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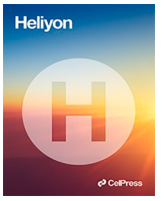

Research article

# Comparative performance of liquid chromatography and spectrophotometry in determining metformin hydrochloride within pharmaceutical formulations

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ABSTRACT

The present study compared the performance of Ultra-high performance liquid chromatography (UHPLC) and UV–Vis spectrophotometry for the quantification of metformin hydrochloride in five commercially available metformin hydrochloride products with different strengths. The metformin hydrochloride was measured in the UHPLC with a mobile phase consisting of a mixture of 0.05 M phosphate buffer solution and methanol (35:65, v/v) with a pH of 3.6. Metformin hydrochloride was determined spectrophotometrically at 234 nm using a mixture of methanol and water as a blank. The methods' linearity for metformin hydrochloride was within the concentration range of (2.5–40 μg/ml) in both techniques. The validation process encompassed assessments of specificity, selectivity, linearity, accuracy, precision, the lower limit of quantification (LLOQ), the lower limit of detection (LLOD), robustness, and system suitability. For the UHPLC validation method, the repeatability and reproducibility (expressed as relative standard deviation) were less than 1.578 and 2.718 %, respectively. The LLOQ for metformin hydrochloride was 0.625 μg/ml, and the LLOD was 0.156 μg/ml. For the UV–Vis spectrophotometric validation method, the repeatability and reproducibility (stated as relative standard deviation) were less than 3.773 and 1.988 %, respectively. The percentage recovery results for the five brands of metformin hydrochloride tablets were (98–101 %) and (92–104 %) for the UHPLC and UV–Vis spectrophotometric methods, respectively. In conclusion, the described methodologies were successfully employed for the quantitative analysis of metformin hydrochloride in different pharmaceutical tablet products.

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## 1. Introduction

Metformin is an antidiabetic drug in the class of biguanides [1–3]. It is most frequently prescribed as an oral medicine to treat type 2 diabetes mellitus, to control blood glucose levels [1,2]. The main mechanism of action entails the reduction of glucose synthesis in the liver, the enhancement of insulin sensitivity, and the improvement of glucose absorption in peripheral tissues [1,4,5]. Metformin supports the reduction of LDL cholesterol, the triglyceride levels and promotes weight loss in some people [1,2,6]. In addition, metformin is also prescribed to treat polycystic ovary syndrome [1–3,7]. There is an early promise indicating the role of metformin as a treatment for cancer [2,8]. In addition, metformin has been linked to a reduced incidence of cardiovascular morbidity and mortality compared to other medications used to lower glucose levels [2,9].

Metformin hydrochloride is chemically referred to as 1,1-dimethylbiguanide hydrochloride, with the molecular formula $C_4H_{11}N_5$ [10,11]. The chemical structure of metformin hydrochloride is displayed in (Fig. 1) [11].

Metformin hydrochloride is available in liquid and tablet form, and administered orally [1]. This medication can be prescribed on its own or in combination with other medications to enhance glycemic control [1]. The physical characteristics of metformin hydrochloride is a crystalline powder, white to off-white, polar molecule, and highly soluble in water [6].

The development and validation of analytical methods play a crucial role in the field of pharmaceutical analysis. This is especially important when dealing with active pharmaceutical ingredients (APIs) such as metformin, to ensure the quality, safety, and efficacy of pharmaceutical products [3,6]. However, there are challenges associated with metformin, such as the potential interference from excipients. These excipients may have similar physical or chemical properties to metformin, which makes it difficult to accurately distinguish and quantify the active ingredient [3,6,12]. It is essential to address potential interference from excipients during the method development and validation for metformin analysis to ensure the specificity and reliability of the analytical method [3,6,12]. There are limited literature related to the use of UV–visible spectrophotometry techniques to quantify metformin hydrochloride in pharmaceutical formulations [12–15]. Additionally, several UHPLC techniques have been developed and validated for the assessment of metformin hydrochloride in both bulk form and pharmaceutical products, either as a separate compound (2,6,16) or in combination with other medications [10–19]. The main focus of this study was to conduct a comparative analysis between the UHPLC and the more cost-effective UV–Vis spectrophotometry methods for the quantification of metformin hydrochloride in tablet formulation. This study aimed to provide analytical chemists, researchers, and pharmaceutical scientists with the essential resources to ensure the accuracy and precision of analytical results in pharmaceutical formulations. This will be achieved by presenting a comprehensive and structured guide for the development and validation of analytical methods, specifically tailored for metformin hydrochloride medications. The methodologies could be applied in quality control analysis and assessing the stability of metformin hydrochloride.

## 2. Experimental

### 2.1. Materials

#### 2.1.1. Drug standards and pharmaceutical products

Metformin standard powder (metformin base, Molekula®, UK) was used to prepare the standard stock solution and standard serial dilutions. Galvus Met® 1000 mg tablet (Novartis Pharma Produktion Gmbh, Germany), Glucophage® 1000 mg tablet (Merck Santé s.a.s, France), metformin Hexal® 1000 mg tablet (Hexal AG, Germany), Metfor® 500 mg tablet (Tabuk Pharmaceuticals Mfg.Co., Saudi Arabia), Omformin® 500 mg tablet (National Pharmaceutical Industries Co. (SAOG) (NPI), Oman) were analyzed. Some of the products were purchased from local Saudi Arabia pharmacy and some were obtained from the pharmacy of the National Guard-Health Affairs Hospital. The information regarding the pharmaceutical products analyzed is available in (Table 1).

#### 2.1.2. Reagents and chemicals

Potassium phosphate monobasic ≥98.0 % was purchased from Sigma–Aldrich® (USA) and Methanol CHROMASOLV™, for HPLC, ≥99.9 %, from Honeywell (USA). Phosphoric acid ≥85.0 % was obtained from Sigma–Aldrich® (USA). A Milli-Q® water ultra-pure water system (Milli-Q® Integral 5 (Millipore®, USA) was used to obtain HPLC grade water.

