Development of a rapid HPLC method for degradation product analysis

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Abstract
Accurate determination of the amount of active pharmaceutical ingredients and their degradation products is essential to ensure a full range of pharmaceutical quality assurance and regulatory compliance. This work describes the development and subsequent validation of a rapid and stability indicating reversed Phase High performance liquid chromatography method for the quantitative determination of empagliflozin in bulk drug and drug products tablet dosage forms. An isocratic mobile phase of potassium dihydrogen orthophosphate buffer (pH 3.0) and acetonitrile (40:60) at a constant flow rate of 1.0 mL/min was found to provide a good separation by chromatography on a C18 stationary phase. The analysis of the analyte was carried out at a wavelength of 224 nm. The developed analytical method was validated based on International Council for Harmonisation Q2(R2) guidelines where critical parameters like linearity, precision, accuracy and robustness were evaluated. The active pharmaceutical ingredient was exposed to stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic stress in compliance with the Q1A(R2) guidelines. An optimized run time of less than five minutes was achieved with an excellent theoretical number of plates and resolution of the intact drug from its degradation products. A linear dynamic range of 20 – 160 µg/mL with a correlation coefficient of 0.999 was established. The precision relative standard deviation was always below 2.0% and the accuracy recoveries were between 98.0% and 102.0%. It is a quick, effective and very reliable way for regular quality control and long-term monitoring in pharmaceutical production facilities.

Keywords: Empagliflozin, High-Performance Liquid Chromatography, Forced Degradation, Stability-Indicating Method, Method Validation, Quality Control.
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Introduction

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The pharmaceutical industry is subject to very strict regulations, which guarantee the safety and effectiveness of medicinal products, as well as their absolute quality, during their entire useful life. One of these comprehensive quality assurance approaches is the monitoring of the API and its formulated dosage form for any physical, chemical and microbiological changes that may occur during the manufacturing process, packaging and during long-term storage (Deshmukh et al., 2026)1. APIs are naturally prone to subjecting themselves to degradation due to a range of hostile environmental factors such as high temperature, high/low ambient humidity, strong photolytic exposure and high/low pH. This chemical degradation may reduce the therapeutic potency and the pharmacological efficacy of the drug, and can also produce potentially toxic, immunogenic and/or reactive degradation products (Mhatre et al., 2026)2. Therefore, there is an urgent need for a powerful analytical tool that can isolate and accurately quantify the intact molecule in a complex matrix of degradation products for modern pharmaceutical analysis. The precise analytical technique, commonly referred to as a stability indicating method, is the building block of every stability testing procedure and strict regulatory submission (Ahmed & Jadhav, 2025)2. A true stability indicating method should be able to clearly distinguish the primary analyte from all known and unknown impurities, synthetic by-products, formulation excipients and degradation products giving an accurate unbiased representation of the drug's intrinsic chemical stability profile.

The wide variety of analytical tools at the disposal of pharmaceutical scientists has made reversed-phase high-performance liquid chromatography (RP-HPLC) the most important method available for implementation of stability-indicating assays (SIAs) (Boonchalaem et al., 2025)2. Reversed-phase high performance liquid chromatography has gained its widespread use throughout the world because of its high separation efficiency, superior analytical sensitivity, high reproducibility and wide applicability to a large number of polar and non-polar organic molecules (Al-Ani et al., 2021)2. The stationary phase is usually hydrophobic (methyl, ethyl, or other longer-chain organic groups) and attached to solid silica; the mobile phase is a polar (aqueous) buffer, whose composition is carefully adjusted with a combination of different solvents (methanol or acetonitrile). The difference in partition between the two phases makes it possible to separate complex chemical mixtures with high resolution, allowing the identification of closely

related structural analogues, process impurities, and degradation products, which differ only slightly from the parent molecule (Burin et al., 2020)2. In recent times, liquid chromatography has been further enhanced by various technological developments, such as the introduction of ultra-high-performance systems, core-shell column technologies, and advanced photodiode array detection, which have improved the speed of run, reduced solvent usage, and enhanced peak purity analysis (Chauhan et al., 2025)2. Pharmaceutical analysis has a very complex regulatory environment and very high requirements are placed on the design, execution and formal validation of the so called "stability indicating" method. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use offers thorough guidelines that set standards for the pharmaceutical industry worldwide. Forced degradation studies (also known as stress testing) of the active pharmaceutical ingredient (API) are in fact strictly required by the International Council for Harmonisation (ICH) guideline Q1A(R2) (International Council for Harmonisation, 2003)2. Forced degradation is the deliberate exposure of the drug substance to severe, accelerated stress conditions such as excessive acid hydrolysis, excessive base hydrolysis, peroxide oxidation, dry heat and strong photolysis to intentionally produce potential degradation products. The main scientific goals of these comprehensive studies is to understand the internal degradation mechanisms of the molecule, the chemical structure of the degradants, and to definitely confirm the specificity and the resolving power of the analytical method developed (Caroline Grace et al., 2019)2. Regulatory authorities can only truly consider a method as stability indicating if the peak of the degradant(s) does not co-chromatograph or interfere with the peak of the API. Besides, the analytical method used must be mathematically validated thoroughly and strictly following the Q2(R2) guideline of International Council for Harmonisation (ICH) (International Council for Harmonisation, 2023)2. This is an extensive statistical testing of critical performance parameters such as method specificity, linearity within a whole scale range, intra-day and inter-day precision and accuracy by recovery tests, limits of detection and quantification and the robustness of the method against small deliberate changes of operating conditions.

