"DEGRADATION BEHAVIOR AND IMPURITY PROFILING OF BULK DRUGS AND THEIR FORMULATION OF SOME SELECTED ANTI-DIABETIC DRUGS"

Synopsis of the PhD Thesis

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1. Abstract

A simple, precise, accurate, specific, linear, rugged and robust method was developed and validated for the estimation of degradation impurities of Empagliflozin and Dapagliflozin in API and tablet formulation. All the degradation Impurities are observed and well separated in same developed method. Unknown impurity formed during stability studies was isolated using preparative HPLC and structure was characterized by NMR and Mass spectroscopy studies. Empagliflozin and Dapagliflozin and its tablet formulation is more sensitive towards acid and Alkali degradation. This method is specific as no interference is observed because of excipients and degradation products. This study investigates the degradation behaviour and impurity profiles of bulk drugs and their formulations for specific anti-diabetic medications. Understanding how these drugs degrade and the impurities that may form is vital for ensuring their potency and safety in the treatment of diabetes. The research contributes to the quality and stability assessment of these essential medications.

2. Introduction

Degradation behaviour[1]

Degradation behaviour refers to the chemical and physical changes that occur in a drug substance over time, under various environmental conditions, such as temperature, humidity, light, and pH. Understanding the degradation behaviour is crucial for determining the drug's stability and shelf-life. Several types of drug degradation can occur:

a. Chemical Degradation: This involves chemical reactions that lead to the formation of degradation products. Common chemical degradation pathways include hydrolysis, oxidation, photolysis, and isomerization.

b. Physical Degradation: This includes changes in physical characteristics like particle size, polymorphism, and crystallinity, which can affect drug stability.

c. Microbial Degradation: Some drugs can be susceptible to microbial contamination and degradation, leading to impurities.

d. Environmental Degradation: Factors such as exposure to moisture, light, or temperature variations can contribute to drug degradation.

Impurity profiling [2-4]

It is description of the identified and unidentified impurities present in a typical batch of API produced by a specific controlled production process. The main reasons for the increasing interest to develop impurity profiles of bulk drug substances are as follows:

- Development of a new drug or a new technology for manufacturing an existing drug.

- Identify suggested structures for the impurities so can be synthesized and thus provide final evidence for structures previously determined by spectroscopic methods.
- The material synthesized can be used as an 'impurity standard' during development of a selective method for the quantitative determination of the impurity.
- In case of major impurities, the synthesized or isolated material can be subjected to toxicological studies thus greatly contributing to the safety of drug therapy.
- For drug authorities the impurity profile of a drug substance is a good fingerprint to indicate the level and constancy of the manufacturing process of the bulk drug substance. Impurity profiling is a crucial aspect of pharmaceutical research and development, particularly when it comes to bulk drugs, also known as active pharmaceutical ingredients (APIs). Impurities can arise at various stages of drug synthesis and manufacturing, and their presence can impact the safety, efficacy, and quality of the final pharmaceutical product. Therefore, a systematic approach to impurity profiling is essential to ensure that bulk drugs meet the required standards and regulatory guidelines.

3. Definition of the problem [5-9]

The effective management of diabetes mellitus, a global health concern, relies significantly on the quality, safety, and efficacy of anti-diabetic drugs. In this context, the degradation behaviour and impurity profiling of bulk drugs, particularly for selected anti-diabetic medications, play a critical role in ensuring product quality, regulatory compliance, and patient well-being. However, there exists a gap in comprehensive research and analysis in this domain, hindering the development of robust formulations and sustainable manufacturing processes for these essential drugs.

The primary issues and challenges that this research project aims to address include:

- **Degradation Behaviour Understanding:** The degradation behaviour of bulk drugs used in anti-diabetic formulations is not thoroughly explored, especially concerning variations in environmental conditions, storage, and manufacturing processes. The lack of comprehensive data impedes the development of stable formulations with extended shelf-lives.
- **Impurity Profiling:** The identification, quantification, and control of impurities in anti-diabetic drugs are pivotal for ensuring their safety and efficacy. A comprehensive impurity profile for specific anti-diabetic drugs is often lacking, leading to potential safety concerns and regulatory hurdles.
- **Formulation Optimization:** Designing effective drug formulations that maintain stability, bioavailability, and therapeutic efficacy is a complex challenge. There is a need for

research that optimizes the formulation of selected anti-diabetic drugs to address patient-specific requirements and improve treatment outcomes.

- **Regulatory Compliance:** Meeting regulatory requirements, such as those set forth by agencies like the FDA and EMA, is critical for drug approval and market access. Comprehensive degradation studies and impurity profiling are essential to ensuring compliance with these stringent standards.
- **Patient Well-being:** The quality and reliability of anti-diabetic drugs directly impact patient health and quality of life. Addressing the aforementioned issues is crucial for minimizing risks associated with suboptimal formulations or impurities in these medications.

4. Objective and Scope of work

The aim of the work undertaken is to identify process impurities and degradation impurities present in selected drugs and formulations used in the treatment of Diabetes**.**

Specific Objectives are as follows

- To develop and validate method for selected anti-diabetic drugs as per ICH Guidelines
- To carry out stress degradation study of selected drugs as per ICH guidelines.
- To isolate major degradation related impurities by Preparative Techniques
- To characterize major impurities by IR, ^{1}H NMR and Mass Spectroscopy

Scope of the research work:

- Isolation and characterization of impurities is required for acquiring and evaluating data that establishes biological safety which reveals the need and scope of impurity profiling of drugs in pharmaceutical research.
- Anti diabetic drugs may also have Impurities like process related impurities, degradation impurities in bulk drugs as well as in formulations.
- Work on Impurity profiling of Sodium glucose co-transporter 2 (SGLT2) inhibitors category of Anti-diabetics like Empagliflozin, Dapagliflozin etc, is one of the such aspect and need to be done.
- So, in present work, aimed to develop an analytical method which is capable to identify impurities present in above listed drugs and enable us to isolate and characterize those impurities as well.

5. Original contribution by the thesis

The entire work in this synopsis, as well as the thesis, is original. An extensive literature review was done to identify different types of process related impurities and degradation impurities in different class of Anti-diabetic drugs. To analyse concept of

degradation behaviour and impurity profiling of bulk drugs Rp-HPLC method was developed for Api and Marketed formulation. Forced degradation study was perform according to ICH guidelines in different conditions. Degradation impurity was isolated by using preparative HPLC and isolated impurity was characterized by IR, MS and ^{1}H NMR. Therefore, this research project seeks to bridge the existing knowledge gaps by conducting comprehensive studies on the degradation behaviour, impurity profiling, and formulation optimization of selected anti-diabetic drugs. The outcomes of this research will contribute to the development of safer, more effective, and compliant anti-diabetic drug formulations, ultimately enhancing the well-being of patients suffering from diabetes worldwide

6. Methodology of Research, Results / Comparisons

- **Chemicals and Reagents:**

  Empagliflozin was purchase from Simson Pharma, Mumbai. Dapagliflozin was purchase from BENZCHEM Enterprise, Vadodara. Acetonitrile (ACN), Methanol (MeOH), Hydrochloric Acid(HCL), Sodium Hydroxide(NaOH),30 % Hydrogen Peroxide were purchased from Thermo Fischer Scientific India Ltd ,Mumbai.