### 2.2. Instrumentations

The UHPLC, (1290 Infinity, Agilent Technologies) was used for the analytical method development and validation of metformin hydrochloride. The liquid chromatography system consisted of a 1290 Quaternary Pump, 1290 Sampler, 1290 Thermostatted Column Compartment (TCC), and 1290 Variable Wavelength Detector (VWD). The instrument software (Openlab EZChrom) version A.01.05

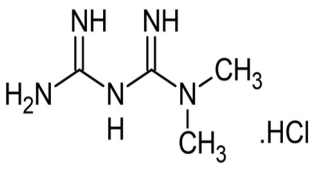


**Fig. 1.** Structure of the metformin hydrochloride.

**Table 1**
List of tested pharmaceutical products of metformin hydrochloride tablets.

| Name of Product | Strength | Active Ingredient | Type of Pharmaceutical Product | MFG | EXP | Manufacturer |
|---|---|---|---|---|---|---|
| Galvus Met® | 50 mg/1000 mg | Vildagliptin/Metformin HCL | Film-coated Tablet | 09.22 | 02.24 | NOVARTIS |
| Glucophage® | 1000 mg | Metformin Hydrochloride | Film-coated Tablet | 06.22 | 05.25 | MERCK |
| Metformin HEXAL® | 1000 mg | Metformin Hydrochloride | Film-coated Tablet | 10.22 | 09.24 | HEXAL AG (Germany) |
| Metfor® | 500 mg | Metformin Hydrochloride | Film-coated Tablet | 06.22 | 06.25 | Tabuk Pharmaceutical Mfg. Co. |
| Omformin® | 500 mg | Metformin Hydrochloride | Film-coated Tablet | 08.22 | 08.25 | NPI Pharma (Oman) |

was employed for the data collection and recording. Additionally, a UV Spectrophotometer (model UV-1800, Shimadzu Corporation) was implemented for the absorbance measurement of metformin hydrochloride. The data acquisition and recording were produced by UV Probe software version 2.34.

### *2.3. Mobile phase preparation*

The mobile phase used in this study was composed of a 0.05 M buffer solution and methanol (35:65, v/v) in isocratic conditions. A 0.05 M buffer solution was prepared in a 1 L volumetric flask by dissolving 6.8 g of potassium dihydrogen phosphate in HPLC-grade water. In total, 350 ml of phosphate buffer solution was transferred to a 1 L glass bottle, followed by 650 ml of methanol. The mixture was mixed using a super-nuova multi-place stirrer (Thermo Fisher Scientific Inc.) for 5 min and the final pH was adjusted to 3.6 using concentrated phosphoric acid. The mobile phase was subsequently filtered through a membrane filter (regenerated cellulose filter membrane 47 mm (pore size 0.45 μm), Agilent Technologies), sonicated, and degassed in an ultrasonic bath (Branson® 500) for 10 min.

### *2.4. Preparation of stock standard solution*

The metformin hydrochloride standard solution was prepared by dissolving 1000 mg of metformin standard powder in 1 L volumetric flask with a mixture of methanol: water (50:50 v/v), gently shaking the flask to dissolve the solution completely [13]. The final concentration of the metformin hydrochloride stock standard solution was (1.00 mg/ml).

### *2.5. Preparation of metformin hydrochloride standard serial dilution*

#### *2.5.1. Preparation of standard calibration curve samples*

Aliquots from the metformin hydrochloride stock standard solution (1.00 mg/ml) were added to a (50:50, v/v) mixture of methanol: water to get the calibration standard solutions over the range of (2.5–40 μg/ml) to a complete volume of 100 ml [13]. Each serial solution was mixed very well. The final concentrations of the calibration standard solutions were 2.5, 5.0, 10.0, 15.0, 20.0, and 40.0 μg/ml. The glass vial (2 ml screw vial 8 mm, Thermo Fisher Scientific Inc.) was filled with the standard solution and injected into the UHPLC system. The cuvette was filled with the test solutions to be measured through the UV–Vis spectrometry.

#### *2.5.2. Preparation of quality control samples*

Aliquots from the metformin hydrochloride stock standard solution (1.00 mg/ml) were added to (50:50, v/v) mixture of methanol: water to get low, medium, and high-quality control samples over the range of (2.5–40 μg/ml) to complete the volume to 100 ml [13]. Each serial solution was mixed very well, and the final concentrations were 3.00, 13.00, and 30.0 μg/ml (two solutions prepared for each concentration). The glass vial (2 ml screw vial 8 mm, Thermo Fisher Scientific Inc.) was filled with standard solution and injected into the UHPLC system, and a cuvette was used to measure the test solutions by UV–Vis spectrometry.

### *2.6. Preparation of pharmaceutical samples assay test of tablet formulation*

Ten tablets of Galvus Met®, Glucophage®, metformin Hexal®, Metfor®, and Omformin® were accurately weighed separately

**Table 2**
The average weight of 10 tablets of tested pharmaceutical products of metformin hydrochloride.

| Name of Product | Strength | Active Ingredient | Average weight of 10 tablets (mg) |
|---|---|---|---|
| Galvus Met® | 50 mg/1000 mg | Vildagliptin/Metformin HCL | 1191.06 |
| Glucophage® | 1000 mg | Metformin Hydrochloride | 1069.0 |
| Metformin HEXAL® | 1000 mg | Metformin Hydrochloride | 1045.5 |
| Metfor® | 500 mg | Metformin Hydrochloride | 542.3 |
| Omformin® | 500 mg | Metformin Hydrochloride | 596.0 |

using the analytical balance (XS64, Mettler-Toledo®) and the average weights were recorded, all the details in (Table 2). The ten tablets of each product were crushed separately to a fine powder, and the weight equivalent to the average weight of the tablet was taken [3,12,13]. The powder was transferred to an amber volumetric flask and diluted up to the mark with a mixture of methanol: water (50:50, v/v), and the flask was gently shaken to homogenize the solution [11]. The metformin concentration assay test solution was (1.00 mg/ml) for all pharmaceutical products. The mixture was mixed for 30 min at a speed of 300 RPM using a super-nuova multi-place stirrer (SP135930-33, Thermo Fisher Scientific Inc). The sample was immediately filtered through a syringe (BD 10 ml, Luer-Lok™ Tip) connected with a syringe filter (Nalgene™ syringe filter 25 mm (0.45 μm) PES, Thermo Fisher) into a 10 ml glass tube. A 2000 μl of the sample was pipetted using a micropipette (1000 μl, Eppendorf Research® plus) into a 100 ml amber volumetric flask and diluted to the mark with a mixture of methanol: water (50:50, v/v), gently shaken to homogenize the solution. The final concentration was (20.0 μg/ml). Five solutions were prepared for analysis. The six glass vials (2 ml screw vial 8 mm, Thermo Fisher Scientific Inc.) were labeled and 20 μl of the sample was injected into the UHPLC system. For the UV–Vis spectroscopy, the cuvette was filled with the test solution.