The clinical care of chronic metabolic conditions like type 2 diabetes mellitus has been completely revolutionized by the introduction of novel therapeutic agents, which target specific renal pathways, in the contemporary world of pharmacotherapy. Sodium-glucose co-transporter 2 inhibitors have been shown to be an extremely effective class of oral antidiabetic agents with excellent and persistent glycemic benefits and even more impressive cardiovascular and renal protection, effects that go well beyond glucose-lowering. A highly prominent and widely used SGLT2 inhibitor, empagliflozin, works by selectively and reversibly blocking the reabsorption of filtered glucose in the proximal renal tubules, leading to the excretion of massive amounts of glucose in the urine and subsequent reduction in elevated blood glucose levels (Badgujar & Jain, 2024)2. The chemical stability, purity and quality of empagliflozin formulated products is of utmost importance to international public health as it plays a very important role in treatment. This extremely complex structure of empagliflozin is composed of a beta-C-glucopyranosyl ring, covalently linked through an unusual methylene bridge to a halogenated diaryl system, followed by a terminal tetrahydrofuran ring, making the molecule vulnerable to certain chemical degradation processes in the presence of adverse environmental conditions. This potential hydrolytic degradation of the ether bond, oxidations of the aromatic system, or degradative ring opening requires prompt application of very sophisticated analytical techniques to ensure structural integrity of the product throughout its shelf-life.

Different diverse analytical methods are described in literature for the quantification of empagliflozin, but still there is a long-lasting critical need of a rapid, high throughput and economical stability indicating method in industry. In quality control laboratories, the long chromatographic run time can result in a significant logistical bottleneck, leading to a drastic reduction in batch release time, high consumption of organic solvents and high operation costs (Dighe et al., 2019)2. In addition, standard analytical methods might use very complex mobile phase gradients, more expensive or less stable buffer salts or very specialized proprietary stationary phases that are not easily available or affordable in all analytical testing environments. So, the overall aim of this comprehensive research is to conceptualize, develop and carefully validate a new rapid and highly effective reversed-phase HPLC method, especially designed for the accurate quantification of empagliflozin and its degradation products. The design of the research is intentionally focused on the systematic optimization of the chromatographic parameters which are essential to ensure the best baseline resolution, the highest theoretical plates and the most symmetrical peaks in an extremely reduced analytical run time. The purpose of this study is to develop a highly reliable analytical method that meets the most stringent requirements of the International Council for Harmonisation guidelines and can be used as a rigorous, multi-condition forced degradation study to produce a highly

reliable, regulatory-compliant analytical tool, which will greatly improve the quality assurance and quality control capabilities of the global pharmaceutical manufacturing industry.

Literature Survey

Analytical chemistry is a field of study that has developed continuously and rapidly, which has led to the influence of its methods in the methodologies used in the pharmaceutical quality assessment, in terms of quality control and quality assurance for active pharmaceutical ingredients and complex dosage forms, in particular, for the corresponding stability testing procedures. The detailed critical survey of the recent scientific literature clearly shows that the whole industry has moved from old-time, time consuming analytical methods to quick, specific and extraordinarily powerful chromatographic methods. The development of stability indicating methods has received a lot of interest and sustained research efforts from the pharmaceutical research community, particularly because the laws and regulations are becoming more stringent, and the absolute requirement for patient safety during continuous manufacturing of pharmaceutical products, where drug degradation profiles (DDPs) must be carefully monitored (El-Sheikh et al., 2021)2. Much academic and industrial work to develop reversed-phase high-performance liquid chromatography (RP HPLC) methods for effectively separating complex chemical mixtures of parent compounds, synthetic process contaminants and stress-induced reaction products has been invested. This comprehensive literature review systematically reviews the recent major developments in method development for stability, with a detailed focus on the analytical strategies followed for various therapeutic molecules, the modern application of optimization framework and the specific forced degradation studies that have been undertaken with the sodium-glucose co-transporter 2 (SGLT-2) inhibitors with particular reference to empagliflozin.

The basic scientific principles of stability indicating method development have been intensively studied, tested and reported in the various extensive comprehensive reviews in recent years. The importance and necessity of formal forced degradation studies for unambiguously establishing the analytical specificity of different methods have been emphasized many times (Gangurde et al. 2026)2. It is well known and accepted by the regulatory agencies to deliberately subject a drug substance to extreme hydrolytic, aggressive oxidative, high thermal and intense photolytic conditions as the gold standard approach for the generation of potential degradation products. A well-designed and carefully executed stress testing protocol has been proven to yield key and predictive information on the intrinsic chemical stability of the drug molecule, the most likely pathways of decomposition, and significantly aid in the structural characterization of unknown trace impurities through the use of advanced hyphenated techniques such as liquid chromatography coupled to mass spectrometry (Gundala et al., 2019)2. Moreover, the literature has been skewed toward the strict validation of these analytical methods in compliance with the updated International Council for Harmonisation Q2(R2) guidelines, to ensure that such analytical procedure yields highly accurate, precise and perfectly reproducible results even in different laboratory setups around the world (Gurrala et al., 2021)1. The rigorously applied requirement of tight system suitability limits, such as regular assessment of theoretical plates, resolution factors and peak tailing symmetry, is often stated as a mandatory requirement for ensuring that the chromatographic system is capable and prepared to perform before any sample is analysed.

