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Applications of ION Pair HPLC in Determination of Metformin Hydrochloride and Linagliptin in Tablet

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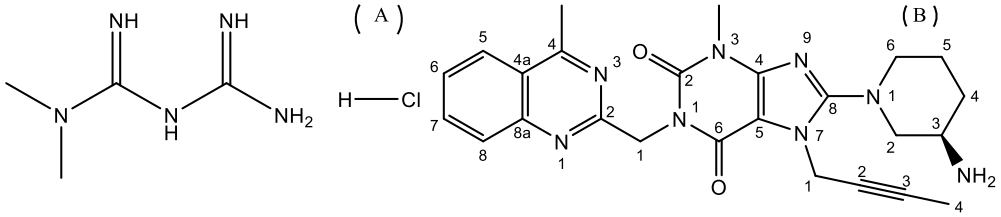
Abstract: Introduction: The combination form of metformin and linagliptin improves glucose metabolism in treating type 2 diabetes. Objective: Establishing an analytical process to determine metformin and linagliptin in tablets by reverse-phase high-performance liquid chromatography (Rp-HPLC). Methods: Various parameters, including detection wavelength, mobile phase, stationary phase, SDS and ion concentration, pH value, flow rates, sample injection volumes, and column temperatures, were systematically investigated to optimize the chromatographic procedure. Results: Optimized chromatographic conditions for determining metformin and linagliptin are followed: Zorbar eclipse plus C18 column (100 x 4.6 mm, 3.5 µm), PDA detector (detection wavelength of 226 nm). The mobile phase was a mixture of methanol, acetonitrile, and phosphate buffer solution containing 1.75 mM SDS at pH 6.8 (7:33:60, v/v/v); Flow rate was 1 mL/min; Sample injection volume was 10 µl; and the column oven temperature was set at 40ºC. The linear ranges of metformin and linagliptin are 7.66 - 45.97 µg/mL and 1.25 - 7.52 µg/mL, respectively (R^2> 0.999). A metformin and linagliptin quantification method has been successfully established and validated by the Rp-HPLC method. Conclusion: The procedure was developed and validated according to ICH guidelines and applied to determine metformin and linagliptin in combination tablets.

Keywords: Metformin hydrochloride, Linagliptin, Rp-HPLC, Validation

I. INTRODUCTION

Metformin hydrochloride (MET, Figure 1a) and linagliptin (LNG, Figure 1b) are commonly used for treating type 2 diabetes mellitus. Metformin is an oral hypoglycemic agent that decreases glucose production in the liver and increases insulin sensitivity in muscle and adipose tissue. Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that increases insulin secretion and decreases glucagon secretion, lowering blood glucose levels. It is essential to develop a reliable method for simultaneously quantifying these drugs in tablets to ensure drug quality and efficacy [1],[2].



Metformin hydrochloride Linagliptin

Figure 1: Chemical structure of MET (a) and LNG (b)

Several analytical methods have been reported for the simultaneous analysis of the combination form of two antidiabetic drugs, including Rp-HPLC methods [1], [2], [3], [4], [5]. These reported Rp-HPLC techniques have common drawbacks: metformin does not interact strongly with the stationary phase and, therefore, is not significantly retained or separated. As a result, it was diluted out of the column along with the solvent, resulting in a non-selective method. The published method [1] had a short analysis time, but the detection wavelength for metformin and linagliptin was less sensitive, and the criteria of chromatographic system compatibility were not validated. In this study, significant changes in chromatographic conditions are meticulously researched to determine the optimal values for the Rp-HPLC parameters to achieve specificity, accuracy, precision, and repeatability for quantifying MH and LNG in tablets. The method was established according to the general ICH guidelines [6].

II. MATERIALS AND METHODS

2.1. Chemicals

The chemicals and reagents employed in the chromatographic analysis were of high quality and met the required standards. Methanol (Merck) was used as the organic component in the mobile phase. Ultrapure water, obtained from Pall Casada III water filtration system, adhered to rigorous chromatography standards and was utilized as a solvent. Sodium dodecyl sulfate (SDS, Sigma-Aldrich) satisfied the analysis requirements. Metformin hydrochloride (97% purity) was supplied by the Institute of Drug Testing in Ho Chi Minh City. Similarly, linagliptin (97% purity) was purchased from Toronto Research Chemicals, Canada.

