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Review Paper

Improvements in RP-HPLC Techniques for Co-determining Dapagliflozin and Sitagliptin: Development of Methods, Validation, and Eco-friendly Practices

Adithya H M.*, Harsha Tripathy, Chandanam Sreedhar, Manju V, T. Srinivasa Rao, Maheen fathima

Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, Bangalore, Karnataka-560064..

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ABSTRACT

The increase in the use of fixed-dose combinations of dapagliflozin and sitagliptin in different parts of the world has amplified the need for effective and sensitive dosing and regulatory-compliant analytical methods to support quality control, stability tests, and pharmaceutical development. RP-HPLC is the application of choice when using simultaneous quantification since it is precise, reproducible and has the capability of being used in a variety of formulation matrices. Although many approaches have been reported, the current literature displays substantial differences in terms of chromatographic conditions, degree of validation, greenness evaluation and Quality by Design (QbD). The review summarizes developments in RP -HPLC methods to simultaneously estimate dapagliflozin-sitagliptin between 2010 and 2025, with a focus on mobile-phase optimization, column-choice, detection methods and stability-indicating techniques. Special attention is given to the validation of methods in accordance with ICH Q2(R1), the measures of chromatographic performance, analytical eco-scale/AGREE greenness and new variations of reducing solvents as well as environmentally friendly analytical chemistry. Particular focus on the study is given to the validation of the analytical methods according to the ICH Q2(R1) requirements, the rigorous evaluation of the metrics of the chromatographic performance, the greenness evaluation based on eco-scale and AGREE scoring along with the discussion of the new ways to decrease the usage of solvents to ensure the sustainability of analytical chemistry.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a rapidly spreading global health problem that affects

***Corresponding Author:** Adithya H M.

Address: Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, Bangalore, Karnataka-560064...

Email ✉: jatiny841@gmail.com

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millions of individuals worldwide [1–3]. Patients are predisposed to serious complications of the cardiovascular, kidney, and nervous systems that encourage this condition or the constant increase in blood sugar levels [2, 4, 6]. Several recent estimates show that the number of diabetes cases will continue to grow significantly globally in the coming decades [1,3,4], which makes it clear that proper treatment strategies and sound quality standards in medical practice and pharmaceutical manufacturing are an absolute necessity.

Combined fixed-dose regimens of antidiabetic drugs, such as dapagliflozin and sitagliptin, are gaining popularity because of their distinct and complementary mechanisms of action [8–12]. Dapagliflozin is an inhibitor of Sodium-Glucose Co-Transporter 2, which is effective in reducing glucose uptake into the kidney, causing a rise in the level of urinary glucose excretion [9, 13,18]. Sitagliptin, a Dipeptidyl Peptidase-4 inhibitor, on the other hand, increases incretin hormone concentrations, including GLP-1 and GIP, and increases insulin secretion in response to glucose in pancreatic beta cells [9, 14, 18,20].

Taken together, these treatments help to increase glycemic control, and the mechanisms of SGLT2 and DPP-4 inhibitors are also associated with a low risk of hypoglycemia, which can be a significant advantage over certain monotherapies [18–20]. It is essential that the active ingredients are quantifiable, and both measures are performed in parallel to ensure the quality of the products, regulatory compliance, stability, and therapeutic efficacy. [9, 21]. The use of parallel chromatographic measurements, in contrast to sequential methods, has the benefits of reducing the cumulative assay time, limiting the amount of solvent needed, and greatly reducing the overall resource requirement [22, 23]. This strategy simplifies the entire investigation process and offers a broad analytical perspective at a glance within a single evaluation.

Several reverse-phase HPLC procedures have been reported for the simultaneous determination of dapagliflozin and sitagliptin [9, 24]. These protocols tend to combine multicomponent analysis and stability-indicating techniques. As a case in point, stability-monitoring RP-HPLC methods have been adopted to decouple dapagliflozin and sitagliptin with their degradation products in multi-drug combinations [9, 21, 24]. These techniques can be used in everyday quality control procedures. Owing to its impressive sensitivity, selectivity, reproducibility, and versatility in a broad selection of pharmaceutical preparations, reverse-phase high-performance liquid chromatography remains the preferred method when the simultaneous assay of multiple analytes is required [25–29]. In RP-HPLC, the sample is fractionated based on its affinity for the hydrophobic C18 stationary phase [9, 30–37]. By fine-tuning the organic-aqueous mobile phases, a clear retention behavior can be achieved, and the peak resolution can be significantly enhanced. [9, 33, 38, 39].

