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Research Article

Development and Validation of an LC–MS/MS Method for the Quantitative Determination of Vildagliptin in Analytical Standard Solutions Using Linagliptin as an Internal Standard

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Abstract

An LC–MS/MS procedure was developed and evaluated for measuring vildagliptin in analytical standard solutions, with linagliptin serving as the internal standard. Reference materials dissolved in acetonitrile were used to produce the calibration and quality-control (QC) solutions, and six independently prepared placebo samples were examined for excipient-related interference. An ACE C18 column (50 mm × 2.1 mm, 5 μm) was operated under gradient conditions at 0.3 mL min-1 with 10 mM ammonium acetate and acetonitrile as the aqueous and organic components; each contained 0.01% formic acid. Positive-ion electrospray and multiple-reaction monitoring were used for detection. Across the validated interval of 0.125–4.0 ng mL^{-1}, the calibration model gave R^2 = 0.9951. Instrumental LOD and LOQ values in the injected solutions were 0.028 and 0.084 ng mL^{-1}, respectively. The highest intra-day or inter-day precision value was 2.15% RSD. At the three QC levels, mean recovery was 99.12–101.7%, and recovery RSD was 2.00–3.29% (n = 6). Each chromatographic cycle lasted 7.5 min. No interfering vildagliptin MRM peak was detected in the placebo preparations, although ion suppression and enhancement were not measured quantitatively. The validation applies to prepared analytical solutions and does not establish performance in dosage forms or biological matrices.

Keywords: Vildagliptin, linagliptin, LC-MS/MS, analytical method validation, quantitative determination

Linagliptin'in İç Standart Olarak Kullanıldığı Analitik Standart Çözeltilerde Vildagliptin Miktar Tayini: LC-MS/MS Yönteminin Geliştirilmesi ve Validasyonu

Öz

Bu çalışmada, linagliptin'in iç standart olarak kullanıldığı, analitik standart çözeltilerde vildagliptin tayinine yönelik bir LC–MS/MS yöntemi geliştirilmiş ve değerlendirilmiştir. Kalibrasyon ve kalite kontrol (QC) çözeltilerinin hazırlanmasında asetonitril içerisinde çözülmüş referans materyaller kullanılmış ve yardımcı madde kaynaklı girişim açısından bağımsız olarak hazırlanan altı plasebo numunesi incelenmiştir. Kromatografik ayırım, 0,3 mL dk^{-1} akış hızında ve gradyan elüsyon koşullarında çalıştırılan bir ACE C18 kolon (50 mm × 2,1 mm, 5 µm) kullanılarak gerçekleştirilmiş; sulu ve organik mobil faz bileşenleri sırasıyla 10 mM amonyum asetat ve asetonitril'den oluşmuş ve her iki bileşene de %0,01 formik asit eklenmiştir. Deteksiyon için pozitif iyon elektrosprey ve çoklu reaksiyon izleme (MRM) teknikleri kullanılmıştır. Valide edilen 0,125–4,0 ng mL^{-1} aralığı boyunca, kalibrasyon modeli R^2 = 0,9951 değerini vermiştir. Enjekte edilen çözeltilerdeki enstrümantal LOD ve LOQ değerleri sırasıyla 0,028 ve 0,084 ng mL^{-1} olarak bulunmuştur. En yüksek gün içi veya günler arası kesinlik değeri %2,15 RSD olarak hesaplanmıştır. Üç farklı QC seviyesinde ortalama geri kazanım %99,12–101,7 ve geri kazanım RSD'si %2,00–3,29 (n = 6) olarak belirlenmiştir. Her bir kromatografik döngü 7,5 dakika sürmüştür. İyon baskılanması ve artışı kantitatif olarak ölçülmemiş olmasına rağmen, plasebo preparatlarında vildagliptin MRM piki ile girişime yol açan herhangi bir sinyal saptanmamıştır. Bu validasyon hazırlanan analitik çözeltiler için geçerli olup, dozaj formlarındaki veya biyolojik matrislerdeki performansı teşkil etmemektedir.

Anahtar Kelimeler: Vildagliptin, linagliptin, LC-MS/MS, analitik yöntem validasyonu, miktar tayini

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1. Introduction

Type 2 diabetes mellitus involves persistent hyperglycemia arising from insulin resistance together with progressive impairment of pancreatic β-cell function. Vildagliptin is an orally administered glucose-lowering agent that inhibits dipeptidyl peptidase-4 (DPP-4). Reduced degradation of endogenous incretin hormones, particularly glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide, enhances glucose-dependent insulin secretion and limits inappropriate glucagon release [1–5]. Reliable measurement of vildagliptin is therefore relevant to pharmaceutical analysis and to investigations in which the drug is quantified in more complex samples.

