



**Establishment of Degradation behaviour of Metformin, Dapagliflozin and Saxagliptin by LC-MS/MS and prediction of their degradation pathways**

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**ABSTRACT**

**Introduction:** The present study examined the deterioration structures of Metformin, Dapagliflozin and Saxagliptin under varying levels of stress were studied regarding the ICH's (International Conference on Harmonization) guidelines. The degradation products and probable fragmentation mechanisms investigated using LC-MS/MS, a liquid chromatography-tandem mass spectrometry method.

**Methodology:** The mobile phase was made up of isocratic elution, acetonitrile in a 70:30 volume ratio, and water containing 0.1% formic acid. For separation, an Agilent, Zorbax C<sub>18</sub> column (150x4.6mm, 5µm) was chosen. The analysis of an ionization mass spectrophotometer used curtain gas flow at 20 psi and a source temperature of 400 °C. By integrating column eluting into the apparatus for Q1 and MRM scan, drug fragments of metformin, dapagliflozin, and Saxagliptin were subjected to mass spectrometric analysis.

**Result:** Metformin, Dapagliflozin, and Saxagliptin were shown to have retention times of 2.01, 6.1, and 3.95 minutes, accordingly. The calibration curve of peak region versus maintaining focus for metformin, dapagliflozin & Saxagliptin was linear in the vicinity of 0.5-1.5 µg/ml with a r<sup>2</sup> of 0.999. Different validation parameters were used, and the findings show that they are all within acceptable limits. Studies on force deterioration in various environments, including UV light, H<sub>2</sub>O<sub>2</sub>, acid, and base.

**Conclusion:** It can be used to characterize the degradant products of Metformin, Dapagliflozin, and Saxagliptin. The proposed method was straightforward, exact, specific, robust, quick, and sensitive.

**Keywords:**

Metformin, Dapagliflozin, Saxagliptin, Force degradation studies, Liquid chromatography-Mass spectrometry, Characterization

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**INTRODUCTION:**

Determining a drug's shelf life, identifying decomposition byproducts, & establishing the pathways for deterioration are the main goals of studying a drug's stability. <sup>[1]</sup>MET is a member

of the biguanide class, which lowers blood sugar by reducing hepatic glucose synthesis and raises hormone sensitivity by improving peripheral glucose absorption and use. Glucose-sodium ratio DAP inhibits SGLT2 (SGLT2), It is in charge of the majority of the reabsorption of filtered glucose from the lumen of the proximal tubule. DAP enhances urine glucose excretion by decreasing SGLT2, which also lowers the kidney glucose threshold. These effects minimize the reabsorption of filtered glucose. [2], [3] SXG is an oral hypoglycemic medicine that is a member of the drugs belonging to the dipeptidyl peptidase-4 (DPP-4) inhibitor class. It has been used to help people with type 2 diabetes improve their glycemic control together with exercise and diet. [4] A review of the literature finds that many analytical techniques, including RP-HPLC, Spectrophotometric, and LC-MS techniques, have been reported either alone or in conjunction with other medications in pharmaceutical dose forms. [5-16] As per our literature review, till date no method is reported for the degradant generated under various stress conditions as per recommendation of ICH. LC-MS is an analytical technique, which applies LC as a method for separation and MS as a system for detection. LC-MS has a broad scope of use in different areas. Therefore, the LC-MS approach is utilized in the current investigation to characterize the degradant products of metformin, dapagliflozin, and saxagliptin.

## **MATERIALS AND METHODS:**

### ***Instrumentation***

Solid state thermal stress experiments were conducted in a oven with hot air (Kesar control system). Optical constancy investigations in a photo stability chamber were conducted (Kesar control system). Analytical balance (Shimadzu ATX-224) was used for weighing. For LC-MS/MS experiments, a chromatographic Shimadzu LC 20-AT system with MS/MS ABSciX API 2000 was employed.

### ***Chemicals and reagents***

Acetonitrile of the LC-MS grade, water, and methanol were utilized in the experiments. The Gujarat, India-based Alembic Pharmaceuticals Ltd. gave away samples of Metformin. Dapagliflozin was given as a gift sample by Zydus Cadila Pvt Ltd, Gujarat, India. The source of Saxagliptin medication was Montage Laboratories Pvt Ltd.

### ***Chromatographic conditions***

The chromatographic separations were completed using Agilent's Zorbax (150 x 4.6 mm, 5 $\mu$ m) C<sub>18</sub> column. A volume-to-volume ratio of 70:30 was used for the mobile phase, which was made up of water with 0.1% formic acid and acetonitrile. Flow rate for the delivery of mobile phases was 1 ml/min. 20 $\mu$ l syringe were used for the chromatographic injection. In these conditions, it was shown that retention time of metformin, dapagliflozin, and saxagliptin were 2.01, 6.1, and 3.95 minutes, correspondingly. The chosen runtime was ten minutes. The column was maintained at 35 °C.

### ***Preparation of mobile phase***

Acetonitrile and water in a 70:30 v/v ratio with 0.1% formic acid as the portable stage were used. Acetonitrile with water 70:30 is used as diluent.

### ***Preparation of standard solution***

10mg of MET, 10mg of DAP, and 10mg of SXG were weighed precisely and transferred into separate 100ml volumetric flasks that were clean and dry before being diluted by 3/4 volume and sonicated for 5 minutes. The volume was set to the appropriate level using diluent. 1 ml of the substance was added to a 100 ml volumetric flask of each of the aforementioned stock solutions to form the final concentration of the 1µg/ml MET, 1 µg/ml DAP, and 1 µg/ml SXG working standard solution.

### ***Force Degradation Studies***

In order to define the inherent stability properties of the active ingredients, stress testing was mandated by the ICH guideline, Stability testing of new drug substances and products (ICH Q1A (R2)). This study's objective was to use the suggested methodology to conduct stress degradation investigations on the MET, DAP, and SXG.

