

Stability-Indicating Method Development Using UV-Visible Spectrophotometry And HPLC For Selected Antidiabetic Drugs: A Comprehensive Review

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder requiring long-term pharmacotherapy with chemically diverse antidiabetic agents. The increasing use of single-drug and fixed-dose combination products necessitates reliable analytical procedures capable of assuring identity, strength, purity and stability. A stability-indicating analytical procedure should selectively and accurately quantify the active pharmaceutical ingredient in the presence of degradation products, process-related impurities and formulation excipients. This review critically discusses the principles and practical considerations involved in developing UV-visible spectrophotometric and high-performance liquid chromatographic (HPLC) methods for selected antidiabetic drugs, including metformin, sulfonylureas, DPP-4 inhibitors, and SGLT2 inhibitors. Emphasis is placed on analytical target profile, selection of wavelength and solvent for UV methods, chromatographic column and mobile-phase selection, pH optimization, detection, forced degradation, system suitability, and validation. The contemporary ICH Q2(R2) and Q14 frameworks are incorporated to explain validation and the development of science- and risk-based analytical procedures. Published applications involving metformin, gliclazide, glipizide, glimepiride, sitagliptin and dapagliflozin demonstrate the practical value of these approaches. Emerging trends including analytical quality by design, design of experiments, green analytical chemistry, diode-array detection, chemometrics and LC-MS-based degradation-product characterization are also discussed. The review provides a structured framework for selecting and developing fit-for-purpose stability-indicating procedures for antidiabetic pharmaceutical products.

Keywords: Antidiabetic drugs; stability-indicating method; UV-visible spectrophotometry; HPLC; RP-HPLC; forced degradation; analytical method development; method validation; ICH Q2(R2); ICH Q14.

INTRODUCTION

Diabetes mellitus is a major chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, impaired insulin action, or both. Pharmacological treatment includes chemically and mechanistically diverse agents such as biguanides, sulfonylureas, DPP-4 inhibitors and SGLT2 inhibitors. The increasing use of combination therapy has created additional analytical challenges because active pharmaceutical ingredients can differ markedly in polarity, pKa, chromophore properties and degradation behaviour.

Pharmaceutical analytical procedures are essential for assuring the identity, strength, quality, purity and

stability of medicines. For stability testing, the analytical procedure must be capable of distinguishing the intact drug from substances generated during degradation. UV-visible spectrophotometry and HPLC remain two important techniques in pharmaceutical analysis. UV methods are rapid, economical, and simple, whereas HPLC provides chromatographic separation and therefore generally offers greater selectivity for stability-indicating applications.

Modern analytical development has increasingly moved from empirical trial-and-error experimentation toward science- and risk-based development. ICH Q14 addresses analytical procedure development, while ICH Q2(R2) establishes the current framework

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for analytical procedure validation. This review integrates these principles with published analytical applications involving selected antidiabetic drugs.

Stability-Indicating Analytical Procedures

A stability-indicating analytical procedure is capable of measuring the active pharmaceutical ingredient accurately and selectively in the presence of degradation products, impurities, excipients and other potential interferences. The principal objective is not simply to obtain an acceptable assay value, but to demonstrate that changes in drug quality can be detected and quantified appropriately during stability studies.

Stability-indicating procedures are particularly important because pharmaceutical products may undergo chemical or physical changes during manufacturing, storage, transportation and use. Temperature, humidity, light, oxygen, pH and interactions with formulation components can influence degradation.

Forced degradation studies are commonly used during method development to challenge the specificity of the analytical procedure. Acid, alkaline, neutral, oxidative, thermal and photolytic stresses may be investigated according to the chemical structure and expected degradation pathways of the selected drug.

Selected Antidiabetic Drug Classes

Metformin hydrochloride, a biguanide, is among the most widely used oral antidiabetic agents. Its high polarity and basic character can make conventional reversed-phase retention challenging and require careful optimization of aqueous conditions, pH and stationary phase. Sulfonylureas such as glimepiride, gliclazide and glipizide stimulate pancreatic insulin secretion and generally possess more hydrophobic structures than metformin. Their chromatographic behaviour can therefore be markedly different from that of metformin, an important consideration for simultaneous methods. DPP-4 inhibitors include sitagliptin, vildagliptin, saxagliptin, linagliptin and alogliptin. These compounds have diverse structures and ionization properties, and analytical methods must be adapted accordingly. SGLT2 inhibitors include dapagliflozin, empagliflozin, canagliflozin, ertugliflozin and remogliflozin. Stability-indicating

chromatographic procedures have been reported for several of these agents, including methods coupled to UV, DAD and mass spectrometric detection.