The analytical literature on vildagliptin includes reviews of its physicochemical properties, pharmaceutical analysis, and available validation approaches [6–8]. Individual procedures have employed voltammetry [9], high-performance thin-layer chromatography [10], and HPLC with UV or photodiode-array detection [11–17]. The HPLC-based studies encompass assays of vildagliptin alone and simultaneous measurements with other antidiabetic agents in bulk materials, synthetic mixtures, and finished dosage forms. These methods were designed for concentration ranges and sample preparation requirements associated with their respective applications and cannot be compared solely on the basis of instrumental sensitivity.

Relevant studies further illustrate the different analytical roles assigned to vildagliptin and linagliptin. Shah et al. validated linagliptin as a target analyte, together with empagliflozin, after mixed-mode solid-phase extraction of human plasma [18]. A separate HPLC study addressed simultaneous measurement of dapagliflozin, vildagliptin, and metformin in a tablet formulation [19]. Kim et al. developed a multi-analyte LC–MS/MS procedure for screening 26 antidiabetic drugs in dietary supplements [20], whereas Pontarolo et al. validated a HILIC–MS/MS method for simultaneous determination of vildagliptin and metformin in human plasma [21]. These procedures incorporated matrices, analyte assignments, extraction steps, calibration ranges, and validation designs that differ from those required for prepared analytical solutions.

The present study developed and evaluated an LC–MS/MS procedure for vildagliptin in calibration standard and quality-control (QC) solutions. Linagliptin was used only as a non-isotopically labelled internal standard and was not independently validated as a second target analyte. Its positive-ion response, distinct MRM transition, and chromatographic separation from vildagliptin enabled analyte to internal standard peak-area ratios to be calculated. Placebo preparations containing representative pharmaceutical excipients were examined for detectable chromatographic interference. The study did not address degradation products, impurities, commercial-tablet extraction, or biological-matrix analysis; applications in those settings would require separate sample-preparation and validation studies.

2. Material and Methods

2.1. Chemicals and Reagents

Certified vildagliptin and linagliptin reference materials (purity ≥ 99.0%) were supplied by Sigma-Aldrich (USA), together with analytical-grade ammonium acetate and formic acid. Merck (Germany) supplied the HPLC-grade acetonitrile. Water was purified with a Milli-Q system (Millipore, USA), and the reagents were used without additional treatment

2.2. Instrumentation and LC-MS/MS Conditions

Measurements were obtained with an Agilent 6420 triple-quadrupole LC–MS/MS instrument. An ACE C18 column (50 mm × 2.1 mm, 5 μm) held at 35 °C provided the chromatographic separation. The aqueous eluent (A) was 10 mM ammonium acetate with 0.01% (v/v) formic acid, and the organic eluent (B) was HPLC-grade acetonitrile with the same formic-acid content. Table 1 gives the time-dependent solvent composition. Elution proceeded at 0.3 mL min^{-1}, using a 5 μL injection and a 7.5-min cycle.

Table 1. Gradient elution program for the liquid chromatography method.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	95	5
1.0	95	5
1.5	50	50
2.0	10	90
3.0	10	90
3.5	50	50
4.0	95	5
7.5	95	5

Ion production used positive-mode electrospray. Source settings were a drying-gas temperature of 300 °C, a drying-gas flow of 10 L min^{-1}, a nebulizer pressure of 40 psi, and a capillary potential of 4000 V. MRM acquisition included a quantitative and a confirmatory transition for vildagliptin. The cell-accelerator voltage was 7 V; compound-specific transitions, fragmentor voltages, and collision energies are listed in Table 2.

Table 2. Optimized MRM parameters for vildagliptin and linagliptin (IS).

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Polarity
Vildagliptin	304.0	154.0	100	14	Positive
Vildagliptin	304.0	96.9	100	32	Positive
Linagliptin (IS)	472.9	419.9	170	24	Positive

The internal standard signal was generated from linagliptin at m/z 472.9 → 419.9. This transition was distinct from those of vildagliptin, and linagliptin also produced an adequate response in the positive-ion method and a separate chromatographic peak. These properties allowed the analyte/IS area ratio to be calculated without transition overlap. Nevertheless, linagliptin is neither isotopically labelled nor co-eluting with vildagliptin; complete correction of compound-specific matrix effects was therefore not inferred from its use.

