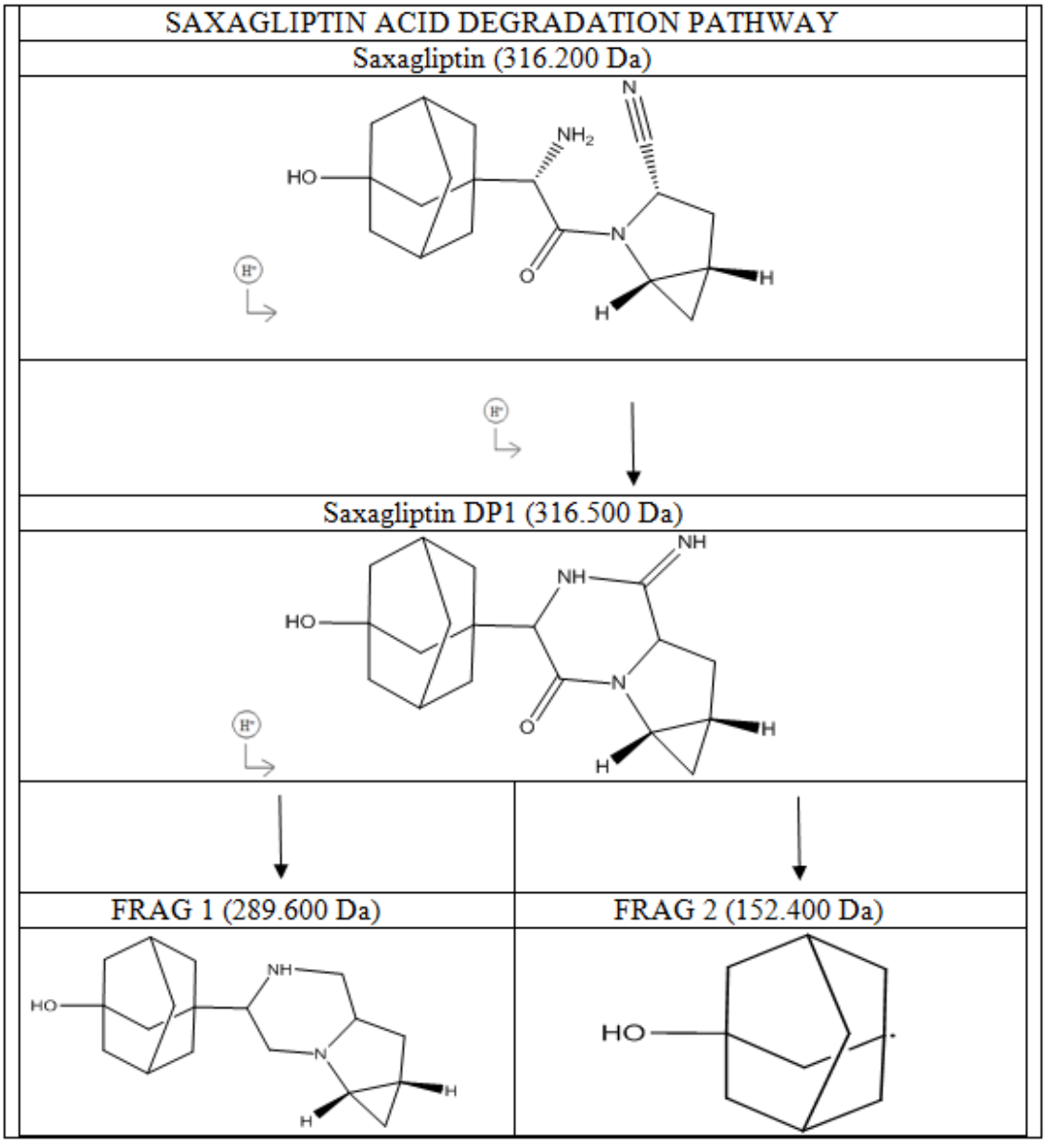
Mass Spectra of basic degradation solution of Dapagliflozin