UV-Visible Spectrophotometric Method Development

UV-visible spectrophotometry measures absorption of ultraviolet or visible radiation by chromophore molecules. The Beer-Lambert relationship is expressed as $A = \epsilon bc$, where A is absorbance, ϵ is molar absorptivity, b is optical path length and c is concentration. Method development normally begins with selection of a suitable solvent, preparation of a representative drug solution and spectral scanning to identify a suitable analytical wavelength. The solvent should provide adequate solubility and stability while exhibiting minimal absorbance at the selected wavelength. For combination products, simultaneous-equation, absorbance-ratio, derivative, ratio-derivative and chemometric approaches may be used when spectra overlap. However, the absence of chromatographic separation means that UV methods require particularly careful assessment of specificity when degradation products are expected.

HPLC Method Development

Reversed-phase HPLC is widely applied to pharmaceutical assay and stability testing. Method development involves selection of the stationary phase, aqueous component, organic modifier, pH, flow rate, column temperature, injection volume, detection wavelength and run time. C18 columns provide broad applicability, although C8, phenyl, cyano, HILIC and ion-exchange phases may be appropriate for specific analytes. Methanol and acetonitrile are commonly used organic modifiers. For ionizable compounds, mobile-phase pH can substantially influence retention, selectivity and peak shape. Detection wavelength should provide adequate response for the analyte while maintaining acceptable baseline behaviour. Diode-array detection can provide additional spectral information and may support peak-purity assessment. System suitability should evaluate parameters such as retention time, theoretical plates, tailing factor, resolution and injection precision according to the method's intended use.

Forced Degradation Studies

Forced degradation is a deliberate stress-testing strategy used to generate degradation products and evaluate whether an analytical procedure can distinguish them from the active drug. It commonly considers acid, alkaline, neutral hydrolysis, oxidation, thermal stress and photolysis. Stress conditions should be scientifically justified and optimized. Excessive degradation can generate secondary products that are not representative of plausible degradation pathways, whereas insufficient degradation may not adequately challenge the method. The objective is therefore to obtain meaningful degradation without unnecessarily destroying the parent drug. For a stability-indicating HPLC method, chromatograms should demonstrate a well-resolved main peak and, where degradation occurs, separated degradation peaks. Where appropriate, peak purity, mass balance and complementary spectrometric characterization can strengthen the assessment.

Analytical Quality by Design and ICH Q14

ICH Q14 provides a modern framework for analytical procedure development. An analytical target profile (ATP) defines what the procedure must measure and the required performance. Risk assessment can then be used to identify critical method attributes and critical method parameters.

Design of Experiments can evaluate interactions among factors such as organic-phase percentage, buffer pH, flow rate and column temperature. Responses may include resolution, retention time, tailing factor, efficiency and assay. This approach can reduce unnecessary experimentation and provide a more systematic understanding of method performance.

An analytical quality-by-design workflow can be summarized as: Analytical Target Profile → risk assessment → method variables and performance criteria → experimental design → optimization → validation → control strategy and lifecycle management.

Validation of Stability-Indicating Methods

ICH Q2(R2) provides the current framework for validation of analytical procedures. The validation strategy should be aligned with the intended analytical purpose. Specificity or selectivity is particularly important for stability-indicating procedures. The method should distinguish the analyte from degradation products, impurities, excipients and other active ingredients. Linearity and range establish the relationship between analytical response and concentration over the intended working interval. Accuracy is commonly investigated by recovery experiments, while precision includes repeatability and, where relevant, intermediate precision. LOD and LOQ may be important for impurity or degradation-product methods. Robustness assesses the effect of small deliberate variations in method parameters. Solution stability should be investigated where samples or standards are held for significant periods before analysis. System suitability should be defined to ensure that the analytical system performs adequately before and during routine use.