In recent years, scientists have developed highly effective reversed-phase HPLC methods for analyzing multi-drug antidiabetic preparations [9, 21, 40–52]. Such approaches can simplify the entire investigation process by providing a comprehensive analytical overview in a single assessment. [53] Moreover, stability-indicating protocols for RP-HPLC have been effectively established that can separate these drugs and their degradation products in multi-drug formulations, thus showing the versatility of the technique in regular quality control adopted procedures [9, 21, 26]. For instance, dapagliflozin has been tested in combination with linagliptin and metformin, further emphasizing the strength and wide applicability of this method [51].

In addition to the effort to develop analytical performance, an apparent change to green analytical chemistry has occurred, necessitated by



the need to reduce environmental effects [31, 32, 54,58]. The large volumes of toxic organic solvents used in traditional chromatographic separations pose major safety concerns. [31, 32, 54, 55, 59–64]. The environmental sustainability of analytical protocols is routinely assessed with respect to cutting-edge frameworks which includes White Analytical Chemistry [65–68] and a set of eco-evaluation instruments such as the Analytical Eco-Scale [65, 69, 70], AGREE [54, 65, 68–70], GAPI [65, 66, 68–70] as well as RGB models [54, 66–70] to reduce the environmental impact of analytical processes. All these strategies aim to eliminate or reduce the number of hazardous chemicals, reduce waste, make energy consumption more straightforward and increase sustainability without the loss of analytical performance [32, 54, 55, 57, 71–76]. This practice is in line with global international health and environmental concerns.

Regardless of these technological advancements, high levels of heterogeneity in chromatographic conditions and the rigor of validation procedures are still reported in the literature [28, 31]. Many methods in the literature often do not provide a detailed evaluation of greenness or conduct systematic robustness tests [28, 31]. Therefore, the use of QbD is becoming a primary focus for regulatory authorities. This guarantees that methodologies are designed in a reliable manner and from a holistic perspective. In which the life cycle of the methodology and incorporation of QbD in every step of development are assured [7781].

This necessitates a systematic review to unite the current RP-HPLC methods and provide a stringent review of the analytical and environmental performance, offering a unified analytical framework.

This review aims to provide a comprehensive and current evaluation of reverse-phase HPLC methods established between 2010 and 2025 for

the simultaneous determination of dapagliflozin and sitagliptin [9, 14, 21, 49, 50, 52, 82]. In this study, chromatographic parameter assessment and validation of the results were conducted according to the ICH Q2 guidelines. [9, 83–87]. The use of forced degradation tests and environmentally safe methods of analysis were stated, and this study also sets new directions that reinforce sustainability in the field [9, 52, 85, 86, 88]. Analytical techniques that adequately meet regulatory demands in pharmaceutical analysis must be developed to ensure the safety, efficacy, and quality of a product before it is made available to patients. [77–81].

Method Development Strategies

Stationary Phase Selection

Selection of the right stationary phase is very critical in the context of the mechanization of the column in respect of chromatography. The octadecylsilane (C18)-bonded silica column is the most widely employed and versatile, possessing outstanding resolving power and unwavering reproducibility in reverse-phase chromatography [9, 28, 30, 8991]. The C18 reversed-phase allows for the effective separation of both polar and non-polar analytes based on their hydrophobicity [30]. In several studies, researchers have resolved dapagliflozin and sitagliptin on standard C18 columns (250 mm × 4.6 mm, 5 µm) to achieve efficient chromatographic functions that offer improved peak resolution and reproducibility, which is crucial for everyday analytical work. [9, 21]. With careful system tuning, a high throughput can be achieved [9, 49, 92] and limited backpressure [77, 93–95]. Furthermore, core-shell particle columns have also started gaining increasing interest in the industry [23, 30, 96–103]. This is more efficient and has a shorter run time; hence, it is best suited to the demanding nature of



modern high-throughput analogous analytical processes [\[30, 96–103\]](#).

Mobile Phase Formulation and Adoption of Environmentally Friendly Solvents.

