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FORCED DEGRADATION STUDIES OF EMPAGLIFLOZIN UNDER ICH STRESS CONDITIONS

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Abstract

Forced degradation studies show how a drug substance breaks down under hydrolytic, oxidative, thermal and photolytic stress, and they underpin the development of stability-indicating methods. This narrative review sets out the regulatory basis for such studies under ICH guidance, the chemistry of empagliflozin that makes some stress routes more likely than others, and the experimental design, chromatographic and mass spectrometric tools used to separate and identify degradants. It also considers how stress results feed into specifications, validation and shelf-life decisions. The review is based on published guidance, reviews and research papers and reports no new experimental data. The main limitations are inconsistent stress conditions between laboratories and incomplete mass balance.

1. INTRODUCTION

Empagliflozin is a selective inhibitor of sodium-glucose co-transporter 2 in the renal proximal tubule, and it lowers blood glucose by increasing urinary glucose excretion independently of insulin [1, 2]. After trials showed reductions in cardiovascular death, in hospitalisation for heart failure and in the progression of kidney disease in people with type 2 diabetes and established cardiovascular disease, its use widened considerably [3, 4]. It is dispensed as low-dose film-coated tablets, taken once daily for years [5]. A product with that profile has to stay intact on the shelf, and the pharmaceutical scientist needs to know

how it will behave if it does not. Real-world exposure is long, and tablets sit in pharmacies, homes and handbags through hot summers and humid monsoons, so the question of what happens to the molecule over time is far from abstract [6, 7]. Clinical benefit is only as good as the dose that actually reaches the patient, and a degraded product delivers less of it.

Forced degradation, also called stress testing, answers part of that question. The drug substance, and later the drug product, is deliberately exposed to conditions harsher than those of storage, such as strong acid and alkali, oxidising agents, heat, humidity and light, so that degradation occurs quickly enough to study [8, 9]. The purpose is not to estimate shelf life. It is to find out which degradation pathways are possible, to generate samples that contain the likely degradants, and to prove that the analytical method can separate the parent compound from them [10, 11]. The approach has a long history in pharmaceutical analysis, and the early recommendations of Singh and Bakshi are still widely quoted as a practical starting point, with later reviews and industry consortia refining the details [10, 12, 13]. Stress testing is now treated as a core scientific activity of development, not a regulatory formality, because it shapes decisions on formulation, packaging and analytical control long before the first long-term stability sample is pulled.

ICH stability guidance requires stress testing as part of the stability package for a new substance, though it gives little detail about how to carry it out [8, 14]. As a result, practice varies. This review describes the regulatory basis, the structural features of empagliflozin that influence its likely degradation, the design of each stress condition, the analytical strategy for resolving and identifying degradants, and the use of the results in validation and specification setting. A summary of the main points is given in table 1. The article is based on published guidance, reviews and research papers and reports no experimental data of its own. Throughout, the terms degradation product and degradant are used interchangeably for compounds that arise by chemical change of the drug substance, and distinguished from process impurities that carry over from synthesis [15, 16].

Table 1: Stress conditions and analytical checks discussed for forced degradation of empagliflozin

Topic|focus	Key point from the published guidance and literature	Ref.
Regulatory basis ICH stability guidance	Stress testing required to establish degradation pathways and demonstrate method specificity.	[8, 10]
Impurity thresholds ICH Q3B(R2)	Reporting, identification and qualification thresholds depend on maximum daily dose.	[15, 16]
Mutagenic impurities ICH M7	Structure-based assessment of degradants for DNA-reactive potential.	[17]
Acid and base hydrolysis 0.1 M starting point	Neutralise before analysis; C-glycosidic bond expected to resist mild hydrolysis.	[1, 9, 18]
Oxidation peroxide and radical initiators	Benzylic methylene is a plausible site; peroxide and azo initiators may give different products.	[19, 20]
Thermal and humidity solid state	Arrhenius extrapolation approximate; excipient interactions may appear only in mixtures.	[21, 22, 23]
Photolysis ICH Q1B	Minimum visible and ultraviolet exposure with dark control; light source details debated.	[14, 24, 25]
RP-HPLC with PDA specificity	Gradient separation of parent from degradants; peak purity supports specificity.	[11, 26, 27]
Mass balance response factors	Assay plus degradants should approach 100 per cent; response factors may differ.	[10, 13, 28]
LC-MS and NMR structure elucidation	Accurate mass and fragmentation locate the site of change; NMR gives definitive structure.	[28, 29]