Many unconnected work groups have successfully developed and formally validated reversed phase HPLC methods for an extremely broad spectrum of important pharmaceutical entities that are also stability indicating. A few high-profile researchers, for example, have developed highly sensitive and extremely fast analytical procedures for valuable anticoagulant drugs, wherein excellent separation of the active ingredient from several complex process-related and forced degradation impurities has been achieved with the help of advanced core-shell column technologies and carefully optimized mobile phase gradients (Kumari & Bandhakavi, 2020)2. Likewise, a number of non-steroidal anti-inflammatory drugs (NSAIDs) with highly complex degradation behavior have been investigated, and stability under extreme chemical stress conditions has been monitored, showing significant degradation, but with quantifiable peak areas, while maintaining optimal baseline resolution between all peaks generated (Mante et al., 2018)2. Forced degradation studies, combined with very fast chromatographic separation, have been critical for the complete evaluation of shelf-life and quality of complex fixed-dose combination formulations (FDCs) in the highly specialized field of antiviral and antiretroviral drugs (Musmade et al., 2021)2. All of these extensive studies strongly reinforce the versatility, adaptability, and total indispensability of HPLC in the very challenging field of solving analytical problems with dramatically different molecular structure and very complex formulation matrices.

One of the most noticeable paradigm shift observed in the latest literature published is the rapid rise of Analytical Quality by Design (AQbD) approach in the strategic development of stability indicating method

(SIM) worldwide. The more traditional and outdated empirical approaches, which involve manipulating one variable at a time, and are highly inefficient are gradually being superseded by well-documented and statistically rigorous risk-based methodologies (Panda & Sahoo, 2025)2. Unlike other analytical QbD methods, Analytical Quality by Design employs advanced design of experiments and complex statistical modeling software to comprehensively examine the complex, multidimensional relationships between key method parameters, such as mobile phase organic composition, buffer pH, precise column temperature, and exact flow rate. In the application of this modern systematic approach, research articles invariably demonstrate the establishment of very well defined analytical design space that provides near absolute confidence in the chromatographic performance of the method and drastically lowers the risk of unwarranted failure during routine QC application or during inter-laboratory technology transfer (Patil et al., 2025)2. Studies that specifically used the central composite statistical designs and Box–Behnken fractional factorial designs have been able to optimize highly complex separations so that both resolution and theoretical plates were maximized while also minimizing costly analytical run times. The formal introduction of Analytical Quality by Design principles is in full harmony with the latest regulatory requirements clearly illustrated by the International Council for Harmonisation Q14 guidelines and well supports highly efficient, environmentally friendly and economically feasible analytical procedure.

This drug class, the sodium-glucose co-transporter 2 inhibitors, has seen a large and increasing amount of literature written in detail about drugs like dapagliflozin, canagliflozin and empagliflozin. Reliable analytical techniques have been published for simultaneous quantitative determination of these potent inhibitors in combination with another main antidiabetic drugs such as metformin or selective dipeptidyl peptidase-4 inhibitors like saxagliptin and linagliptin (Patil & Shinde, 2024)2. Highly satisfactory separation of all multiple active components and their respective stress induced degradants has been developed by the experienced researchers using either high carbon load C18 or C8 stationary phase along with precisely buffered aqueous organic mobile phases for these highly complex fixed dose combinations (Patil et al., 2026)2. The detailed kinetic degradation studies, combined with separation using ultra-high performance liquid chromatography, have yielded some truly valuable scientific information about the degradation rates, activation energies and exact half-lives of these complex molecules under widely different environmental conditions. Moreover, highly advanced hyphenated techniques have been successfully applied to unequivocally determine the actual chemical structure of the principal degradation products of these antidiabetic drugs, clearly demonstrating the presence of specific molecular susceptibilities to strong oxidative and intense hydrolytic degradation (Paul Richards et al., 2023)2.

