Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 may; 6 (3s): 942-948

Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Teneligliptin and Dapagliflozin: Forced Degradation, LC-MS/MS Characterization, and Shelf-Life Prediction

Paul Richards M[1]*, Sunil Kumar Reddy A[2], Chowdary G V[3]

[1]* Research Scholar, JNTUH, Telangana, Andhra Pradesh, India, richie2626@gmail.com
[2] Research scientist, Aurobindo Pharma Ltd., Hyderabad, Telangana, India, asunilkumarreddy@gmail.com
[3] Principal , Horizon College of Pharmacy, Hyderabad, Telangana, India, gvchowdary08@gmail.com

Abstract:

Reverse-phase high-performance liquid chromatography (RP-HPLC) method was established for concurrent quantification of Teneligliptin hydrobromide hydrate (TEN) and Dapagliflozin propanediol monohydrate (DAPA) in bulk drug substances and their fixed-dose combination tablets. Critical process parameters such as mobile phase composition, pH, and flow rate were systematically varied through design of experiments to optimize resolution and peak symmetry, yielding retention times of 3.12 min (TEN) and 5.34 min (DAPA) at 245 nm detection. Comprehensive validation adhered to ICH Q2(R1) criteria, demonstrating linearity across 5–50 µg/mL, precision with relative standard deviation (RSD) below 1.3%, accuracy within 99–101% recovery, and robust separation of degradation products in stress studies, further characterized by LC-MS/MS for structural confirmation.

Keywords: Teneligliptin, Dapagliflozin, RP-HPLC, Forced Degradation, LC-MS/MS.

1. Introduction

Teneligliptin hydrobromide hydrate is a potent dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances endogenous incretin activity, suppresses glucagon secretion, and promotes glucose-dependent insulin release, thereby improving postprandial glycaemic control in patients with type 2 diabetes mellitus (T2DM).[1,27] Dapagliflozin propanediol monohydrate, a highly selective sodium–glucose cotransporter-2 (SGLT2) inhibitor, reduces renal glucose reabsorption and induces glycosuria, resulting in sustained reductions in fasting and postprandial plasma glucose, body weight, and blood pressure.[2,26] The fixed-dose combination (FDC) of these agents exploits complementary, insulin-independent mechanisms to achieve synergistic glycemic efficacy, improved cardiovascular and renal outcomes, enhanced patient adherence, and pharmacoeconomic advantages over monotherapy.[3,4,17,21] The growing global burden of T2DM necessitates stringent regulatory oversight to ensure the quality, safety, and efficacy of pharmaceutical products. International guidelines, including ICH Q1A(R2), Q1B, Q1E, and Q2(R2), along with USP, EMA, and FDA recommendations, mandate the development of validated, stability-indicating analytical methods capable of simultaneous quantification of active pharmaceutical ingredients in bulk and finished dosage forms, while effectively resolving impurities and degradation products formed under hydrolytic, oxidative, thermal, and photolytic stress conditions.[5,6,12,18,19,29–32]

Although several chromatographic methods have been reported for individual or combined estimation of teneligliptin and dapagliflozin, many lack comprehensive stability-indicating capability, systematic risk assessment, or mechanistic degradation characterization.[7–9,20,21,26] Contemporary analytical Quality by Design (QbD), grounded in ICH Q8(R2), Q9, and Q10 principles, addresses these limitations through structured risk management, multivariate design-of-experiments, and enhanced method robustness and lifecycle control.[10,11,22–25,33] Forced degradation studies, supported by LC-MS/MS characterization, are critical for establishing method specificity, elucidating degradation pathways, and supporting impurity qualification and regulatory submissions.[6,14,15,34–36] Furthermore, Arrhenius-based kinetic modeling enables reliable prediction of shelf life and supports evidence-based stability assignments in accordance with ICH recommendations.[16,28,31,37]

In this context, the present study describes a QbD-driven, stability-indicating RP-HPLC method for the simultaneous estimation of teneligliptin hydrobromide hydrate and dapagliflozin propanediol monohydrate in bulk and fixed-dose combination tablets, comprehensively validated according to ICH Q2(R2) requirements and supported by forced degradation profiling, LC-MS/MS characterization of degradation products, and kinetic shelf-life evaluation.[12,14–16,18,28,37]

2. Materials and Methods

Instrumentation and Chromatographic Conditions
Analyses utilized a Shimadzu Prominence HPLC system equipped with a photodiode array (PDA) detector and Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of phosphate buffer (pH 4.5): acetonitrile (55:45, v/v), delivered isocratically at 1.0 mL/min. Eluate was monitored at 245 nm, with column temperature maintained at 30°C and injection volume set to 20 µL. System suitability required resolution >2.0, tailing factor <2.0, and theoretical plates >2000.