2. CHEMISTRY AND LIKELY LIABILITIES

Empagliflozin is a C-glucoside, meaning that the glucose ring is attached to the aromatic core through a carbon-carbon bond and not through the oxygen link found in natural O-glycosides [1, 30]. This difference is the

key to the drug's design, as the C-glycosidic bond resists the glucosidases that would cleave an O-glycoside, and it also resists simple acid hydrolysis far better than an O-glycoside does. Other features include a chlorinated diarylmethane skeleton, an aryl ether linking the phenyl ring to a tetrahydrofuran ring, and four free hydroxyl groups on the glucitol moiety [1, 2]. That robustness helps explain why the class tolerates oral dosing, and it also frames the expectations for stress testing: a molecule built to survive the gut and the liver will not necessarily crumble in the laboratory. Reasonably forcing conditions are therefore needed before anything happens, which makes the choice of severity a real scientific judgement [1, 18].

From such a structure a formulation scientist can predict where trouble may arise. Aryl ethers are generally robust but can be cleaved under forcing acidic conditions. Benzylic carbons, in this case the methylene bridge between the two rings, are classic sites for radical autoxidation and for attack by peroxides [19, 20]. The multiple hydroxyl groups can take part in esterification with acidic excipients or trace impurities such as formic acid in polyethylene glycols, and in dehydration under thermal stress [21, 22]. None of these predictions replaces experiment, but they guide the choice of conditions and the interpretation of unknown peaks. Chemists sometimes use a simple exercise to organise these ideas: list each functional group, ask which stress is known to attack it, and then check whether the experimental data show the expected product. Where the data and the prediction disagree, the surprise is often the most useful result of the entire study [19, 28].

Physical form also matters. The solid state may influence chemical stability through water content, crystallinity and the way molecules pack, and studies of solid-state reactivity show that amorphous or poorly ordered regions often degrade faster than the crystalline bulk [22, 23]. For a low-dose tablet, the excipients far outweigh the active ingredient by mass, so compatibility studies with fillers, binders and lubricants form part of the picture [21]. Regulatory assessment documents describe the commercial product and the relevant quality attributes, including the tests used to control degradation products at release and during shelf life [5]. Surface area and particle size can also change reactivity, as can the manufacturing process, since milling, granulation and compression introduce local heat, moisture and mechanical stress [21, 23]. These factors are one reason why the same active substance can show different stability in two different formulations.

Since empagliflozin is intended for long-term use in a population that is often older and takes several medicines, impurity control carries some weight. Degradation products that exceed identification or qualification thresholds need structural characterisation and a safety justification, and any with a structural alert for mutagenicity are assessed under a separate framework [15, 16, 17]. Establishing early which degradants form under what conditions allows the developer to design the formulation, the pack and the specification in a rational way, rather than discovering a new peak in the final stability study. A short statement on safety belongs here. Most degradants will be present at low levels and with structures close to the parent, which suggests similar pharmacology and toxicology, yet this cannot be assumed without assessment [15, 16]. Packaging selection later builds on the same information, and a pack with a low moisture vapour transmission rate can compensate for modest hydrolytic sensitivity [6, 7].

3. DESIGNING THE STRESS CONDITIONS

The aim of stress is to produce meaningful but not excessive degradation. Most authors recommend targeting roughly 5 to 20 per cent loss of the parent compound, which is enough to generate detectable degradants without driving secondary and tertiary reactions that would never occur in real storage [9, 10, 13]. Pushing degradation to 50 per cent or more tends to create artefacts. Conversely, if no degradation is seen after reasonable exposure, the substance can be declared stable under that stress and the experiment stopped, a point made in the guidance and in several reviews [8, 31]. The range is a convention and not a

law. Some groups prefer to describe the extent of stress by the conditions used and report the loss that resulted, which at least makes the experiment reproducible [9, 13]. What matters is that the extent of degradation is stated, since a method shown to resolve peaks at 2 per cent degradation says little about its behaviour at 15 per cent.