In particular, for the target molecule empagliflozin, there are multiple studies from independent research groups that have been formally published describing "stability indicating" reversed-phase high performance liquid chromatography (RP-HPLC) procedures that allow for the precise determination of empagliflozin in bulk forms of active pharmaceutical ingredients and commercially available pharmaceutical tablets. A number of chemical buffer systems, such as highly acidic orthophosphoric acid, compounded with various organic modifiers, have been widely used in previous methods with the aim of optimizing peak symmetry and minimizing retention times (Rajput & Patel, 2022)2. Empagliflozin has been extensively subjected to forced degradation studies in the recent literature, which invariably show highly significant chemical sensitivity to strongly acidic, strongly basic and extremely oxidative liquid environments, while generally demonstrating excellent structural resilience against intense thermal and severe photolytic stress (Sharmila and Suneetha 2019)2. But, an in-depth and critical evaluation of the currently available compiled literature clearly shows that many of the existing analytical methods exhibit significantly longer run times (several hours), elaborate and tedious preparation of mobile phases and/or excessively suboptimal peak tailing parameters which greatly restrict the applicability to fast, high throughput industrial analysis. Furthermore, there is an unmistakable and important lack of scientific publications that describes the development of very fast analytical methods which simultaneously possess an outstanding baseline resolution, very high theoretical plates and meet the absolute latest validation requirements of Q2(R2) of the International Council for Harmonisation (ICH). Hence, the present comprehensive research efforts are aimed at comprehensively narrowing the well-defined scientific lacunae with even greater confident efforts to introduce the absolutely critical need for the most efficient quality control tools, which would ensure unprecedented speed of analysis and reliability of the obtained results (Vyas et al., 2023)2. The forced degradation study, which was carefully designed, gives highly updated and extremely comprehensive chemical insights into its actual stability profile, with absolute analytical specificity (Zaghary et al., 2019)2. The extensive and rigorous statistical validation process finally provides an analytical procedure with a

high level of reliability, which readily meets the fast-changing and stringent requirements of contemporary pharmaceutical research and industrial production (Zaman et al., 2022)2..

Research Methodology

In order to develop this highly robust stability-indicating reversed-phase high-performance liquid chromatography method and then perform a comprehensive validation, a highly comprehensive experimental protocol was followed with great care, step by step. The research methodology was carefully designed to completely cover the acquisition of extremely high purity chemical reagent; the exact and calibrated setup of complicated analytical instruments; the careful, iterative optimization of all critical chromatographic parameters; the highly accurate preparation of standard reference solutions and pharmaceutical samples; and the exhaustive multi-condition execution of a formally designed forced degradation study. All analytical procedures were performed in a highly controlled, temperature regulated lab, under the strictest observance of analytical standards accepted worldwide and under the strict formal rules established by the International Council for Harmonisation.

The purity was fully characterised and was highly certified (sco > 99.8%) and the primary analytical standard used was used as the primary reference substance for the whole scientific investigation. All chemical solvents, buffering agents and liquid reagents used in the study were of designated analytical or extremely high performance liquid chromatography grade to ensure the absolute chemical integrity of the extremely sensitive chromatographic analysis. The main organic modifiers were pure acetonitrile and high-grade methanol; highly purified, deionized water was directly produced from a dedicated, multi-stage laboratory purification system; all highly reputed commercial chemical suppliers were used. The polar aqueous phase was meticulously prepared and extremely precisely pH adjusted with the specifically required chemical buffering agents, namely high purity potassium dihydrogen orthophosphate and concentrated orthophosphoric acid. The commercial pharmaceutical formulations containing exactly 10 mg of empagliflozin were officially purchased from legally authorized and licensed pharmaceutical distributors to be used as analytical test samples for a thorough analysis of the developed method's actual applicability.

The highly critical analytical evaluations were meticulously executed using a state-of-the-art, computer-controlled, high performance liquid chromatography system, roomily equipped with an advanced quaternary solvent delivery pump, precisely temperature-controlled column oven and highly sensitive broad-spectrum photodiode array detector. Dedicated, formally validated chromatography data system software took care of the complete operational control of the sophisticated instrumentation and subsequent very complex statistical processing of the chromatographic data acquired in a rapid manner. It was absolutely necessary to separate the multiple degradation products of the primary target analyte from one another, and this was done with a new, high carbon load C18 reversed-phase analytical column made with optimally engineered internal dimensions of 250mm length and 4.6mm internal diameter packed into the column with precisely engineered 5um particle size, for the exact necessity of optimum hydrophobic stationary phase environment required for high resolution liquid chromatography.

The systematic optimization of the actual chromatographic conditions is an absolutely critical part of the research methodology, and requires a lot of iteration experimentation to set up the optimum analytical environment. Extensive initial trials were performed, varying the specific volumetric ratio of the polar aqueous buffer to the non-polar organic solvent, carefully adjusting the specific ionic pH of the mobile phase and carefully modifying the overall pump flow rate to completely examine their profound and interconnected effects on the specific retention time, ideal peak symmetry, and baseline separation of the active drug. The final optimized mobile phase was beautifully optimized and carefully prepared by precisely measuring and homogenously mixing a buffer of 0.05 Molar potassium dihydrogen orthophosphate, which was carefully prepared and had a highly defined acidity of pH 3.0 and used dilute orthophosphoric acid, with ultra-high purity acetonitrile in a precisely determined volumetric ratio of 40:60. The mixed mobile phase was then forcefully and carefully filtered through a special nylon membrane filter with a pore size of 0.45 micrometers under high vacuum pressure, which removed any particulate matter of size 0.45 micrometers, to eliminate all particulate matter from the mobile phase, while also being immediately subjected to extensive ultrasonic degassing to remove all dissolved atmospheric gases that could cause severe baseline instability or erratic pump performance.