### 2.7. Determination of wavelength ($\lambda_{max}$)

A two ml aliquot of the stock solution with a concentration of (1.00 mg/ml) was diluted to a final volume of 100 ml using a mixture of methanol and water (50:50 v/v) [13]. The absorbance of the resulting solution was measured in the scanning mode over a wavelength range of 200–400 nm, using a blank solution consisting of a mixture of methanol and water as a reference. The maximum absorbance ($\lambda_{max}$) of metformin hydrochloride was at 234 nm, and this value was subsequently used as a parameter in the development of the UHPLC method.

## 3. Method development, optimization, and validation

This paper describes the development and validation of UV–vis spectrophotometric method compared to the UHPLC for the metformin hydrochloride in pharmaceutical formulations.

### 3.1. Reverse-phase ultra high-performance liquid chromatographic (RP-UHPLC)

#### 3.1.1. Method development

A simple, precise, sensitive, and accurate reverse-phase ultra-high-performance liquid chromatographic (RP-UHPLC) for the estimation of metformin hydrochloride was developed. Several mobile phases were initially tested to obtain an appropriate chromatogram (Table 3). The mobile phase used in the optimized method was based on selectivity, sensitivity, and acceptable chromatographic parameters of the produced peaks [3,6]. The chromatographic separation of metformin was achieved using ZORBAX Eclipse Plus C18 Rapid Resolution (4.6 × 100 mm, 3.5 μm, Agilent HPLC Column). The detection wavelength was 234 nm using a mobile phase (C) consisting of a mixture of 0.05 M buffer solution: methanol (35:65, v/v) pH 3.6 at a flow rate of 0.80 ml/min (Table 3). The separation was achieved with an injection volume of 20 μl at 25 °C with a run time of 4.0 min.

#### 3.1.2. Method validation

The proposed method was validated based on the International Conference on Harmonization (ICH) guidelines (ICH, 2005), including selectivity, specificity, accuracy, linearity, precision, limit of quantification (LOQ), limit of detection (LOD), robustness, and system suitability [20].

*3.1.2.1. Specificity/selectivity.* Selectivity is the ability of an analytical method to discriminate between the analyte and potential interfering substances in the sample [3,11]. The evaluation of selectivity demonstrated that there was no significant interference detected with other ingredients at the retention time of the analyte [5]. The retention time of metformin Hydrochloride was 1.205 min.

Specificity is based on the response to a single or specific analyte [3,11,13]. This method was very specific to detect only the metformin hydrochloride analyte.

*3.1.2.2. Linearity and range.* Various aliquots of the standard solution of metformin were prepared from the stock solution and analyzed to determine the linearity of the proposed method. The analysis of the drug showed linearity in the range of (2.5–40 μg/ml) with a correlation coefficient ($r^2 \geq 0.999895$).

**Table 3**
Mobile phases have been tested during method optimization.

| # | M.P. Composition | pH | Rt min | Result |
|---|---|---|---|---|
| M.P. (A) | methanol-water (30:70 v/v) | 7.60 | 1.179 | Broad peak with tailing |
| M.P. (B) | methanol-water (30:70 v/v) + (0.1 %) formic acid | 2.73 | 1.374 | Inverse peak |
| M.P. (C) | $KH_2PO_4$-methanol (35:65 v/v) | 3.60 | 1.205 | Narrow symmetrical peak |

Note. M.P. = Mobile Phase, Rt = Retention time.

*3.1.2.3. Accuracy and precision.* The intraday accuracy and precision study (repeatability) were done by preparing a set of calibration standard curve samples and twelve replicates of each of the QC samples of each concentration low (03.00 μg/ml), medium (13.00 μg/ml), and high (30.00 μg/ml)), all analyzed in a day. In addition, the interday accuracy and precision study (reproducibility) were done by preparing a set of calibration standard curve samples and six replicates of each of the QC samples of each concentration low (03.00 μg/ml), medium (13.00 μg/ml), and (30.00 μg/ml) high concentration, and analyzed over three days. The mean, % recovery, SD, and % RSD were calculated for both the intraday and interday validation, as well as for the back-calculated value of the standard calibration curve [3,11,13].

*3.1.2.4. Lower limit of detection (LLOD) and lower limit of quantification (LLOQ).* The LLOD is the lowest amount of analyte that can be detected in the sample. The LLOQ is the lowest amount of analyte that can be quantitatively determined by suitable precision and accuracy in the sample [3,11,13]. The LLOD and LLOQ were determined experimentally by the analysis of standard reference solutions and measuring signal/noise ratios (S/N). The mean of these six S/N ratios, the theoretical LLOD (S/N $\geq$ 3), and the theoretical LLOQ (S/N $\geq$ 10) were calculated.

*3.1.2.5. Robustness.* The performance of the chromatography system was guaranteed. The peak area, the peak shape (symmetry), and the retention time were reproducible during the method validation and analysis of the large number of batches consisting of 45 samples which indicates its reliability during the analysis. In addition, the analysis carried out by several analysts ran different batches that indicate the reproducibility of the results.

*3.1.2.6. System suitability testing.* The system suitability test was performed following the ICH guidelines to confirm the method's suitability for the accurate and precise quantification of metformin hydrochloride. Six replicates of a standard solution containing metformin hydrochloride (30 μg/ml) were injected.

### *3.2. UV–Visible spectrophotometry*

#### *3.2.1. Method development*

A method of simple, rapid, economical, and sensitive UV–visible spectrophotometry was improved for metformin hydrochloride determination in tablet formulation. It is determined by spectrophotometric analysis at 234 nm using a mixture of methanol and water (50:50 v/v) as a blank in (Fig. 2).

#### *3.2.2. Method validation*

The proposed method was validated following the ICH guidelines [20]. A similar approach for the method validation was used as for the UHPLC method.

## 4. Application of the proposed method for pharmaceutical formulation

The assay test of the different strengths of the metformin hydrochloride tablet was done to find out the actual amount of active ingredient in the tablet. A set of six calibration standards, six QCs, and six assay samples were prepared and analyzed using both RP-UHPLC and UV–Vis spectrophotometry.