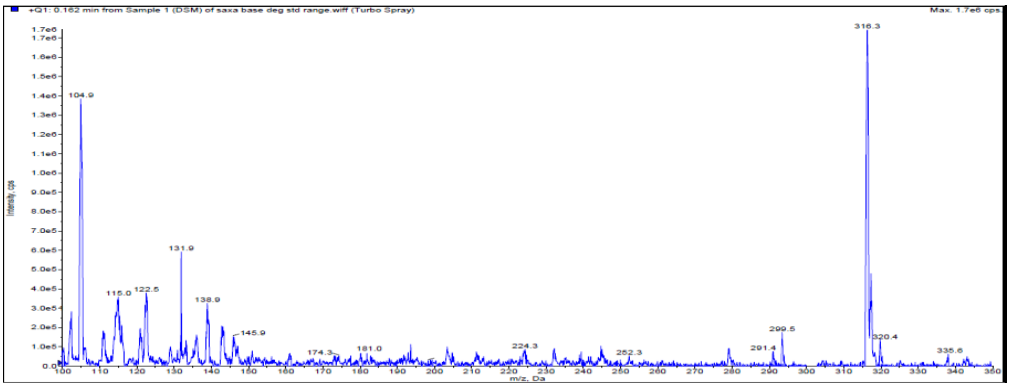
Dapagliflozin basic Degradation Pathway



Mass Spectra of acidic degradation solution of Saxagliptin



Saxagliptin acidic Degradation Pathway



Mass Spectra of basic degradation solution of Saxagliptin

