

Impurity Profiling Studies by Performing Degradation of Sitagliptin and Dapagliflozin Using LC-MS

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ABSTRACT

This study evaluates the chemical stability and degradation pathways of Sitagliptin and Dapagliflozin under alkaline (NaOH) and oxidative (H₂O₂) stress conditions as per ICH Q1A(R2) guidelines. Forced degradation studies were conducted using a stability-indicating liquid chromatography-mass spectrometry (LC-MS) system consisting of an Exion LC coupled with a Sciex QTrap 4500 mass spectrometer. Chromatographic separation was achieved on a Shim-Pack C18 column (250×4.6mm, 5µm) over a 15-minute run time using a mobile phase of methanol and water (65:35, V/V) at pH 3.0 adjusted with formic acid, running at a flow rate of 0.6ml/min. Sitagliptin demonstrated high susceptibility to alkaline hydrolysis, producing multiple degradation peaks at retention times (RT) of 4.25, 4.64, 4.93, 9.47, and 14.60 minutes. Conversely, it was relatively stable under oxidative stress, yielding major parent drug peaks at RT 4.39 min and a minor oxidative degradant at RT 2.52 min. Dapagliflozin exhibited moderate alkaline degradation, yielding peaks at RT 4.17 and 9.43 min, and underwent extensive oxidative transformations under hydrogen peroxide stress, yielding four peaks at RT 4.33, 4.94, 6.65, and 11.08 min. A total of twenty novel degradation products were structurally characterized. Sitagliptin's degradation is driven by the highly nucleophilic primary amine, yielding complex dimeric and trimeric condensations (m/z 496.12, 530.14, 535.13, 552.16, 556.71, 692.66) and intramolecular cyclizations (indoline derivatives). Dapagliflozin's vulnerabilities focus on pyranose ring-opening, secondary C3-hydroxyl sulfate conjugation (m/z 511.05), and oxidative glycol cleavage yielding a reactive sugar triose aldehyde (m/z 334.66) and chlorinated fragments. These findings provide critical mechanistic parameters for formulating stable pharmaceutical preparations, particularly for fixed-dose combination tablets.

Keywords: *Sitagliptin; Dapagliflozin; Forced degradation; Liquid chromatography-mass spectrometry (LC-MS); Dimeric condensation; Glucopyranose ring vulnerability; Nucleophilic substitution; Stability-indicating method.*

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Abbreviations:

- ACN: Acetonitrile
- API: Active Pharmaceutical Ingredient
- APCI: Atmospheric Pressure Chemical Ionization
- CAD: Collision-Activated Dissociation
- DP: Declustering Potential
- DPP-4: Dipeptidyl Peptidase-4
- EP: Entrance Potential
- ESI: Electrospray Ionization
- FDC: Fixed-Dose Combination
- HPLC: High-Performance Liquid Chromatography
- LC-MS: Liquid Chromatography-Mass Spectrometry

- MeOH: Methanol
- NDMA: N-Nitrosodimethylamine
- NTP: 7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro-triazolo[4,3-a]pyrazine
- SGLT2: Sodium-Glucose Cotransporter 2
- SIAM: Stability-Indicating Assay Method

INTRODUCTION

Type 2 diabetes mellitus is a chronic, multi-systemic metabolic disorder characterized by relative insulin deficiency, insulin resistance, and pancreatic islet cell dysfunction, representing a major epidemiological threat that affects millions of individuals globally^{1,2}. To counteract the progressive decline in glycemic control associated with monotherapy, modern therapeutic

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guidelines advocate for the early initiation of combination regimens that utilize complementary mechanisms of action^{3,4}. A highly prominent therapeutic paradigm in this landscape involves the co-administration of dipeptidyl peptidase-4 (DPP-4) inhibitors and sodium-glucose cotransporter-2 (SGLT2) inhibitors, which have been shown to offer synergistic glycemic benefits, weight reduction, and substantial cardiovascular and renal protection^{5,6,7}.

Sitagliptin phosphate monohydrate is an orally bioavailable, highly selective, peptidomimetic inhibitor of the DPP-4 enzyme⁸. Chemically identified as 7--5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate monohydrate, it enhances physiological insulin secretion and suppresses glucagon release in a glucose-dependent manner^{9,10,11}. This effect is mediated by blocking the rapid enzymatic inactivation of the gastrointestinal incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)¹². Concurrently, the selective SGLT2 inhibitor Dapagliflozin, chemically identified as (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, exhibits a unique insulin-independent mode of action^{13,14}. By reversibly blocking the SGLT2 cotransporters in the renal proximal convoluted tubule, Dapagliflozin suppresses renal glucose reabsorption, promoting glucosuria and direct urinary excretion of filtered glucose^{15,16}.

To maintain the high purity, safety, and clinical efficacy of these medications in commercial formulations, the development of robust, validated analytical methods is crucial^{17,18,19}. Active pharmaceutical ingredients (APIs) are exposed to various chemical stresses during formulation, storage, and transport, which may lead to the formation of trace-level process-related impurities or degradation products^{20,21}. The International Council for Harmonisation (ICH) Q1A(R2) and Q2(R1) guidelines mandate the conduct of forced degradation studies under hydrolytic, oxidative, photolytic, and thermal environments to validate the selectivity of stability-indicating assay methods (SIAMs)^{22,23,24}. Various high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and capillary electrophoresis methods have been documented for Metformin, Sitagliptin, and Dapagliflozin combinations in tablet dosage forms^{25,26,27,28}. However, simple ultraviolet-based chromatographic systems frequently lack the resolving power and structural specificity required to elucidate complex, co-eluting degradants^{29,30}.