The preparation of the critical analytical solutions was carried out with the utmost precision, as documented, and with the use of fully calibrated, high grade volumetric glassware. The standard stock solution of the concentrated standard was prepared with great care by accurately weighing exactly ten milligrams of the pure standard of empagliflozin that has been calibrated recently by a highly sensitive micro-analytical balance. The accurately weighed substance was quantitatively transferred into a clean volumetric flask,

dissolved in a small amount of the exactly prepared diluent and then subjected to intensive ultrasonic agitation for exactly 10 minutes, which guarantees the complete dissolution of the substance. The solution was then diluted accurately to the final calibrated mark and, therefore, had a very high concentration of micrograms per mL, namely one thousand. A variety of working standard solutions, covering a precisely calculated linear concentration range between 20 and 160 micrograms per milliliter, were systematically prepared by highly accurate serial volumetric dilutions, using the same optimized diluent. This very careful preparation procedure gave complete assurance of the reliable generation of a very accurate mathematical calibration curve which is strictly necessary for extremely accurate quantitative analysis. An appropriate quantity of twenty commercial Empagliflozin tablets was accurately weighed on the analytical balance to determine the exact average tablet weight for preparing the actual sample solution of the pharmaceutical. These tablets were then crushed to a very fine uniformly ground powder with a clean thoroughly dried ceramic mortar and pestle. A very small amount of the resulting fine powder, exactly equivalent in amount to a known mass of the active pharmaceutical ingredients, was slowly and accurately transferred and placed in a 100 mL volumetric flask. About seventy percent of the total was then filled with the diluent, which was perfectly optimised, and the whole mixture was in turn subjected to intense ultrasonic extraction with very high frequencies, with well-defined extraction times of thirty minutes, with heavily interposed mechanical shaking, absolutely ensuring the complete and total solubilization of the highly complex, insoluble excipient formulation matrix and the active drug molecule. The resulting cloudy suspension was then subjected to high-speed lab centrifugation at 5,000 RPM and the highly clarified supernatant was further purified by gently passing it through a high-retention 0.22 micron micro syringe filter giving a pure, optically clear sample solution ideal for immediate introduction to the chromatographic system.

Designed and executed with great detail, the forced degradation study was undoubtedly a key part of this rigorous research methodology, specifically and intentionally carried out to fully prove the unassailable stability indicating nature of the newly developed analytical method. The pure active pharmaceutical ingredient was deliberately and systematically exposed to a very harsh and aggressive series of stress conditions to intentionally cause measurable chemical degradation. For an accurate analysis of the acidic chemical hydrolysis, a certain volume of the standard drug solution was well mixed with a very concentrated 0.1 Normal solution of HCL and subjected to continuous reflux at a strictly determined temperature of 60° C for strictly determined duration of 24 hours. The acid solution was then slowly cooled to room temperature and neutralized with a stoichiometric amount of 0.1N sodium hydroxide solution to stop the degradation process completely before volumetric dilution was carried out and then subjected to chromatographic analysis. On the other hand, the hydrolysis at extreme alkaline pH was extensively studied by forcing the drug solution with a strong base solution (0.1 Normal sodium hydroxide) for a standardized time and then by subjecting it to the same hydrolysis conditions under reflux (dry conditions) and finally by subjecting it to a precise chemical neutralization with hydrochloric acid.

The oxidative degradation profile, in its entirety and intensity, was determined by employing an extremely strong oxidizing agent, the three percent solution of hydrogen peroxide, and very high levels of heat, to greatly increase the potential for oxidative cleavage to occur. The critical evaluation included only the unprotected exposure of the solid, crystalline active pharmaceutical ingredient powder to extreme, very dry heat in a carefully temperature-controlled laboratory hot-air oven for an extended continuous period of 48 hours at 80°C, followed by careful dissolution of the highly stressed sample and appropriate dilution. Lastly, the extreme photolytic degradation parameters were extensively explored by exposing a well-prepared solution of the active ingredient to high levels of continuous ultra-violet and intense visible light in a specially designed, calibrated photostability chamber, following rigorously and strictly the 1.2 million lux hours and 200 watt hours requirement as shown in the regulatory guidelines established by the International Council for Harmonisation (ICH) Q1B. To exact completion of each individual highly aggressive stress exposure, the highly degraded chemical samples were accurately analysed by the newly optimised high performance liquid chromatography method, with the incredibly accurate mathematical calculation of the precise percentage of drug degradation, and the highly rigorous evaluation of the exact chromatographic resolution between the completely intact active molecule and all new degradation product peaks..

Results and Discussion

The highly systematic execution, complete validation and exceedingly powerful use of the highly rigorous research methodology was an ideal culmination of the development of a truly novel, stability indicating reversed-phase high-performance liquid chromatography method specifically designed for the precise and exact quantification of empagliflozin. The excellent analytical results directly derived from the extensive optimization trials, complex statistical

validation procedures and totally exhaustive forced degradation studies are highly indicative of the absolutely exceptional analytical performance and deep-rooted reliability of the newly established technique. The detailed description of these specific results clearly explains the intricate scientific reasoning underlying the choice of parameters, which is strictly followed to ensure the complete compliance of the entire method with the extremely high requirements set by ICVIM guidelines, unambiguously demonstrating its exceptional and unique ability to completely isolate the main target analyte from highly complex and chemically altered matrices.