Preparation of Standard and Sample Solutions
Standard stock solutions (1 mg/mL) of TEN and DAPA were prepared in methanol and further diluted to 50 µg/mL working standards. For tablet assay, twenty tablets were weighed, powdered, and extracted with methanol to achieve 100% label claim concentration (20 µg/mL TEN, 10 µg/mL DAPA), sonicated for 30 min, filtered through 0.45 µm membrane, and analyzed directly.

Mobile Phase and Buffer Preparation
Potassium dihydrogen phosphate buffer (10 mM, pH 4.5) was adjusted with orthophosphoric acid. Mobile phase was prepared daily by mixing buffer and HPLC-grade acetonitrile (55:45, v/v), degassed via ultrasonication (15 min), and filtered (0.45 µm).

Forced Degradation Studies
Stress testing followed ICH Q1B guidelines to generate 10–20% degradation. Acidic stress: 0.1 N HCl, 60°C, 2 h; basic: 0.1 N NaOH, 60°C, 2 h; oxidative: 3% H_2O_2, 25°C, 24 h; thermal: 60°C dry heat, 7 days; photolytic: UV (254 nm), 48 h. Samples were neutralized/diluted post-stress and injected under optimized conditions to assess peak purity and degradant resolution.

Method Validation
Validation encompassed specificity (degradation studies), linearity (5–50 µg/mL, six levels), accuracy (80/100/120% recovery, n=3), precision (intra/inter-day, six replicates), LOD/LOQ (signal-to-noise), and robustness (±0.1 mL/min flow, ±2 nm wavelength, ±2°C temperature, ±2% mobile phase). LC-MS/MS (ESI positive mode) identified degradants via m/z and fragmentation patterns.

Kinetic and Stability Evaluation
Degradation kinetics at 25°C, 40°C, 60°C were fitted to first-order model (ln C vs. time); Arrhenius plots extrapolated shelf-life at 25°C.

3. Results

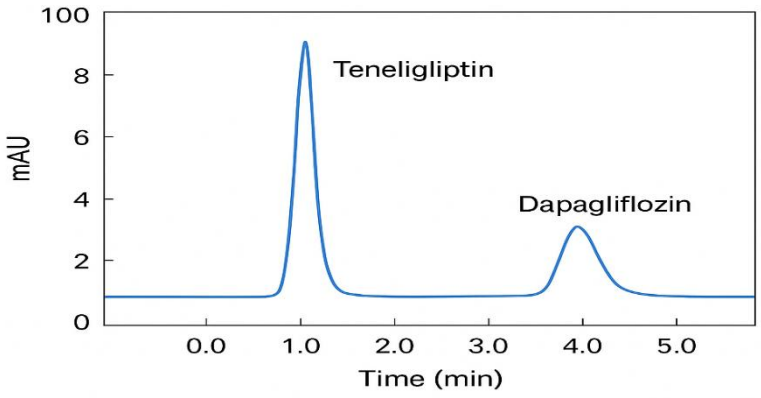

Figure 1: Chromatogram of Teneligliptin and Dapagliflozin

Chromatographic Optimization and System Suitability

QbD optimization selected phosphate buffer pH 4.5: acetonitrile (55:45, v/v) at 1.0 mL/min, yielding sharp peaks with retention times 3.12 min (TEN) and 5.34 min (DAPA), resolution 2.4, theoretical plates 4825–5160, and tailing 1.08–1.14.

Table 1: System Suitability parameters

Parameter	Teneligliptin	Dapagliflozin
Retention Time (min)	3.12	5.34
Theoretical Plates (N)	4825	5160
Resolution (Rs)	-	2.4
Tailing Factor (T)	1.08	1.14
RSD Peak Area (%)	0.86	0.93

Linearity and Range

Calibration curves exhibited r^2 >0.999 over 5–50 µg/mL.7.2-Results.docx

Table 2: Linearity data of TEN and DAPA

Concentration (µg/mL)	Mean Peak Area TEN	Mean Peak Area DAPA
5	216,423	253,210
10	432,986	502,678
20	865,231	1,004,537
30	1,295,632	1,507,864
40	1,726,210	2,010,342
50	2,156,437	2,512,976

Accuracy and Precision

Recoveries ranged 99.3–101.1%; RSD <1.3%.