2.3. Preparation of Calibration Standards and Quality Control (QC) Solutions

Accurately weighed vildagliptin and linagliptin reference materials were dissolved separately in acetonitrile to obtain 1.0 mg mL^{-1} primary stocks, which were kept at 4 °C. Dilution with the mobile phase produced vildagliptin calibrators at 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 ng mL^{-1}. Independently prepared QC solutions represented low (0.25 ng mL^{-1}), medium (1.0 ng mL^{-1}), and high (2.0 ng mL^{-1}) levels. The stated concentrations are those of the solutions delivered to the instrument. Neither commercial tablets nor biological samples underwent extraction or other sample preparation in this study; the placebo samples were examined only for possible excipient interference.

2.4. Method Validation Procedure

Validation of vildagliptin measurement was planned with reference to ICH Q2(R2) [22]. The experiments addressed specificity, calibration linearity and range, LOD, LOQ, repeatability, intermediate precision, and analytical recovery. Peak-area ratios were calculated with the linagliptin signal, but no separate validation was performed for linagliptin as an analyte. The protocol did not encompass biological-matrix validation, matrix-dependent preparation procedures, or a quantitative assessment of ionization effects.

Six placebo preparations were inspected for a vildagliptin-related MRM response. A linear model was fitted to the six calibrator levels spanning 0.125–4.0 ng mL^{-1}. Approximate signal-to-noise ratios of 3:1 and 10:1 served as the respective bases for LOD and LOQ estimation. At the LOQ, six measurements were used to confirm precision and bias below 10%. Same-day repeatability comprised six measurements at every QC level, whereas intermediate precision covered the three QC levels on three successive days. Recovery was likewise determined from six measurements per level. Percentage recovery was obtained from the ratio of calculated to nominal concentration multiplied by 100, and its dispersion was reported as %RSD.

The evaluation bases and acceptance criteria that were explicitly documented in the available validation records are summarized in Table 3. ICH Q2(R2) does not prescribe universal numerical limits for the coefficient of determination, precision RSD, or percentage recovery; these limits should be defined in a validation protocol according to the intended purpose of the analytical procedure. Consequently, numerical limits that were not documented in the source validation records were not retrospectively attributed to ICH.

Table 3. Validation parameters and evaluation criteria applied to the reported analytical procedure.

Parameter	ICH Q2(R2)-aligned evaluation basis	Criterion or reporting basis used in this study
Specificity	Assessment of interference from relevant sample components	No analyte-related MRM signal at the vildagliptin retention time in six placebo preparations
Linearity and range	Evaluation across the intended range using at least five concentration levels and an appropriate regression model	Six levels from 0.125 to 4.0 ng mL^{-1}; regression equation and R^2 were reported. No separate numerical R^2 limit was documented in the source protocol
LOD	Signal-to-noise ratio of approximately 3:1 when the signal-to-noise approach is used	S/N approximately 3:1
LOQ	Signal-to-noise ratio of at least 10:1 and confirmation of suitable performance at the quantification limit	S/N at least 10:1; six replicate measurements with precision and bias below 10%
Repeatability and intermediate precision	Reporting of variability using standard deviation or relative standard deviation under the specified conditions	Intra-day and inter-day %RSD values were reported; no additional numerical acceptance limit was documented in the source protocol
Accuracy	Comparison of measured and accepted reference values across the reported range	Mean calculated concentration, mean recovery, and recovery %RSD were reported at three QC levels (n = 6); no separate numerical recovery interval or RSD limit was documented in the source protocol

3. Results and Discussion

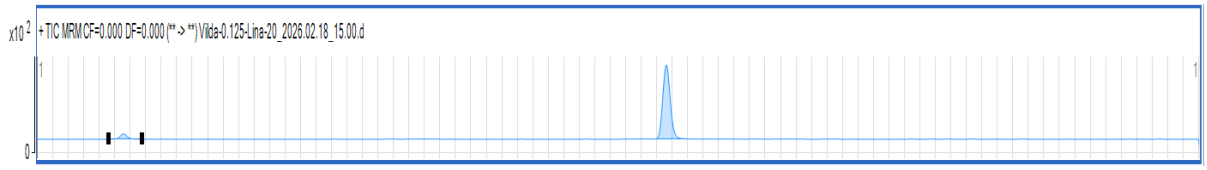
3.1. Chromatography and Specificity

The vildagliptin and linagliptin signals appeared at approximately 0.56 and 4.06 min, respectively, during the 7.5-min chromatographic cycle. Figure 1 shows chromatograms from three positions within the validated range, allowing the retention times and peak profiles to be viewed. The concentration–response relationship is reported separately in Figure 2.