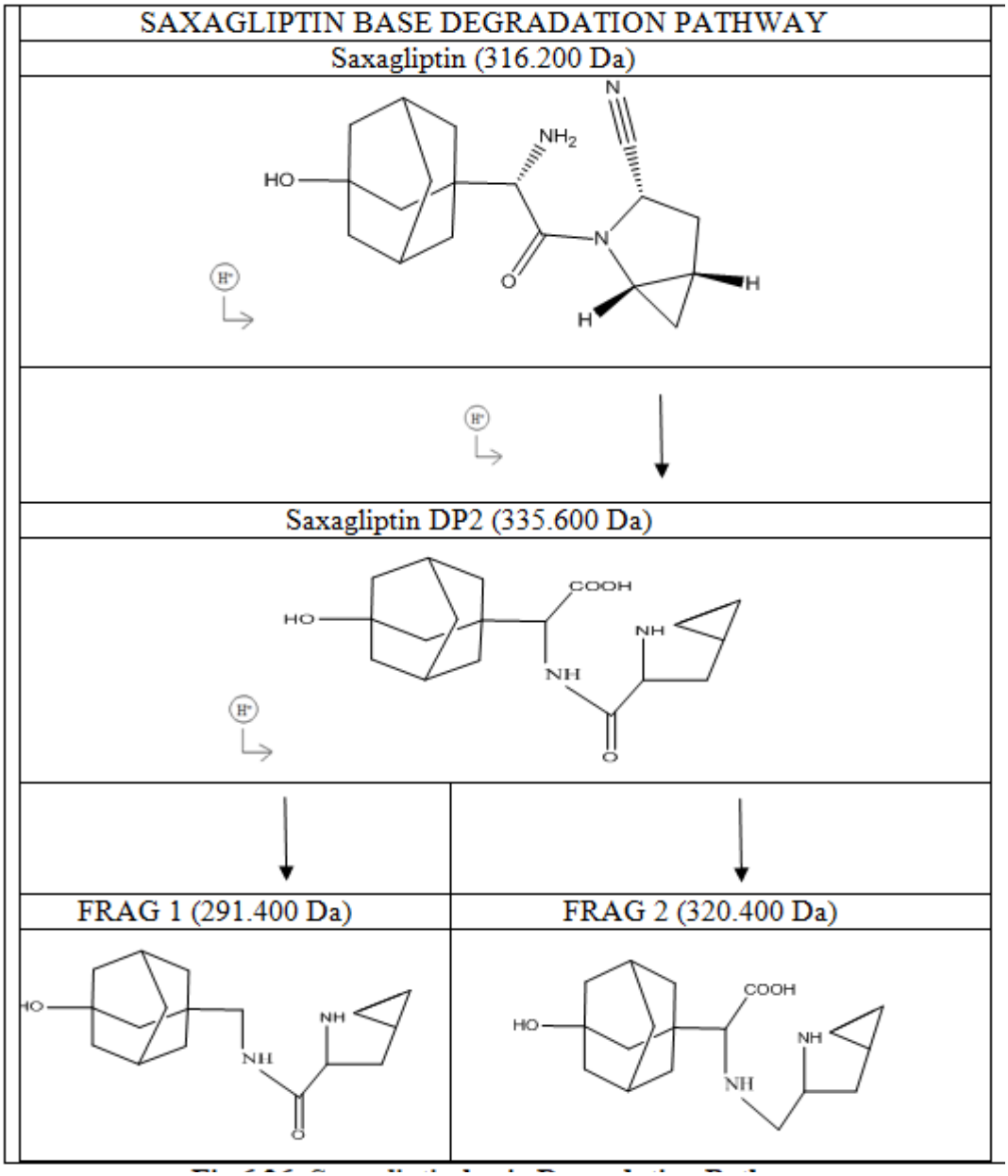


Fig 6.26: Saxagliptin basic Degradation Pathway

METHOD VALIDATION

Specificity

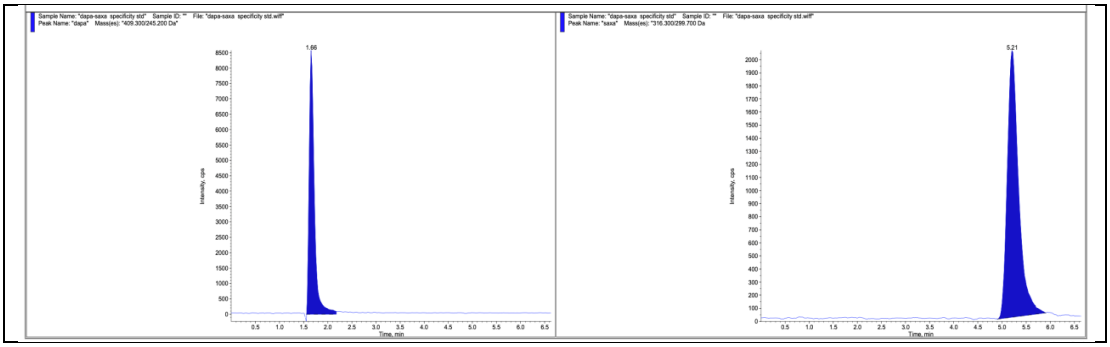
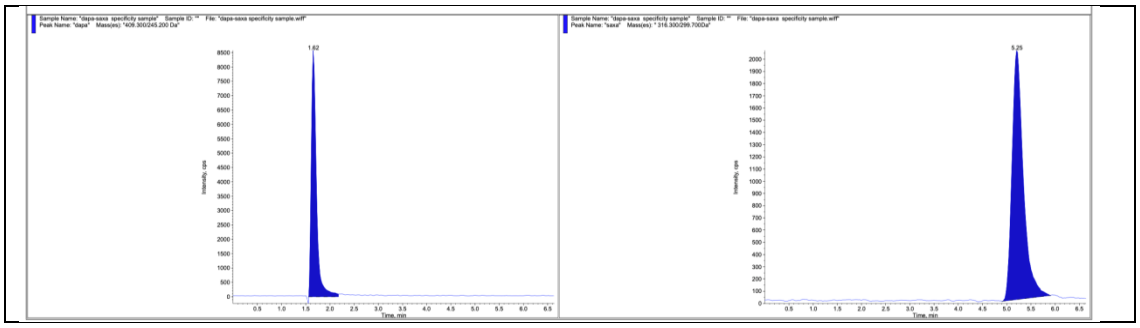
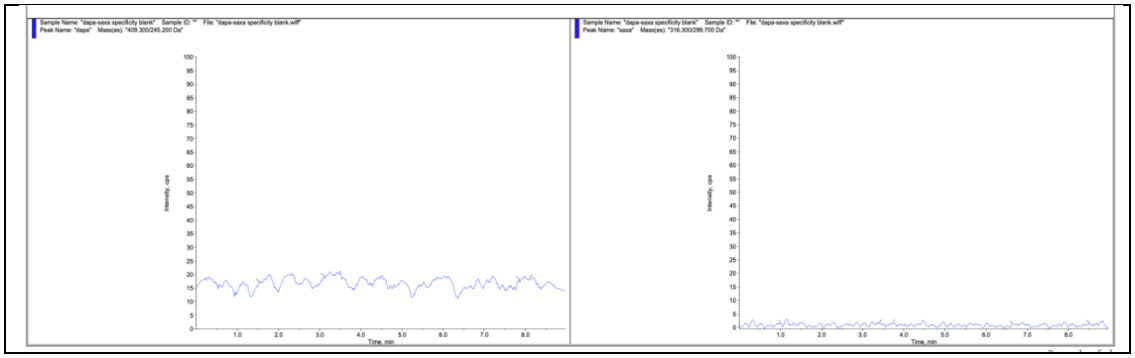