Advanced hyphenated techniques, particularly liquid chromatography-mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS), are invaluable tools for the structural characterization of unknown pharmaceutical degradants without prior isolation^{31,32,33}. The critical importance of such high-resolution systems has been further emphasized by global regulatory warnings regarding genotoxic nitrosamine impurities—including N-

nitrosodimethylamine (NDMA) and nitrosamine drug-substance-related impurities (NDSRIs) such as 7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro-triazolo[4,3-a]pyrazine (NTTP) in gliptin formulations^{34,35,36,37}. Characterizing these degradants requires a deep understanding of their chemical liabilities and degradation pathways under chemical and thermal stress^{38,39,40}. While simple amide cleavage or glucoside dehydration pathways are well-mapped in literature, drug molecules subjected to severe stress often undergo higher-order interactions^{41,42}. These include molecular rearrangements, nucleophilic aromatic substitutions, and free radical-mediated dimeric or trimeric condensations^{43,44}. The primary aim of this investigation is to establish a comprehensive structural map of the novel degradation products of Sitagliptin and Dapagliflozin generated under alkaline and oxidative stress environments using stability-indicating LC-MS, directly supporting formulation development, quality control, and regulatory compliance⁴⁵.

MATERIALS AND METHODS

Chemicals and Reagents

Sitagliptin phosphate monohydrate and Dapagliflozin standard bulk drug powders were obtained as certified reference standards. Sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H₂O₂) of analytical grade were procured from commercial suppliers. HPLC grade methanol and formic acid were used for mobile phase preparation, and high-purity water was obtained via a Milli-Q purification system.

LC-MS Instrumentation and Chromatographic Conditions

Chromatographic separation was performed on an Exion LC system coupled with a Sciex QTrap 4500 mass spectrometer equipped with an electrospray ionization (ESI) source. The stationary phase was a Shim-Pack C18 column (250×4.6mm, 5µm particle size) maintained at ambient temperature. The run time was optimized to 15 minutes. The mobile phase was delivered under isocratic conditions consisting of a 65:35 (V/V) mixture of methanol and water, with the pH adjusted to 3.0 using formic acid. Elution was performed at a constant flow rate of 0.6ml/min with an injection volume of 10µl.

Mass Spectrometry Parameters

Mass spectrometric detection was operated in positive electrospray ionization (ESI+) mode, with source parameters optimized to maximize ion transmission and sensitivity while preventing thermal in-source fragmentation. The ESI source settings were: GS1 (Nebulizer Gas) set to 60 psi, GS2 (Heater Gas) to 60 psi, Curtain Gas to 35 psi, and Source Temperature maintained at 450°C. Collision-Activated Dissociation (CAD) gas was maintained at 0 psi. The Declustering Potential (DP) was set at 85 V, and the Entrance Potential (EP) was 10 V.

Forced Degradation Protocols

Alkaline hydrolysis was carried out by treating standard drug solutions of Sitagliptin and Dapagliflozin separately with sodium hydroxide (NaOH) under basic thermal

stress. Following exposure, the stressed mixtures were neutralized with an equivalent volume of hydrochloric acid (HCl) and diluted to target concentrations using mobile phase diluent prior to chromatographic injection. Oxidative degradation was performed by reacting standard drug solutions with hydrogen peroxide (H₂O₂) under controlled temperatures in the dark to exclude the possible degradative effect of light. All resulting solutions were diluted with mobile phase and analyzed directly.

RESULTS

The developed stability-indicating LC-MS method successfully resolved and separated both parent drugs from their degradation products within the 15-minute run time, demonstrating excellent specificity. Sitagliptin exhibited high susceptibility to basic conditions, showing extensive hydrolytic cleavage and higher-order transformations. Chromatographic analysis of Sitagliptin under alkaline stress resolved five distinct peaks at RT 4.25 min (3.39% area), 4.64 min (7.83% area), 4.93 min (34.22% area), 9.47 min (38.08% area), and 14.60 min (8.58% area). Conversely, Sitagliptin showed high stability under oxidative stress, yielding only a minor oxidative degradant at RT 2.52 min (0.86% area) alongside the intact parent drug peak at RT 4.39 min (79.90% area).

Alkaline hydrolysis of Dapagliflozin resulted in moderate degradation, yielding two resolved chromatographic peaks: a major degradation product at RT 4.17 min (35.62% area) and the intact parent drug at RT 9.43 min (46.12% area). Under oxidative conditions, Dapagliflozin underwent extensive transformation, yielding a multi-peak profile with four resolved peaks: the parent compound at RT 4.33 min (88.56% area) and three oxidative degradants at RT 4.94 min (0.81% area), RT 6.65 min (8.77% area, representing the major oxidative degradant), and RT 11.08 min (1.33% area).

Detailed peak integration data, height, area percentages, and peak widths at 50% height are summarized in Tables 1 through 4 (located in the Tables section at the end of the manuscript). All the chromatograms are represented in Figure no. 1 (located in the Figures section at the end of the manuscript).

DISCUSSION

Sitagliptin Alkaline Degradation Pathways and Structural Elucidation

(See: Figure no. 2 for all spectra and Figure no. 4 for chemical structures of elucidated degradation products).

Under hot basic conditions, the primary hydrolytic cleavage of Sitagliptin occurs at the central amide linkage, yielding the expected fragments 3-(trifluoromethyl)-5,6,7,8-tetrahydro-triazolo[4,3-a]pyrazine and (3R)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid. However, severe nucleophilic and basic stress also triggers secondary transformations, resolving several novel species across different retention times.