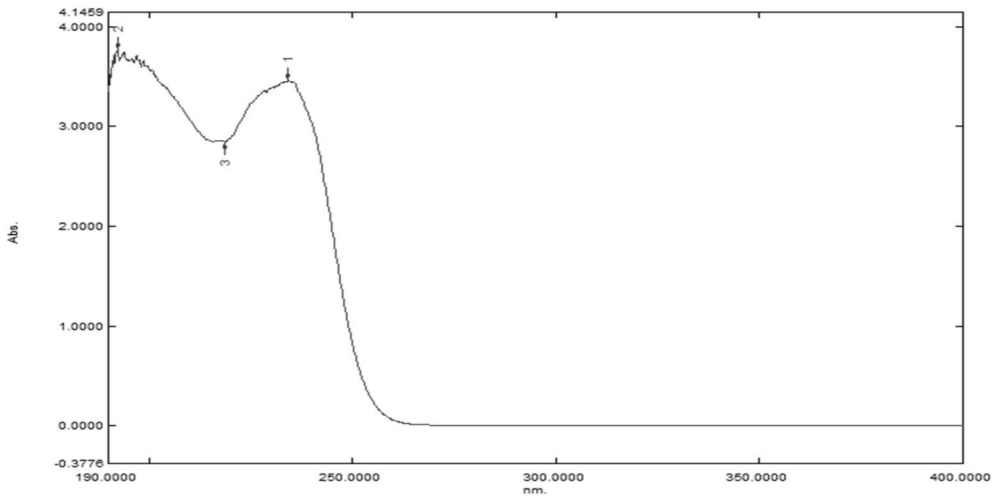


**Fig. 2.** The UV–Vis spectrum of metformin hydrochloride showed $\lambda_{max}$ in 234 nm.

## 5. Result and discussion

### 5.1. Reverse-phase ultra high-performance liquid chromatographic (RP-UHPLC)

#### 5.1.1. Method validation

The most fundamental challenge in our study was to develop and validate different methods using different instruments and applying the same range of the standard calibration curve samples. The proposed UHPLC was validated following ICH guidelines [20].

*5.1.1.1. Linearity.* The validated UHPLC method showed excellent linearity with a correlation coefficient ($r^2 \geq 0.999895$) (Table 4), and (Fig. 3).

*5.1.1.2. Accuracy and precision.* The method was applicable to determine metformin hydrochloride with high sensitivity. The validation data indicated that the UHPLC method in aqueous solutions is sensitive, accurate, and precise. The repeatability and reproducibility (expressed as %RSD) were less than 1.578 and 2.718 %, respectively. The % recovery of metformin hydrochloride ranged from (98–100 %), which was in the accepted range of (90–110 %), (Table 5).

The back-calculated concentration of six sets of calibration curve standards for metformin hydrochloride (n = 6) was measured to ensure the accuracy of analyzed data. The %RSD ≤2.485. The % recovery of metformin hydrochloride ranged from (98–101 %), (Table 6).

*5.1.1.3. Lower limit of detection (LLOD) and lower limit of quantification (LLOQ).* The chromatography technique demonstrated the LLOD and LLOQ of 0.156 μg/ml and 0.625 μg/ml, respectively (Figs. 4–6).

*5.1.1.4. Robustness.* To challenge the method, small changes were made to the chromatographic conditions such as column temperature, flow rate, injection volume, and pH of the mobile phase. Each of these conditions was altered in separate runs using a standard solution of metformin hydrochloride (30 μg/ml). The results obtained from these experiments indicated that there were no significant differences observed in terms of retention time, peak area, standard deviation (SD), and percent relative standard deviation (%RSD) in (Table 7).

*5.1.1.5. System suitability testing.* The number of theoretical plates and tailing factor (symmetry) were determined using a standard solution containing metformin hydrochloride (30 μg/ml). The results obtained from this evaluation were found to be acceptable, meeting the specified criteria for system suitability in (Table 8).

### 5.2. UV–visible spectrophotometry

#### 5.2.1. Method validation

The UV–Vis spectrophotometry was validated according to the ICH guidelines [20].

*5.2.1.1. Linearity.* The results indicated excellent linearity with a correlation coefficient ($r^2 \geq 0.99733$) (Table 9), & (Fig. 7). Beer's law equation was obeyed in the range of (2.5–40 μg/ml), [12,13].

*5.2.1.2. Accuracy and precision.* Due to the limitation of the UV Probe software version 2.34, we used the SPSS program to analyze the data for the method validation and assay test by applying the (1/x) Weighted Linear Regression equation. The method is simple, rapid, precise, accurate, and cost-effective. All parameters were within the acceptable limits of the ICH guidelines [20]. The result of repeatability and reproducibility (expressed as %RSD) were ≤3.773 and 1.988 %, respectively. The % recovery of metformin hydrochloride was in the range of (92–104 %), (Table 10).

The back-calculated concentration of the six sets of calibration curve standards for metformin hydrochloride (n = 6) was measured to ensure the reliability and accuracy of the analyzed data. The %RSD was ≤3.133 %. The % recovery of metformin hydrochloride ranged from (93–104 %), (Table 11).

**Table 4**
Summary of the validation parameters for the UHPLC method.

| Validation Parameters | Metformin HCL analysis by UHPLC |
|---|---|
| Linearity range (μg/ml) | (2.50–40.00) |
| Correlation coefficient ($r^2$) | ≥0.999895 |
| % Recovery | (90–110 %) |
| Repeatability and Reproducibility % RSD | Error <10 % |
| Lower Limit of detection (LLOD) | 0.156 μg/ml |
| Lower Limit of quantification (LLOQ) | 0.625 μg/ml |

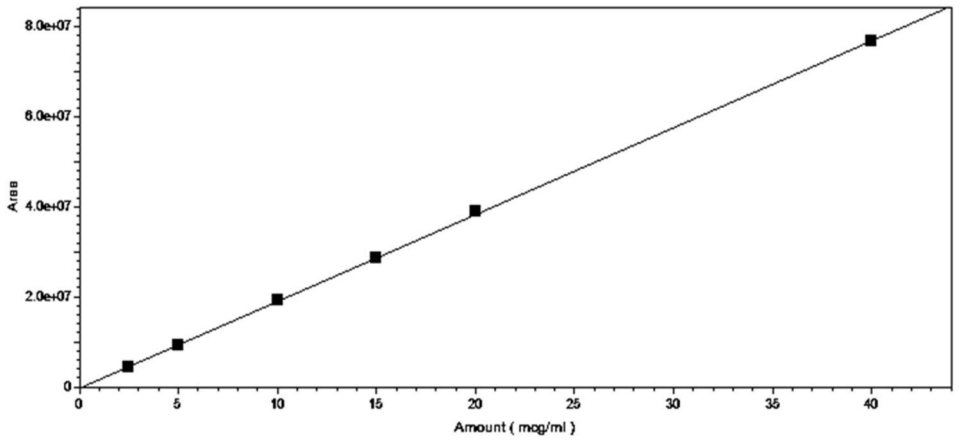


**Fig. 3.** Metformin hydrochloride calibration curve (2.50–40.00 μg/ml) in a mixture of methanol: water (50:50, v/v).

**Table 5**
Summary of the precision results of the proposed UHPLC method.

| Validation | Metformin Concentration (μg/ml) | Mean | % Recovery | SD | % RSD |
|---|---|---|---|---|---|
| Repeatability | 3.00 | 2.968 | 98.927 | 0.047 | 1.578 |
| | 13.00 | 12.840 | 98.771 | 0.062 | 0.484 |
| | 30.00 | 29.922 | 99.739 | 0.069 | 0.229 |
| Reproducibility | 3.00 | 3.026 | 100.859 | 0.082 | 2.718 |
| | 13.00 | 13.042 | 100.321 | 0.134 | 1.027 |
| | 30.00 | 30.245 | 100.817 | 0.121 | 0.402 |