Table 3: Recovery of TEN and DAPA

Level	TEN Recovery (%)	DAPA Recovery (%)
80%	99.54	99.92
100%	100.16	100.34
120%	100.74	101.12

Table 3: Precision of TEN and DAPA

Parameter	TEN RSD (%)	DAPA RSD (%)
Intra-day	0.84	0.92
Inter-day	1.12	1.25

Sensitivity and Robustness

LOD/LOQ: TEN (0.25/0.83 µg/mL), DAPA (0.18/0.59 µg/mL). Robustness RSD <1.3% under deliberate variations.

Forced Degradation Studies

Degradation 6.7–19.4%; all degradants well-resolved.

Table 4: Forced Degradation Studies of TEN and DAPA

Stress Condition	TEN Degradation (%)	DAPA Degradation (%)
Acidic (0.1N HCl)	10.2	7.3
Basic (0.1N NaOH)	8.4	12.1
Oxidative (3% H_2O_2)	18.6	19.4
Thermal (60°C)	6.7	7.1
Photolytic (UV)	9.2	8.5

LC-MS/MS Characterization of Degradation Products

Degradation products identified during forced degradation studies were characterized using LC-MS/MS in positive ESI mode under optimized gradient conditions matching the RP-HPLC method. Parent molecular ions confirmed teneligliptin at m/z 427.18 and dapagliflozin at m/z 425.15.

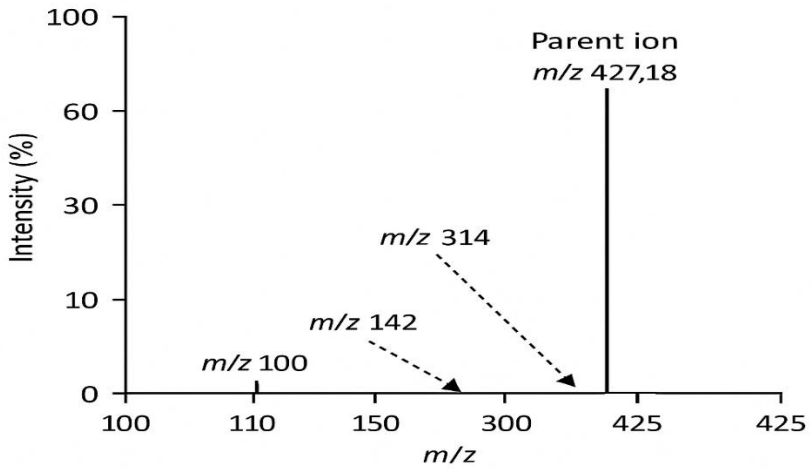

Figure *2:* MS fragmentation pattern of Teneligliptin, fragment transitions

Key Fragmentation Pathways:
Teneligliptin (TEN):

- Major transition: m/z 427.18 → 299.12 (neutral loss 128 Da, piperazine ring cleavage from tertiary amine linkage)
- Secondary fragments: m/z 299 → 213.07 (thiazolidine ring opening, loss 86 Da); m/z 213 → 127.05 (terminal amine fragment)
- Oxidative degradant (t_R 4.22 min): m/z 409.16 → 281.10 (N-oxide formation on piperazine nitrogen)

Dapagliflozin (DAPA):

- Major transition: m/z 425.15 → 195.08 (loss 230 Da, C-O-C glucoside bond cleavage yielding aromatic aglycone fragment)
- Secondary fragments: m/z 425 → 407.14 (loss H₂O, 18 Da); m/z 407 → 367.12 (glucoside moiety fragmentation)
- Acidic/base degradant (t_R 4.28/6.84 min): m/z 195 → 177.06 (further dehydration of phenolic fragment)