Fig6.34: Chromatogram of Dapagliflozin and Saxagliptin Standard



Chromatogram of Dapagliflozin and Saxagliptin Sample



Chromatogram of Dapagliflozin and Saxagliptin Blank

Linearity and Range

The linearity for Dapagliflozin and Saxagliptin were assessed by analysis of standard solution in range of 0.5-1.5µg/ml and 0.25-0.75 Dapagliflozin and Saxagliptin respectively. Correlation co-efficient for calibration curve Dapagliflozin and Saxagliptin was found to be 0.997and 0.999respectively.

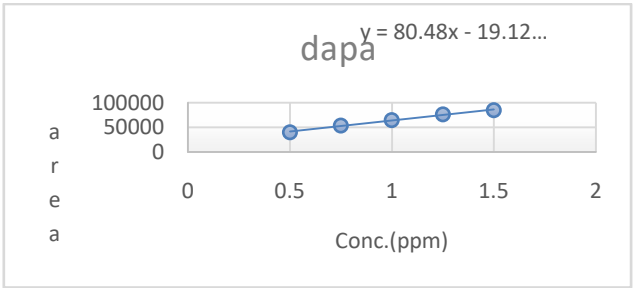
The regression line equation for Dapagliflozin and Saxagliptinare as following:

For Dapagliflozin and Saxagliptin: **y = 80.48x –19.12 and y = 6880x –438.3**

Linearity Data for Dapagliflozin

Sr.No	Concentration (µg/ml)	Area
1	0.50	40218.835

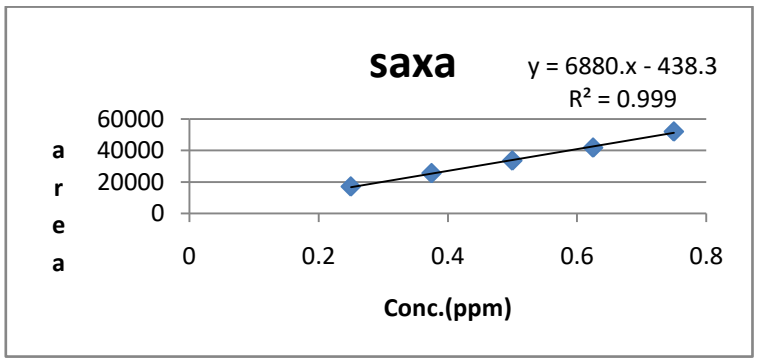
2	0.75	53503.872
3	1.00	64793.893
4	1.25	76675.038
5	1.50	85106.095