**Table 6**
Summary of the accuracy and precision results of the proposed UHPLC method.

| | Metformin Concentration (μg/ml) | Mean | % Recovery | SD | % RSD |
|---|---|---|---|---|---|
| Back Calculated Values | 2.50 | 2.466 | 98.656 | 0.061 | 2.485 |
| | 5.00 | 4.943 | 98.857 | 0.044 | 0.887 |
| | 10.00 | 10.036 | 100.362 | 0.031 | 0.305 |
| | 15.00 | 15.077 | 100.513 | 0.086 | 0.570 |
| | 20.00 | 20.201 | 101.003 | 0.107 | 0.531 |
| | 40.00 | 40.035 | 100.086 | 0.236 | 0.589 |

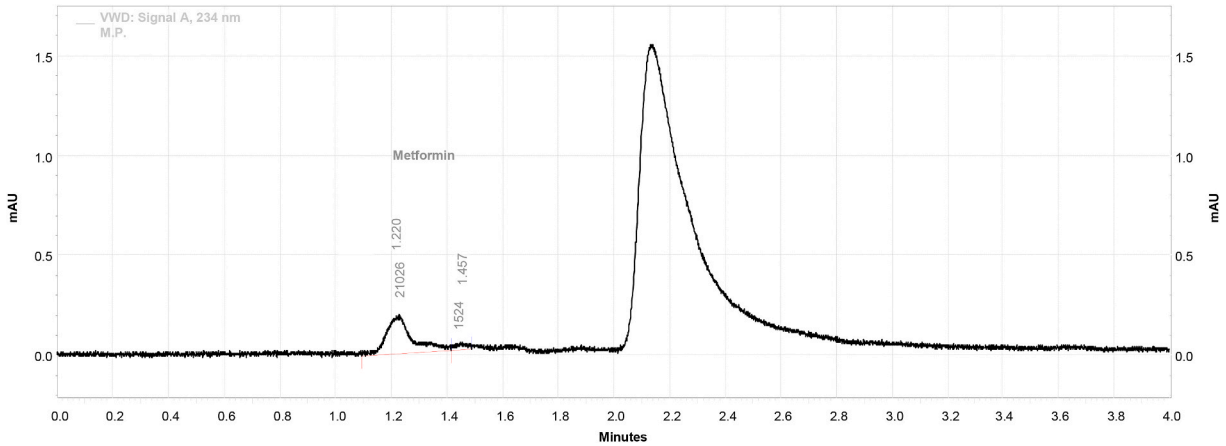


**Fig. 4.** UHPLC chromatogram of the blank sample.

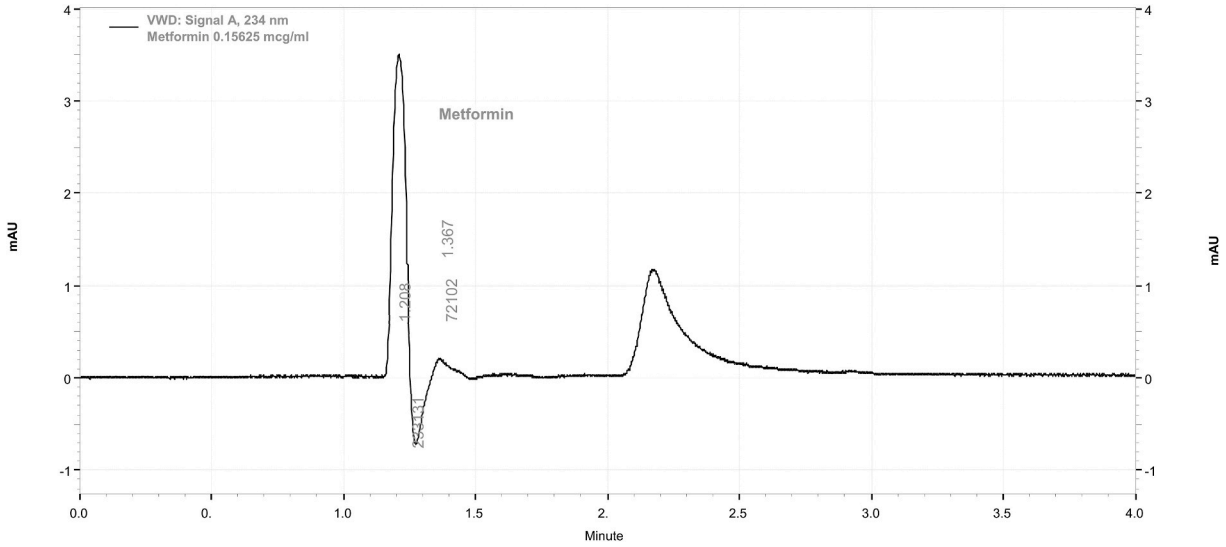


**Fig. 5.** The lower limit of detection (LLOD) of metformin hydrochloride 0.156 μg/ml.

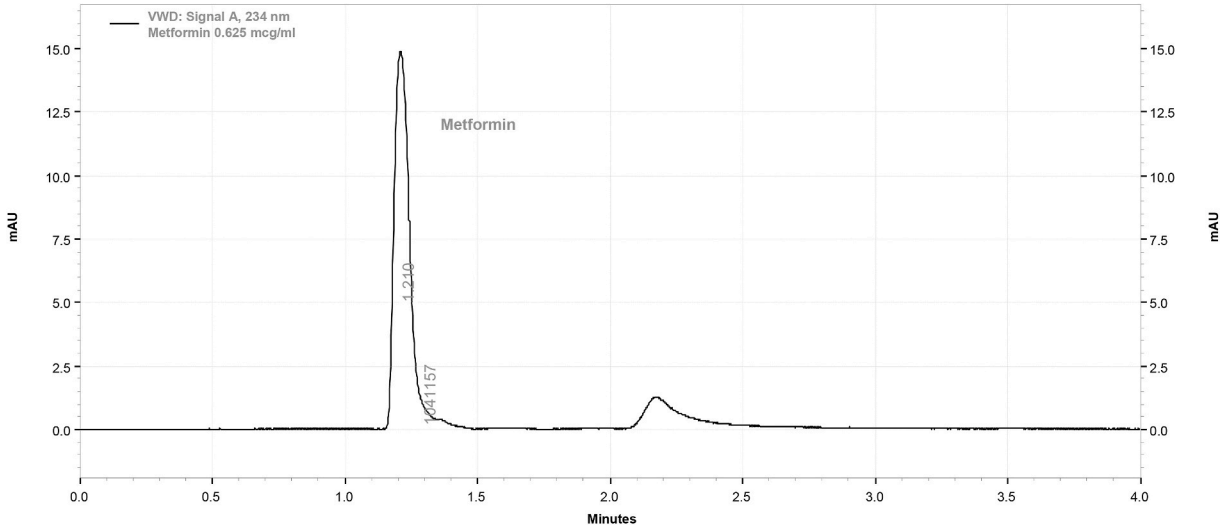


**Fig. 6.** The lower limit of quantification (LLOQ) of metformin hydrochloride 0.625 μg/ml.

### 5.3. Assay results of marketed formulations

#### 5.3.1. UHPLC method

The five pharmaceutical products of metformin hydrochloric tablets, namely Galvus Met®, Glucophage®, metformin Hexal®, Metfor®, and Omformin® were analyzed by the UHPLC method. The peak area of the sample solution was calculated and the amount of metformin hydrochloride present in the tablet formulation was calculated by the calibration curve. The assay results are summarized in (Table 12). The percentage content of active ingredients in the five brands of metformin hydrochloride tablets showed acceptable values of (96%–101 %), complying with the ICH acceptance criteria of (90%–110 %). However, there was a variation in the recovery percentage results between the five pharmaceutical products. Glucophage® (Merck Santé s.a.s., France) obtained the highest recovery percentage of (101.651 %) in comparison to Omformin® (National Pharmaceutical Industries Co. (SAOG) (NPI), Oman) with the lowest recovery percentage of (96.896 %) in (Table 12).

The chromatogram showed the separation of metformin hydrochloride. Glucophage® (1000 mg tablets) and Omformin® (500 mg tablets) at (20 μg/ml) in (Figs. 8 and 9).

#### 5.3.2. UV–Vis spectrophotometric method

UV–Vis Spectrophotometric method was implemented for the determination of the five pharmaceutical products of metformin

**Table 7**
Robustness results for metformin hydrochloride according to peak area and retention time.

| Run # | Parameter | Condition | Metformin (30.00 μg/ml) | | Metformin (30.00 μg/ml) | |
|---|---|---|---|---|---|---|
| | | | Peak area Statistical | Analysis | Retention Statistical | Time Analysis |
| 1 | Column temperature (°C) | 30 | 57198117 | Mean = 56824654 | 1.194 | Mean = 1.1920 |
