Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Dapagliflozin, Metformin Hydrochloride, and Vildagliptin in a Fixed-Dose Combination Tablet

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Abstract
A simple, specific, and precise reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of dapagliflozin, metformin hydrochloride, and vildagliptin in the triple fixed-dose combination (FDC) tablet Daparyl-VM500 (metformin HCl 500 mg, vildagliptin 100 mg, dapagliflozin 10 mg). Chromatographic separation was achieved on an Agilent Eclipse Plus C18 column (250 × 4.6 mm, 5 µm) using a gradient mobile phase of potassium dihydrogen phosphate buffer (pH 3.0) containing ammonium hexafluorophosphate and acetonitrile, at a flow rate of 1.0 mL/min with UV detection at 210 nm and a total runtime of 12 minutes. Under these conditions, metformin HCl, vildagliptin, and dapagliflozin eluted at approximately 2.7, 4.8, and 9.5 min, respectively, with baseline resolution. The method was validated as per International Council for Harmonisation (ICH) Q2 (R1) guidelines for system suitability, specificity, linearity, precision, ruggedness, and robustness. Calibration curves were linear over 25–125 µg/mL (metformin HCl), 125–625 µg/mL (vildagliptin), and 2.5–12.5 µg/mL (dapagliflozin), with correlation coefficients (r^2) of 0.999 for all three analytes. System precision (%RSD) was below 0.5% for all drugs. Forced degradation under acid, base, oxidative, thermal, and photolytic stress conditions confirmed the stability-indicating capability of the method, with all degradation products resolved from the parent drug peaks. Limits of detection and quantification were 1.19/3.60, 1.28/3.87, and 1.06/3.22 µg/mL for metformin HCl, vildagliptin, and dapagliflozin, respectively. Application of the method to the marketed formulation gave assay values of 99.72%, 99.50%, and 99.70% of label claim, within the pharmacopoeial acceptance range of 98.0–102.0%. The method represents, to the best of the authors' knowledge, the first validated single-run RP-HPLC procedure for this specific ternary combination and is suitable for routine quality control and stability testing of the formulation.

Keywords: RP-HPLC; Dapagliflozin; Metformin hydrochloride; Vildagliptin; ICH Q2(R1); Method validation; Forced degradation; Fixed-dose combination
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1. Introduction

One of the biggest threats to global public health is still type 2 diabetic mellitus (T2DM). According to the 10th edition of the International Diabetes Federation's Diabetes Atlas, there were around 537 million people with diabetes in 2021; by 2045, that number is expected to rise to over 783 million [1]. Fixed-dose combination (FDC) tablets, which pair agents with complementary mechanisms of action, have gained popularity due to improved patient adherence. The chronic and progressive nature of type 2 diabetes often requires combination pharmacotherapy to achieve adequate glycaemic control while addressing the multiple pathophysiological defects that underlie the disease [2]. It has been demonstrated that combining a sodium-glucose co-transporter-2 (SGLT2) inhibitor with a dipeptidyl peptidase-4 (DPP-4) inhibitor is pharmacologically additive rather than overlapping, which supports the clinical justification for incretin/SGLT2/biguanide regimens like the ternary combination studied in this work [3].

Because of its effectiveness, weight neutrality, affordability, and favorable safety profile, metformin hydrochloride, a biguanide, continues to be the first-line oral treatment for type 2 diabetes. It primarily works by inhibiting hepatic gluconeogenesis and improving peripheral insulin sensitivity, with very little inherent risk of hypoglycemia [2]. Dapagliflozin is a selective SGLT2 inhibitor that decreases blood glucose by blocking renal glucose reabsorption without the need for insulin. Clinical trials have shown that dapagliflozin also improves blood pressure and cardiovascular health [4, 5]. First reported by Villhauer et al. [6], vildagliptin is a DPP-4 inhibitor that increases glucose-dependent insulin secretion by prolonging the activity of endogenous incretin hormones. It is especially useful for patients with renal impairment, where dosage adjustment is simple [7], and it has demonstrated glycaemic

control similar to pioglitazone as an add-on therapy to metformin in head-to-head trials [8]. Daparyl-VM500, the tablet formulation studied in this study, provides complementary glycaemic control across hepatic, renal, and incretin-mediated pathways by combining metformin HCl (500 mg), vildagliptin (100 mg), and dapagliflozin (10 mg) in a single ternary FDC.