- Hydrolytic breakdown in an acidic environment: One milliliter of standard into a 100 ml volumetric flask, solutions was pipetted from the prepared stock solution. In order to stop further degradation, one milliliter of addition of 1N HCl was kept at room temperature for many hours, and then neutralized with 1ml of 1N NaOH. With diluent, the volume was adjusted to the proper level.
- Hydrolytic degradation under alkaline conditions: 1 ml of standard solutions into a 100 ml volumetric flask by pipetting them out of the prepared stock solution. To avoid further deterioration, one milliliter of 1N NaOH was added and held for hours at room temperature before being neutralized with One milliliter of 1N HCl. With diluent, the volume was adjusted to the proper level.
- Oxidative degradation: From the prepared stock solution, one milliliter of the standard solutions was pipetted into a 100 ml volumetric flask. 1 ml of 30% H<sub>2</sub>O<sub>2</sub> was added, and it was kept for several hours. With diluent, the volume was adjusted to the proper level.
- Thermal induced degradation: Performed by taking 50mg of standard drug powder in a petridish. The petridish was kept in oven at 100 degree for hours. The stock solution was created from this. After dissolving 10 mg of powder into 100 ml of volumetric flask, the volume was adjusted. Using diluent, 1 ml of the solution was transferred into a 100 ml volumetric flask, and the volume was then increased to the necessary amount (1 µg/ml).
- Photo degradation: Performed by taking 50mg standard drug powder in petridish. The petridish spent an hour in the photo stability chamber. The stock remedy was ready. 100 ml of a volumetric flask were filled with 10 mg of the aforementioned powder, and the remaining volume was adjusted with diluent. 1 ml of the aforementioned solution should be transferred into a 100 ml volumetric flask before being diluted (1 µg/ml) to the necessary volume.

### ***RP-HPLC Studies on Stressed Samples:***

The degradation samples were collected, and HPLC with UV detection was used to conduct the analysis. All of the deteriorated samples were injected into the column after being diluted with a diluent. Acetonitrile and phosphate buffer (pH 3.0), mixed 50:50 ratios in the mobile phase, and were run on a C<sub>18</sub> column (Hypersil 5  $\mu$ m, 250 x 4.60 mm, i.d.) at a flow rate of 1 ml/min. A fixed 220nm wavelength was used for detection. Drugs and degradant were successfully separated. Quantitative analysis was applied to each sample that had been stressed. To characterize all degradant products, the established approach was expanded to include mass spectrometry with liquid chromatography. For the LC-MS experiments previously created LC technique was altered by substituting water for the buffer.

### **RESULTS:**

Several physical and chemical properties of MET, DAP and SXG were available from the literature. The analytical method was developed to select preliminary chromatographic conditions. Various tests were carried out for this goal by altering the proportion and make-up of the mobile phase.

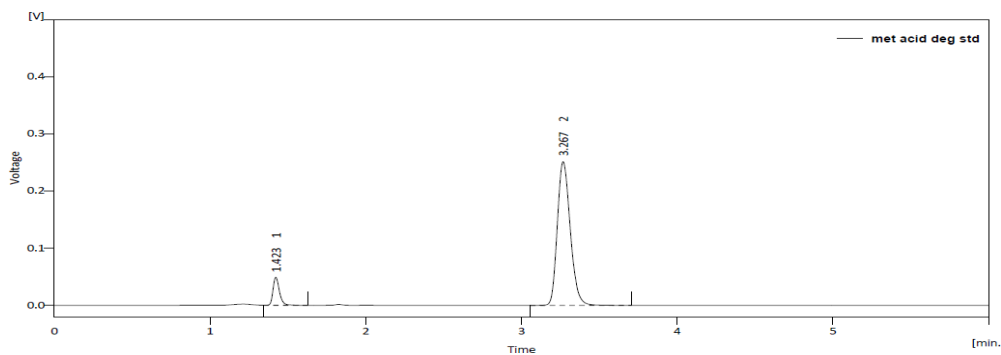
The drugs were subjected to acidic, alkali, oxidative, thermal and photolytic conditions. Here MET shows instability in acidic, alkali and oxidative, DAP and SXG shows instability in acidic and alkali conditions, while all three drugs remained stable in thermal and photolytic conditions (Table 1).

**Table 1: Result of Quantitative determination of degradant samples**

Stress Conditions	Metformin		Dapagliflozin		Saxagliptin	
	Exposure	%deg.	Exposure	%deg.	Exposure	%deg.
Acid Hydrolysis (1N HCl)	6 hr	11.39	7 hr	6.48	6 hr	49.45
Basic hydrolysis (1N NaOH)	8 hr	19.86	9 hr	9.18	10 hr	19.22
Oxidative degradation (30% H <sub>2</sub> O <sub>2</sub> )	4 hr	24.61	48 hr	--	72 hr	--
Photo degradation	72 hr	--	96 hr	--	72 hr	--
Thermal degradation (100°C)	72 hr	--	96 hr	--	72 hr	--

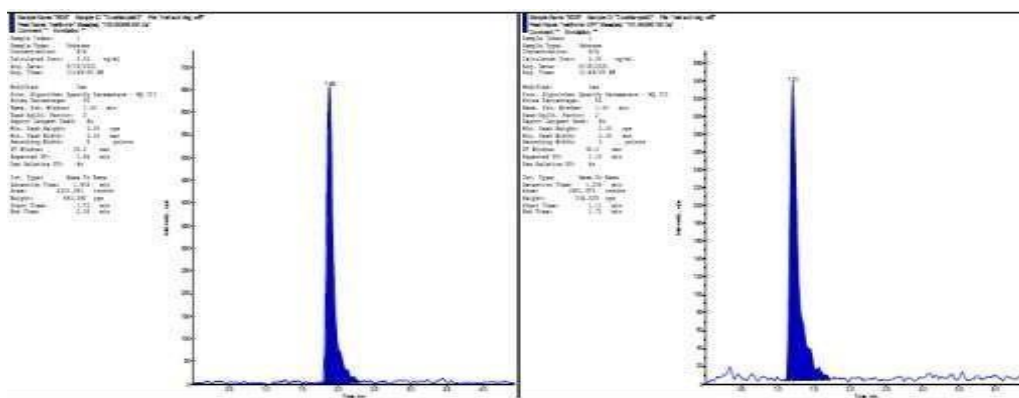
According to ICH criteria, the LC-MS method was used to characterize degradation products and analyze MET, DAP, and SXG. The systematic process optimization was completed after achieving the symmetric peak shape with good resolution. LC-UV and LC-MS chromatograph were compared in Figure 1, 2 and 3.