- **Instrumentation**

  Chromatographic analysis was carried out on automatic liquid chromatography Model LC-2010 (Shimadzu, Japan), Pump-single pump systems using UV-VIS Detector with Software-LC Solution to acquire and process the data. Reversed-Phase YMC ODS A C-18 (150mm x 4.6mm) column was used as stationary phase, Semi micro analytical balance (Sartorius CD2250, Germany), pH tutor(313927, Eutech Instruments), Ultrasonic cleaner (D 120/1H, Trans-O- Sonic) and Nylon membrane filters (0.22 µm, 47 mm D) were used in the study.

- **Chromatographic conditions:**

  An HPLC system (make: Simadzu, model- LC-2010) which is operated using a software, LC Solution, fitted with YMC ODS A C-18 (150mm x 4.6mm) Column and UV Detector (at 224 nm for Empagliflozin and 273 for Dapagliflozin) was used for the analysis. The mode was isocratic. A mixture (50:50) of Acetonitrile and Water was used as mobile phase. The mobile phase was filtered through a 0.22 µm nylon membrane filter and degassed prior to use.

- **Method Validation**

  The method was validated according to ICH guidelines for validation of analytical procedures in order to determine linearity, sensitivity, accuracy and precision for each

analyte.

- **System suitability**

  The system suitability was evaluated by five replicate analysis of drug at specific concentration. The column efficiency, resolution, and peak asymmetry were calculated for the standard solutions.

- **Forced Degradation studies:**

  Forced degradation experiments were carried out on Empagliflozin and its marketed formulation under various conditions explained in ICH guideline Q1A (R2), namely, acid, alkali, wet heat, dry heat, and oxidative and photolytic conditions.

6.1 Impurity Profile of Empagliflozin

Proper selection of method depends upon the nature of the sample, its molecular weight and solubility. Empagliflozin were dissolved in polar solvent, so the developed method of estimation was called as reverse phase high performance liquid chromatography. To develop a rugged and suitable HPLC method for the quantitative determination of Empagliflozin the analytical condition was selected after consideration of different parameters such as diluent, solvents for mobile phase and mobile phase composition and other chromatographic conditions. Preliminary trials were taken with different composition of Acetonitrile and water. The column selection has been done by backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution. After evaluating all these factors, YMC ODS A C-18 (150mm x 4.6mm) column was found to be giving satisfactory results. The selection of Acetonitrile and water were based on chemical structure of both the drugs. Best results were obtained with, Acetonitrile: Water. For the selection of organic constituent of mobile phase, Acetonitrile: water was chosen to reduce the longer retention time and to attain good peak shape. Therefore, final mobile phase composition consisting of a mixture of Acetonitrile: Water (50:50), set at a flow rate 1 ml/min was selected for the chromatographic analysis (Fig. 2). Empagliflozin and its marketed formulation was subject to stress condition in all conditions but degradation shows only in acidic (Fig. 3,5), alkaline (Fig. 4,6) respectively, The degradation products in acidic and alkali condition of both pure form and marketed formulation were observed on same R_f value so it can be concluded that same degradation product was made in pure form and marketed formulation. The acid degradation impurity denoted as DP1 and alkali degradation impurity denoted as DP2. Comparison of degradation in API and marketed formulation is given in Table 1.

Sr.No	Condition	Temperature (°C)	Time duration for degradation	% Degradation observed in API	% Degradation observed in Marketed Formulation
1	2 N HCL	60	24 hr	48.54	38.45
2	2 N NaOH	60	24 hr	31.79	30.76
3	30 % H_2O_2	60	24 hr	No degradation	No degradation
4	Thermal	60	24 hr	No degradation	No degradation
5	Photolytic	Sunlight	7 days	No degradation	No degradation