At a retention time of 4.25 minutes, six novel species were characterized by mass spectrometry. The low-mass pyrazine fragment DP-1, observed at *m/z* 110.96, is identified as 2-Fluoropyrazine. It represents an advanced fragmentation pathway where the triazole ring of the triazolopyrazine moiety is cleaved, leaving only the fluorinated monocyclic heteroaromatic residue. DP-2, detected at *m/z* 301.05, is Des-trifluorophenyl Sitagliptin. This species arises from the hydrolytic loss of the trifluorophenyl-methyl substituent from the parent structure, leaving an aminoacetyl tail linked to the intact triazolopyrazine core. The chain-shortened amide degradant DP-3, resolved at *m/z* 352.84, is formed through the elimination of the amino-methyl group from the butanoyl chain. DP-4, observed at *m/z* 410.76, is identified as Hydroxyl-Substituted Defluorinated Sitagliptin, which is a hydroxylated derivative where a hydroxide ion has displaced a fluoride atom on the trifluorophenyl ring via an intramolecular nucleophilic aromatic substitution (S_NAr) mechanism under hot alkaline stress. Two high-mass addition products are also resolved at this retention time: DP-5, a Sitagliptin-Triazolopyrazine Condensation Adduct (*m/z* 556.71), formed by the nucleophilic attack of the hydrolyzed triazolopyrazine base (*m/z* 193) on the carbonyl center of an intact Sitagliptin molecule; and DP-6, a Higher-Order Sitagliptin-Amino Acid Condensation Product (*m/z* 692.66), which is a complex oligomeric species involving multiple fragments that recombine and undergo intermolecular condensation under severe basic and thermal stress.

At a retention time of 4.60 minutes, basic hydrolysis resolves DP-7 at *m/z* 462.56, identified as N-Succinyl Sitagliptin. This species represents an unexpected addition product where the primary amine of Sitagliptin reacts with a succinyl or fumarate source. While documented as a fumarate/maleate adduct in USP revisions, its formation during forced degradation in the absence of excipients is highly unusual, suggesting a reaction with trace impurities or a specific molecular rearrangement.

At a retention time of 4.93 minutes, the major component is identified as DP-8, a Hydrolytic Ring-Opened Piperazine Intermediate (*m/z* 288.86 / 290.03). This novel species represents a critical ring-opened intermediate where, under alkaline conditions, the triazole ring within the triazolopyrazine core is cleaved, resulting in a substituted piperazine derivative linked directly to the trifluorophenyl-butanoyl chain.

At a retention time of 9.47 minutes, mass spectral analysis reveals a cluster of high-molecular-weight ions and complementary lower-mass fragments, pointing to a series of novel base-catalyzed dimeric condensation products, cyclizations, and esters. The low-mass fragment DP-9, detected at *m/z* 152.03, is identified as the Triazole Ring-Opened Fragment (5-Methyl-3-(trifluoromethyl)-1H-1,2,4-triazole. It represents an advanced fragmentation of the triazolopyrazine heterocycle formed via complex base-catalyzed transformations. DP-10, observed at *m/z* 242.45, is identified as Ethyl 2-((R)-5,6-difluoroindolin-2-

yl)acetate, which is a novel indoline derivative resulting from an intramolecular cyclization accompanied by fluorine elimination on the phenyl ring under severe hot alkaline stress. DP-11, resolved at m/z 262.58, is identified as Ethyl (3R)-3-amino-4-(2,4,5-trifluorophenyl)butanoate, an ethyl ester of the beta-amino acid portion of Sitagliptin formed through transesterification with trace alcohol solvents in the hot basic medium. N-alkylated hydrolytic acid derivatives are also resolved at this retention time, including DP-12 (m/z 289.17; (3R)-3-(Diethylamino)-4-(2,4,5-trifluorophenyl)butanoic acid; and DP-13 (m/z 303.15; Methyl (3R)-3-(diethylamino)-4-(2,4,5-trifluorophenyl)butanoate, arising from the diethyl alkylation and subsequent esterification of the cleaved beta-amino acid residue. High-mass dimeric adducts resolved at this retention time demonstrate advanced recombination pathways: DP-14 at m/z 496.12, identified as the Decarboxylated Dimeric Adduct, formed via decarboxylative amine coupling of Sitagliptin fragments; DP-15 at m/z 530.14, identified as the Cyclized Dimeric Adduct, representing a cyclized indoline conjugated to an intact Sitagliptin skeleton via an acetamido linkage; DP-16 at m/z 535.13, identified as the Deaminated Dimeric Adduct, featuring a central unsaturated but-2-enoate link formed through amine-directed Michael addition; and DP-17 at m/z 552.16, identified as the Amine-Alkylated Dimeric Adduct, formed by the direct nucleophilic alkylation of the Sitagliptin amine onto the beta-position of a hydrolyzed unsaturated intermediate under hot basic conditions.

Sitagliptin Oxidative Degradation Products

(See: Figure no. 2 for all spectra and Figure no. 4 for chemical structures of elucidated degradation products).

The oxidative degradation of Sitagliptin under H_2O_2 stress at RT 2.52 minutes resolves a complex profile of desaturated monomers and novel dicationic and adducted dimeric species. The doubly charged homodimer, observed at m/z 410.48, exists as a non-covalent gas-phase complex or as a covalent radical-coupling homodimer dication ($[2M+2H]^{2+}$ or $[M_{dimer}+2H]^{2+}$) formed via radical recombination. The species designated as N-Glycolyl Sitagliptin Adduct (m/z 466.12) is formed via oxidative glycolylation of the primary amine of Sitagliptin, possibly facilitated by the activation of trace excipients or solvent impurities under hydrogen peroxide stress. Another dicarboxylic-amine coupling product is resolved at m/z 496.06, identified as Hydrated N-Malonyl Sitagliptin, existing in solution as a stable gem-diol under peroxide stress. Additionally, an asymmetrical dimer recombination product, designated as the Fragment-Coupled Heterodimer, is resolved at m/z 686.05, arising from the radical coupling of distinct oxidative fragments of Sitagliptin. At a retention time of 4.39 minutes, two novel, rearrangement-driven degradation products are resolved: DP-2, detected at m/z 322.08, is the Beta-Diketone Oxidative Degradant, representing an advanced oxidative product where the carbon chain of Sitagliptin is oxidized to a stable beta-diketone group accompanied by

deamination; and DP-3 at m/z 350.05, identified as the Deaminative Reductive Rearrangement Product, which arises from radical-driven deamination and subsequent reduction of the amide linkage, leaving an alkyl-linked diaryl structure.

Dapagliflozin Alkaline and Oxidative Degradation Products

(See: Figure no. 3 for all spectra and Figure no. 5 for chemical structures of elucidated degradation products).