(a)



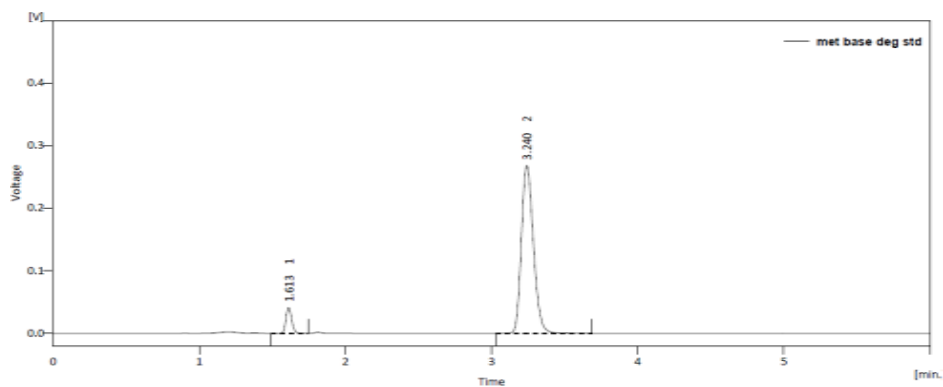
	MET	Deg
Rt	1.423min	3.267min

(a)



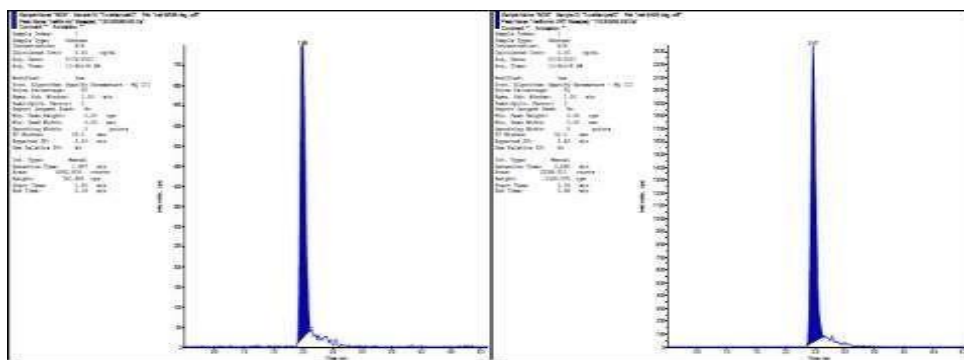
	MET	Deg
Rt	1.935min	1.209min

(b)



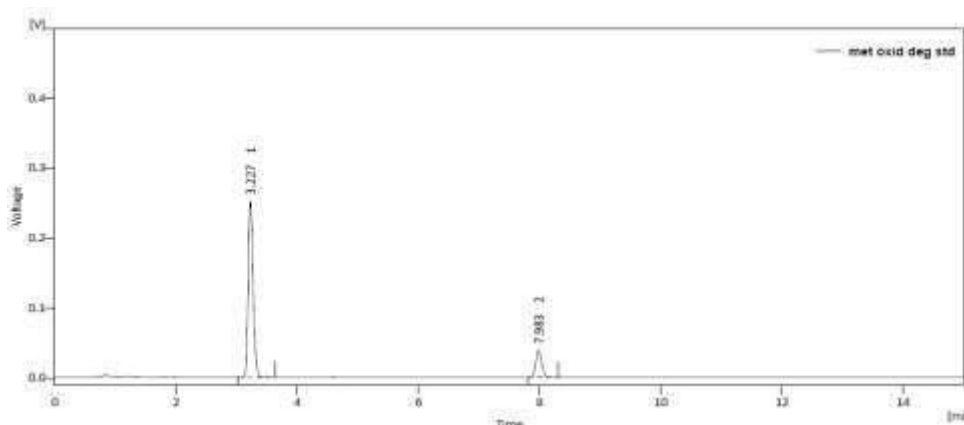
	MET	Deg
Rt	1.613min	3.240min

(b)



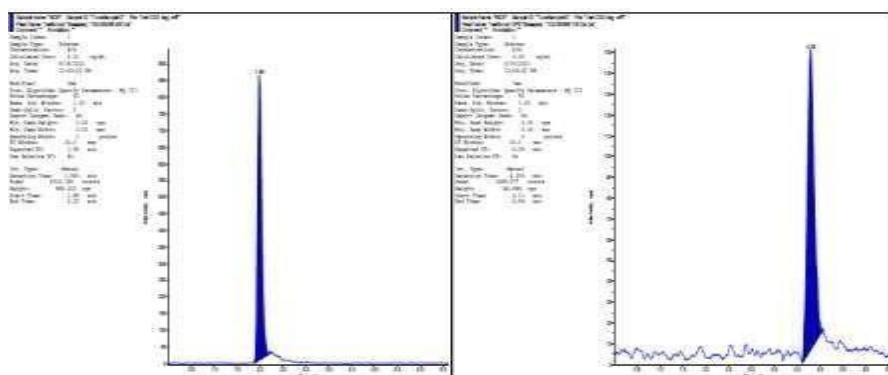
	MET	Deg
Rt	1.987min	2.465min

(c)



	MET	Deg
Rt	3.227min	7.983min

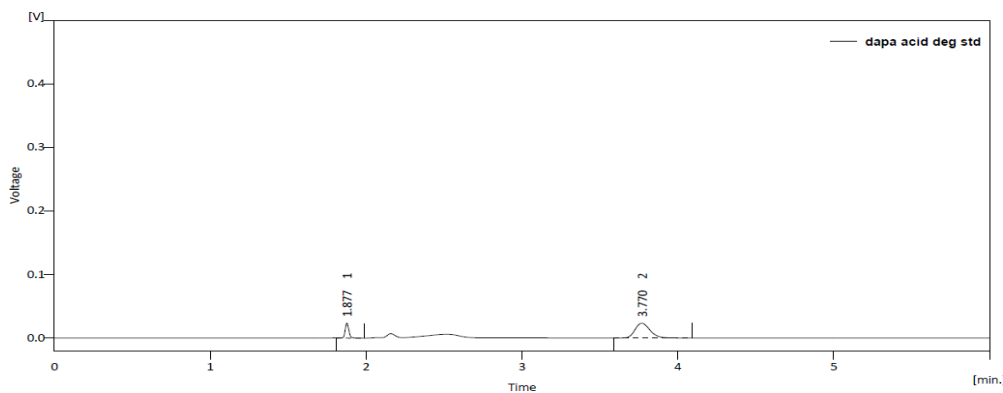
(c)