2.2. Chromatographic conditions

The chromatographic conditions employed for determining metformin and linagliptin were as follows: a Zorbar Eclipse Plus C18 column with dimensions of 100 x 4.6 mm, a particle size of 3.5 µm and photodiode array (PDA) detector at the wavelength of 226 nm. The mobile phase contained a mixture of acetonitrile, methanol, 20 mM phosphate buffer solution, and 1.75 mM SDS at a pH of 6.8 at a volumetric ratio of 7:33:60. The flow rate was maintained at 1 mL/min and 10 µL sample injection volume was used. The column oven temperature was precisely set at 40°C to optimize the separation process of the target compounds.

2.3. Preparation of the buffer solution

The buffer solution was prepared by dissolving 2.72 g KH_2PO_4 in 500 mL demineralized water and adding 8.8 mL of a 200 mM SDS solution. The total volume was carefully adjusted to 1000 mL and mixed well. The 2M KOH solution was incrementally added and stirred until the desired pH of 6.8. Subsequently, the buffer mixture was filtered through a 0.45 µm nylon filter and degassed for 15 minutes.

2.4. Preparation of the standard solution

Two stock standard solutions of MET (1000 µg/mL) and LNG (500 µg/mL) were prepared in 50% methanol. The stock solutions were diluted with mobile phase to obtain the working standard solution at 25 µg/mL concentrations and 5 µg/mL for MET and LNG, respectively.

2.5. Preparation of the sample solution

Twenty tablets of different strengths (2.5/500 mg, 2.5/850 mg, and 2.5/1000 mg) were selected. The tablet film-coating was weighed after being removed and finely ground in a ceramic mortar. An aliquot of drug powder equivalent to the calculated average weight of a single tablet was transferred into a 100 mL volumetric flask. Approximately 70 mL of methanol was added, ultrasound for 30 minutes, and set to the mark. The resulting mixture was then centrifuged at 6000 rpm for 15 min. The supernatant was carefully collected, appropriately diluted with the mobile phase, and filtered through a 0.45µm membrane filter. Finally, the filtered solution was ready for subsequent chromatographic analysis.

2.6. Analytical method establishment

The experimental setup utilized an Agilent Technologies chromatography system, model 1260 Infinity, with a PDA detector. The chromatography conditions included a C18 column (100 x 4.6 mm, 3.5 µm), a mobile phase comprising a mixture of acetonitrile and water (with a 1 mM SDS ion-pairing agent and 10 mM buffer solution) at the ratio of 40:60 (v/v); a flow rate of 1 mL/min, a sample injection volume of 10 µL, and a column temperature set at 40ºC.

Initially, MET and LNG standard solutions were used to survey and select appropriate detection wavelengths. Subsequently, mobile phase investigations were performed using various combinations of methanol, acetonitrile, and H_2O with different buffer systems (CH_3COONH_4, KH_2PO_4) to achieve optimal separation and chromatographic parameters, ensuring sharp and balanced peaks.

The ion pair formation factors included SDS ranging from 0.5 to 2.0 mM in the mobile phase and the ion concentration ranging from 5 to 25 mM in the buffer. Furthermore, the pH value was surveyed between 5.0 and 6.8. Alongside these considerations, various technical parameters of the HPLC system, such as the flow rate within the range of 0.8 to 1.4 mL/min, the sample injection volume from 5 to 25 µL, and column temperature ranging from 30 to 40°C were assessed.

Stationary phase investigations involved the assessment of three different columns (i) Zorbar Eclipse Plus C18 (100x4.6 mm; 3.5µm), (ii) Pursuit XRs C18 (150x4.6 mm; 5 µm), and (iii) Gemini® NX-C18 (250x4.6 mm; 5 µm), based on parameters such as retention time, peak area, asymmetry coefficient, resolution, and number of theoretical plates.