Table 1. Degradation data of Empagliflozin in API and Marketed Formulation

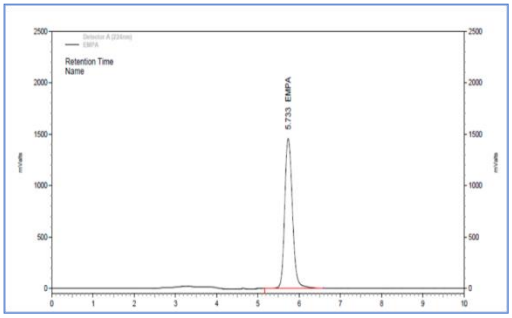

Fig. 2 Chromatogram of Empagliflozin

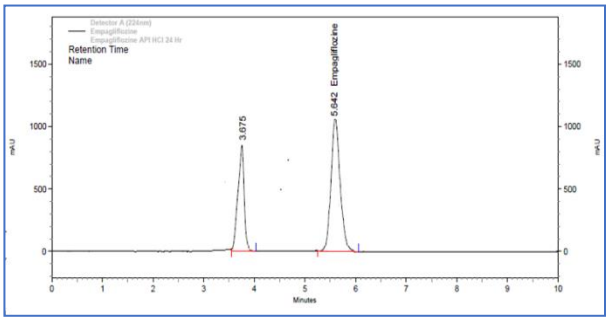

Fig. 3 Chromatogram of Acid degradation

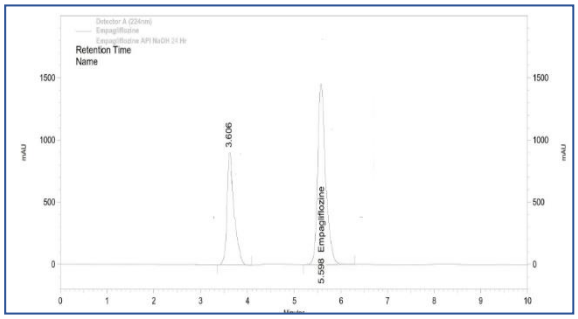

Fig. 4 Chromatogram of Alkali degradation

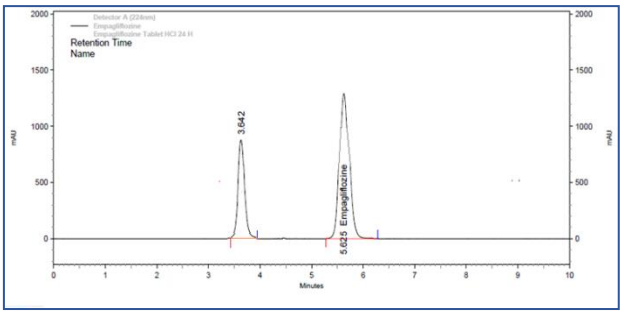

Fig.5 Chromatogram of Acid degradation of marketed formulation

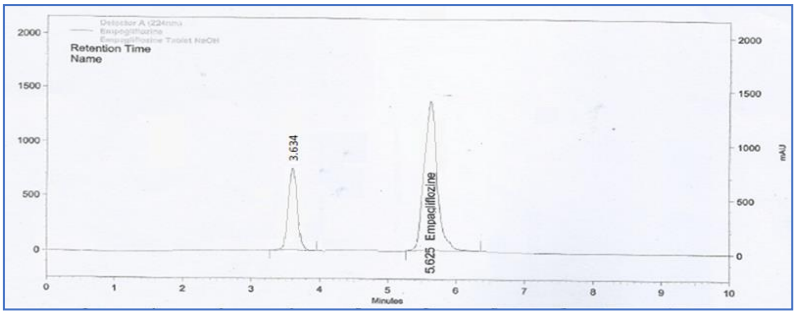

Fig. 6 Chromatogram of Alkali degradation in marketed formulation

6.2 Detection, Isolation and Characterization of Empagliflozin Degradation Impurities (DP1 and DP2)

The preparative HPLC is used for isolation of degradation product of acid (DP1) and alkali (DP2) by using same developed condition. Isolated degradation product was characterized by using IR, Mass and ^{1}H NMR. The retention time for DP1 is 3.67 min and DP 2 is 3.606 min.

Proposed Structure Elucidation of Impurities

Chemically Empagliflozin is 2S,3R,4R,5S,6R)-2-[4-Chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol, with empirical formula $C_{23}H_{27}CLO$ and it molecular weight is 450.91 gm/mol. When drug is degrade in acidic condition it forms DP1 with Empirical formula $C_{23}H_{29}CLO_8$ and Molecular weight is 468.92 g/mol. Standard values of different bonds in IR spectrum show in Table 2.

Functional Group	Standard Range (cm^{-1})	Observed value (cm^{-1})
-OH	3200-3600	3421.7
Ar- C-H	2850-2970	2929.7
C-O-C (Ether linkage)	1040-1280	1279.5

Table 2. IR Standard range of DP1

MS spectra of DP1 shown *m/z* at 469.1 [M^+] as base peak. The Product ion peaks at *m/z* 449 (loss of water molecule), *m/z* 434 (Loss of – Cl), *m/z* 416 (Loss of – Cl from *m/z* 449), *m/z* 346 (Loss of tetrahydrofuran ring), *m/z* 313(loss of -OH group), *m/z* 327 (loss of water molecule), *m/z* 169 (loss of – $C_6H_{11}O_5$molecule).The positive mass spectrum for DP1 is shown in Fig. 7. The possible MS fragmentation of DP1 in acidic condition shown in Fig.8.

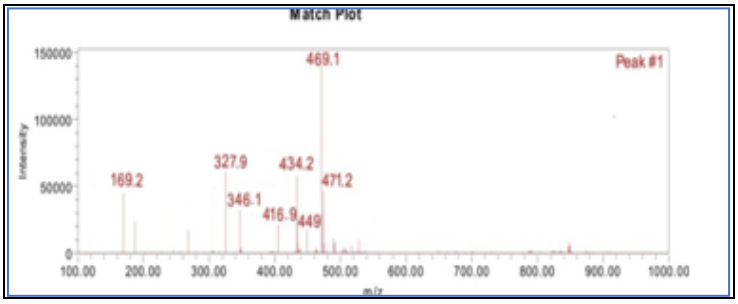

Fig. 7 ESI (+ve) mass Spectrum for DP1

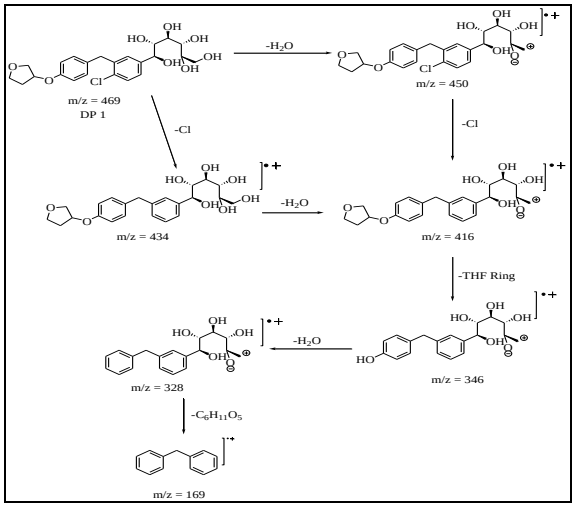

Fig. 8 Possible Mass fragmentation of DP1 in Acidic Condition

^{1}H Analysis has been done in DMSO at 400 MHz. The chemical shift values reported on δ scale in ppm with respect to TMS (0.00 ppm). ^{1}H NMR of Empagliflozin acid impurity DP1 shown in Fig. 9.^{1}H NMR values shown in Table 3 confirms structure of DP1.Based on previous data structure of DP1 was shown in Fig. 10. IUPAC name of DP1 is (1S,2S,3R,4R,5S)-1-(3-((S)-4-((S)-tetrahydrofuran-3-yloxy)benzyl)-4-chlorophenyl)hexane-1,2,3,4,5,6-hexaol.

Chemical Shift (δ ppm)	Number of product	Multiplicity	Assignment
3.08-3.24	5H	Multiplet	open sugarmoiety
3.41-3.71	7H	Multiplet	Tetrahydrofuran ring
3.53	4H	Multiplet	-CH_2
4.03	1H	Singlet	-OH
4.44-4.95	5H	Singlet	-OH
7.10-7.38	7H	Multiplet	Ar-H

Table 3. ^{1}H NMR assignment of acid degraded Product DP1

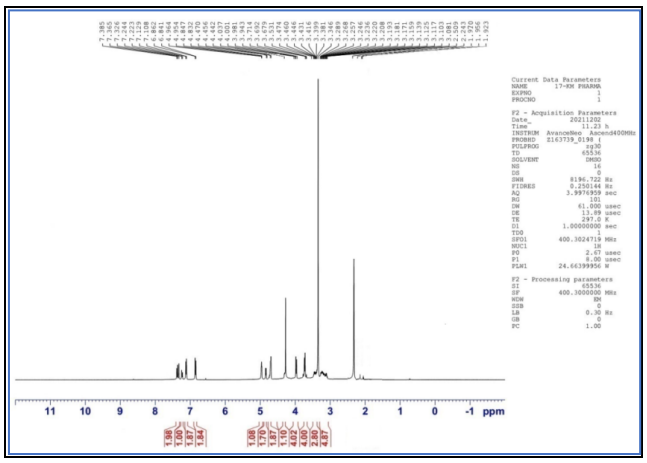

Fig. 9 ^{1}H NMR of Empagliflozin Acid Impurity DP1

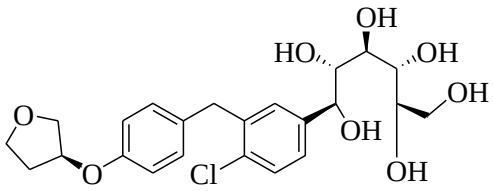

Fig. 10 Possible structure of DP1

On the basis of gain in molecular weight of DP1 in comparison to pure drug, in acidic hydrolysis proton attack on oxygen atom present in sugar moiety so corbocation is formed and ring opening is formed because of Hydroniom ion attack on Corbocation. Further loss of proton from that position form new structure of DP1 .Possible degradation pathway was shown in Fig. 11**.**