| | | | 56967465 | | 1.192 | |
| | | | 56961649 | SD = 268221.6 | 1.191 | SD = 0.0011 |
| | | | 56518237 | | 1.192 | |
| | | | 56534969 | % RSD = 0.4720 | 1.191 | % RSD = 0.0919 |
| | | | 56767487 | | 1.192 | |
| 2 | Flow rate (ml/min) | 0.9 | 50956415 | Mean = 50742178 | 1.066 | Mean = 1.0677 |
| | | | 50894100 | | 1.068 | |
| | | | 50919486 | SD = 213868.2 | 1.068 | SD = 0.0008 |
| | | | 50452485 | | 1.068 | |
| | | | 50537318 | % RSD = 0.4215 | 1.068 | % RSD = 0.0765 |
| | | | 50693266 | | 1.068 | |
| 3 | Injection value (μl) | 18 | 51695466 | Mean = 51373063 | 1.201 | Mean = 1.2020 |
| | | | 51315331 | | 1.202 | |
| | | | 51571848 | SD = 233477.1 | 1.202 | SD = 0.0006 |
| | | | 51046812 | | 1.202 | |
| | | | 51234057 | % RSD = 0.4545 | 1.203 | % RSD = 0.0526 |
| | | | 51374862 | | 1.202 | |
| 4 | pH of M.P. | 3.5 | 57035010 | Mean = 56675050 | 1.200 | Mean = 1.2000 |
| | | | 56583688 | | 1.200 | |
| | | | 56944343 | SD = 257438.9 | 1.201 | SD = 0.0006 |
| | | | 56373615 | | 1.200 | |
| | | | 56524280 | % RSD = 0.4542 | 1.200 | % RSD = 0.0527 |
| | | | 56589363 | | 1.199 | |

Note. M.P. = Mobile Phase.

**Table 8**
System suitability parameters.

| Parameter | Observed value | Limits |
|---|---|---|
| No. of theoretical plates | 1875 | > 1000 |
| Tailing factor (symmetry) | 1.37 | < 1.5 |
| Regression equation | y = 5.22229e-007x + 0.460672 | |
| Correlation coefficient ($r^2$) | 0.999944 | ~1 (0.99–1.00) |

**Table 9**
Summary of the validation parameters for UV–vis spectrophotometry method.

| Validation Parameters | Metformin HCL analysis by UV–Vis spectrophotometry |
|---|---|
| Linearity range (μg/ml) | (2.50–40.00) |
| Correlation coefficient ($r^2$) | ≥0.99733 |
| %Recovery | (90–110 %) |
| Repeatability and Reproducibility % RSD | <10 % |

hydrochloric tablets. The absorbance of the test sample solution was determined and the amount of metformin hydrochloride present in the tablet formulation was measured by employing the calibration curve. The assay results are summarized in (Table 13). The percentage content of active ingredients in the five brands had acceptable values of (100%–105 %), complying with the ICH acceptance criteria of (90%–110 %). There was also a variation in the recovery percentage results between the five pharmaceutical products analyzed in that method. Glucophage® (Merck Santé s.a.s., France) obtained the highest recovery percentage of (105.151 %) in comparison to Omformin® (National Pharmaceutical Industries Co. (SAOG) (NPI), Oman) with the lowest recovery percentage of (100.839 %).