Finally, the analytical procedure was built through careful selection of the values of each experimental factor, ensuring the method is suitable and meets the expected analytical requirements such as selectivity, sensitivity and chromatographic parameters such as peak sharpness, symmetry, tailing coefficient and resolution between peaks.

2.7. Analytical method validation

The optimized method for the determination of MET and LNG was validated following the guidelines of the International Conference on Harmonization (ICH) Q2 (R1) [6]. The assessed criteria consisted of the system suitability, specificity, repeatability, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ).

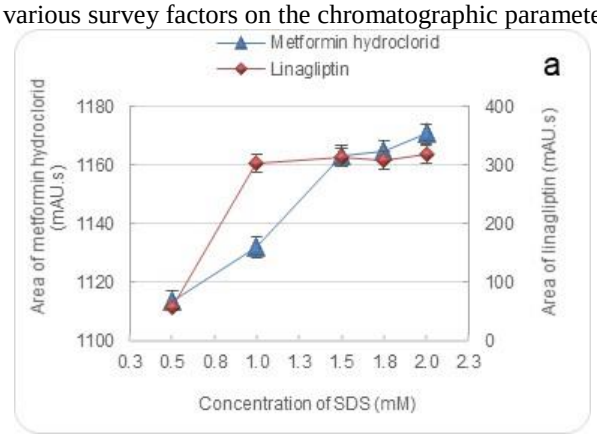
III. RESULTS AND DISCUSSION

3.1. Chromatographic method development

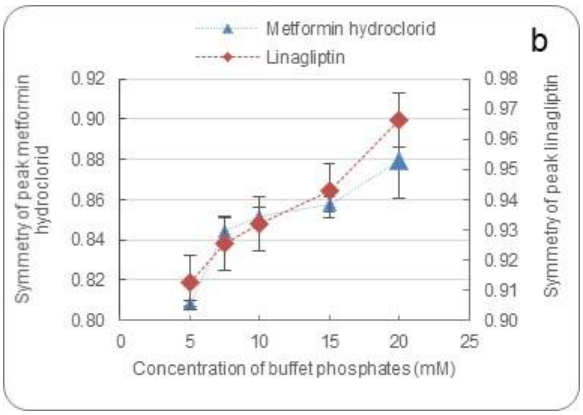
This optimization process necessitated carefully adjusting various critical parameters, including the mobile phase composition, pH, temperature, stationary column selection, and flow rate. Quantitative analysis of MET presented a formidable challenge in Rp-HPLC utilizing a C18 column due to its polar nature, characterized by a low logarithm of the partition coefficient (log P) of -1.43, signifying a high degree of polarity. In contrast, LNG exhibits relatively lower polarity, with a log P value of 2.4. Consequently, LNG can be effectively retained on the C18 column as previously documented literature. The distinctive chemical characteristics of MET and LNG lead to successful separation within complex mixtures.

Some prior studies have reported the efficacy of Rp-HPLC using the C18 column for retaining and separating MET and LNG. However, repeating the published parameters under current experimental conditions brought unexpected results wherein MET failed to retain the column and then co-eluted with the solvents and excipients.