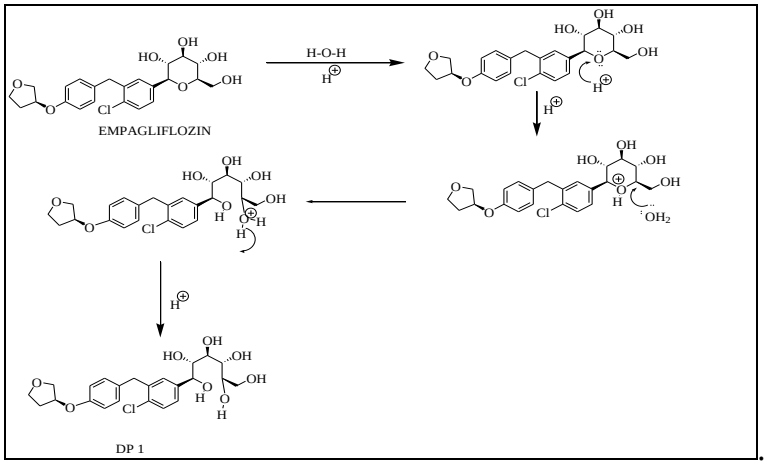

Fig. 11 Possible degradation pathway DP1

In Alkali condition it forms DP2 with Empirical formula $C_{23}H_{25}CLO_7$ and Molecular weight is 448.89 g/mol. Standard values of different bonds in IR spectrum show in Table 4.

Table 4. IR standard range of DP2

Functional Group	**Standard Range (cm^{-1})**	**Observed value (cm^{-1})**
-OH	3200-3600	3474.3
Ar- C-H	2850-2970	2958.2
C-O-C (Ether linkage)	1040-1280	1237.5
C=0	1650-1820	1752.7

MS spectra of DP2 shown *m/z* at 449.3 [M^+] as base peak. The Product ion peaks at *m/z* 443 (loss of water molecule), *m/z* 399 (Loss of – Cl), *m/z* 363 (Loss of – C_4H_7O from *m/z* 443), *m/z* 313 (Loss of – OH from *m/z* 363), *m/z* 285 (Loss of-CHO from *m/z* 313), *m/z* 169 (loss of -$C_5H_9O_3$ group from *m/z* 285), The positive mass spectrum for DP2 is shown in Fig. 12.

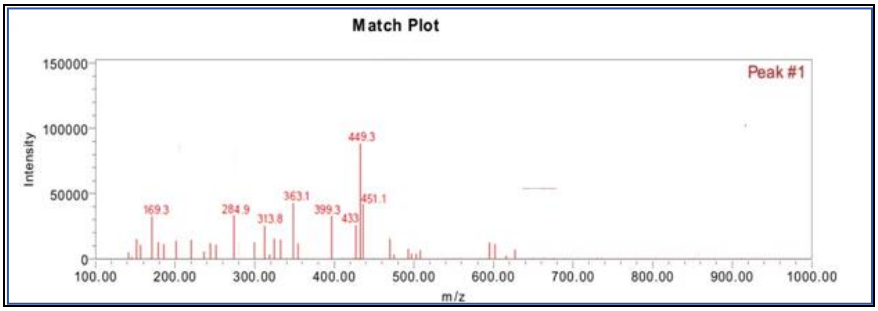

Fig. 12 ESI (+ve) mass spectrum for DP2

^{1}H NMR of Empagliflozin acid impurity DP2 shown in Fig. 13.^{1}H NMR values shown in Table 5 confirms structure of DP2. Based on previous data structure of DP2 was shown in Fig. 14.

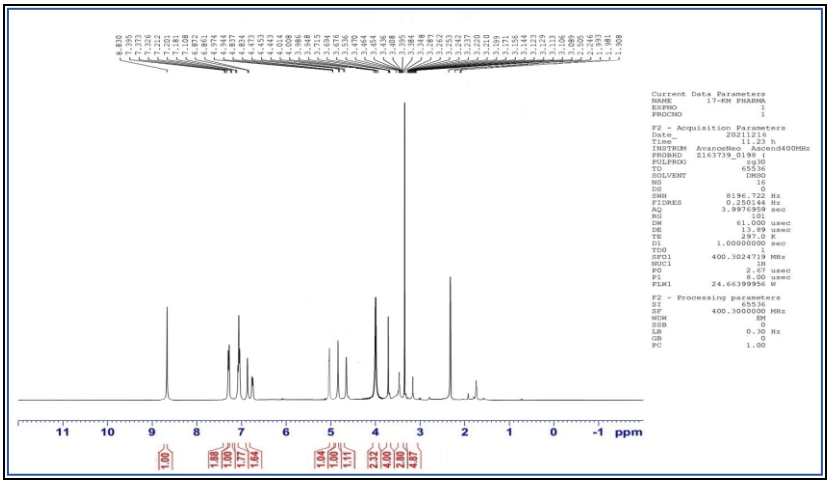

Fig. 13 ^{1}H NMR of Empagliflozin Acid Impurity DP2

Table 5. ^{1}H NMR Assignment of Acid degraded Product DP2

Chemical Shift δ ppm	Number of product	Multiplicity	Assignment
3.40-3.47	5H	Multiplet	open sugarmoiety
3.53-3.98	7H	Multiplet	Tetrahydrofuran ring
4.01	2H	Singlet	-CH_2
4.47-4.97	3H	Singlet	-OH
7.10-7.39	7H	Multiplet	Ar-H
8.830	1H	Singlet	-CHO

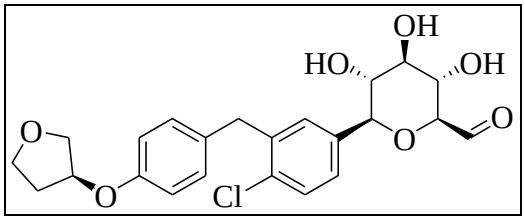

Fig. 12 Possible structure of DP2

In alkali hydrolysis molecular weight is loss as compare to pure drug so in Alkali hydrolysis in presence of sodium hydroxide two hydrogen ion is removed and oxygen convert in to Aldehyde it means Alcohol convents in to Aldehyde in formed DP2. Possible degradation pathway of DP2 was shown in fig. 13. IUPAC name of DP2 is 2S,3S,4R,5R,6S)-6-(3-(4-(tetrahydrofuran-3-yloxy)benzyl)-4-chlorophenyl)-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-carbaldehyde.