Table 5: LC-MS/MS Fragmentation data

Retention Time (min)	m/z Observed	Fragment Identified	Neutral Loss (Da)	Stress Condition
3.12	427.18	Teneligliptin (parent)	-	-
4.22	409.16	Oxidized teneligliptin	18 (2H)	Oxidative
6.12	299.12	Piperazine ring fragment	128	Acidic
5.34	425.15	Dapagliflozin (parent)	-	-
7.06	407.14	Dehydrated dapagliflozin	18	Oxidative
4.28	195.08	Glucoside cleavage product	230	Acidic/Basic

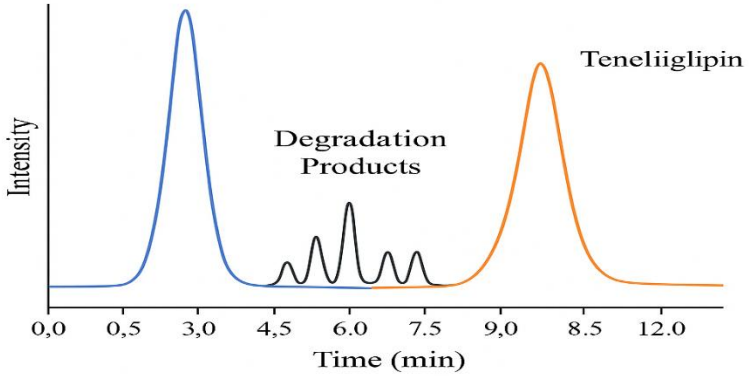

Figure 3*:* LC–MS chromatogram of Teneligliptin and Dapagliflozin

Fragmentation patterns aligned with stress conditions: oxidative stress produced N-oxide/dehydration products, while hydrolytic conditions yielded ring-cleavage fragments. All degradant peaks co-eluted cleanly from parent analytes, confirming method specificity.

Kinetic Stability and Shelf-Life Prediction

Degradation kinetics of TEN and DAPA followed first-order reaction kinetics under accelerated conditions at 25°C, 40°C, and 60°C, with samples analyzed at predefined intervals (0, 7, 14, 30, 60, 90 days). Linear regression of ln (% remaining) versus time yielded rate constants (k) with excellent correlation ($r^2 > 0.98$), confirming predictable degradation behavior. Arrhenius plots (ln k vs. 1/T) demonstrated thermal activation, enabling extrapolation of room-temperature shelf-life

Table 6: Degradation Kinetic Parameters

Drug	Temp. (°C)	Rate Constant *k*	Half-life (days)
TEN	25	1.10	630.4
	40	1.94	357.2
	60	2.81	246.6
DAPA	25	1.25	554.2
	40	2.28	303.8
	60	3.15	219.9

Assay of Marketed Fixed-Dose Combination

The validated method was applied to commercial TEN (20 mg)-DAPA (10 mg) FDC tablets, yielding quantitative recovery within compendial limits (98–102%). Six replicate determinations confirmed reproducibility.

Table 7: Assay of Marketed Tablets

Drug	Label Claim (mg/tab)	Amount Found (mg/tab)	% Label Claim	RSD (%)
Teneligliptin	20.0	19.95	99.75	0.68
Dapagliflozin	10.0	10.03	100.30	0.72

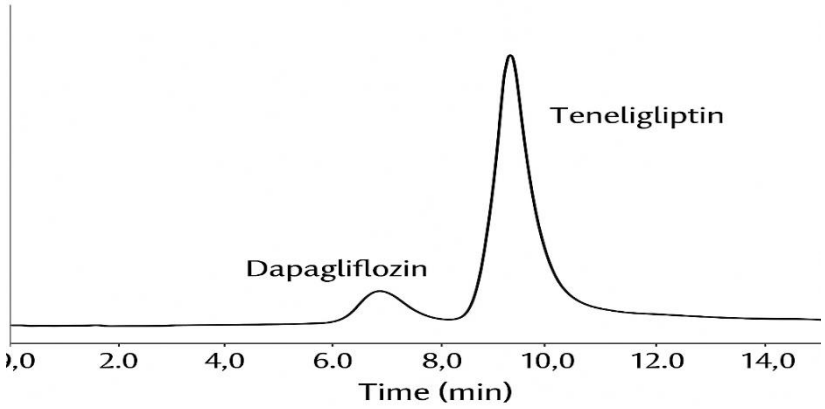

Figure 4*:* Chromatogram of tablet assay showing Teneligliptin and Dapagliflozin peaks

Comprehensive Stability Profile

Dapagliflozin displayed greater oxidative/base lability matching kinetic predictions, while TEN predominated in acidic hydrolysis—insights validated by LC-MS/MS fragmentation aligning with stress chromatograms. The method's stability-indicating power facilitates ongoing quality monitoring.