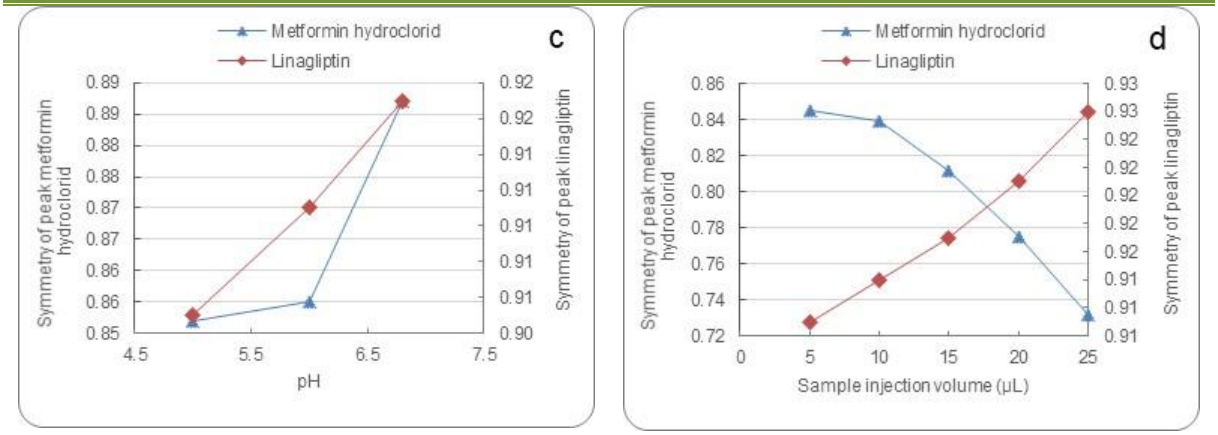
To ensure the analytical method's requisite specificity, accuracy, and precision, it becomes imperative to establish rigorous criteria for mobile phase selection, ensuring complete separation from interfering constituents such as excipients. Subsequently, a viable solution to overcome this challenge was achieved through ion-pair chromatography by introducing SDS. This modification effectively resolved the issue, facilitating the reliable and precise quantification of MET and LNG in combined preparation form. Figure 2 illustrates the impact of various survey factors on the chromatographic parameters.



Effect of SDS concentration



Effect of buffer concentration



Affect of pH Effect of sample injection volume

Figure 2: Influence of survey factors on chromatographic parameters of MET and LNG signals

The investigation focused on optimizing separation conditions, evaluating varying SDS ratios, buffering capacity across different pH levels, and incorporating organic components within the mobile phase. Results obtained at a 1.75 mM SDS concentration revealed consistent and stable peak area signals for MET and LNG(Figure 2a). Notably, increased pH enhanced the chromatographic signals for both analytes, with pH 6.8 and 20 mM KH_2PO_4 (Figure 2b and 2c). Careful consideration was given to the proportion of organic solvent in the mobile phase to prevent precipitation when combined with SDS. The buffering solution 20 mM KH_2PO_4 at pH 6.8 exhibited retention enhancement of MET within the chromatography column. Other factors were thoroughly determined, such as column temperature of 40^oC and injection volume 10 µL (Figure 2d). A wavelength of 226 nm was selected for the analytical signal recording of MET and LNG. The optimal mobile phase composition consisted of a mixture of 60% buffer solution (comprising 20 mM KH_2PO_4 and 1.75 nM SDS in water, pH adjusted to 6.8 with KOH) mixed with 33% acetonitrile và 7% methanol. The stationary column was the Zorbar Eclipse Plus C18 column (100 x 4.6 mm; particle size 3.5 µm), which is evident in the chromatogram in Figure 3.