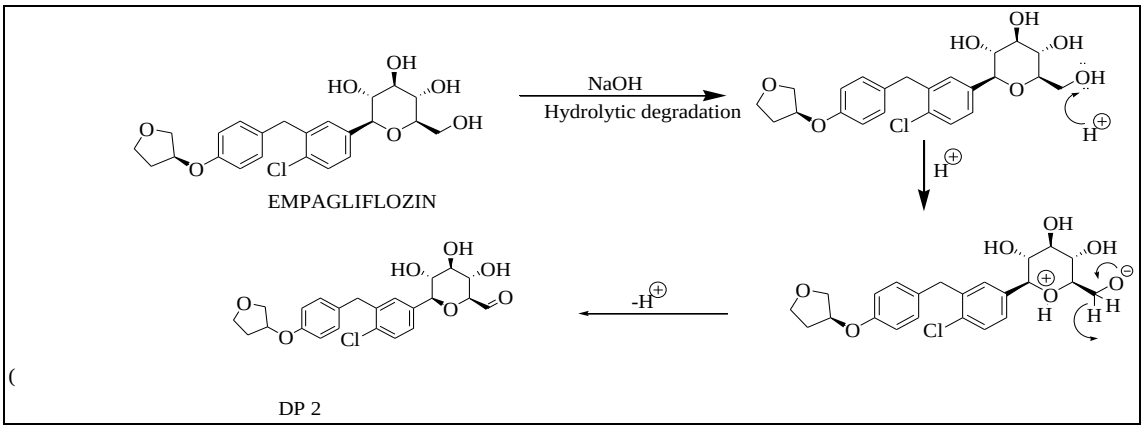

Fig. 13 Possible degradation pathway DP2

6.3 Impurity Profile of Dapagliflozin

Same method is followed like Empagliflozin in YMC ODS A C-18 (150mm x 4.6mm) column shows satisfactory results. Final mobile phase composition consisting of a mixture of Acetonitrile: Water (50:50), set at a flow rate 0.7 ml/min was selected for the chromatographic analysis.). Dapagliflozin and its marketed formulation was subject to stress condition in all conditions but degradation shows only in acidic (Fig. 15,17), alkaline (Fig. 16,18) respectively, the degradation products in acidic and alkali condition of both pure form and marketed formulation were observed on same R_f value so it can be concluded that same degradation product was made in pure form and marketed formulation. The acid degradation impurity denoted as DP1 and alkali degradation impurity denoted as DP2. The Retention Time of Dapagliflozin in developed method was found to be 5.30 min shown in Fig.14 Comparison of degradation in API and marketed formulation is given in Table 6.

Sr.No	Condition	Temperature (°C)	Time duration for degradation	% Degradation observed in API	% Degradation observed in Marketed Formulation
1	2 N HCL	60	24 hr	41.56	52.77
2	2 N NaOH	60	24 hr	43.84	45.43
3	30 % H_2O_2	60	24 hr	No degradation	No degradation
4	Thermal	60	24 hr	No degradation	No degradation
5	Photolytic	Sunlight	7 days	No degradation	No degradation

Table 6. Degradation data of Dapagliflozin in API and Marketed Formulation

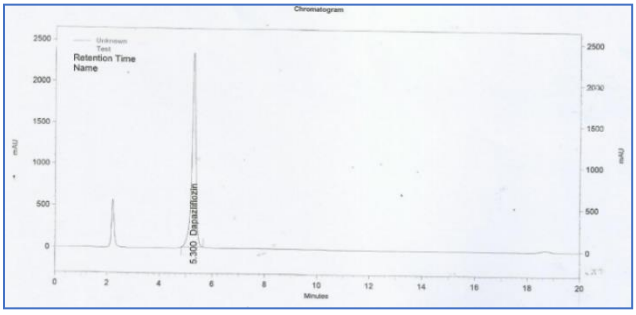

Fig. 14: Chromatogram of Dapagliflozin API

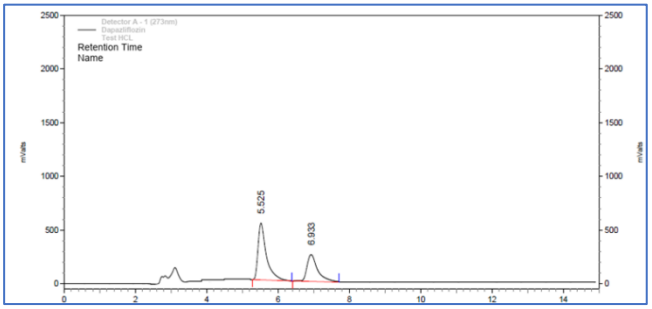

Fig. 15 Chromatogram of Acid degradation

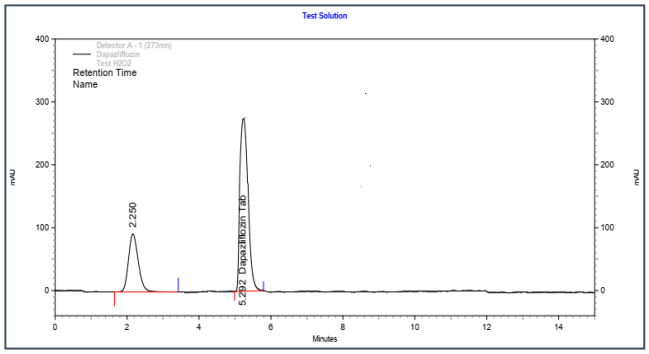

Fig. 16 Chromatogram of Alkali degradation

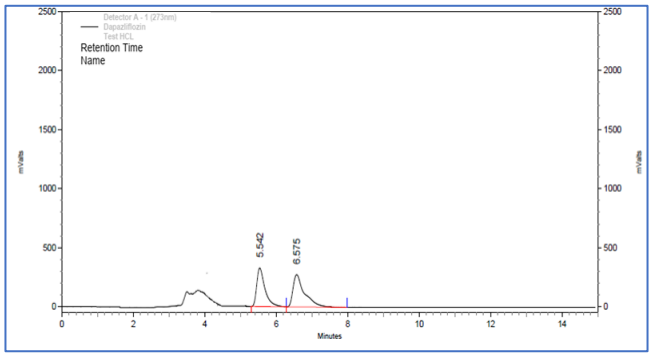

Fig.17 Chromatogram of Acid degradation of marketed formulation

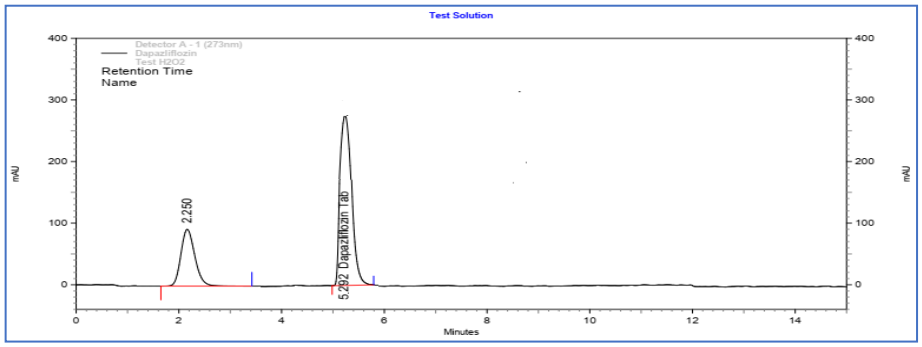

Fig.18 Chromatogram of Acid degradation of marketed formulation

6.4 Detection, Isolation and Characterization of Dapagliflozin Degradation Impurities (DP1 and DP2)

The preparative HPLC is used for isolation of degradation product of acid (DP1) and alkali (DP2) by using same developed condition. Isolated degradation product was characterized by using IR, Mass and ^{1}H NMR. The retention time for DP1 is 6.570 min and DP 2 is 2.250 min.