Representative Applications to Antidiabetic Drugs

Published studies demonstrate the application of stability-indicating HPLC to combinations and individual antidiabetic drugs. Methods have been described for simultaneous determination of metformin with gliclazide or glipizide, including forced-degradation evaluation. Multivariate experimental-design approaches have been used for development of stability-indicating HPLC procedures for glimepiride. Dapagliflozin has been evaluated by stability-indicating RP-HPLC and by LC/DAD and MS/MS approaches for chemical-stability and degradation-product investigations.

UV-visible methods have also been reported for antidiabetic combinations, including multicomponent formulations containing metformin, sitagliptin and dapagliflozin. Such methods can be useful for rapid routine assay when specificity has been adequately established. For complex degradation profiles, however, chromatographic or hyphenated techniques generally provide more direct separation and characterization.

Comparison of UV and HPLC Approaches

UV-visible spectrophotometry offers simple instrumentation, rapid analysis, low solvent consumption and relatively low operating cost. Its main limitation is limited inherent separation capability. HPLC requires greater instrumentation, maintenance and solvent use, but provides chromatographic separation and is therefore particularly valuable when degradation products or multiple active ingredients must be resolved.

The appropriate technique should be selected according to the analytical target, sample complexity, required selectivity, available instrumentation and regulatory expectations. A UV method should not be labelled stability-indicating solely because it produces precise assay results; supporting specificity evidence is necessary.

Green Analytical Chemistry and Future Perspectives

Environmental considerations are increasingly relevant to pharmaceutical analytical development. Reduction of organic solvent volume, shorter run times, smaller columns, safer solvents, miniaturization and efficient sample preparation can reduce environmental burden.

Future method development is likely to incorporate analytical quality by design, chemometrics, automated optimization, high-resolution mass spectrometry, diode-array detection, miniaturized

chromatography and lifecycle management. For fixed-dose antidiabetic combinations, integrated workflows combining UV screening, chromatographic separation and complementary spectrometric characterization may provide efficient and scientifically defensible analytical control.

CONCLUSION

Stability-indicating analytical procedures are essential for assuring the quality and stability of antidiabetic drug substances and pharmaceutical products. UV-visible spectrophotometry remains valuable for rapid and economical quantitative analysis, whereas RP-HPLC provides a strong platform for separation of active drugs from degradation products and formulation components. The development process should be scientifically justified, beginning with an analytical target profile and understanding of drug chemistry, followed by rational method selection, optimization, forced degradation and validation. ICH Q2(R2) and Q14 provide an appropriate contemporary framework for validation and development. Published analytical applications involving metformin, sulfonylureas, DPP-4 inhibitors and SGLT2 inhibitors demonstrate the broad applicability of UV and HPLC approaches. Future stability-indicating procedures are expected to increasingly integrate quality-by-design concepts, green analytical principles, chemometrics and hyphenated analytical techniques to improve selectivity, robustness, sustainability and scientific understanding.

Parameter	UV-Visible	HPLC
Instrument cost	Relatively low	Relatively high
Analysis time	Very short	Moderate
Solvent consumption	Low	Moderate to high
Separation capability	Limited	High
Degradation-product separation	Usually limited	Strong
Routine assay	Suitable	Suitable
Stability-indicating use	Requires supporting specificity evidence	Particularly suitable

Method complexity	Low	Moderate to high
Maintenance	Low	Higher

Table 1. General comparison of UV-visible spectrophotometry and HPLC

Stress condition	Purpose	Analytical observation
Acid hydrolysis	Assess acid susceptibility	Parent-drug loss and degradation peaks
Base hydrolysis	Assess alkaline susceptibility	Parent-drug loss and degradation peaks
Neutral hydrolysis	Assess aqueous stability	Changes under neutral aqueous conditions
Oxidation	Assess oxidative susceptibility	Oxidative degradation products
Thermal stress	Assess temperature sensitivity	Thermal degradation profile
Photolysis	Assess light sensitivity	Photodegradation products

Table 2. Typical forced-degradation study design**ACKNOWLEDGEMENTS**

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

M. Pavan Kumar: conceptualization, supervision, literature review, and manuscript preparation. Dr. M. Kishore Babu: supervision, academic guidance, and critical review. M. Rakshitha: literature collection and manuscript preparation. M. Lakshmi Kusuma: literature survey and compilation. K. Yashwanth:

literature survey and data compilation. S.D. Nagur Shareef: literature review and editing. D. Mahima Kumari: literature survey, reference compilation and editing. All authors reviewed and approved the manuscript.

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