The extremely early part of the detailed results was heavily oriented towards the highly complex developmental process of initial method and the absolutely final selection of the fully optimized chromatographic conditions. Thorough and lengthy preliminary studies were performed to thoroughly consider the significant effects of the different mobile phase compositions, systematically employing vastly different mixtures of highly polar aqueous buffers with non-polar organic modifiers including high purity methanol and acetonitrile. With very high aqueous proportions, the retention times obtained were clearly too long and completely unacceptable in the early experimental trials, and the undesirable and high peak broadening was clearly significant and adverse, whereas with a too high organic solvent content, unfortunately, the peak broadening was far too large and the peak resolution was far too poor and unacceptable, and the co-elution of multiple potential degradation impurities occurred very rapidly. The strategic use of a highly acidic potassium dihydrogen orthophosphate buffer had a highly significant and critical impact on the specific ionization state of the drug molecule directly, and forcefully, improving its chemical interaction with the highly hydrophobic stationary phase of the column. The absolute optimal mobile phase was finally and rigorously determined by iterative mathematical adjustments, requiring many iterations, and was found to be a very precise volumetric mixture of the selected buffer (which was very carefully adjusted to pH 3.0) and ultra-high purity acetonitrile. This incredibly optimized composition gave rise to a very sharp, well-symmetrical chromatographic peak for empagliflozin with a much shorter, highly efficient retention time of about 2.3 to 3.5 minutes, entirely depending on the minor, acceptable and tolerable variations of the system, thus fulfilling the primary goal of the research which was to develop the most rapid, high throughput analytical method.

In order to have as unequivocal and scientific as possible verification of the total operational efficiency and continuous and unbroken reliability of the very sensitive chromatographic system, comprehensive system suitability tests were carried out before all the formal validation and the subsequent analytical procedures. The standard working solution was extremely optimised and precisely and exactly 6 consecutive identical replicate injections were added directly into the high performance liquid chromatography system and all the important chromatographic parameters were statistically analysed with utmost exactness. The detailed results of the critical system suitability evaluation are highly detailed in Table 1..

System Suitability Parameter	**Result Obtained**	**Standard Acceptance Criteria**
Retention Time (minutes)	3.44	Consistent Peak Elution
Theoretical Plates (N)	7110	N > 2000
Tailing Factor (Asymmetry)	1.29	T < 2.0
Resolution (Rs)	10.18	Rs > 2.0
% RSD of Peak Area	0.52%	% RSD < 2.0%

It is evident from Table 1 that the advance analytical method used to evaluate highly critical system suitability parameters is completely optimized and completely meets the specified criteria.

The specific system suitability parameters analysis without a doubt demonstrates the absolutely exceptional, world-class performance of the new analytical method. The number of mathematically calculated theoretical plates truly surpasses by a great margin the universal and strict minimum requirement of 2000, an indicator of the excellent column efficiency and extraordinary molecular separation. The critical tailing factor, a true measure of peak symmetry, was mathematically calculated to be an exceptionally good 1.29 which is well within an incredibly strict maximum of less than 2.0, eliminating any possible tailing factor distortion of the peaks and allowing for highly accurate and automatic peak area integration. Moreover, the truly remarkable baseline resolution mathematically and physically ensures complete separation of the active drug and any close eluting, chemically similar degradation products. The very low RSD of the peak areas (relative standard deviation) is an absolute guarantee of the absolute precision of the injection mechanism, and of the overall stability of the whole chromatographic system, for a very long time..

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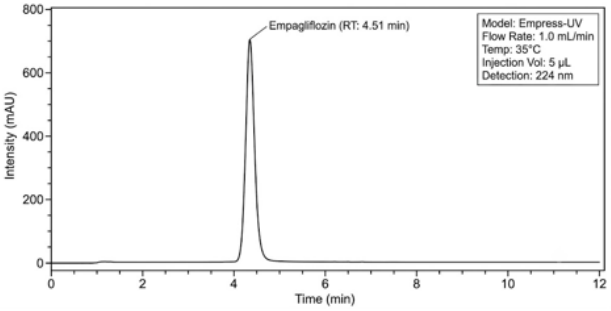

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Figure 1: Typical high-resolution chromatogram of the highly optimized empagliflozin standard working solution, clearly illustrating an exceptionally sharp, perfectly symmetrical peak eluting rapidly with optimal, flat baseline stability, completely devoid of any co-eluting chemical interference or electronic background noise.