Proposed Structure Elucidation of Impurities

Chemically Dapagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol, with empirical formula $C_{21}H_{25}ClO_6$ and it molecular weight is 408.9 gm/mol. When drug is degraded in acidic condition it forms DP1 with Empirical formula $C_{23}H_{27}ClO_7$ and Molecular weight is 450.14 g/mol. In Alkali condition it forms DP2 with Empirical formula $C_{21}H_{25}CLO_7$ and Molecular weight is 424.87 g/mol. Standard values of different bonds of Acidic degradation product DP1 in IR spectrum show in Table 6.

Functional Group	Standard Range (cm^{-1})	Observed value (cm^{-1})
- OH	3200-3600	3339
-C-H	2850-2970	2877
- C = O Ester Carbonyl	1650-1820	1722
C-O-C (Ether linkage)	1040-1280	1036

Table 2. IR Standard range of DP1

MS spectra of DP1 shown *m/z* at 450.3 [M^+] as base peak. The Product ion peaks at *m/z* 405 (loss of $CH_3CH_2O^-$), *m/z* 391 (loss ofCH_3COO^-), *m/z* 2 (Loss of CH_3CH_2 CH_2OH), *m/z* 202 Loss of $CH_6CH_{10}O_6$) *m/z* 168 (Loss of – Cl from *m/z* 202). The positive mass spectrum for DP1 is shown in Fig. 19. The possible MS fragmentation of DP1 in acidic condition shown in Fig.20.

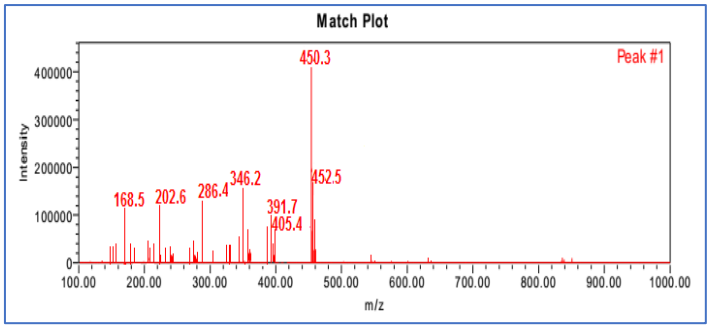

Fig. 19 ESI (+ve) mass Spectrum for DP1

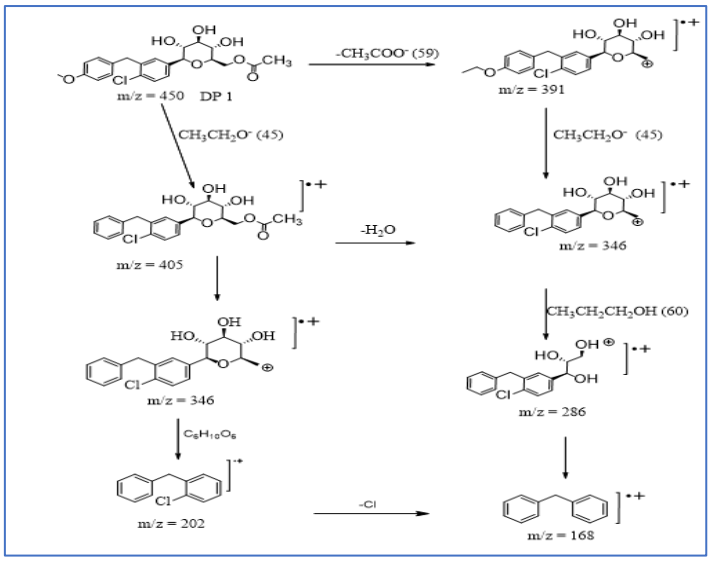

Fig. 20 Possible Mass fragmentation of DP1 in Acidic Condition

^{1}H Analysis has been done in DMSO at 400 MHz. The chemical shift values reported on δ scale in ppm with respect to TMS (0.00 ppm). ^{1}H NMR of Empagliflozin acid impurity DP1 shown in Fig. 21.^{1}H NMR values shown in Table 7 confirms structure of DP1.Based on previous data structure of DP1 was shown in Fig. 22. IUPAC name of DP1 is (2R,3S,4R,5R,6S)-6-(3-(4-ethoxybenzyl)-4-chlorophenyl)-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-yl)methyl acetate.

Chemical Shift δ ppm	**Number of product**	**Multiplicity**	**Assignment**
1.29-1.32	3H	Triplate	-CH_3
2.19	3H	Singlet	-CH_3
3.11-3.51	5H	Multiplet	Sugar Moeity
3.71- 4.08	6H	Multiplet	-CH2
4.51.5.00	3H	Doublet	-OH
6.83-7.40	7H	Multiplet	Ar-H

Table 7. ^{1}H NMR assignment of acid degraded Product DP1

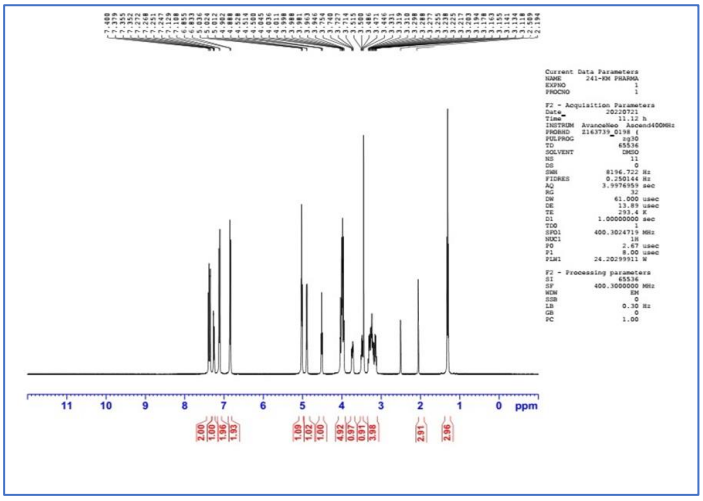

Fig. 21 ^{1}H NMR of Empagliflozin Acid Impurity DP1

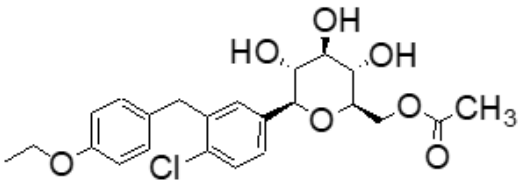

Fig. 22 Possible structure of DP1

Formation of DP1, sugar moiety in the target molecule undergoes gradual decomposition to form formaldehyde /acetaldehyde and acetic acid in different proportions. The formed acetic acid involved in the esterification/Acetylation of dapagliflozin under the existing hot acidic condition. Possible degradation pathway was shown in Fig. 23**.**