	MET	Deg
Rt	1.983min	4.293min

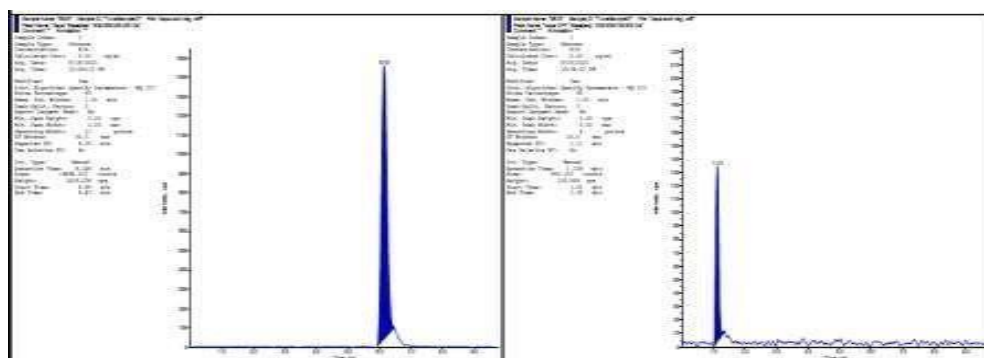
**Fig.1 MET chromatograms for LC-UV and LC-MS under (a) acidic, (b) base, and (c) oxidative hydrolysis**

(a)



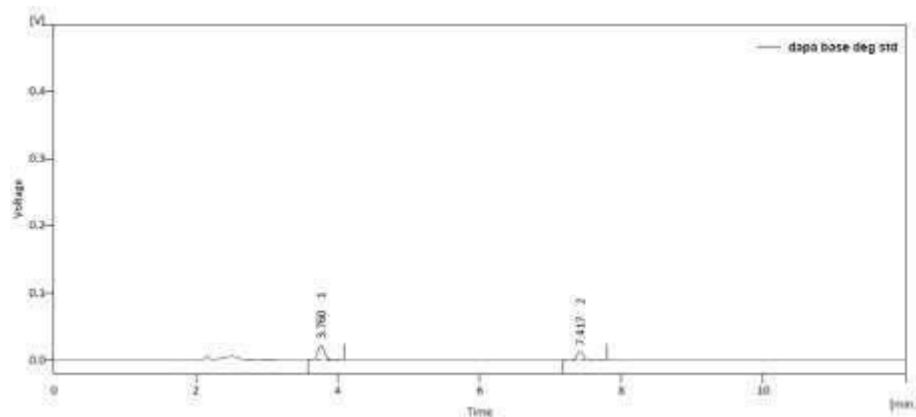
	DAP	Deg
Rt	1.877min	3.770min

(a)



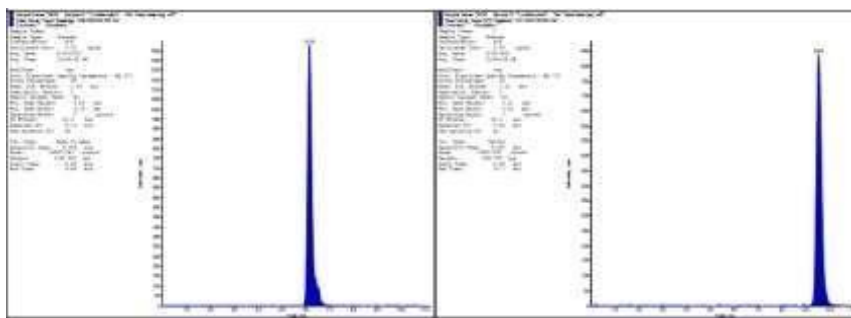
	DAP	Deg
Rt	6.148min	1.125min

(b)



	DAP	Deg
Rt	3.760min	7.417min

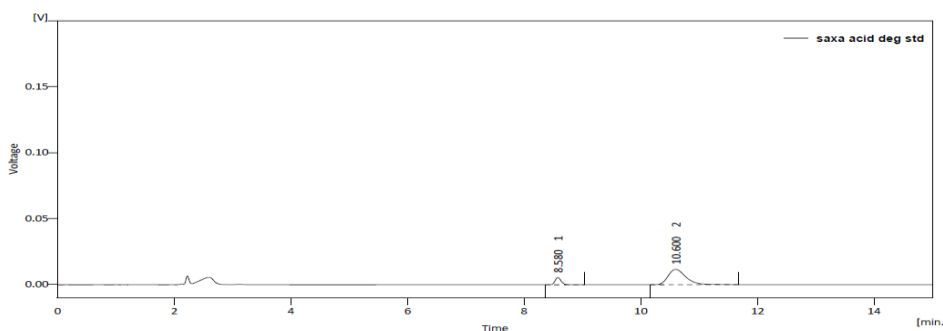
(b)



	DAP	Deg
Rt	6.156min	9.537min

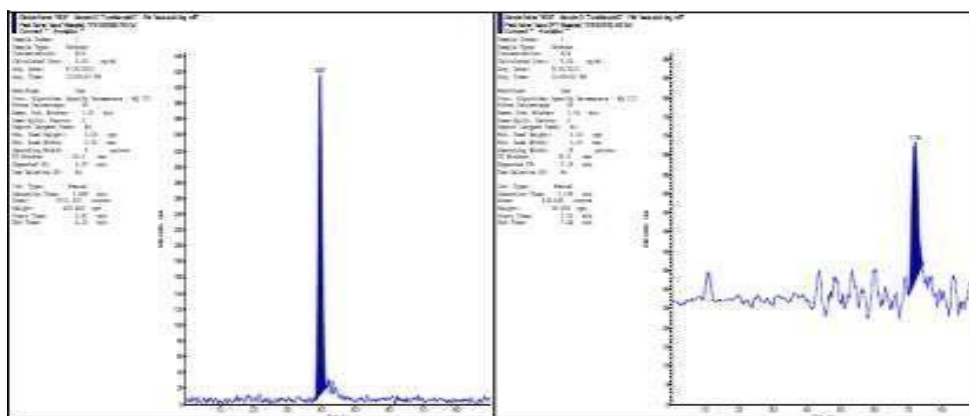
**Fig. 2 DAP chromatograms for LC-UV and LC-MS analysis under (a) acidic hydrolysis and (b) base hydrolysis**