It is important to select the correct mobile phase composition because it has a strong impact on the chromatographic resolution and environmental impact of the analysis [\[31, 54, 63, 64, 104–108\]](#). In standard reverse-phase HPLC, the mobile phase is typically an aqueous mixture with a minor percentage of an organic solvent, typically acetonitrile or methanol [\[9, 27, 31, 109–112\]](#). The optimal pH values in the sample were approximately 3.0–4.5—the range which were used to buffer the sample to enhance peak form and maintain the stability of the analyte [\[9, 28, 113–117\]](#). As the current trend is towards green analytical chemistry, scholars are persistently substituting acetonitrile with greener solvents such as ethanol or aqueous rich solutions in view to reduce the overall toxicity of the process while reducing the level of waste solvent [\[31, 32, 54–58\]](#). In addition, the paper also notes that green solvents can be easily integrated into the normal operations of bioanalytical processes without affecting the quality of the results [\[15, 31, 32, 54, 55, 118, 119\]](#). This method is directly compatible with mass spectrometric detection using volatile buffers such as ammonium acetate [\[85, 86, 95, 116, 120–124\]](#), which permits further expansion of the method to increase its overall versatility.

Choosing the wavelengths of detection and detector technologies is important.

The UV-visible spectrophotometric method remains the preferred method for the concurrent determination of dapagliflozin and sitagliptin because of its simplicity and cost-effectiveness. [\[13, 125, 126\]](#). In the literature, we found that the optimal wavelengths for detecting the two compounds were between 210 nm and 270 nm, the precise area in which the two substances are more

absorbent [\[9, 15, 49, 125, 127–130\]](#). The high absorbance of both compounds is demonstrated at a wavelength of 260 nm, which has been established by many researchers as a good compromise that provides good sensitivity and specificity [\[9, 131, 132\]](#). Photodiode array detectors have several important benefits, the most prominent being their spectral scanning capability [\[133–137\]](#). Peak purity analysis was conducted to ensure that there were no co-eluting impurities or degradation products [\[134–136, 138–142\]](#). LC-MS/MS provides unmatched selectivity and sensitivity in bioanalytical studies [\[30, 143–147\]](#). Nonetheless, they are more advanced techniques and are not commonly applied in daily quality control practices [\[147, 148\]](#).

Run Time Optimization

Reducing the analysis time is important for enhancing laboratory productivity and reducing solvent consumption [\[23, 31, 32, 61, 142, 149–151\]](#). Several strategies have been adopted in the literature [\[56, 58, 63, 103, 152–158\]](#). Through precise fine-tuning of the pool ratios in the mobile phase, dapagliflozin and sitagliptin were fully separated within 5–10 minutes [\[21, 49, 50\]](#). It was run at flow rates of 0.6 to 1.0 mL/min with gradient profiles [\[9, 21, 49, 50, 159–160\]](#). This can be achieved by reducing the column length and particle size or using core-shell technology to allow the analysts to run tests faster without affecting the resolution [\[96–99, 103, 154, 161–164\]](#). These advancements have significantly enhanced the efficiency, speed, resolution, and overall utility of chromatographic methods for routine pharmaceutical applications [\[30, 31, 99, 103, 162\]](#). Reducing the reaction time not only increases the overall efficiency [\[32, 54\]](#) but is also in line with the ideals of green chemistry [\[31, 54, 142\]](#), which aims to reduce the energy [\[31, 54\]](#) and the amount of solvent [\[31, 54, 142\]](#) used.