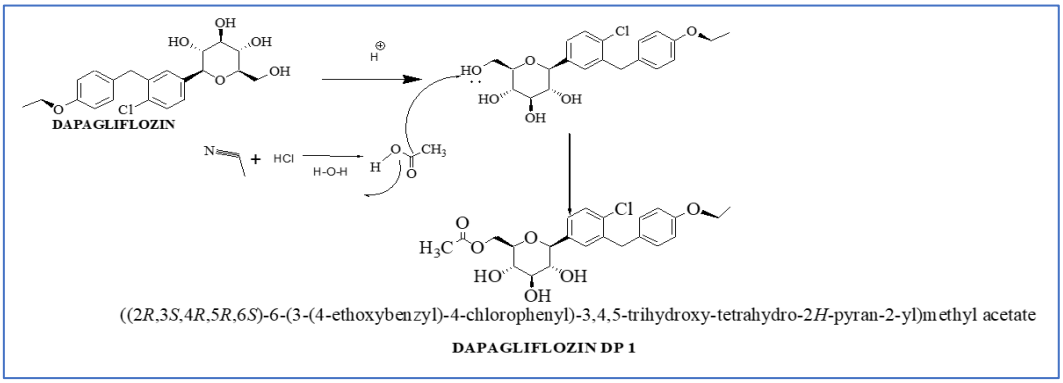

Fig. 23 Possible degradation pathway DP1

In Alkali condition it forms DP2 with Empirical formula $C_{23}H_{25}CLO_7$ and Molecular weight is 448.89 g/mol.Standard values of different bonds of Acidic degradation product DP2 in IR spectrum show in Table 8.

Functional Group	**Standard Range (cm^{-1})**	**Observed value (cm^{-1})**
- OH	3200-3600	3354
C-O-C (Ether linkage)	1040-1280	1170
C=0	1650-1820	1677

Table 8 : IR Standard range of DP2

MS spectra of DP2 shown *m/z* at 424.2 [M^+] as base peak. The Product ion peaks at *m/z* 406 (loss of water molecule), *m/z* 389 (Loss of – Cl), *m/z* 371 (loss of water molecule from *m/z* 389) *m/z* 326 (Loss of – OCH_2CH_3 from *m/z* 371), *m/z* 308 (Loss of – OH from *m/z* 326),, *m/z* 169 (loss of -$C_5H_9O_3$ group from *m/z* 308), The positive mass spectrum for DP2 is shown in fig.24. Possible MASS fragmentation is shown in figure 25.

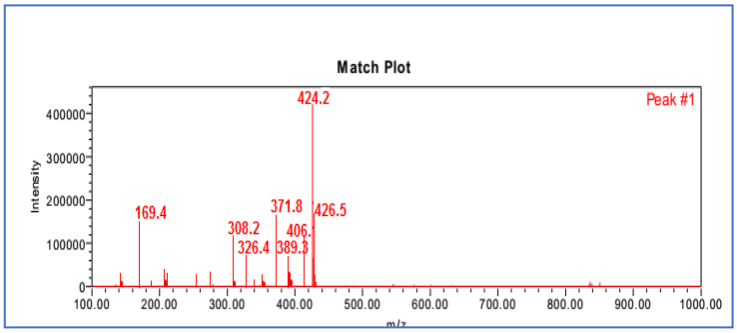

Fig. 25: ESI (+ve) mass Spectrum for DP2

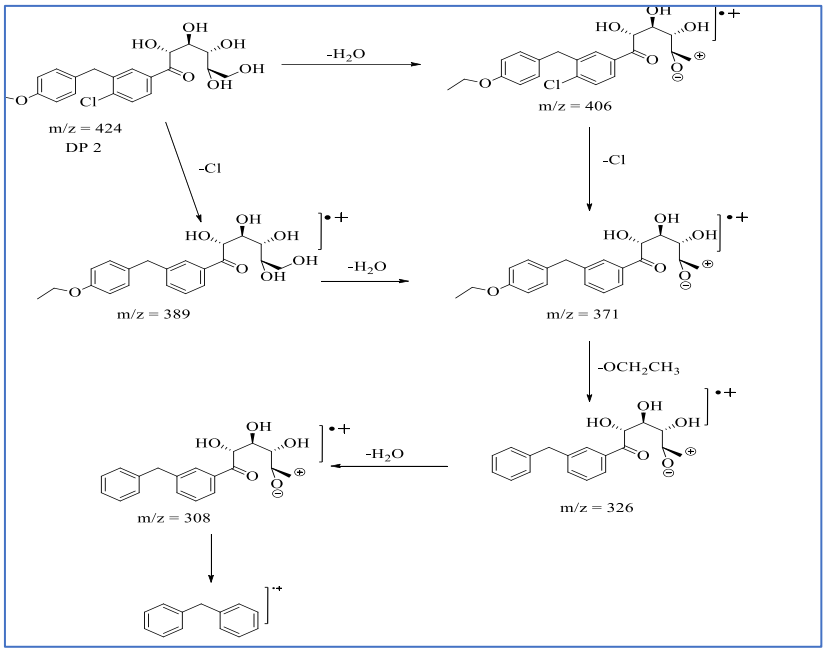

Fig. 26 Possible Mass fragmentation of DP2 in Alkali Condition

^{1}H NMR of Dapagliflozin acid impurity DP2 shown in fig.27. ^{1}H NMR values shown in table 9 confirms structure of DP2. Based on previous data structure of DP2 was shown in fig.28. IUPAC name of DP2 is (2R,3S,4R,5S)-1-(3-(4-ethoxybenzyl)-4-chlorophenyl)-2,3,4,5,6-pentahydroxyhexan-1-one.