Hydrolysis is normally tested in acid, alkali and neutral solution. A common starting point is 0.1 M hydrochloric acid and 0.1 M sodium hydroxide, at room temperature first and then at elevated temperature if little happens, with samples withdrawn at intervals and neutralised before analysis [9, 13, 18]. Solubility of the drug in the medium is a practical issue, and a cosolvent such as methanol or acetonitrile is often added, although the organic solvent can itself take part in reactions such as methanolysis. For a drug with a robust C-glycosidic bond, conditions at the harsher end of the range may be needed to see any change [1, 18]. Time and temperature are adjusted stepwise. A sensible plan starts mild, sampling after a few hours, and moves to more forcing conditions only if the molecule survives, so that over-degradation is avoided [18, 31]. Neutral hydrolysis in water, which is seldom emphasised, deserves a place because the real tablet meets moisture long before it meets a strong acid or base.

Oxidative stress usually employs hydrogen peroxide at concentrations from 0.1 to 3 per cent, but peroxide mimics only one oxidation mechanism, the nucleophilic or electrophilic attack of a peroxy species. Radical autoxidation is better modelled by azo initiators such as azobisisobutyronitrile, and a few laboratories now run both, because they can give different products from the same molecule [19, 20]. Metal-catalysed oxidation can be examined by adding trace iron or copper. A drug with a benzylic methylene group makes this work particularly relevant, since radical pathways attack such positions readily [19, 20]. Oxidation is difficult to control experimentally because the reaction can continue after sampling unless the oxidant is quenched or diluted away. Some laboratories add catalase or sodium bisulphite to stop the reaction at the chosen time, although the quenching agent can interfere with detection and must be checked in the blank [19, 26].

Thermal stress is applied to the solid at temperatures above the accelerated storage condition, for example 60 to 80 °C, with and without added humidity, as well as to solutions. Results are interpreted through the Arrhenius relationship, although the extrapolation from stress to storage temperature is only approximate and fails when the mechanism changes with temperature [8, 23]. High-humidity exposure, such as 75 per cent relative humidity or more, reveals hydrolysis in the solid state and the role of absorbed water. In the presence of excipients, the Maillard-type interactions or esterification can appear only at this stage, which is why drug-excipient mixtures are often stressed alongside the pure substance [21, 22]. Kinetic modelling is sometimes used to extend these data. Where degradation has been followed at several temperatures, the rate constants can be fitted to give an activation energy, which allows a first estimate of behaviour at storage conditions and provides a useful consistency check on the mechanism [6, 23].

Solid-state and solution stress answer different questions and are best read together. In solution, molecules collide freely and reaction rates are governed mainly by pH, temperature and the concentration of reagents, which makes the results easy to model but less like the tablet in the pack. In the solid, reactions depend on contact between particles, moisture sorbed on surfaces and the mobility of molecules in disordered regions, so degradation is often slow and uneven [22, 23]. A thorough programme therefore stresses both, and treats disagreement between them as information about the mechanism rather than as an inconsistency to be explained away [12, 32]. Combined with real-time data, the two stress modes help decide which test results can safely be dropped from routine monitoring [8, 33].

Photostability follows ICH Q1B, which calls for exposure to a minimum of 1.2 million lux hours of visible light and 200 watt hours per square metre of near-ultraviolet energy, with

a dark control to separate light effects from heat [14]. Critics point out that the guideline sets exposure levels but is less clear about the light source, the sample presentation and what to do when only the drug substance, and not the final pack, is examined [24, 25]. A molecule with a chlorinated aromatic ring may undergo dehalogenation under ultraviolet light, which is a reason for including the test even if the product is later packed in an opaque container [24]. Practical points matter too. Samples should be protected from stray light during preparation unless light is the variable under study, and the dose of near-ultraviolet energy should be measured with a calibrated actinometer or radiometer rather than assumed from the lamp rating [14, 24].

Controls are as important as the stressed samples. Each experiment should include an unstressed sample, a blank containing only the stressor, and a placebo when the product is examined, so that peaks arising from reagents, solvents or excipients are not mistaken for degradants [9, 10, 26]. Neutralisation and dilution must be done consistently, and the time between stopping the reaction and injecting the sample should be recorded, as some degradants are themselves unstable. These details are tedious but explain many of the disagreements between laboratories reporting stress studies of the same compound [13, 31]. Where laboratory notebooks record these details, differences between sites can often be traced to small procedural variations and corrected without repeating the entire study [9, 12].