None of the six placebo preparations produced a detectable analyte-related MRM peak at 0.56 min. Thus, the tested excipients caused no observable chromatographic interference at the vildagliptin retention time. This observation concerns peak interference only, because the experiment did not measure ion suppression or enhancement.

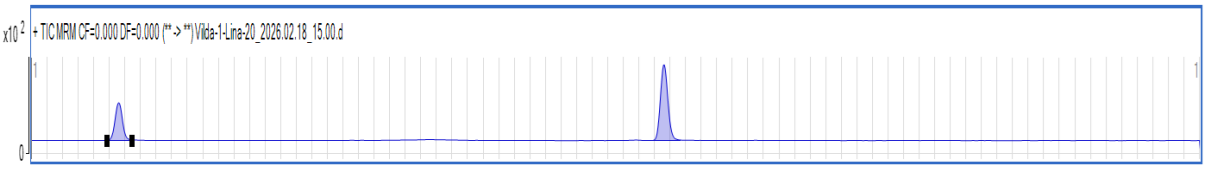
(A) 0.125 ng mL^{-1}

Vildagliptin RT ~ 0.56 min; linagliptin (IS) RT ~ 4.06 min



(B) 1.0 ng mL^{-1}

Vildagliptin RT ~ 0.56 min; linagliptin (IS) RT ~ 4.06 min



(C) 4.0 ng mL^{-1}

Vildagliptin RT ~ 0.56 min; linagliptin (IS) RT ~ 4.06 min

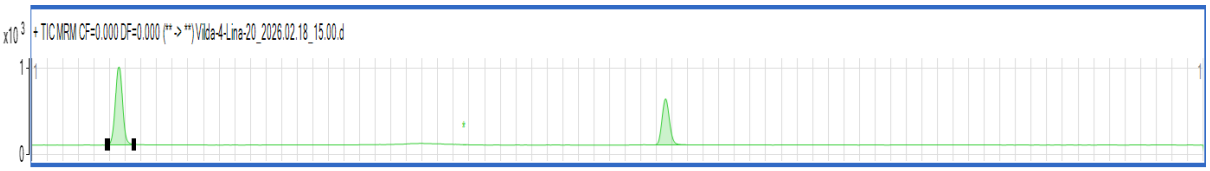


Figure 1. MRM chromatograms recorded for vildagliptin standards at (A) 0.125, (B) 1.0, and (C) 4.0 ng mL^{-1}.

The vildagliptin transition (m/z 304.0 → 154.0) gave a peak near 0.56 min, and the linagliptin transition (m/z 472.9 → 419.9) gave a peak near 4.06 min. Because each chromatogram was exported with separate signal scaling, peak heights should not be compared directly.

None of the six placebo preparations produced a detectable analyte-related MRM peak at 0.56 min. Thus, the tested excipients caused no observable chromatographic interference at the vildagliptin retention time. This observation concerns peak interference only, because the experiment did not measure ion suppression or enhancement.

3.2. Linearity and Calibration Curve

Calibration used six vildagliptin levels: 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 ng mL^{-1}. In Figure 2, nominal concentration is plotted against the vildagliptin/linagliptin area ratio. Regression gave y = 0.4317x + 0.0557 and a coefficient of determination of 0.9951 (Table 4).