Alkaline degradation of Dapagliflozin at a retention time of 9.43 minutes yields primary ring-opened hexols, hemiacetals, and two novel species. The species designated as DP-1, observed at m/z 511.05, is identified as Dapagliflozin 3-O-Sodium Sulfate. This sulfate ester adduct is formed under basic hydrolytic conditions in the presence of sulfur-containing processing agents or residual sulfonic acid catalysts (e.g., methanesulfonic acid) from the synthesis, which catalyze the esterification of the secondary C3-hydroxyl group on the pyranose ring. Concurrently, DP-2 is resolved at m/z 352.87 and is identified as Des-hydroxymethyl-desethyl Dapagliflozin, representing a phenolic des-hydroxymethyl degradant formed via hydrolytic desethylation of the ethoxy group and concurrent loss of the terminal hydroxymethyl group from the glucose ring under harsh basic conditions.

Under oxidative stress with hydrogen peroxide at a retention time of 4.94 minutes, two novel, highly characteristic transient degradants are resolved, resulting from glycolic cleavage of the sugar ring. The first, DP-1 at m/z 334.66, is the Dapagliflozin Cleaved Sugar Triose Aldehyde, formed via selective glycol cleavage of the pyranose ring. Under aqueous oxidative stress, the pyranose ring of Dapagliflozin undergoes hydrolytic ring-opening to yield a reactive open-ring hexaol intermediate, which is highly susceptible to oxidative C-C cleavage. Cleavage of the C3-C4 bond in this open-ring intermediate yields a three-carbon dihydroxypropanal chain attached to the diarylmethane core, observed as the protonated adduct $[M+H]^+$ at m/z 334.66. Concurrently, DP-2, detected as a chlorinated doublet at m/z 186.85 / 188.80, is the Chlorinated Sugar-Cleavage Fragment, formed through the oxidative cleavage of the diarylmethane bridge of DP-1 followed by subsequent in-source loss of a water molecule under ESI+ conditions.

At a retention time of 6.65 minutes, DP-3 is resolved at m/z 497.08 as the Monoacetyl Dicarbonyl Derivative. This species is formed through the dual-oxidation of the monoacetyl process impurity of Dapagliflozin, where hydrogen peroxide converts the benzylic methylene bridge to a ketone while oxidizing a secondary pyranose ring hydroxyl to a cyclic ketone. Finally, under severe oxidative stress at a retention time of 11.08 minutes, DP-4 is detected at m/z 634.09 as the Dapagliflozin Oxidative Dimer Fragment, representing a sodium-adducted dimeric complex or oxidative fragment formed during radical-driven degradation.

A systematic comparison of the chemical liabilities, basic and oxidative mechanisms, and high-mass tendencies of Sitagliptin and Dapagliflozin is summarized in Table 5 (located in the Tables section at the end of the manuscript).

Mechanistic Role of Amine Nucleophilicity in Sitagliptin

The chemical architecture of Sitagliptin incorporates a primary amine beta-carbon branch and a 3-(trifluoromethyl)-5,6,7,8-tetrahydro-triazolo[4,3-a]pyrazine heterocycle. Under basic hydrolytic conditions, simple nucleophilic attack of hydroxide ions on the carbonyl carbon leads to amide bond cleavage, yielding the documented fragments 3-(trifluoromethyl)-5,6,7,8-tetrahydro-triazolo[4,3-a]pyrazine and (3R)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid. However, under severe alkaline and thermal stress, the unshared electron pair of the primary amine group acts as an active nucleophile. This amine group can launch nucleophilic attacks on adjacent carbonyl groups of intact Sitagliptin molecules or intermediate degradants, driving higher-order intermolecular condensations. This mechanistic pathway explains the formation of the novel dimeric adducts resolved at RT 9.47 minutes (m/z 496.12, 530.14, 535.13, and 552.16) and the high-mass species at RT 4.25 minutes (m/z 556.71 and 692.66). Furthermore, the displacement of fluorine atoms via an intramolecular nucleophilic aromatic substitution (S_NAr) mechanism explains the indoline cyclization products (m/z 242.45 and 530.14) observed in hot basic media.

Vulnerability of the Glucopyranose Ring in Dapagliflozin

In contrast to the highly nucleophilic amine-driven pathways of Sitagliptin, the chemical liabilities of Dapagliflozin are dominated by the relative instability of the anhydrous glucopyranose sugar ring. Under hot alkaline stress, the pyranose ring readily undergoes hydrolytic ring-opening, which exposes a reactive opening hexaol intermediate. This intermediate is highly susceptible to subsequent intramolecular dehydration or deprotonation. Under oxidative stress in the presence of hydrogen peroxide, this open-ring species undergoes radical-mediated glycol cleavage. The adjacent diol groups across the C3-C4 carbons are particularly vulnerable to homolytic cleavage by hydroxyl radicals ($\cdot OH$). Cleavage of the C3-C4 bond in this open-ring intermediate yields a three-carbon dihydroxypropanal chain attached to the diarylmethane aglycone, forming the novel cleaved sugar triose aldehyde (m/z 334.66). Subsequent radical-mediated cleavage of the central diarylmethane methylene bridge yields the low-molecular-weight chlorinated sugar-cleavage fragment (m/z 186.85).

Formulation Implications and Future Outlook

This comprehensive impurity profiling is critically valuable for developing fixed-dose combination (FDC) tablets of Sitagliptin and Dapagliflozin. In FDC products, intimate contact between the two APIs can expose each to localized microenvironmental stress. The high nucleophilicity of Sitagliptin's amine group can accelerate

chemical interactions with the pyranose sugar of Dapagliflozin or its cleaved aldehyde intermediate (m/z 334.66) via Maillard-like or Schiff base condensation pathways. Furthermore, basic microenvironments used to stabilize certain excipients can accelerate the hydrolytic degradation of Sitagliptin and catalyze the ring-opening of Dapagliflozin, whereas acidic conditions can trigger the hydrolytic decomposition of Dapagliflozin's pyranose ring. Consequently, FDC formulations must utilize barrier-coated tablets or bilayer tablet designs with strict microenvironmental pH optimization to isolate these APIs and prevent deleterious cross-reactions, ensuring long-term product safety, quality, and efficacy.