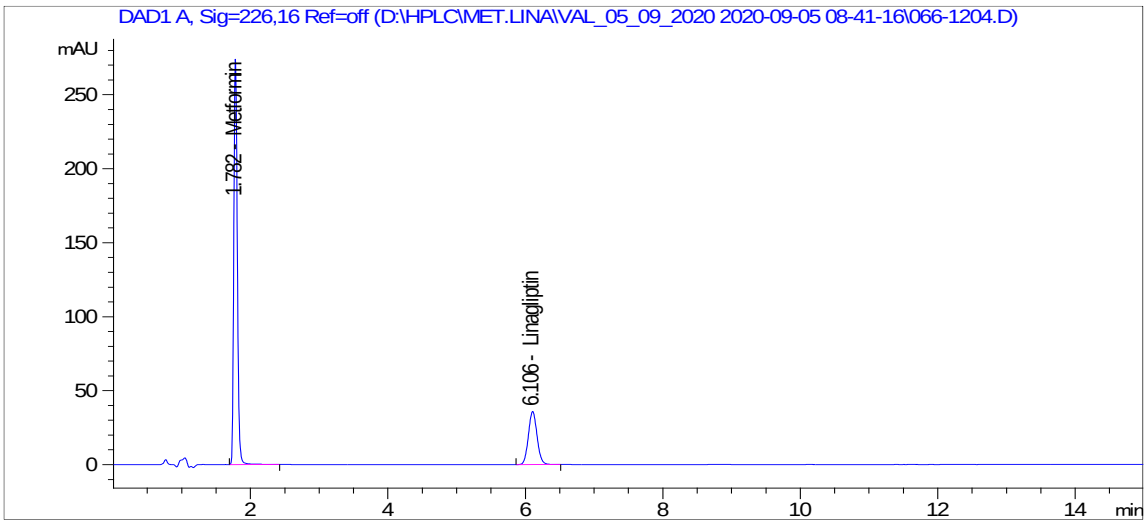


Figure 3: A typical chromatogram of MET 25 ppm and LNG 5 ppm under selected conditions (Zorbar Eclipse Plus C18 column, detection wavelength 226 nm, a mobile phase composition of 60% buffer solution (including 20 mM KH_2PO_4 and 1.75 nM SDS dissolved in water with a pH of 6.8), 33% acetonitrile, and 7% methanol)

3.2. Analytical method validation

3.2.1. Selectivity

The method involves comparing the relative retention time of a reference substance with that of a spiked test sample, along with the evaluation and comparison of the UV spectra and the purity of the chromatographic signals. The obtained results indicated that the peak purity was over 99.99% for each analyte, and the UV spectra are illustrated in Figure 4.

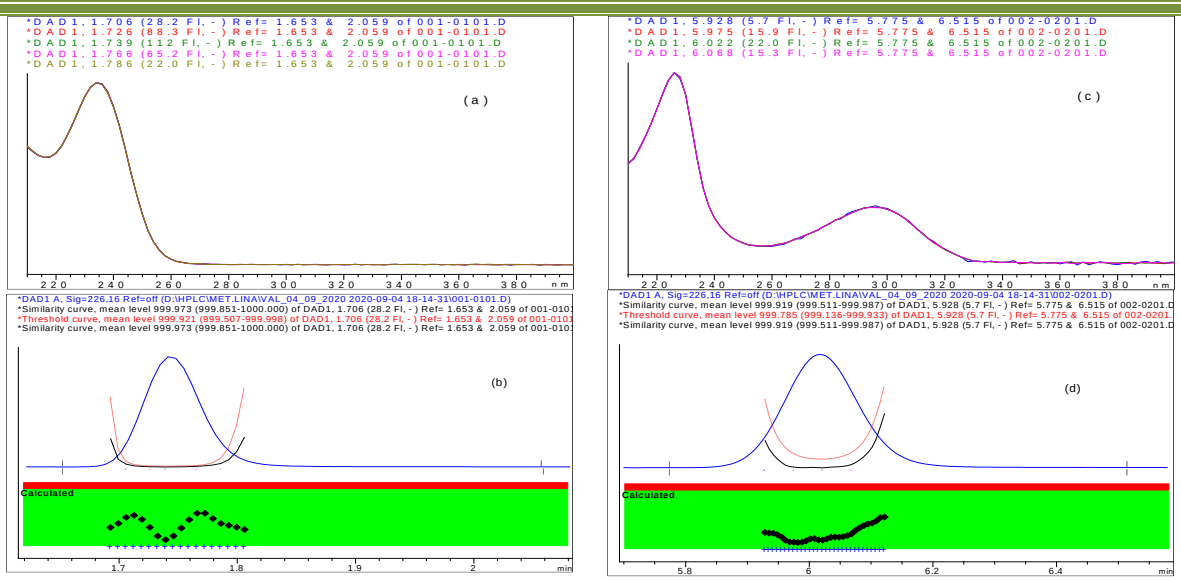


Figure 4: UV spectrum and peak purity at retention time of MET (a, b,) and LNG (c, d)

3.2.2. System suitability

The results from six consecutive injections of the analytes revealed that the investigated parameters remained consistent, facilitating the retention and separation of analytes with retention times of 4.1 and 6.9 min for MET and LNG, respectively. The observed resolution between the two peaks was notably excellent, as evidenced by relative standard deviation percentages (% RSD) of retention times consistently below 0.3%. This low % RSD showed the repeatability of the replicated injections on the Rp-HPLC system.