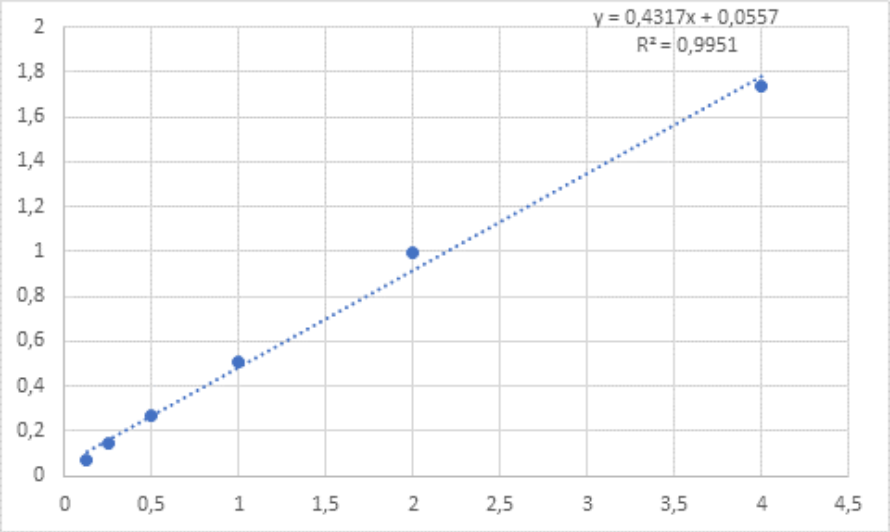


Figure 2. Vildagliptin calibration data from 0.125 to 4.0 ng mL^{-1}, plotted as analyte/linagliptin peak-area ratio against nominal analyte concentration. The fitted equation and R^2 are displayed on the graph.

Table 4. Linear regression statistics for the Vildagliptin calibration curve.

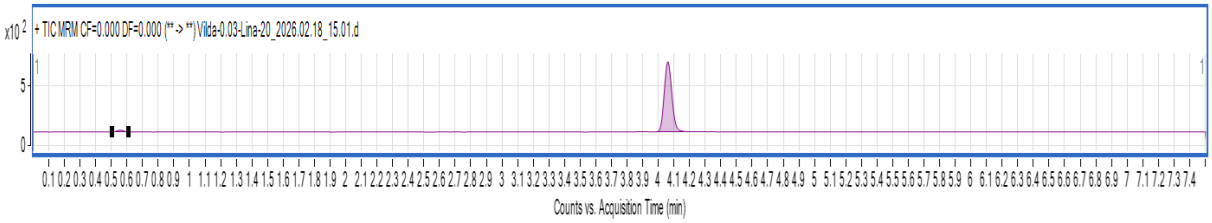
Statistical Parameter	Value
Multiple R	0.9976
R Square (R^2)	0.9951
Adjusted R Square	0.9939
Standard Error	0.0499
Observations (n)	6

3.3. Sensitivity

Signal-to-noise evaluation gave an LOD of 0.028 ng mL^{-1} at approximately 3:1 and an LOQ of 0.084 ng mL^{-1} at no less than 10:1. At 0.084 ng mL^{-1}, both precision and bias were below 10% across six independent measurements. These limits refer to the final solutions analyzed by the instrument.

Figure 3 contrasts a 0.03 ng mL^{-1} solution, included as a visual example of near-LOD detection, with the 4.0 ng mL^{-1} upper calibrator. The lower solution was not a point in the fitted calibration model.

(A) 0.03 ng mL^{-1}



(B) 4.0 ng mL^{-1}

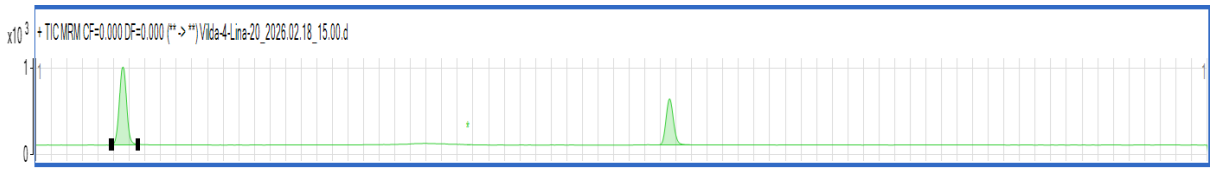


Figure 3. MRM response for (A) a 0.03 ng mL^{-1} vildagliptin solution shown as a near-LOD detectability example and (B) the 4.0 ng mL^{-1} upper calibrator. Only the latter concentration belonged to the quantitative calibration set.

3.4. Precision and Repeatability

Precision experiments used QC concentrations of 0.25 ng mL^{-1} (LQC), 1.0 ng mL^{-1} (MQC), and 2.0 ng mL^{-1} (HQC). Repeatability was derived from six independently measured solutions at each level within one day. For intermediate precision, the same three levels were examined on three consecutive days.

The same-day RSD values were 1.79–1.98%, while the across-day values were 1.88–2.15% (Table 5). The observed variability summarizes method precision for the solutions and conditions examined.

Table 5. Intra-day and inter-day precision for the determination of vildagliptin.