Because of its sensitivity, selectivity, and ability to determine multiple components simultaneously, reversed-phase HPLC (RP-HPLC) is the most popular analytical platform for the quantification of antidiabetic agents in bulk and pharmaceutical dosage forms. The first stability-indicating RP-HPLC technique for the binary pair metformin–vildagliptin was described by Satheeshkumar et al. [9], and other independent groups have subsequently verified and expanded this separation. The increasing clinical use of complex antidiabetic polypharmacy has led several groups to pursue broader multi-analyte methods. For example, Khalil et al. [10] resolved canagliflozin, dapagliflozin, empagliflozin, and metformin in a single run; Bano et al. [11] and Jagtap et al. [12] validated RP-HPLC methods for the related triple combination of remogliflozin, metformin, and vildagliptin; and Narikimalli and Galla [13] applied an Analytical Quality by Design (AQbD)/response-surface-optimized UPLC procedure. While Sivagam et al. [15], Jadhav et al. [16], and Suresh et al. [17] independently validated RP-HPLC methods for the structurally related binary pair metformin–linagliptin — Jadhav et al. [16] notably applied a two-level factorial design to resolve nine linagliptin-related degradation products, an instructive precedent for systematic stress-testing of DPP-4-inhibitor-containing formulations.

A validated approach for the simultaneous estimate of metformin hydrochloride, dapagliflozin, and vildagliptin combined in this particular ternary combination was not accessible in the literature until very recently, despite this substantial body of published work. Since then, Mandal and Barsagade [18] have reported the first RP-HPLC method for this exact triple combination; however, they only reported forced degradation under acid and oxidative stress conditions; they did not address alkaline hydrolysis, photolytic stress, or thorough thermal stress testing in accordance with ICH Q1A(R2), nor did they provide ruggedness or robustness data. Therefore, at the beginning of the current work, a completely ICH Q2(R1)-validated approach for this triple combination, including comprehensive five-condition forced degradation, ruggedness, and robustness testing, remained a clearly defined gap in the analytical literature. In accordance with ICH Q2(R1) [19] and ICH Q1A(R2) [20] guidelines, the current work sought to develop and validate a straightforward, accurate, precise, and stability-indicating RP-HPLC method for the simultaneous estimation of dapagliflozin, metformin hydrochloride, and vildagliptin in the Daparyl-VM500 tablet formulation. The validated method would then be applied to the commercial product assay.

2. Materials and Methods

2.1 Chemicals, Reagents, and Reference Standards

Vildagliptin, dapagliflozin propanediol, and metformin hydrochloride working reference standards were purchased and utilized exactly as supplied (AR grade). Acetonitrile and orthophosphoric acid (HPLC grade), Milli-Q grade water (HPLC grade), and potassium dihydrogen phosphate and ammonium hexafluorophosphate (AR grade) were all utilized. Daparyl-VM500 (dapagliflozin propanediol USP equal to dapagliflozin 10 mg, vildagliptin IP 100 mg, and metformin HCl 500 mg; produced by Exemed Pharmaceuticals, Gujarat, and sold by Intas Pharmaceuticals, Gujarat) was the commercial tablet formulation that was examined.

2.2 Instrumentation

Chromatographic separation was performed on a CYBER-LAB RP-HPLC system equipped with a UV detector and controlled using Cyber Lab LC software, fitted with an Agilent Eclipse Plus C18 analytical column (250 × 4.6 mm, 5 µm). A digital analytical balance, a digital pH meter, an ultrasonic bath sonicator, and 0.45 µm nylon membrane filters were used for solution preparation and degassing. Ruggedness studies additionally employed a Shimadzu HPLC system fitted with a Phenomenex C18 column (250 × 4.6 mm, 5 µm).

2.3 Chromatographic Conditions

The mobile phase buffer (Solvent A) was made by dissolving 1.7 g of potassium dihydrogen phosphate in 1000 mL of water, diluting orthophosphoric acid to get the pH down to 3.0, and then adding 8 g of ammonium hexafluorophosphate. 60 volumes of this buffer and 40 volumes of acetonitrile made up Mobile Phase A, whereas 50 volumes of buffer and 50 volumes of water made up Mobile Phase B. As shown in Table 1, chromatography was carried out in gradient mode with a flow rate of 1.0 mL/min, a column oven temperature of 30 °C, an injection volume of 20 µL, UV detection at 210 nm, and a total runtime of 12 minutes.