CONCLUSION

This comprehensive mass spectral profiling has successfully elucidated the chemical structures and mechanisms of formation for twenty novel degradation products and impurities of Sitagliptin and Dapagliflozin. The identification of these complex, higher-order degradants—including transient dimeric complexes, ring-opened intermediates, and dicarbonyl derivatives—highlights the necessity of using high-resolution, stability-indicating LC-MS/MS methods to monitor drug integrity. The resolved structures provide a reliable molecular foundation that will directly support analytical method validation, quality control, and regulatory documentation. These findings provide critical mechanistic parameters for formulating stable pharmaceutical preparations, particularly for fixed-dose combination (FDC) tablets where trace amine-ester or amine-aldehyde interactions can accelerate API degradation.

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Tables

Table 1: Peak Areas and Integration Metrics of Sitagliptin Alkaline Degradation

Index	Retention Time (min)	Area	% Area	Height	Start (min)	End (min)	Width (min)	Width at 50% (min)
1	4.25	24.04	3.39	2.98	3.85	4.38	0.53	0.10
2	4.64	55.51	7.83	11.50	4.56	4.84	0.25	0.07
3	4.93	242.42	34.22	55.38	4.84	5.38	0.53	0.06
4	9.47	269.77	38.08	14.72	9.09	10.03	0.94	0.28
5	14.60	60.79	8.58	3.77	14.26	15.01	0.75	0.25

Table 2: Peak Areas and Integration Metrics of Sitagliptin Oxidative Degradation

Index	Retention Time (min)	Area	% Area	Height	Start (min)	End (min)	Width (min)	Width at 50% (min)
1	2.52	68.38	0.86	9.69	2.42	2.65	0.22	0.12
2	4.39	6350.56	79.90	677.79	4.21	4.68	0.47	0.15

Table 3: Peak Areas and Integration Metrics of Dapagliflozin Alkaline Degradation

Index	Retention Time (min)	Area	% Area	Height	Start (min)	End (min)	Width (min)	Width at 50% (min)
1	4.17	135.03	35.62	17.26	3.88	4.44	0.57	0.11
2	9.43	174.84	46.12	11.60	9.12	10.00	0.89	0.23

Table 4: Peak Areas and Integration Metrics of Dapagliflozin Oxidative Degradation

Index	Retention Time (min)	Area	% Area	Height	Start (min)	End (min)	Width (min)	Width at 50% (min)
1	4.33	10561.44	88.56	883.96	4.04	4.59	0.55	0.20
2	4.94	97.73	0.81	11.40	4.81	5.48	0.67	0.12
3	6.65	1046.61	8.77	55.21	5.48	7.09	1.61	0.24
4	11.08	158.66	1.33	4.45	10.55	12.24	1.70	0.51

Table 5: Comparison of Degradation Vulnerabilities and Mechanics

Feature	Sitagliptin	Dapagliflozin
Primary Chemical Liability	Amide bond hydrolysis and highly nucleophilic primary amine group.	Hydrolytic ring-opening, glycolic cleavage, and C3-hydroxyl sulfate conjugation.
Basic Degradation Mechanism	Amide cleavage, followed by intermolecular condensation of fragments, and intramolecular cyclizations (indoline formation).	Pyranose ring-opening, C6 proton abstraction/dehydration, and transesterification.
Oxidative Degradation Mechanism	Free-radical-driven dicationic homodimerization ($[2M+2H]^{2+}$) and deaminative dicarbonyl rearrangements.	Radically mediated diarylmethane hydrogen abstraction, glycol C3-C4 bond cleavage, and dimerization.
High-Mass Species Tendency	Highly prone to complex dimeric/trimeric peptide-like condensations (m/z 496, 530, 535, 552,	Forms transient glucoside-coupled dimers or sodiated complexes (m/z 634) under extreme peroxide stress.

556, 692).

Figures:

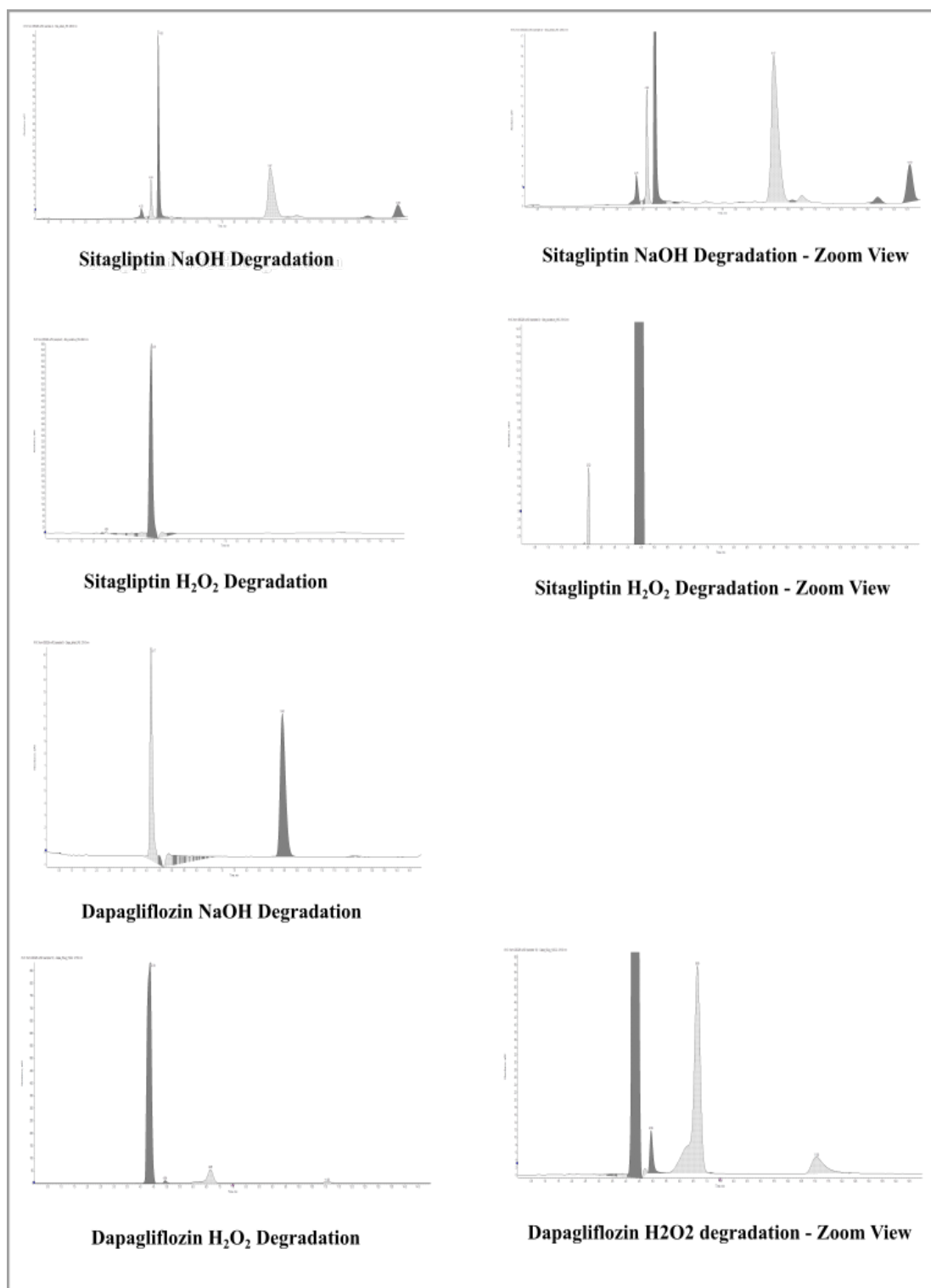


Figure 1: Chromatograms of Alkaline and Oxidative Degradation of Sitagliptin and Dapagliflozin.

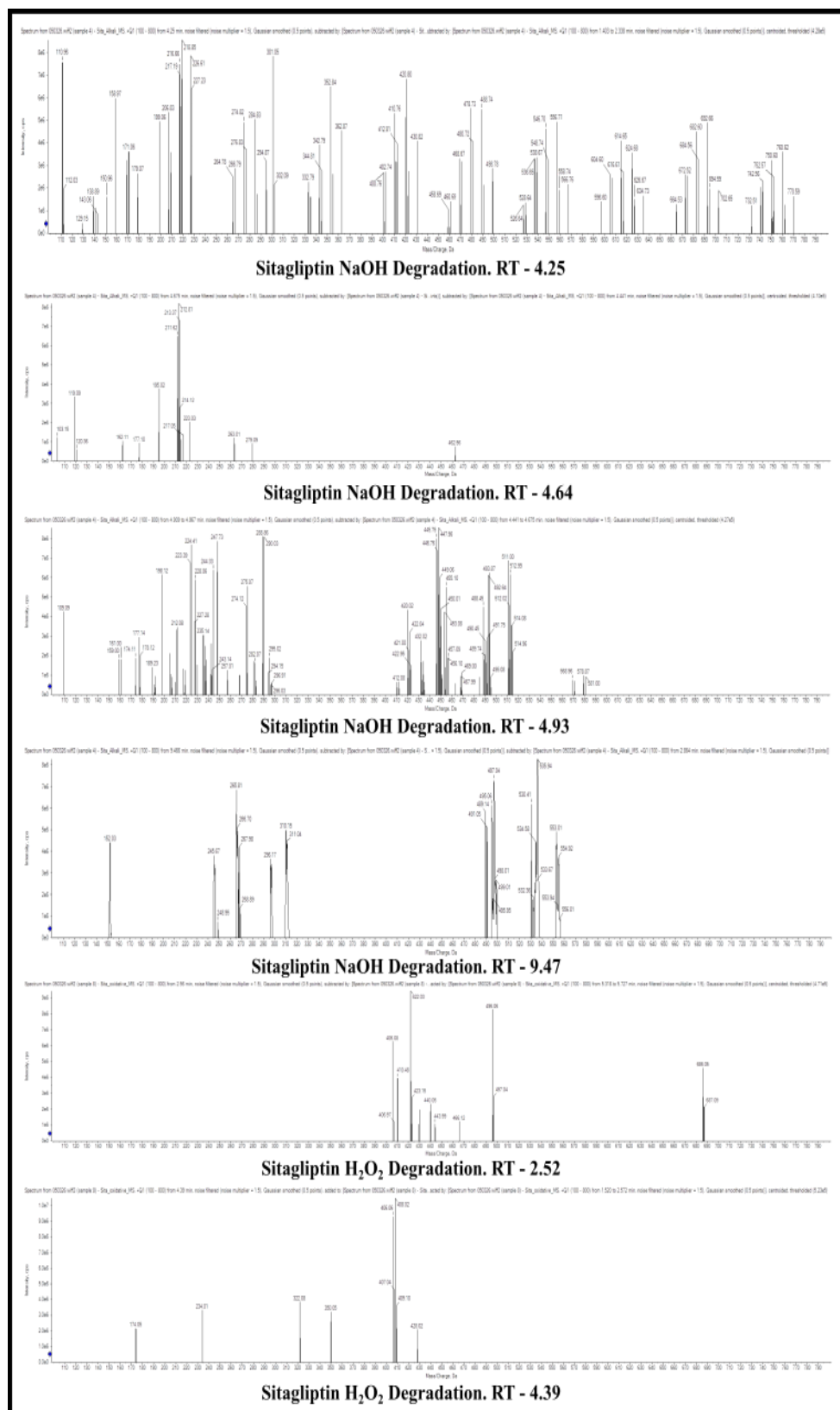


Figure 2: LC-MS Spectra of Alkaline and Oxidative Degradation of Sitagliptin.