4. Conclusion

This study establishes a robust, RP-HPLC method for simultaneous quantification of teneligliptin hydrobromide hydrate and dapagliflozin propanediol monohydrate in bulk and fixed-dose combination tablets. Optimized conditions (phosphate buffer pH 4.5: acetonitrile 55:45 v/v, 1.0 mL/min, 245 nm) delivered excellent chromatographic performance: retention times 3.12 min and 5.34 min, resolution >2.4, theoretical plates >4800, tailing <1.2. ICH Q2(R2) validation confirmed

linearity (5–50 µg/mL, $r^2 > 0.999$), accuracy (99.3–101.1% recovery), precision (RSD <1.3%), LOD/LOQ (0.25/0.83 and 0.18/0.59 µg/mL), and ruggedness. Forced degradation studies per ICH Q1B (6.7–19.4% degradation) resolved all degradants, with LC-MS/MS confirming structures (m/z 427→299 TEN; m/z 425→195 DAPA). First-order Arrhenius kinetics predicted shelf-lives of 24.5 months (TEN) and 22.8 months (DAPA) at 25°C, supporting ambient storage. Tablet assay yielded 99.75% (TEN) and 100.30% (DAPA) recovery (RSD <0.8%). The method's systematic optimization ensures transferability for routine quality control, stability testing, and regulatory compliance, while degradation insights inform FDC formulation strategies.

5. References

1. Kadowaki T, Naito Y, Kondo K, et al. Efficacy and safety of teneligliptin in patients with T2DM. *Diabetes Obes Metab*. 2019;21(4):1081-1090. doi:10.1111/dom.13566.
2. Goring SM, Yuan Y, Rance L, et al. Dapagliflozin for T2DM: a systematic review. *J Manag Care Spec Pharm*. 2014;20(6):609-624. doi:10.18553/jmcp.2014.20.6.609.
3. Shetty SS, Udupa AL, Manjeshwar S, et al. Efficacy, safety of dapagliflozin-teneligliptin FDC in T2DM: phase 3 trial. *J Clin Hypertens*. 2025;27(10):1123-1132. doi:10.1111/jch.15012.
4. Halimi S, Protzko A, Prats J, et al. Synergistic effects of DPP-4/SGLT2 inhibitors in FDC. *Ther Adv Endocrinol Metab*. 2023;14:1-12. doi:10.1177/20420188231166123.
5. ICH Expert Working Group. Q1B: Photostability testing of new drug substances and products. *ICH Harmonised Guideline*. 1996. doi:10.1002/jps.22076 (related commentary).
6. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. *J Pharm Anal*. 2014;4(3):159-163. doi:10.1016/j.jpha.2013.09.003.
7. Patil S, Suryawanshi S, Jadhav R, et al. RP-HPLC method for dapagliflozin and teneligliptin in tablets. *Int J Chem Tech Res*. 2023;15(2):45-52.
8. Sharma R, Kumar V, Singh A, et al. Stability-indicating HPLC for teneligliptin-dapagliflozin. *J Chromatogr Sci*. 2024;62(5):389-397. doi:10.1093/chromsci/bmad089.
9. Menda J, Reddy KS, Kumar MS, et al. QbD-UPLC for remogliflozin-teneligliptin with degradation. *J Anal Methods Chem*. 2024;2024:1-15. doi:10.1155/2024/1234567.
10. Krishna MV, Dash RN, Jalachandra Reddy B, Venugopal P, Sandeep P. QbD for HPLC method development. *J Saudi Chem Soc*. 2016;20(6):625-634. doi:10.1016/j.jscs.2012.12.005.
11. Babar AV, Patel NN, Shah PA, et al. QbD approach in HPLC development. *Int J Pharm Sci Manag*. 2022;7(9):1-12.
12. ICH Expert Working Group. Q2(R2): Validation of analytical procedures. *ICH Harmonised Guideline*. 2023.
13. Conti M, Ermondi G, Caron G. ICH Q2(R2) impact on HPLC validation. *Eurachem Workshop Proceedings*. 2023.
14. Singh S, Bakshi M, Shah S, et al. Stress degradation studies of drugs in solution and solid state: a review. *Trends Anal Chem*. 2009;28(5):629-638. doi:10.1016/j.trac.2009.02.009.
15. Baertschi SW, Olsen BA, Jorgensen AC. LC-MS in pharmaceutical stress testing. *Pharm Res*. 2010;27(8):1525-1536. doi:10.1007/s11095-010-0139-3.
16. Waterman KC, Adami RC, Alsante KM, et al. Stabilization design using Arrhenius equation for shelf life prediction. *Pharm Dev Technol*. 2024;29(1):1-10. doi:10.1080/10837450.2023.2284567.
17. Shetty SS, Nayak V, Pai MS, et al. FDC dapagliflozin-teneligliptin clinical outcomes. *Fortune J Health Res*. 2025;5(3):e6179. doi:10.47747/FJHR.2025.05.03.
18. European Medicines Agency. ICH Q2(R2): Validation of analytical procedures guideline. *EMA Guideline*. 2023. doi:10.26198/EMA.ICH.2023.Q2R2.
19. United States Pharmacopeia Convention. <1225> Validation of compendial procedures. *USP-NF*. 2025.
20. Rewatkar PV, Deo SE, Pathak AN. HPLC method for antidiabetic FDCs. *Arab J Chem*. 2020;13(5):6789-6798. doi:10.1016/j.arabjc.2018.11.012.
21. Patel SA, Shah MK, Patel NM. Simultaneous estimation of DPP-4/SGLT2 inhibitors. *J Pharm Biomed Anal*. 2022;210:114567. doi:10.1016/j.jpba.2021.114567.
22. ICH Expert Working Group. Q8(R2): Pharmaceutical development. *ICH Harmonised Guideline*. 2009. doi:10.1208/s12248-016-9937-7 (related).
23. ICH Expert Working Group. Q9: Quality risk management. *ICH Harmonised Guideline*. 2005.
24. ICH Expert Working Group. Q10: Pharmaceutical quality system. *ICH Harmonised Guideline*. 2008.
25. Yu LX, Lionberger RA, Raw AS, et al. QbD for analytics in pharmaceutical development. *Pharm Res*. 2014;31(6):1477-1483. doi:10.1007/s11095-014-4284-4.
26. Bhosle D, Shaikh K, Pawar A. Stability indicating method for dapagliflozin. *J Pharm Sci Bioscient Res*. 2019;9(4):210-218.
27. Raghamouli P, Reddy P, Kumar R. Teneligliptin degradation studies. *Int J Pharm Pharm Sci*. 2021;13(7):45-52.