Furthermore, the tailing coefficient for the MET and LNG peaks was below 1.25, indicating good peak symmetry (values below 2 are deemed acceptable). Additionally, the number of theoretical plates was higher than 3000 in all chromatographic runs. These findings, systematically shown in Table 1, proved the robustness and reliability of the developed analytical method.

Table 1. System suitability results

		Retention time Rt (min)	Peak area (mAU.s)	Symmetry coefficient	Number of theoretical
MET	Average (n=6)	1.78	978.06	0.81	5602
	RSD (%)	0.09	0.36	0.78	0.63
LNG	Average (n=6)	6.11	316.03	0.91	11309
	RSD (%)	0.16	0.49	0.54	0.70

3.2.3. Linearity

Linearity testing was conducted with the preparation of six increasing concentration levels for each analyte: 1.25, 2.50, 3.75, 5.00, 6.25, and 7.50 µg/mL for LNG and 7.50, 15.00, 22.50, 30.00, 37.50, and 45.00 µg/mL for MET. Subsequently, 10 µL of each test solution was injected into the HPLC system, where the peak area for each analyte was recorded. The average peak areas from two chromatographic injections at each concentration level were calculated in linear regression equations to establish analytical curves with high correlation coefficients (R^2 values of approximately 0.9997 for LNG and 0.9994 for MET). The data of linearity assessment can be observed in Figures 5 and 6.

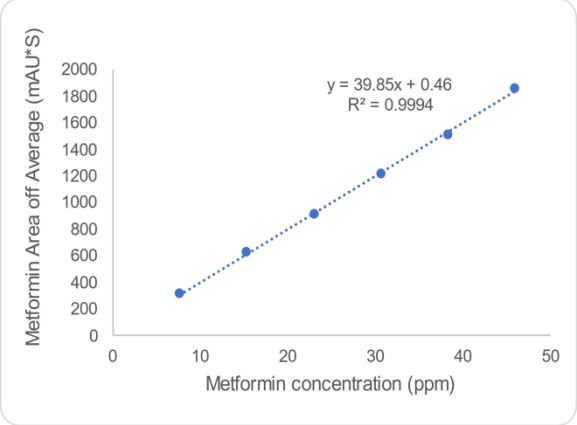


Figure 5: The correlation between concentration and peak area of MET

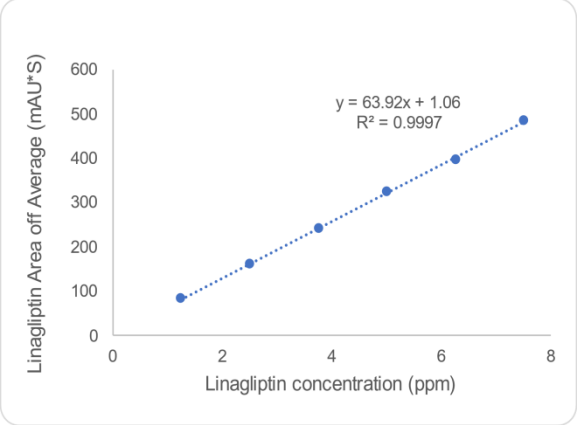


Figure 6: The correlation between concentration and peak area of LNG

3.2.4. LOD and LOQ

The standard deviation and slope of the linear regression equation were used to calculate the limits of detection (LOD) and quantification (LOQ) for analytes. The LOD was found to be 1.92 µg/mL for MET, indicating the lowest concentration that can be reliably detected but not necessarily quantified. The LOQ for MET was calculated as 5.83 µg/mL, signifying the lowest concentration of MET that can be reliably detected and quantified with acceptable precision and accuracy. Similarly, LNG were 0.35 µg/mL and 1.07 µg/mL for LOD and LOQ, respectively.