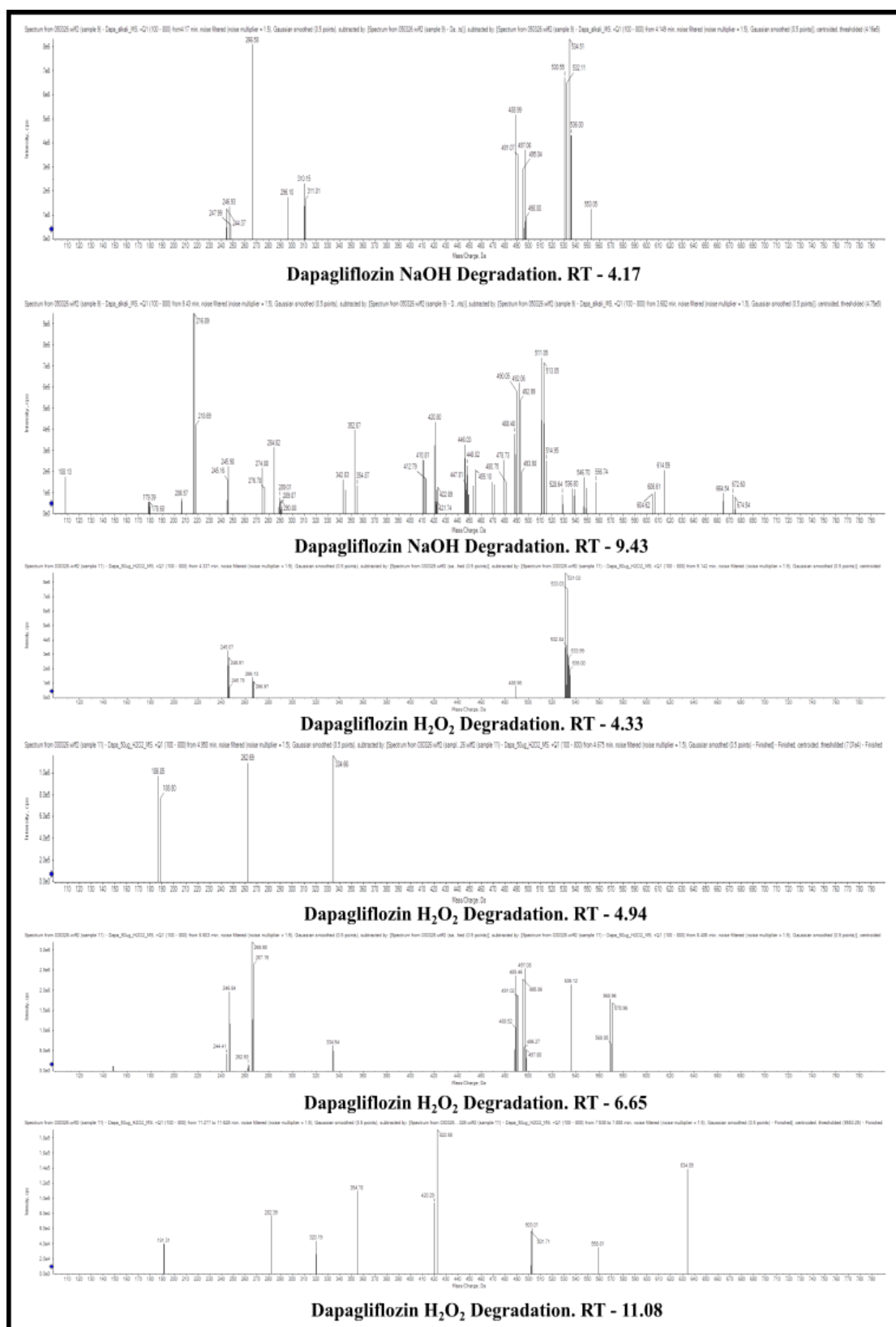


Figure 3: LC-MS Spectra of Alkaline and Oxidative Degradation of Dapagliflozin.