Concentration (ng mL^{-1})	Intra-day Precision (%RSD, n = 6)	Inter-day Precision (%RSD, 3 days)
0.25	1.79	1.88
1.0	1.98	2.15
2.0	1.85	1.98

3.5. Accuracy and Recovery

For each QC level, the concentration calculated in six measurements was compared with the nominal value. The resulting mean recoveries were 99.12–101.7%, with RSD values between 2.00% and 3.29% (Table 6). Because these measurements were made in prepared solutions, they characterize analytical accuracy rather than recovery from an extracted tablet.

Table 6. Accuracy and recovery data for the determination of vildagliptin (n = 6 at each QC level).

Nominal Concentration (ng mL^{-1})	Calculated Concentration (ng mL^{-1})	Mean recovery (%)	Recovery RSD (%)
0.25	0.2478	99.12	2.00
1.0	0.9985	99.85	3.29
2.0	2.034	101.7	2.41

3.6. Validation Context and Role of the Internal Standard

The reported validation characteristics belong to vildagliptin in the prepared solutions. Linearity, sensitivity, precision, and analytical recovery were examined across or within the 0.125–4.0 ng mL^{-1} working range. Linagliptin contributed only the denominator of the analyte/IS area ratio and was not subjected to a second analyte-validation scheme. All performance values therefore describe vildagliptin.

Vildagliptin (m/z 304.0 → 154.0) and linagliptin (m/z 472.9 → 419.9) were distinguished by both transition and retention time (approximately 0.56 and 4.06 min). This separation enabled area-ratio calculation in the standard solutions. It does not, however, show that linagliptin corrects fully for analyte-specific ionization effects, because the internal standard is non-isotopically labelled and does not co-elute with the analyte.

3.7. Comparison with Reported Methods

Conventional RP-HPLC-UV/PDA procedures, including the methods reported by Kumar et al. [14], Prajapati et al. [16], and Narikimalli and Galla [17], were developed for pharmaceutical dosage forms and used concentration ranges and sample-preparation procedures appropriate to those applications. In comparison, previously published LC–MS/MS procedures addressed different analytical settings. Pontarolo et al. determined vildagliptin and metformin in human plasma over 5–500 ng mL^{-1} and included matrix-specific bioanalytical validation [21]. Shah et al. determined linagliptin and empagliflozin following solid-phase extraction from human plasma [18], whereas Kim et al. reported a multi-analyte LC–MS/MS procedure for antidiabetic drugs in dietary supplements [20].

In the current study, the 7.5-min separation and compound-specific MRM transitions were assessed only with calibrators and QC solutions. Published procedures cannot be ranked directly on this basis, since their matrices, analyte assignments, preparation steps, concentration ranges, and validation objectives are not equivalent.

The mass transitions used in LC–MS/MS provide analyte-identification information that differs from UV absorbance detection. Vildagliptin eluted at approximately 0.56 min, whereas linagliptin eluted at approximately 4.06 min. The placebo experiment established the absence of a detectable interfering MRM peak under the tested conditions, but quantitative ion-

suppression or enhancement experiments were not part of the standard-solution validation. Similarly, commercial-tablet extraction, filtration, dilution, and dosage-form recovery were outside the study scope. Extension of the procedure to commercial tablets, co-formulations, degradation samples, or biological matrices would therefore require application-specific sample preparation and validation.

4. Conclusion

The evaluated LC–MS/MS procedure quantified vildagliptin in prepared solutions by reference to a linagliptin internal-standard signal. Over 0.125–4.0 ng mL^{-1}, the regression model produced R^2 = 0.9951; the instrumental detection and quantification limits were 0.028 and 0.084 ng mL^{-1}. Precision remained at or below 2.15% RSD, and six measurements per QC level gave mean recoveries of 99.12–101.7% with recovery RSD values of 2.00–3.29%. No vildagliptin-related interference was detected in the placebo chromatograms. These findings support use with analytical standard solutions under the stated conditions. Separate preparation and validation studies would be necessary before applying the procedure to dosage forms or biological samples.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

All authors contributed significantly to the conception, execution, and documentation of this research. Burcu Eser was responsible for designing and executing the comprehensive method validation protocols to ensure analytical reliability, as well as optimizing the sample preparation procedures. Esra Merve Zanbak Çotaoğlu and Kübra Kılıç conducted the systematic sample preparation steps in the laboratory, managed data acquisition, performed the rigorous statistical data analysis, and provided critical input in the interpretation of the findings. Yıldırım Cesaretli conducted the extensive literature review, and drafted the original manuscript. All authors critically reviewed the manuscript for important intellectual content and approved the final version for publication.

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