Chemical Shift δ ppm	Number of product	Multiplicity	Assignment
1.00-1.45	3H	Triplate	-CH3
3.00-3.58	4H	Multiplet	open sugarmoiety
3.69-4.12	6H	Multiplet	$-CH_2$
4.44-4.92	5H	Doublet	-OH
6.84-7.37	7H	Multiplet	Ar-H

Table 9: ^{1}H NMR Assignment of Alkali degraded Product DP2

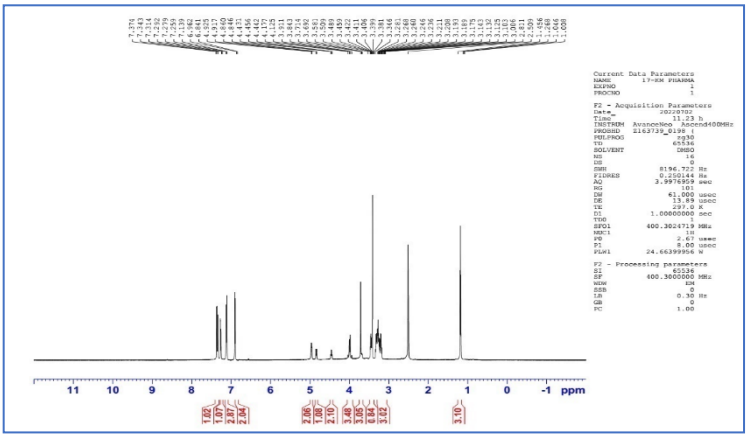

Fig. 27: ^{1}H NMR of Dapagliflozin Alkali Impurity DP2

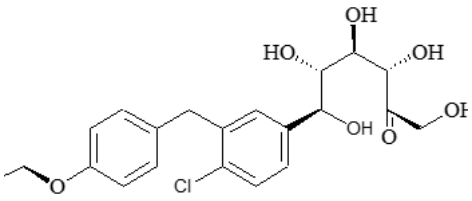

Fig.28: Fig. 22 Possible structure of DP2

In alkali hydrolysis molecular weight is loss as compare to pure drug so in Alkali hydrolysis in presence of sodium hydroxide two hydron ion is removed and oxygen convert in to Aldehyde it means Alcohol convents in to Aldehyde in formed DP2. Possible degradation pathway of DP2 was shown in fig. 29.

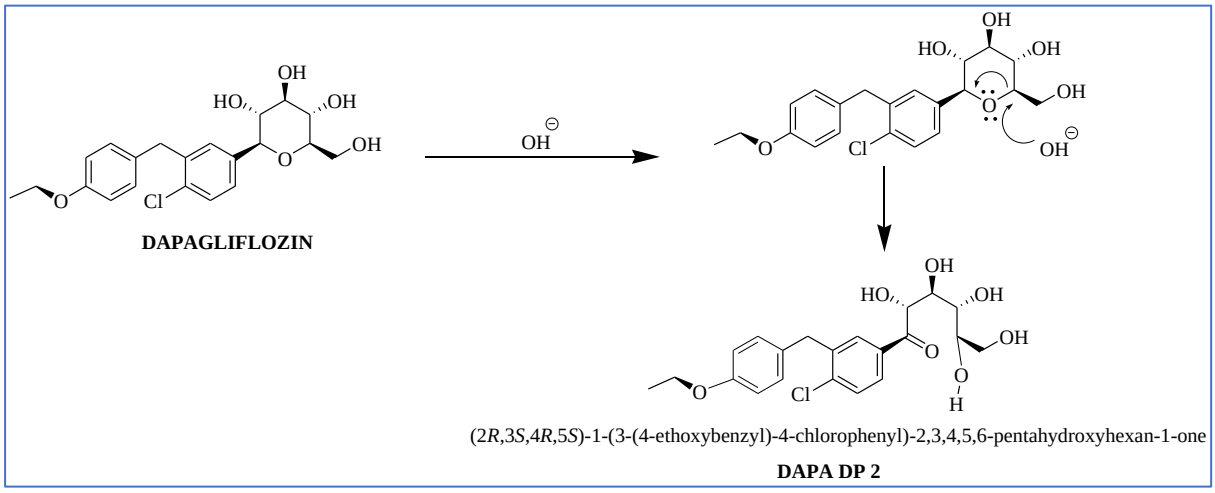

Fig.29: Possible degradation Alkali degradation Impurity DP2

7. Achievements with respect to objectives

The research project successfully identified and quantified degradation impurities present in anti-diabetic drugs categorized from SGLT-2 inhibitors ex. Empagliflozin and Dapagliflozin provides essential information for ensuring the quality and safety of these medications for that degradation behavior of these drugs under various stress conditions (ex. Acidic, alkali, oxidative, photolytic and thermal) was systematically investigated. Both drugs in API form and in its marketed formulation are unstable in acidic and alkaline condition. These degradation impurities are isolated and characterized by different sophisticated techniques like Preparative HPLC, IR, MS and ^{1}H NMR. This has provided insights into the potential pathways of degradation and the identification of degradation products. In summary, the research project on impurity profiling and degradation behaviors of selected anti-diabetic drugs has made significant strides in ensuring the quality, safety, and efficacy of these essential pharmaceuticals. The knowledge gained from this project is vital not only for regulatory compliance but, more importantly, for the well-being and health of the patients who depend on these medications. The ongoing commitment to quality control and safety will remain a central focus for the pharmaceutical industry and regulatory agencies in the years to come.

8. Conclusion

Method is developed for Empagliflozin by using Mobile phase Acetonitrile: water 50:50 (V/V) with flow rate 0.5 ml/min using automatic liquid chromatography Model LC-2010 (Shimadzu, Japan), Pump-single pump systems using UV-VIS Detector at 224 nm with Software-LC Solution to acquire and process the data. Reversed-Phase YMC ODS A C-18 (150mm x 4.6mm). The retention time was found to be 5.733 min. Validation is performed according to ICH Guidelines Q2(R1).The degradation study of Empagliflozin under stressed conditions was examined following ICH guidelines Q1(R2).The API was subjected to oxidative, acidic, alkaline, neutral, photolytic, and thermolytic degradation conditions. The drug was stable in Oxidative, thermal, and photolytic conditions, and no degradation products were observed. However, 2 degradation products were formed in acid (DP-1, RT:2.28 min) and Alkali stress hydrolysis conditions (DP-2, RT:2.25 min). DP1 is formed by attack of H^{+} on oxygen of oxane ring which lead to oxane ring opening in acid degradation condition. DP-2 was formed by attack of -OH with loss of H2 which convert in to aldehyde. Method is developed for Dapagliflozin by using Mobile phase Acetonitrile: water 50:50 (V/V)

with flow rate 0.7 ml/min by using same at 273 nm. The retention time was found to be 5.33 min.2 degradation products formed in acid (DP-1, RT: 6.90 min) and Alkali stress hydrolysis conditions (DP-2, RT: 2.3 min). Formation of DP1, sugar moiety in the target molecule undergoes gradual decomposition to form formaldehyde /acetaldehyde and acetic acid in different proportions. The formed acetic acid involved in the esterification/Acetylation of dapagliflozin under the existing hot acidic condition.DP2 form by attack of OH- which led to oxane ring opening.

9. Paper publication

a. Research paper published in UGC care list group II Approved Journal Chinese Journal of Medical genetics entitled " Structural characterization of Forced degradation impurity of Dapagliflozin ". Vol. 32 Iss. 1(2023). ISSN: 1003-9406.

b. Research paper accepted in World Journal of Pharmaceutical Research entitled "Stability indicating RP-HPLC method for estimation of Empagliflozin in bulk and pharmaceutical dosage form "On dated:06/08/2023. ISSN 2277 – 7105.

c. Review article published in Asian Journal of Pharmaceutical Research entitled "Impurity identification and Characterisation some Anti-Diabetic drugs using of various analytical methods". Vol. 9 Iss. 4 (2019). ISSN: 2231-5691

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