Validation parameter	ICH Q2(R1) Criteria	Typical RP-HPLC Results (2005–2025)	References
Specificity	None of the interference by excipients, impurities or degradation products. Peak purity > 0.99.	Peaks corresponding to dapagliflozin and sitagliptin resolved well with no adversarial co-eluting peaks as verified by PDA spectral analysis and forced degradation experiments.	[9, 21, 49, 121, 165–167]
Linearity	$R^2 \geq 0.990$	Very high linearity ($R^2 = 0.9959999$) in extensive concentration intervals (e.g. 0.5 -50 $\mu\text{g/mL}$).	[9, 42, 49, 92, 130, 160, 166, 168]
Accuracy	98–102% recovery	Recoveries of between 98.5 to 101.5 in pharmaceutical dosage form and spiked matrices.	[9, 49, 52, 166, 168–170]
Precision	%RSD $\leq 2\%$ for intra- and inter-day	Excellent repeatability and reproducibility is indicated by precision values of between 0.3 percent to 1.8 percent (RSD).	[14, 49, 52, 92, 121, 166, 169]
LOD	Signal-to-noise ratio ≥ 3	The normal range of the LOD of dapagliflozin and sitagliptin ranges between 0.02 -0.2 $\mu\text{g/mL}$.	[14, 21, 92, 121, 160, 165, 166, 168–170]
LOQ	Signal-to-noise ratio ≥ 10	Values of LOQ are 0.07 to 0.6 $\mu\text{g/mL}$, and it is appropriate in the case of pharmaceutical and biological samples.	[14, 21, 92, 121, 160, 165, 166, 168–170]
Robustness	Minor deliberate changes do not significantly affect results	Small variations in flow rate, mobile phase pH, temperature depicted less than 2 percent variation in retention time and resolution.	[9, 14, 21, 49, 52, 121, 160, 165, 169]

Eco-Friendly RP-HPLC Procedures for Concurrent Determination of Dapagliflozin and Sitagliptin.

1.The Importance of Green Analytical Chemistry to Pharmaceutical RP -HPLC

Green analytical chemistry has taken center stage over the last couple of years with an attempt to realign the current pharmaceutical analysis towards a more sustainable approach [31, 32, 57, 71, 165]. Traditional chromatographic methods have environmental, occupational, and economic costs [31, 32, 55, 142, 165]. Although reverse-phase high-performance liquid chromatography is an essential tool in the quality control of antidiabetic drugs [151, 166], it is progressively changing to suit the growing regulatory standards [23, 167]. Historically, the procedure uses large volumes of organic solvents which are dangerous and produces a large amount of chemical wastes [31, 32, 55, 142]. These shortcomings are receiving increased attention from regulatory agencies, journal editors, and the pharmaceutical industry [30, 58, 78, 165, 168, 169].

Combined doses, such as dapagliflozin and sitagliptin, are regularly prescribed to treat diabetes on a long-term basis [8–12, 18–20]. Due to the repetitive cycle of analytical testing, the amount of solvent used, and waste increases [22, 23, 31, 32, 54, 55, 59–64, 142]. Consequently, the trend of integrating the concept of greenness in designing RP-HPLC methods has ceased to be an optional procedure but a necessity, scientifically justified and morally necessary [30–32, 54–58, 71–76, 78, 165, 168, 169].

Selection of Eco-Friendly Solvents and Development of Mobile Phase.

In chemical formulae, hazardous organic modifiers are substituted with other elements.

One of the most viable green strategies is to replace hazardous solvents, such as acetonitrile, with environmentally friendly alternatives [31, 32,

54, 55, 63, 104, 170–174]. Ethanol has emerged as the most promising green organic modifier owing to its low toxicity, biodegradability, and renewability [31, 32, 63, 170, 171]. These compounds have desirable chromatographic characteristics [31, 104, 171, 174]. Many studies have demonstrated that when ethanol-water mixtures are used as mobile phases, they provide no better resolution than other solvent systems [31, 110, 175, 176]. With optimal optimization of the system, it provides a high level of peak symmetry and stable retention control with a moderately polar antidiabetic drug [47, 172, 177–182].

Dapagliflozin and sitagliptin have been analyzed using mobile phases based on ethanol [31, 32, 162, 172, 174, 178, 183–185], and volatile buffers are typically used. Examples of such ammonium acetate and ammonium formate have been shown to be highly compatible with UV and PDA detection [53, 116, 120, 186, 187]. It has a significant effect on reducing environmental impacts [31, 32, 162, 172, 174, 178, 183–185].

2.2 Reduction of Solvents and Wasting Minimization

In addition to solvent replacement, green reverse-phase high-performance liquid chromatography focuses on the total number of solvents consumed [31, 32, 56, 63, 157, 188, 189]. This result was achieved by lowering the organic content, reducing the flow rates, and reducing analytical run times [56, 63, 115, 157, 158, 190, 191]. The result of this close adjustment of the mobile phase composition and pH is a high-speed elution in the retention of chromatographic resolution [31, 165, 192–197], which is highly recommended by major journals in analytical chemistry [196].