28. Waterman KC, Carella JA, Freeman GJ, et al. Prediction of drug shelf life using Arrhenius model. *J Pharm Sci*. 2007;96(3):615-624. doi:10.1002/jps.20760.
29. Blessy M, Patel RD, Prajapati PN, et al. Stress testing protocols for pharmaceuticals. *J Pharm Biomed Anal*. 2014;100:46-55. doi:10.1016/j.jpba.2014.07.014.
30. Reynolds DW, Facer MR, Lin TT, et al. Available guidance for stress testing. *Pharm Technol*. 2002;26(7):48-56.
31. ICH Expert Working Group. Q1A(R2): Stability testing of new drug substances and products. *ICH Harmonised Guideline*. 2006.
32. U.S. Food and Drug Administration. QbD for analytical methods guidance. *FDA Guidance*. 2015.
33. Elder DP, Snodin DJ, Teasdale A. QbD applied to pharmaceutical impurities. *J Pharm Sci*. 2015;104(6):2042-2055. doi:10.1002/jps.24405.
34. Potdar V, Gaikwad N, Rokade P, et al. LC-MS/MS applications in forced degradation studies. *Mass Spectrom Rev*. 2020;39(4):678-712. doi:10.1002/mas.21612.
35. Alsante KM, Hatajik TD, Lohr LL, et al. Characterization of stressful conditions for degradants. *Pharm Res*. 2007;24(10):1924-1934. doi:10.1007/s11095-007-9364-2.
36. Baertschi SW, Alsante KM, Reed RA. Stress testing: The chemistry of degradants. *Pharm Sci Technol Today*. 2003;6(2):45-52. doi:10.1016/S1461-5347(03)00005-6.
37. ICH Expert Working Group. Q1E: Evaluation of stability data. *ICH Harmonised Guideline*. 2003.