(a)



	SXG	Deg
Rt	8.580min	10.600min

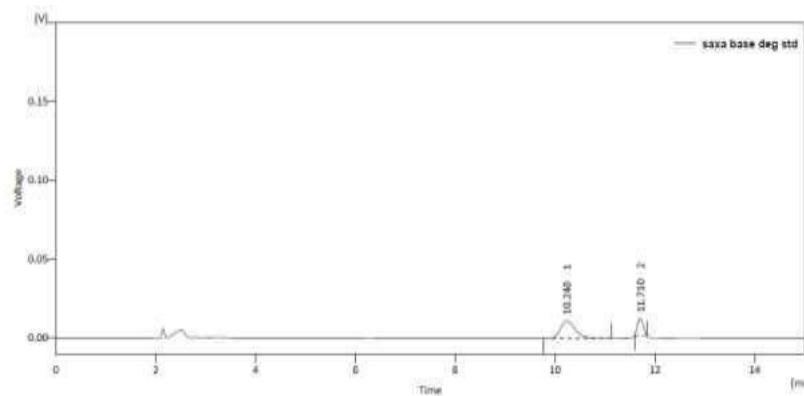
(a)



	SXG	Deg
Rt	3.965min	7.195min

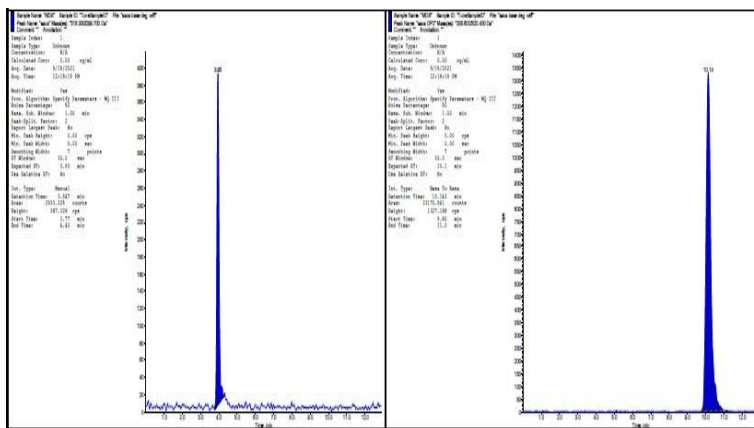


(b)



	SXG	Deg
Rt	10.240min	11.710min

(b)



	SXG	Deg
Rt	3.947min	10.143min

Fig. 3 SXG LC-UV and LC-MS chromatograms under acidic and base hydrolysis, respectively

**Method Validation**

**Specificity:** By contrasting the standard chromatogram with a blank, the specificity of the technique was assessed. The excipients used in formulation and diluent used for study did not interfere with the drug peaks and thus the method is specific. (Figure 4 & 5)