Calibration Curve of Dapagliflozin(0.5-1.5µg/ml)

Linearity Data for Saxagliptin

Sr.No	Concentration (µg/ml)	Area
1	0.25	17056.756
2	0.375	25548.039
3	0.5	33506.757
4	0.625	41743.166
5	0.75	51961.095



Calibration Curve of Saxagliptin (0.25-0.75µg/ml)

Precision

Repeatability

The data for repeatability of peak area measurement for Dapagliflozin and Saxagliptin, based on six measurements of same solution of Dapagliflozin and Saxagliptin are depicted in table respectively. The %RSD for Dapagliflozin and Saxagliptin was found to be 0.674 and 1.889respectively.

Repeatability Data for Dapagliflozin

Dapagliflozin				
Sr. No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	1.00	65221.823 65201.652 64896.241 65207.614 64882.621 66087.521	65249.579±439.655	0.674

Repeatability Data for Saxagliptin

Saxagliptin				
Sr. No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	0.5	32856.149 33204.924 34086.651 32556.41 32984.991 34007.612	33282.790±628.643	1.889

Intraday precision

The data for intraday precision for Dapagliflozin and Saxagliptin is shown in table 6.14 and 6.15 respectively. The % R.S.D. for Intraday precision was found to be 0.913-1.534 for Dapagliflozin and 1.056 – 1.880 for Saxagliptin.

Intraday precision data for Estimation of Dapagliflozin

Sr. No.	**Dapagliflozin**		
	Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	0.5	39365.955±359.310	0.913
2	1.0	65219.404±1000.245	1.534
3	1.5	85437.762±1283.181	1.502

Intraday precision data for Estimation of Saxagliptin

Sr. No.	**Saxagliptin**		
	Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**% R.S.D**

1	0.250	18263.924±343.382	1.880
2	0.500	33141.177±349.861	1.056
3	0.750	53318.806±678.846	1.273

Interday precision

The data for intraday precision for Dapagliflozin and Saxagliptinis shown in table respectively. The % R.S.D. for interday precision was found to be 0.572-1.449 for Dapagliflozin and 0.547-1.327Saxagliptin.

Interday Precision data for Estimation of Dapagliflozin

Sr. No.	Dapagliflozin		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	0.5	39607.378±573.958	1.449
2	1.0	65530.209± 847.147	1.293
3	1.5	83053.019±474.933	0.572

Interday Precision data for Estimation of Saxagliptin

Sr. No.	Saxagliptin		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	0.25	19549.988±106.943	0.547
2	0.5	33860.312± 299.523	0.885
3	0.75	53973.044±716.235	1.327

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 19 respectively. Percentage recovery for Dapagliflozin and Saxagliptinwas100.421-100.699% and 99.638-100.150 respectively.

Recovery Data for Dapagliflozin

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	0.5	0.4	0.404	100.895	100.699 ± 1.228
2		0.5	0.4	0.407	101.818	
3		0.5	0.4	0.398	99.385	

4	100 %	0.5	0.5	0.506	101.179	100.421 ± 0.910
5		0.5	0.5	0.497	99.407	
6		0.5	0.5	0.503	100.677	
7	120 %	0.5	0.6	0.606	101.077	100.437 ± 0.570
8		0.5	0.6	0.600	99.973	
9		0.5	0.6	0.602	100.261	

Recovery Data for Saxagliptin

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	0.25	0.2	0.298	99.201	99.638 ± 0.916
2		0.25	0.2	0.306	101.867	
3		0.25	0.2	0.303	101.085	
4	100 %	0.25	0.25	0.298	99.201	100.280 ± 1.139
5		0.25	0.25	0.306	101.867	
6		0.25	0.25	0.303	101.085	
7	120 %	0.25	0.3	0.298	99.201	100.150 ± 1.471
8		0.25	0.3	0.306	101.867	
9		0.25	0.3	0.303	101.085	

LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD= 3.3 * SD/slope of calibration curve