4. ANALYTICAL STRATEGY

A stability-indicating method is one that measures the active ingredient accurately in the presence of its degradants, impurities and excipients, and that can detect changes in the quantity of each over time [11, 34]. Reversed-phase liquid chromatography with ultraviolet detection is the standard approach for a drug such as empagliflozin, which has aromatic chromophores. Column chemistry, mobile-phase pH, organic modifier and gradient shape are tuned until the parent peak is resolved from the degradant peaks seen in the stressed samples [27, 35]. Gradient elution is usual, because degradants can differ widely in polarity. The word stability-indicating is sometimes applied loosely to any method that has been run on a stressed sample, which is not enough. The method must show that the assay value falls when the drug degrades and that no degradant peak hides under the parent [11, 26]. Method developers therefore treat the stressed mixtures as the central test samples rather than as an afterthought.

Resolution alone does not show that a peak is pure. Photodiode array detection allows peak purity to be assessed by comparing spectra across the peak, and a purity angle below the threshold is taken as support for a single component, though co-eluting compounds with similar spectra can slip through [11, 26]. Orthogonal checks add confidence, for instance repeating the separation on a column with different selectivity or at a different pH, or using mass spectrometry to confirm that the main peak carries a single mass [27, 29]. These steps are part of the argument that the method is specific. Detection wavelength is a further choice that deserves care. A wavelength near the absorption maximum of the parent gives the best sensitivity for the drug, but degradants that have lost the chromophore, for instance through ring opening, may then be invisible, which is another way mass balance can fail [13, 27].

Choice of the chromatographic system deserves a few words. Octadecylsilane columns remain the usual first choice, with phosphate or formate buffers and acetonitrile or methanol as the organic modifier. Volatile buffers such as formate or acetate are preferred when the method will later be coupled to a mass spectrometer, since phosphate salts foul the ion source [27, 35]. Many laboratories now screen several columns and pH values automatically and select the best separation from the stressed mixtures, which saves time and reduces the risk that a degradant is missed because of a single unlucky set of conditions [26, 35]. Selectivity changes caused by pH are especially useful, because ionisable degradants shift their retention while neutral ones stay put [27, 35].

Mass balance is a related check. The sum of the assay value and the levels of the

degradants should come close to 100 per cent of the initial content, and a shortfall suggests that some degradants are not eluting, are not detected at the wavelength used, or have been lost as volatile compounds [10, 13]. Response factors of degradants often differ from that of the parent, so area percentages can mislead. Where reference standards of the degradants are unavailable, relative response factors are estimated by isolating the compound or by using a universal detector, such as charged aerosol or mass spectrometric detection, as a cross-check [13, 28]. Practical limits apply to detection too. Very small degradants, below the reporting threshold, rarely affect conclusions, but when thresholds are strict because of a high daily dose they matter more, and the method then needs adequate sensitivity at the reporting level [15, 16].

Validation follows ICH Q2(R1), which lists specificity, linearity, range, accuracy, precision, detection and quantitation limits, and robustness as the characteristics to be examined, and practical guides show how each is tested on a chromatographic method [34]. For a stability-indicating assay, specificity is demonstrated with the stressed samples themselves. Regulators such as the FDA expect the same, and describe in their guidance how method validation data should be presented [36]. The distribution of reviewed topics in table 1 is shown in fig. 1. The methods literature agrees that the stressed samples should be retained and re-injected during validation and transfer to show that the method continues to perform [26, 37]. Robustness testing, in which pH, temperature, flow rate and organic content are deliberately varied, shows whether critical peak pairs stay resolved when the method is moved to another instrument or analyst [36, 37]. Annotated chromatograms of each stressed sample, filed with the validation report, make later method transfers considerably smoother [26, 37].

Newer chromatographic formats are changing practice. Ultra-high-performance columns with sub-two-micron particles shorten run times and improve resolution, allowing screening of many stressed samples in a day. Their higher pressures and lower dispersion tolerance require adapted instruments, but the principle of resolving all degradants is unchanged, and methods can be transferred between formats using established scaling rules [27, 35]. Supercritical fluid chromatography and two-dimensional liquid chromatography are used in some laboratories as orthogonal checks, mainly where peak purity is in doubt. Such tools are most valuable in laboratories that run many projects, where a single fast platform can serve several programmes [26, 27].