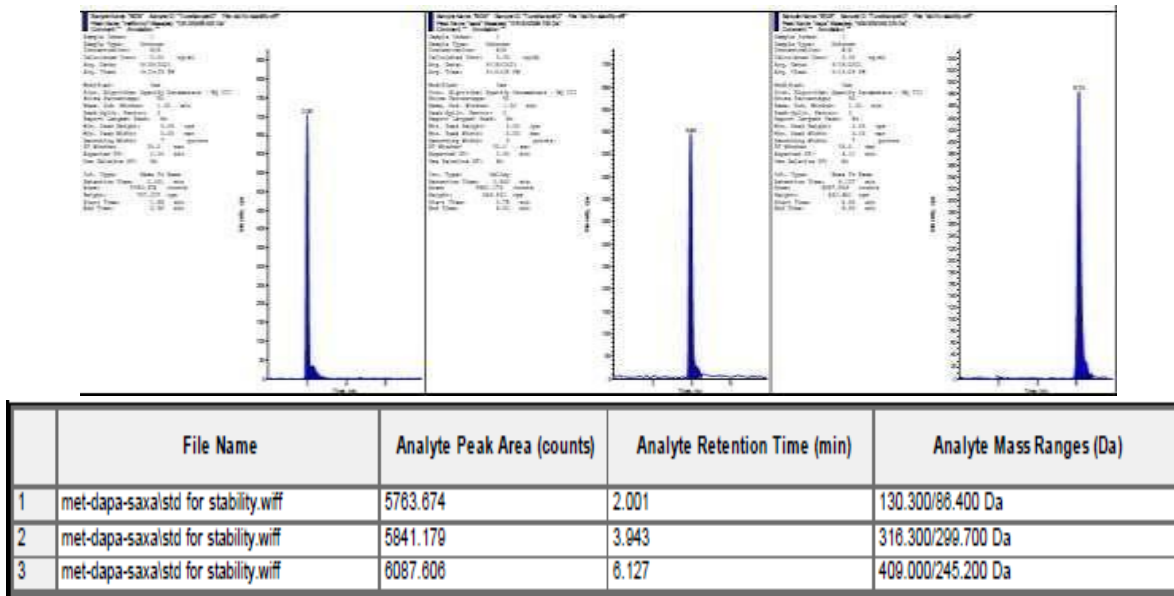


Figure 4: MET, SXG and DAP standard chromatogram

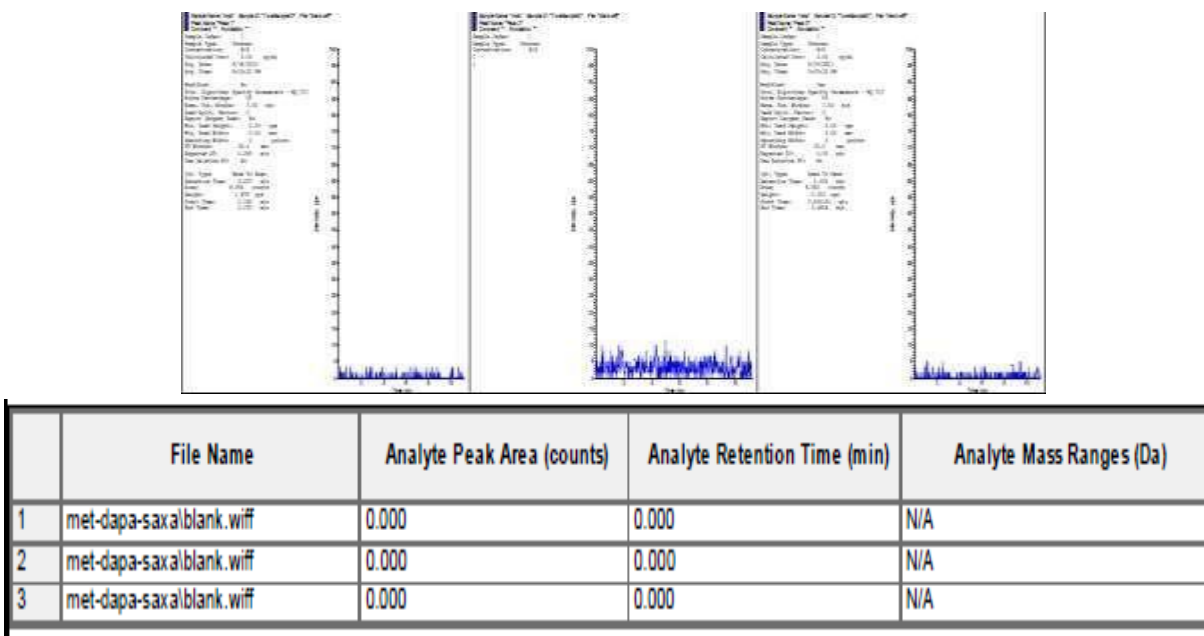


Figure 5: Blank chromatogram

**Linearity:** Each concentration was injected into five separate duplicates. There were chromatograms made. To obtain a calibration curve, peak area was plotted against concentration. Peak area vs. respective MET, DAP, and SXG concentration plots were shown to be linear in the 0.5-1.5 µg/ml range with a coefficient of correlation (r<sup>2</sup>) of 0.999.

**Table 2: Results for Linearity**

Conc. Level	Conc. (µg/ml)	Area of Metformin	Area of Saxagliptin	Area of Dapagliflozin
LOQ	0.25	1669.714	1907.778	1868.103
50%	0.5	3045.138	2911.306	3220.154
75%	0.75	4485.386	4503.96	4697.413
100%	1	5763.674	5841.179	6087.606
125%	1.25	7060.457	7083.452	8093.198
150%	1.5	8122.211	8864.824	9537.534
<b>SD</b>		118.574	154.954	217.321
<b>slope</b>		5091.687	5794.611	6403.730
<b>LOD</b>		0.077 µg/ml	0.088 µg/ml	0.112 µg/ml
<b>LOQ</b>		0.233 µg/ml	0.267 µg/ml	0.339 µg/ml

**Precision:** By producing three different concentrations of standard stock solutions (lower, middle, and higher), the interday and intraday precision were assessed. All solutions were analyzed, in order to record precision. Tables 3 and 4 contain the results.

**Table 3: Intraday precision**

Intraday precision						
Conc.	Metformin		Saxagliptin		Dapagliflozin	
	Avg. area	%RSD	Avg. area	%RSD	Avg. area	%RSD
50%	3083.6	1.316	2906.86	1.102	3321.24	1.079
100%	5942.8	1.116	6486.74	1.637	6161.19	1.781
150%	8252.5	1.454	8732.71	0.887	9191.01	1.802