LOQ=10 * SD/slope of calibration curve

Limit of Detection

Limit of Detection Data for Dapagliflozin and Saxagliptin

Dapagliflozin	Saxagliptin
LOD = 3.3 x (SD / Slope) = 3.3 x (1502.896/45178.274) = 0.110µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (738.867/68803.044) = 0.035µg/ml

Limit of Quantitation

Limit of Quantitation Data for Dapagliflozin and Saxagliptin

Dapagliflozin	Saxagliptin
LOQ = 10 x (SD / Slope) = 10 x (1502.896/45178.274) = 0.333µg/ml	LOQ = 10 x (SD / Slope) = 10 x (738.867/68803.044) = 0.107µg/ml

Robustness

The effect of changes was found to be within the acceptance criteria as shown in table 6.22 and 6.23 respectively. The %RSD should be less than 2%.

Robustness data for Dapagliflozin

Sr No.	Area at Flow rate (- 2.0ml/min)	Area at Flow rate (+ 2.0ml/min)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	106782.862	52915.111	72615.49	57311.266
2	104723.691	53804.634	71283.292	58631.952
3	105562.341	54889.641	72965.622	58013.951
% R.S.D	0.980	1.836	1.228	1.140

Robustness data for Saxagliptin

Sr No.	Area at Flow rate (- 2.0ml/min)	Area at Flow rate (+ 2.0ml/min)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	62244.592	28846.068	38368.127	31168.662
2	61429.374	27937.952	39208.862	32276.981
3	61892.347	28053.641	38598.542	32097.625
% R.S.D	0.661	1.748	1.122	1.868

Analysis of marketed formulation by developed method.

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Remo- V. The results are shown in table

Analysis of Marketed Formulation

Tablet	Label claim		Assay (% of label claim*) Mean ± S. D.	
	Dapagliflozin Saxagliptin	Saxagliptin	% Dapagliflozin Saxagliptin	% Saxagliptin
QTERN 5/10 mg	10mg	5mg	100.328±0.742	100.217± 0.396

CONCLISION

A novel attempt in a field of research has been made to develop and validate stability indicating analytical method via LC-MS/MS. The objective of this study was to study the degradation behaviour of Dapagliflozin and Saxagliptin under acidic, basic, oxidative, photolytic and thermal stress conditions as per prescribed International Conference on Harmonization (ICH) guidelines. Dapagliflozin and Saxagliptin was degraded under acidic and basic stress conditions. Dapagliflozin was degraded under basic and acidic stress condition and Saxagliptin was degraded under acidic and basic stress condition.A total of two degradation products (DPs) were characterized for dapagliflozin and two degradation products (DPs) were characterized for Saxagliptin, and their chromatographic separation was accomplished on Hypersil, BDS, C18, (150mm x 4.6mm, 5µm) column using a mobile phase consisting buffer (pH-5) acetonitrile in a isocratic elution mode. The ion transitions were quantified in positive mode with MRM transition of 409.300→245.200 Da for

Dapagliflozin and 316.300→299.700 Da for Saxagliptin. Retention time of Dapagliflozin and Saxagliptin were found to be 1.6 min and 5.2 min respectively with a flow rate of 1.0 ml/min. Dapagliflozin and Saxagliptin and its degradation products were characterized based on MRM scan mode and fragmentation patterns were obtained from ESI-MS/MS spectra. Structural elucidation of DPs of Dapagliflozin and Saxagliptin was achieved by comparing their fragmentation patterns with that of Dapagliflozin and Saxagliptin The developed method has been validated as per ICH guideline by applying various validation parameters like specificity, linearity and range, accuracy precision and robustness. The LC-MS/MS method developed for the determination of Dapagliflozin and Saxagliptin is found to be specific, linear, sensitive, precise, accurate and robust in nature. The method was successfully validated in terms of specificity, precision, linearity, accuracy and robustness as per ICH guidelines. It can be concluded that the proposed method can be used for routine analysis for estimation of Dapagliflozin and Saxagliptin in its Pharmaceutical dosage form by LC-MS/MS.

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