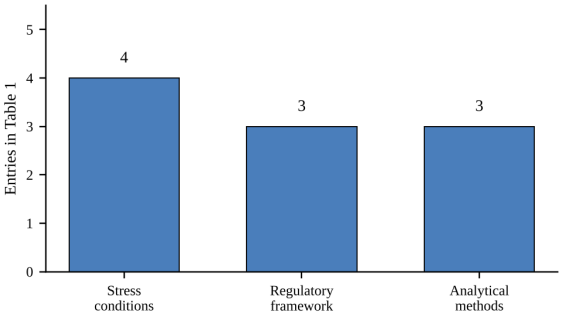


Fig. 1: Number of entries in table 1 by topic group

5. IDENTIFYING AND REPORTING DEGRADANTS

After separation comes identification. Liquid chromatography coupled to high-resolution mass spectrometry gives accurate masses of the degradants and, with tandem fragmentation, information on their structure; comparing the fragmentation of the parent and the degradant localises the site of modification [28, 29]. Typical findings in studies of this kind include masses that differ from the parent by +16 (oxidation), −18 (dehydration) or by loss of a side chain. When structures are uncertain, isolation by preparative chromatography and nuclear magnetic resonance spectroscopy provide a definitive answer, but this demands time and material [28, 29]. Interpretation is helped by knowledge of common transformation types. Oxidation commonly adds oxygen or removes hydrogen, hydrolysis adds water, and thermal stress can remove it, so that a few mass differences cover a large share of observed products [28, 29]. A list of such differences is a handy first aid when a new peak appears.

Not every peak needs full characterisation. ICH Q3B(R2) sets reporting, identification and qualification thresholds based on

maximum daily dose, so that degradants appearing in real stability studies above the thresholds must be identified and justified [15, 16]. Degradants seen only under extreme stress are generally not controlled in specifications, but they remain valuable in method development and in understanding the molecule. Distinguishing the two groups prevents the specification from being cluttered with impurities that never occur in practice, a point that is often misunderstood [9, 38]. Another practical rule concerns timing. Degradants that appear only at late time points under mild conditions are of more concern than those that form quickly under severe stress and then vanish, since the former may reach the thresholds during shelf life [33, 38].

Reference materials pose their own difficulty. Degradants are rarely commercially available at the time they are first seen, and quantifying them against the parent compound can mislead if their response differs. Synthesising or isolating each one is costly, so teams usually reserve this effort for the degradants that exceed identification thresholds or that raise a safety question [15, 16, 29]. Until then, relative retention times and relative response factors serve as working tools, and their limits should be stated alongside the numbers so that readers do not over-interpret small differences between early and late samples [13, 28]. A poorly characterised degradant that is nevertheless carried through years of testing becomes a recurring burden for analysts and reviewers alike [29, 38].

Mutagenic potential deserves its own consideration. ICH M7 describes how to assess impurities, including degradation products, for DNA-reactive potential using structure-based computational methods and, if needed, bacterial mutagenicity testing [17]. Forced degradation helps by showing which degradants could form, so that the in silico assessment can be done for real structures in place of speculation. Degradants that are formed in practical storage conditions receive the most attention. Those formed only under severe stress, and not under long-term conditions, are usually not subjected to the full evaluation [17, 38]. Where in silico tools flag a structural alert, bacterial mutagenicity assays are done on the isolated or synthesised compound, and the acceptable intake is derived from the toxicological data. Quite often the alert is not confirmed, which lets the team manage the degradant as an ordinary impurity [17].

Findings from stress studies also feed into packaging and storage statements. If a drug proves sensitive to light, the pack is chosen accordingly. If it is vulnerable to moisture, a desiccant or a low-permeability blister may be justified. Real-time and accelerated stability data collected under ICH storage conditions then confirm that the control strategy works, with shelf life derived from the statistical evaluation described in ICH Q1E [8, 33]. In this sense, forced degradation is an early warning system for the later and longer studies. Records are the final piece. Results should be collected in a way that links each stress condition, sample and chromatogram to the conclusion, which makes later regulatory queries easier to answer and avoids repeating work [8, 36]. Pharmacopoeial monographs, where they exist, add public specifications and test methods that the stress study can be compared against, though they are updated more slowly than the research literature [36, 37].