3.Column technology and Instrument optimization

The selection of high-performance stationary phases is a wise decision that should be considered



to promote sustainable chromatographic practices. [31, 32]. C18 phases are the most popular stationary media used to determine dapagliflozin and sitagliptin concurrently [9, 21, 24, 42, 49, 198]. The shift to shorter chromatography columns [103, 116, 199–203] and the introduction of core-shell particle technology [23, 30, 96–103] have resulted in significant greenness of the analytical technique [96, 104, 204] owing to the strength and reproducibility of the results [101, 205–207].

C18 columns with a core-shell design are characterized by significantly high chromatographic effectiveness [96, 99, 101, 204, 206] at extremely low backpressure [97, 99, 101, 206], allowing short analysis run times [96, 97, 99, 101, 204, 206] and less solvent usage [96, 97, 104, 204]. Such benefits directly provide a more sustainable working process [97, 104, 204] without deteriorating the analytical functionality [96, 101] or compliance with ICH validation requirements [9, 165].

4. Strategies for detecting and supporting green analysis.

UV and PDA detectors are the most commonly used in green reverse-phase HPLC, as they are energy-efficient [63], easy to handle [53], and most suitable for routine quality control analysis [40, 53, 208]. In addition, the maximum purity can be measured using PDA detection, which makes the method more specific and reliable for regulatory purposes, and no additional solvents or reagents are required [92, 142, 208]. Although liquid chromatography-mass spectrometry offers the maximum sensitivity, its energy intensity and the necessity of using solvents must be justified with a tight reason, particularly when establishing an alternative analytical procedure that is more sustainability-oriented [73, 105, 209]. The environmental impact of LC-MS/MS can be significant because of the continuous consumption of organic solvents, energy-intensive column

temperature control, and generation of complex waste streams [209]. Some LC-MS/MS methods, despite their high analytical performance, have a moderate environmental impact because of the high-energy demands of mass spectrometry and the consumption of organic mobile phases. [209]. Green analytical chemistry ideologies encourage the reduction of material, energy, and waste usage [30], making more environmentally friendly LC-MS/MS workflows, including those that avoid energy- and solvent-intensive procedures, required [30].

5. Quantitative Greenness Assessment Tools.

In high-impact Q1 journals, authors are required to make more quantitative and objective evaluations of the greenness of a method than qualitative or subjective evaluations. [30, 58, 78, 165, 168, 169]. Tools such as the Analytical Eco-Scale, GAPI, and AGREE provide systematic guidelines for analyzing the environmental performance of analytical methods at each stage of a given workflow. [54, 65, 66, 68–70]. AGREE has received a significant level of editorial support because of its overall consistency with the 12 tenets of green analytical chemistry [31, 32, 54, 119, 157, 165, 179, 210–214], and its scoring system is highly intuitive [31, 32, 54, 157, 165, 210, 214]; therefore, it is suitable for a Q1 journal [32, 54]. Recent RP-HPLC studies that also implemented AGREE or GAPI assessment frameworks have shown significantly better methodological transparency and associated enhancement of results in peer review [63, 213, 215–217].

6. Investigating the Benefits and Disadvantages of Green Reverse-Phase HPLC.

The benefits of green RP-HPLC include the reduction of the negative ecological footprint [31, 32, 55, 58, 61, 63, 64, 110, 162, 165, 218], enhancement of the safety of the laboratory [31, 32, 58, 61, 110, 162, 165], reduction of the costs of solvent waste



disposal [31, 32, 63, 162], and achievement of regulatory sustainability [58, 165]. However, it also has challenges, including an increase in mobile-phase viscosity in the case of ethanol [31, 32, 64, 219–221] and a lower ability to elute highly hydrophobic materials [31, 32, 222], which need to be addressed through extensive, systematic approach development [31, 32, 223, 224].

The reviewed studies show that when correctly designed, eco-friendly RP-HPLC methods for analyzing dapagliflozin and sitagliptin can be as functional as their conventional counterparts [9, 15, 31, 32, 47, 54, 55, 57, 71–76, 96, 101, 110, 118, 119, 165, 172, 175–182] and have a significant positive impact on the environment [31, 32, 54–58, 61, 63, 64, 110, 162, 165, 218]

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