**Table 4: Interday precision**

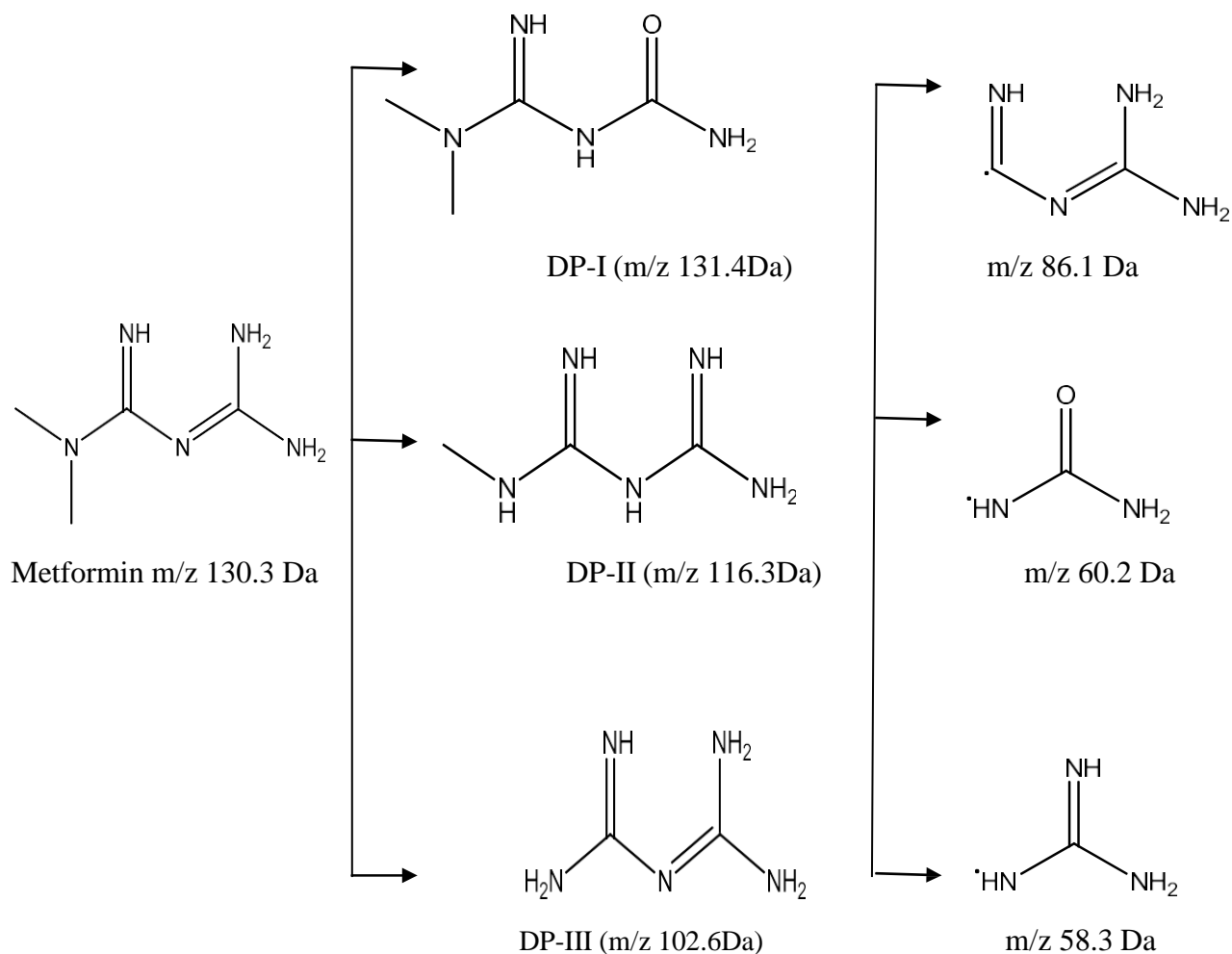
Interday precision						
Conc.	Metformin		Saxagliptin		Dapagliflozin	
	Avg. area	%RSD	Avg. area	%RSD	Avg. area	%RSD
50%	3162.517	1.309	2703.918	1.445	3416.108	0.917
100%	6133.02	1.956	6527.601	1.673	6274.638	1.156
150%	8316.449	1.216	8849.61	1.379	9251.516	1.195

**Robustness:** This was accomplished by making a minimal adjustment to the chromatographic conditions, which were discovered to be unaffected by adjustments include a 2% a shift in the mobile phase minor components and a 0.2 change in flow rate. The outcomes are depicted in Table 5.

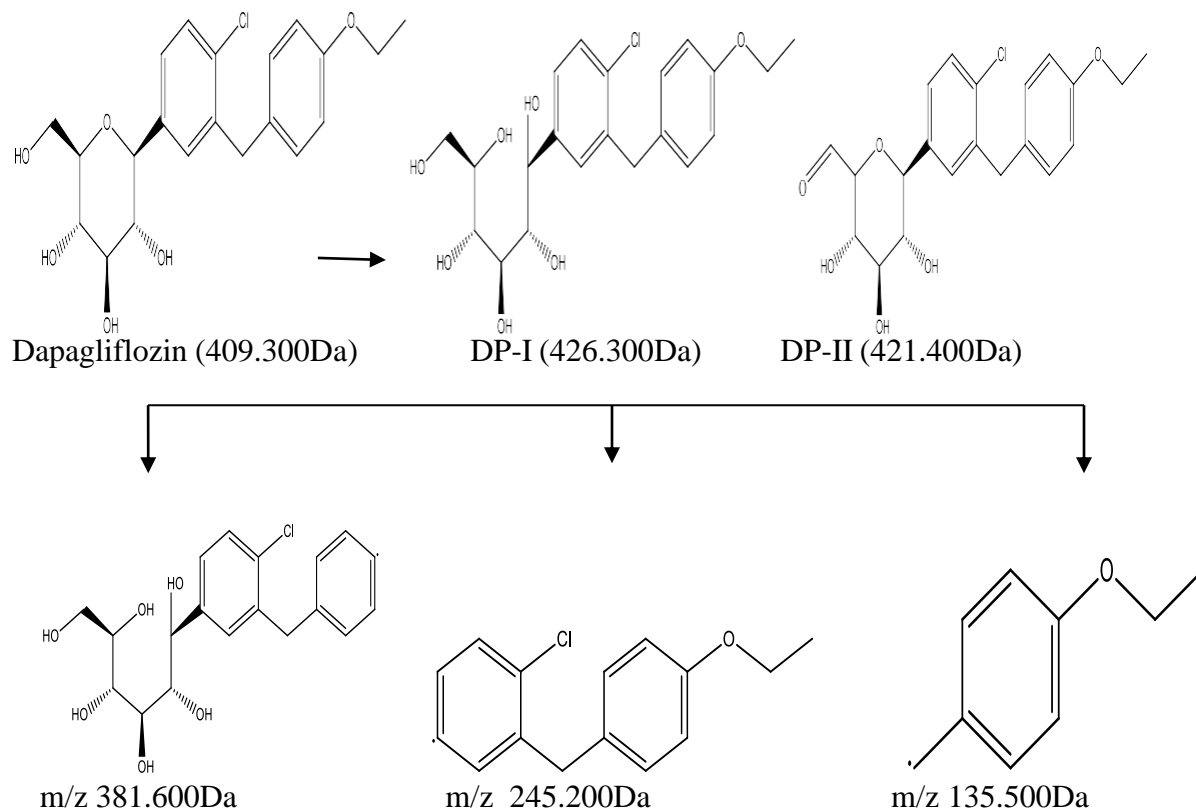
**Table 5: Result of Robustness**

Changes in condition	MET Area	%RSD	DAP Area	%RSD	SXG Area	%RSD
Flow rate (+2ml/min)	4633.409	1.358	5678.935	1.389	4574.993	1.719
Flow rate (-2ml/min)	5756.140	1.757	6397.212	1.089	6666.378	1.365
Mobile Phase Composition (+2)	5120.295	1.918	6082.272	1.118	5559.855	1.493
Mobile Phase Composition (-2)	5601.319	1.037	6260.566	1.845	6258.348	1.171