Software has become a routine partner in this work. Programs that predict likely degradation pathways from a structure, or that compare spectra and retention behaviour across stress conditions, speed up the first pass at assignments. They remain suggestions, and the final structural proposal still rests on the analyst's judgement and on the fragmentation data. Even so, a structured record of predicted and observed degradants is a useful teaching tool for junior scientists who are learning to think about reactivity [28, 29]. Used well, they free time for the harder task of deciding what each result means for the product.

6. LIMITATIONS AND FUTURE DIRECTIONS

Several weaknesses affect the forced degradation literature, empagliflozin included. Stress conditions are not standardised, so a study using 1 M acid at reflux and another using 0.1 M acid at room

temperature may reach opposite conclusions about the same compound [10, 31]. Reports frequently omit details such as extent of degradation, mass balance, peak purity or the identity of degradants, which prevents comparison. Reviews of the field repeatedly call for fuller reporting and for consistent stress levels [9, 13, 32]. Different brands and formulations of the same drug complicate matters further, since generic and innovator products may use different excipients and so produce different degradation profiles under identical stress [21, 22]. Clear reporting standards would address much of this at little cost.

A second limitation is relevance. Harsh stress can generate degradants that never form under storage, and conversely a degradant that forms slowly in the solid state, perhaps through interaction with an excipient, may not appear in solution experiments at all [21, 23]. Stress testing of the drug substance alone also misses formulation effects. For this reason, stressing the product and the substance in parallel, and comparing both with real-time data once available, gives a more reliable picture [8, 32]. The literature on empagliflozin products specifically is smaller than that on the molecule itself. Because most published work comes from academic and quality-control laboratories, data from industry development files are largely unavailable, which limits how far the literature can be generalised [6, 7].

Regulatory expectations are also moving. Regulators and industry have been discussing a more lifecycle-oriented view of analytical procedures, in which method knowledge gained during forced degradation is kept and used when changes are made later [34, 36, 37]. In such a view, stress studies are not a single event at the start of development but a body of knowledge that is revisited when a new supplier, site or pack is introduced. The practical consequence is better documentation, which costs little at the time and saves effort later. Training analysts to document unexpected observations in the same way as expected ones would also help future readers of the literature. Reviews of the field suggest that this shift is already visible in how well-run laboratories organise their records [32, 37].

Future work could improve this field in several ways. Wider use of orthogonal detection, mass balance reporting and stress protocols agreed across laboratories would make published studies easier to compare [13, 32]. Predictive tools based on chemical reactivity and kinetics may reduce the experimental burden, as ideas from kinetic treatments of drug stability become more accessible [23]. Finally, greater transparency about stressed-sample chromatograms, including unassigned peaks, would let other groups build on the work and would support regulators and quality teams who need confidence that methods remain suitable throughout the product lifecycle [34, 37].

7. CONCLUSION

Forced degradation of empagliflozin is best seen as a structured piece of chemical reasoning supported by experiment. The molecule's C-glucoside core, aryl ether and benzylic methylene point to oxidation, strong acid and light as the most probable routes, while the stress design, controls and analytical checks described in ICH guidance and in methodological reviews decide whether the evidence is trustworthy [1, 8, 19]. A method that separates the drug from every degradant seen under properly graded stress, and that passes peak purity and mass balance checks, can reasonably be called stability-indicating [10, 11]. None of this replaces measurement, but it explains why different laboratories, working carefully, tend to see similar patterns.

The weaker points are consistency and relevance. Stress levels differ between laboratories, reports often leave out the data that would let others judge them, and severe conditions can produce degradants of no practical importance [13, 31, 32]. Better reporting, parallel stressing of drug substance and product, and orthogonal identification would improve usefulness, and connecting stress results to real-time stability data keeps the programme honest [8, 33, 36]. Readers should consult product-specific stability reports for quantitative findings, as this review has not tried to reproduce them. Collaboration between academic groups,

regulators and industry on agreed protocols seems the most realistic route to progress.

8. ABBREVIATIONS

ICH-International Council for Harmonisation, SGLT2-Sodium-glucose co-transporter 2, HPLC-High-performance liquid chromatography, RP-HPLC-Reversed-phase HPLC, PDA-Photodiode array, LC-MS-Liquid chromatography-mass spectrometry, NMR-Nuclear magnetic resonance, EMA-European Medicines Agency, FDA-Food and Drug Administration, API-Active pharmaceutical ingredient

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Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

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