### *5.4. Statistical analysis*

To compare the two methods across the five products, we conducted the F-test as the most suited test for the type of data. The F-test assumptions were tested including the sphericity test using Bartlett's test and where violations were observed, a non-parametric test (Kruskal Wallis) was done. We presented the statistics using the F-test since both parametric and non-parametric tests were in agreement (Table 14).

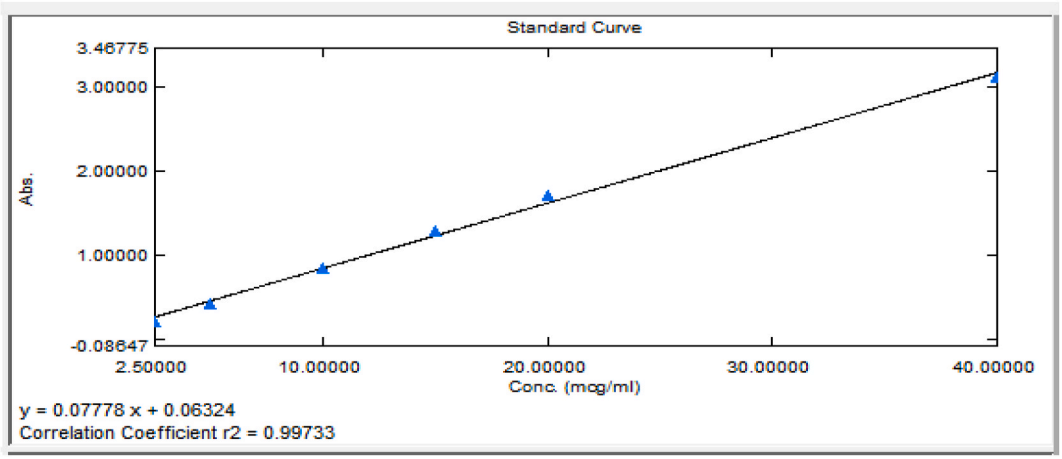


**Fig. 7.** The calibration curve for metformin hydrochloride (2.50–40.00 μg/ml) at 234 nm.

**Table 10**
A summary of the precision results of the proposed UV–vis spectrophotometer method.

| Validation | Metformin Concentration (μg/ml) | Mean | % Recovery | SD | % RSD |
|---|---|---|---|---|---|
| Repeatability | 3.00 | 2.783 | 92.760 | 0.105 | 3.773 |
| | 13.00 | 13.482 | 103.705 | 0.043 | 0.319 |
| | 30.00 | 31.031 | 103.435 | 0.066 | 0.211 |
| Reproducibility | 3.00 | 2.903 | 96.775 | 0.057 | 1.988 |
| | 13.00 | 13.607 | 104.668 | 0.084 | 0.618 |
| | 30.00 | 30.904 | 103.014 | 0.109 | 0.355 |

**Table 11**
A summary of the accuracy and precision results of the proposed UV–Vis spectrophotometer method.

| | Metformin Concentration (μg/ml) | Mean | % Recovery | SD | % RSD |
|---|---|---|---|---|---|
| Back Calculated Values | 2.50 | 2.345 | 93.787 | 0.065 | 2.775 |
| | 5.00 | 5.006 | 100.117 | 0.157 | 3.133 |
| | 10.00 | 10.233 | 102.328 | 0.042 | 0.409 |
| | 15.00 | 15.464 | 103.097 | 0.077 | 0.497 |
| | 20.00 | 20.816 | 104.078 | 0.063 | 0.301 |
| | 40.00 | 38.637 | 96.592 | 0.105 | 0.271 |

**Table 12**
The metformin hydrochloride assay tests analyzed by the UHPLC.

| Name of Product | Strength (mg) | Test Product Concentration (μg/ml) | Average (of six samples) | %Recovery | SD | %RSD |
|---|---|---|---|---|---|---|
| Glucophage® | 1000 | 20 | 20.330 | 101.651 | 0.085 | 0.416 |
| Galvus Met® | 1000 | 20 | 20.216 | 101.081 | 0.105 | 0.518 |
| Metformin Hexal® | 1000 | 20 | 19.676 | 98.383 | 0.076 | 0.387 |
| Metfor® | 500 | 20 | 19.415 | 97.076 | 0.070 | 0.363 |
| Omformin® | 500 | 20 | 19.379 | 96.896 | 0.056 | 0.291 |

## 6. Stability testing

The metformin hydrochloride stability testing was performed on three pharmaceutical products (Glucophage®, Galvus Met®, and Omformin®). Briefly, the metformin products were incubated at 43 °C in the dark and 10 % relative humidity for 4 weeks in a stability chamber (Climate chamber ICH750L, Memmert). After the incubation period, all the stability products were processed according to the previous samples' assay test preparation and analyzed by the UHPLC. The climate parameters (Temperature, Humidity) were selected to simulate the mean daily maximum temperature (°C) in Riyadh between the months of June, July, and August. The stability results showed no significant differences before (Table 12) and after the stability test (Table 15).