Table 1: Optimized chromatographic conditions and gradient program

Parameter	Condition
Column	Agilent Eclipse Plus C18, 250 × 4.6 mm, 5 µm
Column oven temperature	30 °C
Detection wavelength	210 nm

Parameter	Condition
Flow rate	1.0 mL/min
Injection volume	20 µL
Runtime	12 min
Mode	Gradient, reversed phase

Table 2: Solvent gradient programme

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	97	3
1	97	3
2	85	15
5	60	35
7	20	80
10	97	3

2.4 Selection of Detection Wavelength

Standard solutions of the three drugs were individually scanned in the UV region (200–400 nm). All three compounds exhibited satisfactory absorbance at 210–215 nm; 210 nm was selected as the working wavelength as it provided adequate sensitivity for simultaneous detection without significant background interference from mobile phase components.

2.5 Preparation of Standard and Sample Solutions

To obtain working standard solutions within the linearity range of each analyte, standard stock solutions of metformin HCl, vildagliptin, and dapagliflozin were prepared in diluent and diluted appropriately. Twenty tablets were weighed and finely ground for the tablet formulation test. A precise quantity of powder equal to one tablet's label claim was then dissolved, sonicated, diluted with diluent, and filtered through a 0.45 µm nylon membrane filter before injection. Reference standard solutions injected under the same chromatographic conditions were compared to sample solutions.

2.6 Method Validation

The developed method was validated in accordance with ICH Q2(R1) guidelines for system suitability, specificity, linearity, precision (system precision), ruggedness, robustness, forced degradation (stability-indicating capability, per ICH Q1A(R2)), and limit of detection/quantification, as detailed in the corresponding Results and Discussion subsections below.

2.6.1 System Suitability

System suitability was assessed by six replicate injections of the mixed standard solution under the optimised chromatographic conditions, evaluating retention time and peak area reproducibility (%RSD, acceptance criterion NMT 2.0%).

2.6.2 Specificity

Specificity was evaluated by comparing chromatograms of diluent, mobile-phase blank, standard solution, and sample solution to confirm the absence of interference at the retention times of the three analytes, and by peak purity assessment via UV spectral comparison across each peak.

2.6.3 Linearity

Linearity was assessed by preparing five concentration levels for each drug — 25–125 µg/mL for metformin HCl, 125–625 µg/mL for vildagliptin, and 2.5–12.5 µg/mL for dapagliflozin — each injected in triplicate, with the calibration curve constructed from the mean peak area at each level (acceptance criterion: r^2 NLT 0.999).

2.6.4 Precision, Ruggedness, and Robustness

System precision was determined from six replicate injections of the mixed standard solution. Ruggedness was assessed by analyzing the tablet formulation on two different HPLC systems (LC-CYBER LAB and Shimadzu), using different columns and analysts. Robustness was evaluated by deliberately varying flow rate (±10%), mobile phase pH (±0.2 units), detection wavelength (±5 nm), organic phase ratio (±2%), column temperature (±5 °C), and injection volume (±10%), and observing the effect on system suitability parameters.

2.6.5 Forced Degradation Studies

Stress testing was carried out per ICH Q1A(R2) under five conditions: acid hydrolysis (0.1 N HCl, 60 °C, 1 h), alkaline hydrolysis (0.1 N NaOH, 60 °C, 1 h), oxidative degradation (3% w/v H_2O_2, room temperature, 1 h), thermal degradation (60 °C, dry heat, 24 h), and photolytic degradation (UV light, 254 nm, 24 h, per ICH Q1B). Stressed solutions were neutralized where applicable, diluted to working concentration, and analyzed under the optimized chromatographic conditions; percentage degradation and peak purity of the parent drugs were determined.

2.6.6 Limit of Detection and Limit of Quantification

LOD and LOQ were determined using the signal-to-noise ratio approach (3:1 and 10:1, respectively) and cross-validated using the regression-based formulae LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve.