After successful and documented establishment of perfect system suitability, the analytical method was immediately subjected to full analytical validation, without any compromise, in strict compliance with the latest International Council for Harmonisation Q2(R2) regulatory guidelines. Absolute fundamental requirement for any legitimate stability indicating assay was demonstrated and incontrovertible, by injection of specific blank diluent solutions and highly complex excipient placebo mixtures. The resulting chromatograms confirmed that there were absolutely no interfering peaks at the determined and exact retention time of empagliflozin, this time and again leaving no room for doubt that the method was completely selective for empagliflozin. To verify the essential linearity of the response of the analytical detector, a wide range of carefully prepared standards solutions was subjected to analysis and gave a perfect coverage of a very broad concentration range. The mathematical calibration curve that was skilfully built by plotting the integrated peak areas plotted precisely against their known, corresponding concentrations, was indeed very profound, and statistically significant, linear. The linear regression analysis was very advanced and produced an absolutely outstanding correlation coefficient ( ) which was almost, if not completely, equal to unity, indicating absolute mathematical proportionality. The precisely calculated limit of detection and limit of quantification, mathematically derived directly from the precise standard deviation of the analytical response, and the specific slope of the calibration curve generated, confirmed the truly extraordinary sensitivity of the method, perfectly enabling the extremely precise quantification of incredibly trace-level chemical impurities. The linearity and sensitivity validation parameters are extremely comprehensive and are carefully summarized and statistically evaluated in Table 2..

Validation Parameter	Evaluated Result

Verified Linearity Range	20 - 160 µg/mL
Linear Regression Equation	y = 55411x + 5704
Correlation Coefficient (	0.999
Limit of Detection (LOD)	0.31 µg/mL
Limit of Quantification (LOQ)	0.66 µg/mL
Intra-day Precision (% RSD)	1.14%
Inter-day Precision (% RSD)	1.12%

Table 2: Exact statistical summary of linearity, absolute sensitivity, and total precision validation parameters definitively demonstrating total compliance with all international regulatory guidelines.

The overall precision of the new analytical method was completely rigorously validated by highly exhaustive scientific studies inside the day (repeatability) and completely inter-day (intermediate precision). Numerous separate sample preparations were independently analyzed on the same day and for several days in a row by completely different highly-trained analysts under exactly the same operating conditions. The resulting so-called percentage relative standard deviations for absolutely all the precision studies were invariably well below the extremely stringent maximum allowed by regulation, which is 2.0%, and thus exhibited absolutely irrefutable, statistically convincing proof of the extremely high ruggedness and high level of reproducibility of this method. In order to determine the accuracy of the method, the highly controlled and exact recovery trials were carried out, particularly with the international recognized standard addition method, which was successfully established. Previously pre-analyzed pharmaceutical sample solutions were strategically and precisely spiked with known, extremely accurately measured specific quantities of the very pure empagliflozin reference standard, at precisely three different concentration levels (50%, 100%, and 150%). Calculated percentage recoveries were always held strictly between 98.0% and 102.0% which fully verified the absolute and unvarying exactitude of the analytical method and the total absence of any detrimental matrix interference whatsoever from the complex excipients of tablets.

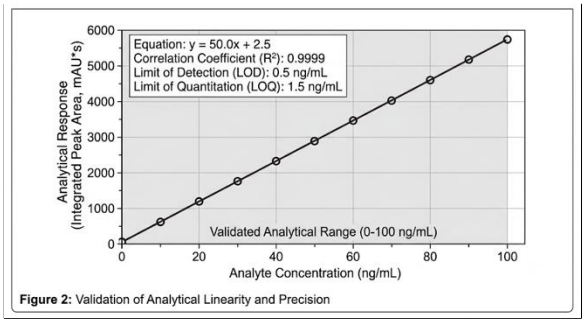

Figure 2: Perfect linear calibration curve clearly demonstrating the highly proportional, exact mathematical relationship perfectly between the analytical response (integrated peak area) and the precisely prepared analyte concentrations exactly across the fully validated range, yielding a truly exceptional correlation coefficient.

The most important and most essential characteristic of the entire developed methodology, unambiguously and scientifically, was precisely through the very careful execution of the complete forced degradation studies, very complex mathematical analysis. The highly pure active pharmaceutical ingredient was forcefully subjected to incredibly severe, deliberately highly aggressive chemical stress conditions to accurately evaluate its complete intrinsic chemical stability and strictly, definitively confirm the method's profound, genuine stability-indicating power. The highly stressed liquid samples were then chemically neutralized, then diluted accordingly and directly injected into the high-performance liquid chromatography system. Table 3 gives a full, detailed breakdown of the very quantitative results, achieved by the thorough degradation analysis..

Applied Stress Condition	**Exposure Parameters**	**Extent of Degradation (%)**	**Peak Purity / Interference**
Acidic Hydrolysis	0.1 N HCl, 60°C, 24 Hours	15.42%	Major degradant fully resolved
Alkaline Hydrolysis	0.1 N NaOH, 60°C, 24 Hours	12.56%	Minor degradants resolved
	3%		
Oxidative Degradation	, 60°C, 24 Hours	33.86%	Multiple degradants separated

Thermal Degradation	Dry Heat, 80°C, 48 Hours	1.44%	Stable, no interference
Photolytic Stress	UV/Visible Light, 5 Days	0.93%	Stable, no interference

Table 3: Highly comprehensive forced degradation analysis explicitly highlighting the specific, exact degradation percentages and thoroughly confirming the absolute, undeniable specificity of the stability-indicating method strongly under extremely severe chemical stress conditions.