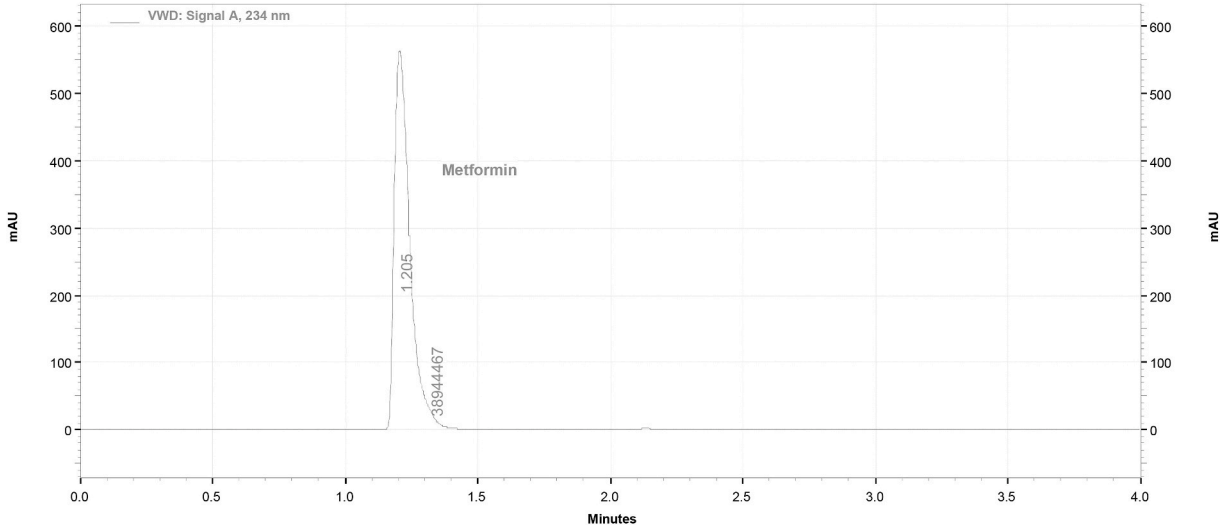


**Fig. 8.** The chromatogram of metformin HCl Glucophage® (Merck, 1000 mg tablets), was at (20.0 μg/ml).

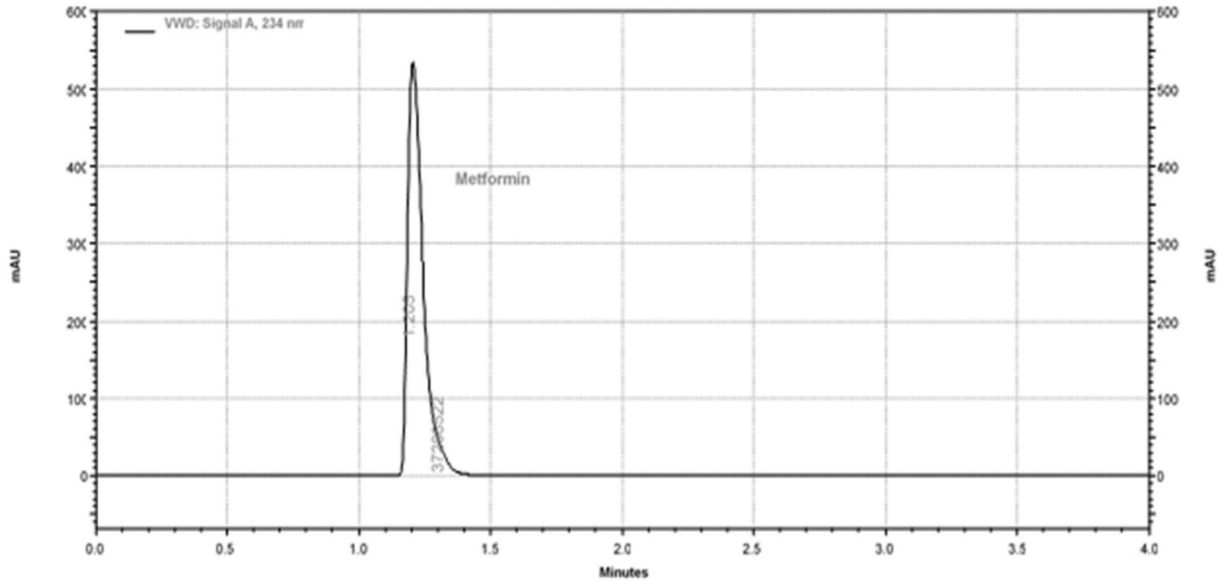


**Fig. 9.** The chromatogram of metformin HCl Omformine® ((NPI), 500 mg tablets), was at (20.0 μg/ml).

**Table 13**
Metformin hydrochloride assay tests were analyzed by the UV–Vis spectrophotometer.

| Name of Product | Strength (mg) | Test Product Concentration (μg/ml) | Average (of six samples) | %Recovery | SD | %RSD |
|---|---|---|---|---|---|---|
| Glucophage® | 1000 | 20 | 21.030 | 105.151 | 0.021 | 0.099 |
| Galvus Met® | 1000 | 20 | 21.028 | 105.141 | 0.078 | 0.371 |
| Metformin Hexal® | 1000 | 20 | 20.434 | 102.168 | 0.007 | 0.034 |
| Metfor® | 500 | 20 | 20.172 | 100.860 | 0.013 | 0.064 |
| Omformin® | 500 | 20 | 20.168 | 100.839 | 0.017 | 0.085 |

## 7. Conclusion

The present study was carried out using different analysis methods validated according to the ICH guidelines. The validated analytical methods enable accurate and sensitive quantification of metformin hydrochloride. The UHPLC method was more accurate,

**Table 14**
The comparison of UHPLC and UV–Vis spectrophotometer results by ANOVA, F-test, and Bartlett's test using STATA® 18.0 software.

| Name of Product | F statistics | df | *P*-value |
|---|---|---|---|
| Glucophage® | 387.33 | 11 | 0.009 |
| Galvus Met® | 232.10 | 11 | 0.533 |
| Metformin Hexal® | 587.01 | 11 | 0.000 |
| Metfor® | 670.04 | 11 | 0.002 |
| Omformin® | 1074.27 | 11 | 0.021 |

**Table 15**
The stability assay tests for the metformin products (Glucophage®, Galvus Met®, and Omformin®) analyzed by the UHPLC.

| Name of Product | Strength (mg) | Test Product Concentration (μg/ml) | Average (of six samples) | %Recovery | SD | %RSD |
|---|---|---|---|---|---|---|
| Glucophage® | 1000 | 20 | 19.904 | 99.519 | 0.089 | 0.447 |
| Galvus Met® | 1000 | 20 | 20.052 | 100.262 | 0.060 | 0.299 |
| Omformin® | 500 | 20 | 19.416 | 97.078 | 0.070 | 0.361 |

precise, linear, and sensitive compared to the UV–Vis Spectrophotometric method; however, the UV–Vis spectrophotometric method is more cost-effective. These analytical methods are applicable in any in-vitro experiments such as quality control and stability tests.

## Data availability statement

The authors declare that all the data is included in the article and there is no additional data available.

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## CRediT authorship contribution statement

**Amani Kurdi:** Methodology, Formal analysis. **Waleed Alhussaini:** Formal analysis, Data curation. **Abdulrahman Alawaji:** Resources, Methodology. **Abdullah Alhudathi:** Resources, Methodology. **Rakan Alharbi:** Investigation, Formal analysis. **Faisal Binsaleh:** Funding acquisition, Data curation. **Yazeed Alghamidi:** Software, Resources. **Abdulkareem Al Bekairy:** Writing – review & editing, Validation. **Abdulmalik Alkatheri:** Writing – original draft, Validation. **Imadul Islam:** Writing – review & editing, Supervision. **Ibrahim Farh:** Methodology, Data curation, Conceptualization. **Ezzeldeen Ghanem:** Supervision. **Mahmoud Mansour:** Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Mahmoud Mansour reports financial support was provided by King Abdullah International Medical Research Center. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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