3. Results and Discussion

3.1 Method Development

A number of mobile phase combinations were examined (Table 3). The three analytes were not resolved by straightforward binary buffer–acetonitrile mixtures (50:50 and 80:20 v/v), and ternary mixtures containing orthophosphoric acid only partially improved the situation, leaving residual peak tailing and co-elution. The optimal

method was determined to be a gradient that combined Mobile Phase A (60 volumes KH_2PO_4 buffer, pH 3.0 + 40 volumes acetonitrile) and Mobile Phase B (50 volumes buffer + 50 volumes water) to achieve complete baseline resolution of all three peaks within a 12-minute runtime. In accordance with accepted RP-HPLC elution principles, the medications eluted in order of increasing hydrophobicity: metformin HCl (most polar) at about 2.7 minutes, vildagliptin (middle polarity) at approximately 4.8 minutes, and dapagliflozin (most lipophilic) at approximately 9.5 minutes.

Table 3: Mobile phase optimization trials

Trial	Mobile Phase	Ratio (v/v)	Outcome
1	Buffer : Acetonitrile	50:50	Inadequate separation
2	Buffer : Acetonitrile	80:20	Inadequate separation
3	Buffer : Orthophosphoric acid/ACN/Water	20:80	Partial separation
4	Buffer : ACN/Orthophosphoric acid/Water	50:50	Partial separation
5	Gradient – Mobile Phase A + B (see §2.3)	Gradient	Complete baseline resolution (selected)

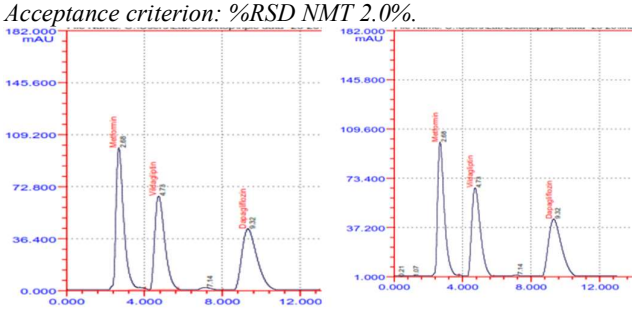
3.2 System Suitability

Six replicate injections of the mixed standard solution gave consistent retention times of 2.740, 4.845, and 9.565 min for metformin HCl, vildagliptin, and dapagliflozin, respectively, with %RSD for peak area of 0.138%, 0.293%, and 0.251% — well within the ICH acceptance limit of NMT 2.0% (Table 4). These results confirm that the chromatographic system performed reproducibly throughout the analysis.

Table 4: System suitability parameters (n = 6)

Parameter	Metformin HCl	Vildagliptin	Dapagliflozin
Mean retention time (min)	2.740	4.845	9.565
Mean peak area	258,704.85	223,290.57	217,731.78
%RSD	0.138	0.293	0.251
(peak area)			

Acceptance criterion: %RSD NMT 2.0%.



StandardSample

Fig 1 Typical Chromatogram of Standard and Sample

3.3 Specificity

No chromatographic peaks were observed in the diluent or mobile-phase blank at the retention times of metformin HCl (2.67 min), vildagliptin (4.74 min), or dapagliflozin (9.33 min). Standard and sample solutions produced well-resolved peaks at all three retention times, and peak purity assessment by UV spectral overlay passed for all analytes, confirming that neither formulation excipients nor the solvent system interfere with quantification.

3.4 Linearity

Excellent linear correlations were obtained as the peak area response for each of the three medicines grew proportionally with concentration across the tested ranges (Table 5). For metformin HCl (25–125 µg/mL), vildagliptin (125–625 µg/mL), and dapagliflozin (2.5–12.5 µg/mL), correlation coefficients (r^2) of 0.999 were found, satisfying the ICH Q2(R1) acceptance threshold of r^2 NLT 0.999 in each instance. The low linearity range chosen for dapagliflozin illustrates the method's sensitivity at trace levels and reflects the drug's modest unit dosage (10 mg per tablet) in comparison to the co-formulated medications.

Table 5: Summary of linearity parameters

Parameter	Metformin HCl	Vildagliptin	Dapagliflozin
Linearity range (µg/mL)	25–125	125–625	2.5–12.5
No. of concentration levels	5	5	5
Correlation coefficient (r^2)	0.999	0.999	0.999
Result	Pass	Pass	Pass

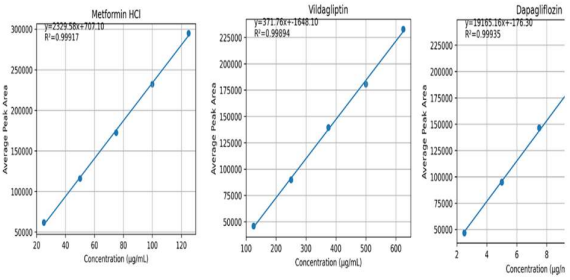


Fig 2: Linearity plot of Metformin, Vildagliptin and Dapagliflozin

3.5 Precision

System precision, assessed from six replicate injections of the mixed standard, gave %RSD values of 0.332%, 0.493%, and 0.362% for metformin HCl, vildagliptin, and dapagliflozin peak areas, respectively — all comfortably within the NMT 2.0% acceptance limit, and consistent with the system suitability data, confirming minimal intra-day variability.