3.2.5. Accuracy and repeatability

Precision reflects how closely the average test results approach the actual value. In precision assessment, simulated sample preparations at three different concentration levels (80%, 100%, and 120% compared to the quantitative concentration) were carried out. Chromatographic analyses were performed on the same and different days under the chosen conditions. The recovery rate (%) and repeatability of the test were calculated. The intra-day average recovery for MET and LNG ranged from 98.27% to 101.00% and 98.20% to 101.57%, respectively. The inter-day values fluctuated from 98.43% to 100.94% for MET and 98.53% to 101.88% for LNG. All relative standard deviation (RSD) measurements were ≤ 2.0% for each experimental batch. A summary of these analysis results is presented in Table 3.

Table 3: The results of accuracy and precision

		Intra-day			Inter-day		
	Added (µg/mL)	Concentration found (µg/mL)	Recovery (%)	RSD (%)	Concentration found (µg/mL)	Recovery (%)	RSD (%)
MET	20	20.20	101.00	0.66	20.19	100.94	1.21
	25	24.58	98.33	0.89	24.71	98.85	0.64
	30	29.48	98.27	0.30	29.53	98.43	0.43
LNG	4	4.06	101.57	0.93	4.08	101.88	1.00
	5	4.99	99.79	0.60	5.00	100.09	0.58
	6	5.89	98.20	1.54	5.91	98.53	1.65

3.2.8. Analysis of commercial drug tablet samples

The described method was applied to analyze tablets containing the active ingredient LNG/MET at a 2.5/1000 mg concentration. The test results yielded average measurements of 101.31% and 104.19% for LNG and 98.45% and 99.32% for MET on sample batches. Statistical analysis showed that both analytes' relative standard deviation (RSD) values were below 2.0%. The assay results, presented in Table 4, demonstrate that the method is selective and capable of accurately quantifying MET and LNG without being influenced by other components in the preparation form.

Table 4. The quantitative results of 2 sample batches

N.o	Analyte	Content on the label	Measured content compared to label (%)	RSD (%)
NC001	MET	1000.00	98.45	1.30
	LNG	2.50	101.31	1.80
NC002	MET	1000.00	99.32	1.16
	LNG	2.50	104.19	1.65

IV. CONCLUSION

The combination of ion pair agent and chromatographic conditions has successfully established optimized parameters for determining metformin and linagliptin. According to the International Conference on Harmonization (ICH) guidelines, the method validation has demonstrated its accuracy, precision, and reproducibility, thus affirming its robustness and reliability. Consequently, this analytical method holds significant utility within the pharmaceutical industry, particularly for the analysis of metformin and its diverse combination products.

REFERENCE

[1]. Vemula, P., D. Dodda, U. Balekari, S. Panga, and C. Veeresham, Simultaneous determination of linagliptin and metformin by reverse phase-high performance liquid chromatography method: An application in quantitative analysis of pharmaceutical dosage forms, *Journal of Advanced Pharmaceutical Technology & Research*, *6(1)*, 2015, 25-8.

[2]. Raj, Moncy, M. Reddy, K. Sunil, M. Chaitanya, M. Priyanka, H. Bindu, P. Palem, and P. Visakhapatnam, Simultaneous determination of metformin hydrochloride and linagliptin by RP-HPLC in bulk and pharmaceutical formulations, *Indo American Journal of Pharmaceutical Research, 4(10)*, 2014, 4047-4053.

[3]. Ahmed, A., G. Khan, B. Mustaqueem, A. Yaasir, and Z. Inamdar, Analytical method development and validation for simultaneous estimation of Metformin and Linagliptin, *International Journal of Research in Advent Technology*, *7(4S)*, 2019, 40-44.

[4]. Varaprasad, C., M. Asif, and K. Ramakrishna, RP-HPLC method for simultaneous estimation of metformin and linagliptin in tablet dosage form, *Rasayan Journal of Chemistry*, *8(4)*, 2015, 426-432.

[5]. Sudharani, N., M. Bollepally, and R.B. Konduri, A validated RP_HPLC method for the simultaneous estimation of metformin hydrochloride & lingliptin in its bulk and pharmaceutical dosage form, *World Journal of Pharmaceutical Research*, *6(17)*, 2017, 365-380.

[6]. Guideline, I.H.J.I.G., Switzerland, Validation of analytical procedures Q2 (R2), 2022.