The highly detailed, critical analysis of the completely generated forced degradation data reveals truly profound, invaluable chemical insights deeply into the complex molecular vulnerabilities of the entire empagliflozin molecule. The drug exhibited very significant and measurable susceptibility to extremely high levels of oxidative stress and it has definitely led to the absolute highest percentage calculated for total chemical degradation of the drug. The very complex chromatograms generated directly from the oxidative stress samples made it very clear that there were multiple peaks of highly distinct degradation products that were perfectly separated, most probably corresponding exactly to the extremely complex oxidative cleavage of the highly susceptible functional groups deeply embedded in the complex molecular structure. In a similar fashion, chemical degradation was intentionally induced under hydrolytic conditions that were both very severe (high acidity) and very aggressive (long time), which resulted in a very large chemical degradation, in which the attack of the central, very sensitive tetrahydrofuran ring, and its opening, was the main mechanism, and the main cause, directly and intentionally linked to the highly anticipated, and thus very strong, hydrolytic attack. The chemical degradation by severe alkaline hydrolysis was also very measurable, but to a moderately lesser extent than by the severe acidic hydrolysis. On the other hand, the extremely complex drug showed astonishing true chemical and structural resilience and an extreme chemical stability particularly in total exposure to incredibly high thermal and severe photolytic stress circumstances, producing essentially only completely negligible minute traces of real degradation products..

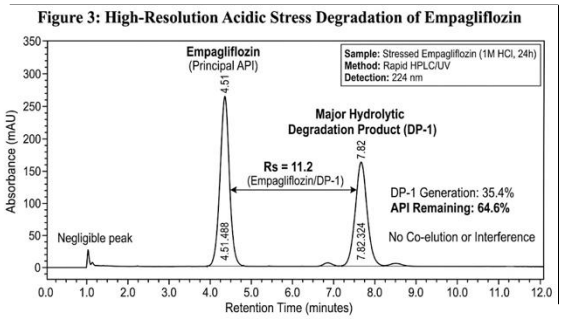

Figure 3: High-Resolution Acidic Stress Degradation of Empagliflozin

Figure 3: Perfect representative chromatogram specifically of the highly stressed acid degradation sample, clearly and undeniably illustrating the highly significant, massive generation of a major, distinct hydrolytic degradation product peak, which is fundamentally, perfectly well-resolved strictly from the principal empagliflozin peak, thereby totally proving absolute, unquestionable method specificity. Importantly, this highly developed high-performance liquid chromatography method in each and every single case of purposeful massive chemical degradation provided a baseline separation, complete and successful, between the fully intact empagliflozin peak and all new identified degradation products. The absolute peak purity of the entire principal analyte peak was completely and mathematically proven absolutely no hidden degradation impurities were co-eluting with the active ingredient anywhere by conducting the advanced photodiode array peak purity analysis. The almost perfect maintenance of truly exceptional theoretical plates, highly and completely symmetrical tailing factors and totally optimal resolution consistently throughout the analysis of the incredibly complex analysis of the highly chemically degraded samples clearly and absolutely demonstrates the truly excellent analytical performance of the method. Therefore, the comprehensive and very precise results from the forced degradation, which were achieved with a truly high degree of care and precision, are truly irrefutable and undeniable scientific evidence of the complete stability indicating properties of the fully new analytical method, which can be used continuously to monitor the exact chemical integrity of empagliflozin through its entire, highly regulated pharmaceutical life cycle.

Conclusion

The very extensive research, complete and exhaustive, resulting in this detail has succumbed to the complete development and really thorough validation of a very novel, very quick and very sensitive stability-indicating reversed-phase high-performance liquid chromatography method specifically developed for the most precise analysis of degradation products. All absolutely critical chromatographic parameters could be optimized in a highly systematic and

mathematically sound manner, that perfectly produced a highly efficient, incredibly rapid procedure, which was completely characterized by extremely high theoretical plates, very best and very optimal peak resolution as well as peak symmetrically tailing factors, which enabled the quantification of the pure active pharmaceutical ingredient to be perfectly performed within an incredibly short analytical run time, and within a very economical analytical time frame. The result was the uncompromising validation procedure performed in absolute, total compliance, expressly and entirely according to the incredibly strict International Council for Harmonisation Q2(R2) guidelines and confirmed the unparalleled linear range, the unparalleled precision, the completely outstanding accuracy and the truly exceptional sensitivity of the method. Besides, the fully comprehensive and highly aggressive forced degradation study provided truly deep and valuable scientific insights into the totally intrinsic chemical stability of the complex molecule – completely confirmed and undeniably demonstrated the complete unrivalled specificity of the method, and the totally unassailable and robust capability to complete isolation of the principal drug from highly complex and very close-lying mixtures of the hydrolytic and severely oxidative degradation products. The complete absence of any absolutely co-eluting chemical interference whatsoever and the strict maintenance of absolute peak spectral purity, mathematically and physically validate the method's truly profound and completely undeniable stability indicating nature. Finally this is one of the most robust, most economically viable and truly exceptionally repeatable analytical methods, which absolutely represents one of the most important and major advances that pharmaceutical quality control laboratories the world over can only witness in modern pharmaceutical analysis, and which is exactly what they are looking for when it comes to routine assay determination and continuous, highly accurate long-term stability monitoring of absolutely critical, lifesaving pharmaceutical formulations, which is, to say the least, a truly indispensable and totally regulatory compliant analytical method..

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