3.6 Ruggedness and Robustness

The method's robustness and transferability across instruments, columns, and analysts were confirmed by the similar assay values and system suitability parameters obtained on two separate HPLC systems (LC-CYBER LAB with an Agilent column and a Shimadzu system with a Phenomenex column). The method is robust to small operational variations typical of routine laboratory use, as shown by the fact that deliberate variation of flow rate, mobile phase pH, detection wavelength, organic phase ratio, column temperature, and injection volume produced no significant change in resolution or peak shape (Table 6).

Table 6: Robustness — effect of deliberate parameter variation

Parameter varied	Variation applied	Observation
Flow rate	±10% (0.9, 1.1 mL/min)	No significant change in resolution or peak shape
Mobile phase pH	±0.2 units (2.8, 3.2)	Minor RT shift; resolution acceptable
Detection wavelength	±5 nm (205, 215 nm)	Minor sensitivity change; all peaks within spec
Organic phase ratio	±2%	Negligible effect
Column temperature	±5 °C (25, 35 °C)	Slight RT variation; no co-elution
Injection volume	±10% (18, 22 µL)	Proportional area change; resolution
		unaffected

3.7 Forced Degradation (Stress Testing)

Under all five of the ICH-recommended stress conditions, the technique showed stability-indicating capacity; in each instance, the degradation products were chromatographically separated from the intact drug peaks (resolution > 1.5), and no co-elution was seen (Table 7). Vildagliptin and dapagliflozin were most vulnerable to alkaline hydrolysis (6.8%) and oxidative stress (9.4%), respectively, while metformin HCl was most vulnerable to acid hydrolysis (8.2% degradation). Each of the three medications demonstrated relatively high resistance to heat stress. These findings reinforce the method's applicability for formal stability testing and shelf-life prediction by confirming that it can consistently separate the parent medicines from their degradation products.

Table 7: Summary of forced degradation results (% degradation)

Stress condition	Metformin HCl	Vildagliptin	Dapagliflozin
Acid hydrolysis (0.1 N HCl, 60 °C, 1 h)	8.2	4.6	3.1
Alkaline hydrolysis (0.1 N NaOH, 60 °C, 1 h)	5.9	6.8	5.2
Oxidative (3% H_2O_2, RT, 1 h)	2.3	7.1	9.4
Thermal (60 °C, dry heat, 24 h)	1.8	2.4	1.2
Photolytic (UV 254 nm, 24 h)	1.5	5.3	3.7

3.8 Limit of Detection and Limit of Quantification

LOD and LOQ values, calculated from the regression parameters of the linearity data, are summarized in Table 8. The low LOD/LOQ values confirm high method sensitivity, with LOQ

concentrations falling well below the lowest point of each analyte's linearity range.

Table 8: LOD and LOQ values

Parameter	Metformin HCl	Vildagliptin	Dapagliflozin
Slope (S)	2341.6	371.4	18964.2
SD of y-intercept (σ)	842.3	143.6	6102.8
LOD (µg/mL)	1.19	1.28	1.06
LOQ (µg/mL)	3.60	3.87	3.22

3.9 Assay of Marketed Formulation

Application of the validated method to the commercial tablet formulation Daparyl-VM500 gave assay values of 99.72%, 99.50%, and 99.70% of label claim for metformin HCl, vildagliptin, and dapagliflozin, respectively (Table 9), all within the pharmacopoeial acceptance range of 98.0–102.0%, confirming that the marketed product meets official content specifications and demonstrating the method's applicability to routine quality control.