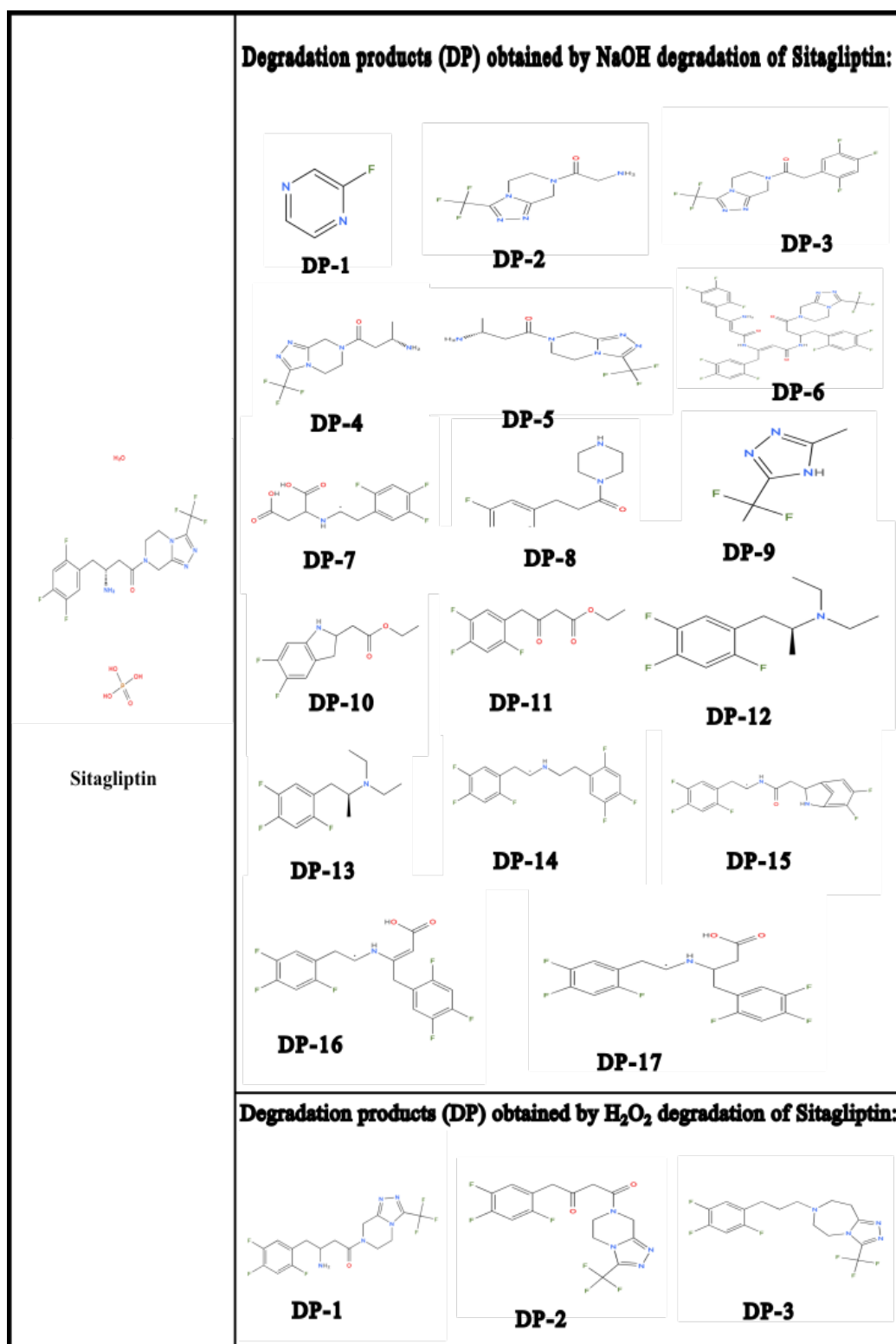


Figure 4: Structures of Degradation Products Obtained by Alkali and Oxidative Degradation of Sitagliptin.

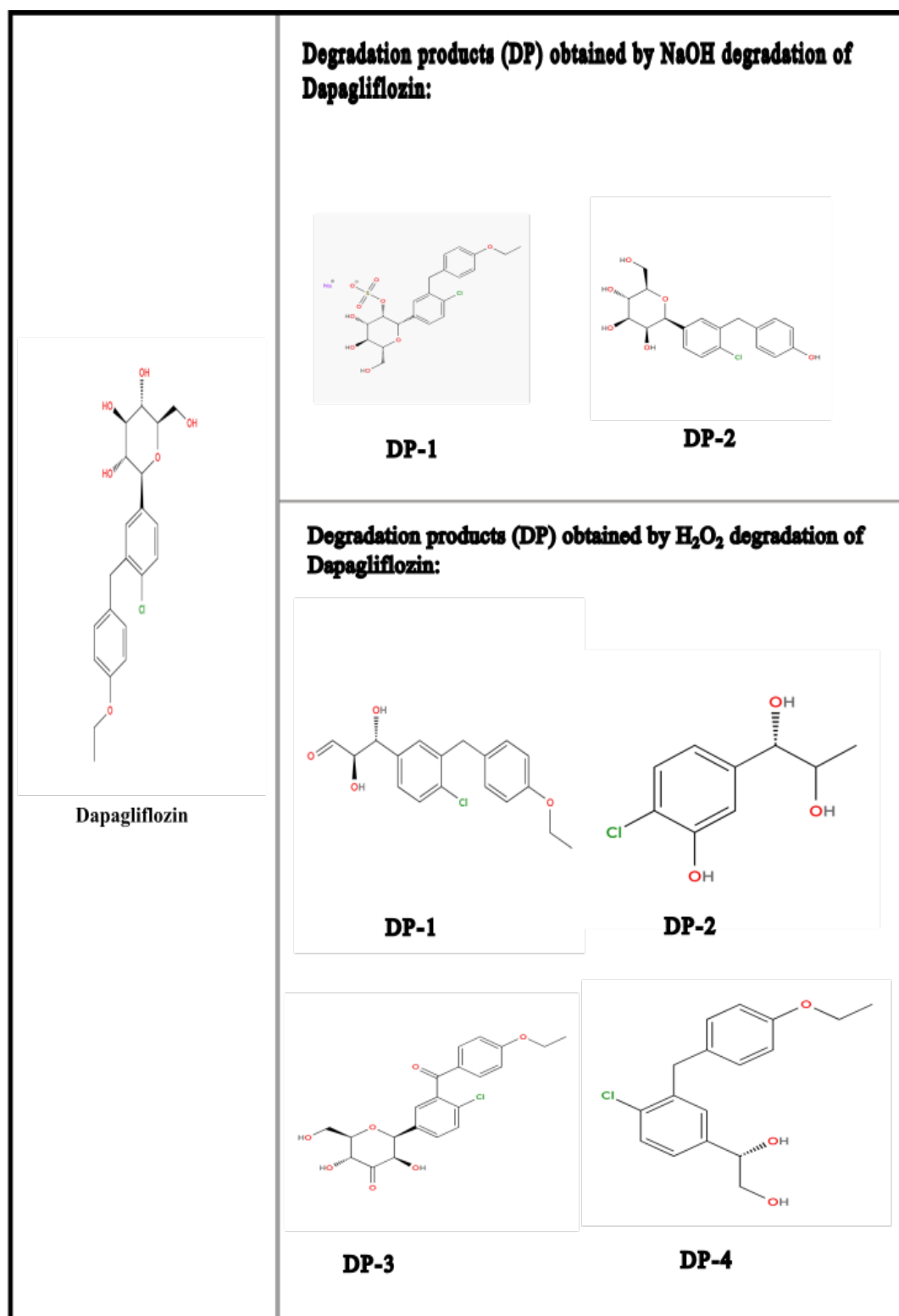


Figure 5: Structures of Degradation Products Obtained by Alkali and Oxidative Degradation of Dapagliflozin.