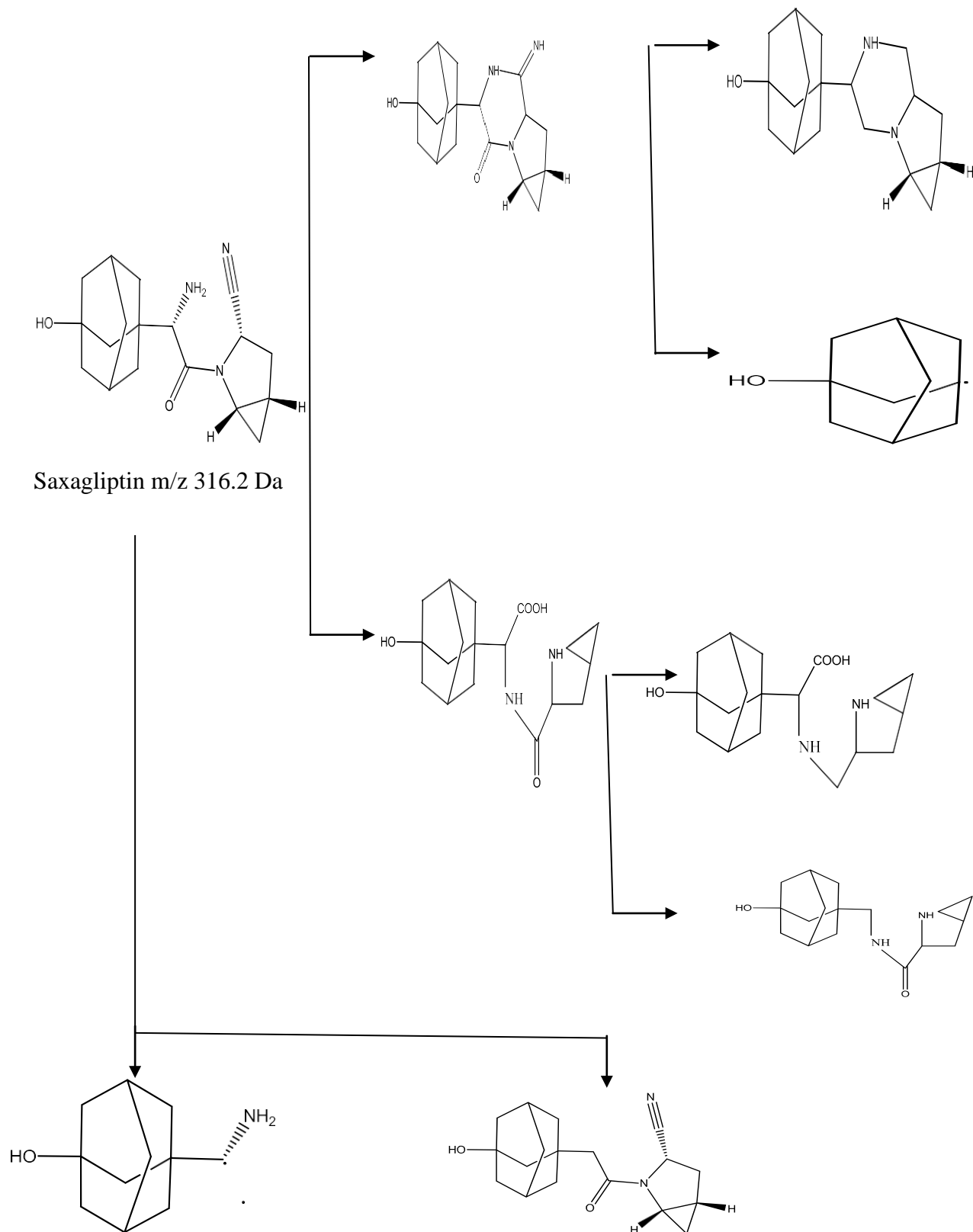
**Degradation Pathways of MET, DAP and SXG**



**Figure 6: Probable fragmentation pathway of MET for formation of deg. I, II and III product under acidic, basic and oxidative hydrolysis**



**Figure 7: Possible DAP fragmentation mechanism for deg. I and II product generation during acidic and basic hydrolysis**



**Figure 8: Probable fragmentation pathway of SXG for formation of deg. I and II product under acidic and basic hydrolysis**

## **DISCUSSION:**

For the purpose of characterizing the degradation byproducts of Metformin, Dapagliflozin, and Saxagliptin, a new LC-MS approach has been devised. Metformin, Dapagliflozin, and Saxagliptin were shown to have retention times of 2.01, 6.1, and 3.95 minutes, respectively. For Saxagliptin, Dapagliflozin, and Metformin, the calibration curve of peak area versus concentration was linear in a range of 0.5 to 1.5 µg/ml with a coefficient of correlation ( $r^2$ ) of 0.999. The validation of several parameters was done, and the results show that every parameter is within acceptable bounds. Studies on forced degradation were conducted under a variety of circumstances, including acid, base, H<sub>2</sub>O<sub>2</sub>, temperature, and UV light. It can be used to characterize the degradant products of metformin, dapagliflozin, and saxagliptin. The suggested approach was straightforward, exact, specific, strong, quick, and responsive.

## **CONCLUSION:**

The proposed approach for separating apart Metformin, Dapagliflozin, and Saxagliptin from bulk formulation was found to be straightforward, accurate, and quick. The mobile phase is inexpensive and easy to prepare. The sample recovery in synthetic mixture was in good agreement with their respective label claim. The three medications' forced degradation experiments were carried out under a variety of degradation settings, comprising oxidative, thermal, photolytic, acidic, alkaline, and other environments. The effectiveness of the suggested method was determined by the resolution of the employed sample peaks. No such thorough and stability-indicating approach has, as far as we are aware, been published for the analysis of drug mixtures containing these three medicines. As a result, it may be quickly and readily used for regular analysis.

## **ACKNOWLEDGEMENTS:**

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### **ABBREVIATIONS**

MET- Metformin HCl

DAP- Dapagliflozin

SXG- Saxagliptin

RP-HPLC- Reverse phase- High performance liquid chromatography

LC-MS- Liquid chromatography Mass spectrometry

Rt- Retention time

ICH- International Conference on Harmonization