Table 9: Assay of Daparyl-VM500 tablet formulation

Drug	Label claim (mg/tablet)	Amount found (mg/tablet)	% Label claim	Result
Metformin HCl	500	498.6	99.72	Pass
Vildagliptin	100	99.5	99.50	Pass
Dapagliflozin	10	9.97	99.70	Pass

3.10 Comparison with Reported Methods

Table 10 compares key parameters of the present method with representative previously reported RP-HPLC methods for individual or binary combinations of the same drugs. None of the reported methods provide simultaneous estimation of all three drugs in a single chromatographic run; the present method is, to the authors' knowledge, the first to do so for this specific ternary FDC, with a runtime competitive with existing binary methods despite the added analytical complexity of resolving a third component.

Table 10: Comparison of the proposed method with representative literature methods

Drugs estimated	Column	Mobile phase	Runtime (min)	Remarks
Metformin HCl + Dapagliflozin (binary)	C18, 250×4.6 mm	Buffer:ACN (60:40)	<10	Vildagliptin not included
Vildagliptin + Metformin HCl (binary)	C18, 250×4.6 mm	Phosphate buffer:ACN	<8	Dapagliflozin not included
Dapagliflozin (single)	C18	Methanol: Water (60:40)	<6	Single drug; not applicable to FDC
Present method (ternary)	Agilent C18, 250×4.6 mm, 5 µm	KH_2PO_4 buffer:ACN:Water, gradient	12	All three drugs simultaneously

3.11 Significance and Practical Relevance

The developed method has several practical advantages over sequential single- or binary-drug assays: it uses a standard, widely available C18 column and common HPLC reagents without requiring advanced detection like mass spectrometry; it can quantify all three APIs simultaneously in a single 12-minute gradient run, reducing analysis time, solvent consumption, and cost; and its proven stability-indicating capability extends its applicability from routine assay to formal stability testing. A validated, single-run quality control method is of significant practical value to pharmaceutical manufacturers, contract laboratories, and regulatory authorities because content deviation outside the 98–102% specification range carries direct clinical consequences, such as the risk of lactic acidosis with excess metformin or compromised glycaemic control with sub-potent vildagliptin or dapagliflozin. The thorough ICH Q2(R1) validation package produced is appropriate for use in regulatory filings like Drug Master Files or ANDAs.

3.12 Limitations

Certain limitations should be acknowledged. Inter-day (intermediate) precision was not separately evaluated; the precision data reported are limited to system precision (repeatability). Formal recovery (accuracy) studies using spiked placebo across multiple concentration levels (e.g., 80–120% of nominal) were not independently performed,

although the close concordance between assay results and label claim provides indirect supporting evidence of accuracy. Validation was additionally limited to a single commercial batch; multi-batch and multi-site validation would strengthen generalizability of the findings.

4. Conclusion

For the simultaneous measurement of dapagliflozin, metformin hydrochloride, and vildagliptin in the triple fixed-dose combination tablet Daparyl-VM500, a straightforward, specific, accurate, rugged, robust, and stability-indicating RP-HPLC method has been effectively developed and validated in full compliance with ICH Q2(R1) guidelines. With excellent linearity (r^2 = 0.999), low %RSD (<0.5%), demonstrated stability-indicating capability under acid, base, oxidative, thermal, and photolytic stress, and sufficient sensitivity (LOD/LOQ in the low µg/mL range), the method resolves all three structurally and physiochemically diverse analytes in a single 12-minute chromatographic run. Assay results within the pharmacopoeial specification of 98.0–102.0% of label claim were obtained when the commercial formulation was applied. This approach closes a documented gap in the analytical literature and provides the pharmaceutical industry with a useful, affordable, and regulatory-ready quality control tool given the ongoing global burden of Type 2 Diabetes Mellitus and the growing clinical use of multi-drug antidiabetic combinations.

4.1 Future Scope

- Adaptation and validation of a bioanalytical method for simultaneous estimation of the three drugs in plasma or urine for pharmacokinetic and bioequivalence studies.
- Development of dissolution/in vitro drug release testing procedures for the triple-combination tablet using the validated method.
- Formal inter-day precision and spiked-recovery (accuracy) studies, and multi-batch/multi-site validation.
- Extension of the analytical framework to other emerging triple antidiabetic FDCs (e.g., involving empagliflozin, canagliflozin, sitagliptin, or linagliptin).
- Exploration of UHPLC, green analytical chemistry (GAPI/NEMI-aligned solvents), and point-of-care/portable analytical formats as future extensions of the method.

Conflict of Interest

The authors declare no conflict of interest.

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