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Development and Validation of HPLC-UV Method for Determination of Metformin Hydrochloride in Tablets Available in the Libyan Market

Osama I. G. Khreit[1], Marwa M. Bader [2], Amna M. Imbarak [2], Khoud M. Yacoub [2], Khawla A. Jomaa [2], Rowaida A. Aboubakr.[1]

[1]*Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Omar AL-Mukhtar University, Libya*

[2]*Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Omar AL-Mukhtar University, Libya.*

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Abstract: A rapid and simple HPLC-UV method has been developed and validated for the estimation of metformin HCl formulated in tablet dosage form, as well as to identify different commercial brands of metformin HCl using BP test Thin Layer Chromatography. The HPLC method was performed on a Reversed-Phase Brownlee Bio C18 column (250 x 4.6 mm, 5 µm) at a 1.0 mL min^{-1} flow rate with UV detection at 236 nm. The mobile phase was conducted in an isocratic manner and contained 52% acetonitrile and 48% aqueous phase (0.1M Potassium dihydrogen orthophosphate). The pH of the aqueous phase was adjusted to 5.5. The validation of the analytical method for the determination of metformin HCl in tablet formulation was performed following parameters, including system suitability, specificity, the limit of quantification, and limit of detection. Peak shapes asymmetries have resulted. The precision was calculated and showed excellent reproducibility (RSD = 0. 1-0.57 %, n=3). The linearity of the method has been tested in the range of 4.0–12.0 µg mL^{-1} for metformin HCl. The limits of detection and quantification have been also established to be lower than 2.7 µg mL^{-1} and 8.0 µg mL^{-1}, respectively. This method is suitable for estimating pharmaceutical formulations with no interference from excipients.

تطوير والتحقق من صحة طريقة HPLC-UV لتقدير الميتفورمين هيدروكلوريد في الأقراص المتوفرة في السوق الليبي

الكلمات المفتاحية:
الكروماتوغرافى؛
ميتفورمين هيدروكلوريد؛
اقراص؛
التحقق من صحة الطريقة.

المستخلص: تم تطوير طريقة HPLC-UV سريعة وبسيطة والتحقق من صحتها؛ لتقدير الميتفورمين هيدروكلوريد في شكل جرعة قرصية، وكذلك لتحديد العلامات التجارية المختلفة للميتفورمين هيدروكلوريد باستخدام اختبار BP كروماتوغرافيا الطبقة الرقيقة. تم إجراء طريقة الكروماتوغرافى السائل عالي الأداء على عمود C18 معكوس الطور (250x 4.6 مم ، 5 ميكرومتر) بمعدل تدفق 1.0 مل دقيقة $^{-1}$ مع الكشف بواسطة الأشعة فوق البنفسجية عند 236 نانومتر. تم إجراء الطور المتحرك بطريقة ثابتة وتحتوي على 52٪ أسيتونيتريل، و 48٪ طور مائي (0.1 مولار بوتاسيوم ثنائي هيدروجين أورثوفوسفات). تم تعديل الأس الهيدروجيني للطور المتحرك إلى 5.5. تم إجراء التحقق من صحة الطريقة التحليلية لتحديد ميتفورمين هيدروكلوريد في شكله الدوائى كأقراص بعد حدود مثل: ملاءمة النظام، والنوعية، وحد القياس الكمي، وحد الكشف، وأشكال الذروة وعدم التناسق تم إنتاجها. وكذلك تم حساب الدقة، وأظهرت استنساخًا ممتازًا (RSD = 0. 1-0.57)٪ وتم اختبار خطية الطريقة في نطاق 4.0–12.0 ميكروغرام مل $^{-1}$ للميتفورمين هيدروكلوريد. أيضا تحديد حدود الكشف، والقياس الكمي أيضًا لتكون أقل من 2.7 ميكروغرام مل $^{-1}$، و 8.0 ميكروغرام مل $^{-1}$ على التوالي.

INTRODUCTION

Diabetes mellitus is a category of metabolic disorders in which blood glucose levels are higher than normal due to insulin deficiency or inappropriate cell response to insulin (Nasri & Rafieian-Kopaei, 2014). Metformin mainly

***Corresponding author:** Osama I. G. Khreit: *osama.khreit@omu.edu.ly* , Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Omar AL-Mukhtar University, Libya.

acts by reducing hepatic glucose production as well as increasing glucose uptake and utilization by muscles, which eventually leads to regulating blood glucose levels. (Foretz, Guigas, & Viollet, 2023). Metformin works by assisting in the restoration of the body's insulin response. It has been used in addition to dietary control and exercise to prevent diabetes in those who are at high risk of developing the disease. It is also used to treat polycystic ovarian syndrome in women. It may help regulate menstrual periods and enhance fertility (Hundal & Inzucchi, 2003). Metformin is a biguanide derivative that contains a lot of guanidine, a hypoglycemic chemical (C. J. Bailey & Day, 1989; Hill, 1771). It is also known as 1, 1 dimethylbiguanide hydrochloride, and comes as a white crystalline powder that is hygroscopic and freely soluble in water, slightly soluble in alcohol, and practically insoluble in acetone and methylene chloride. The melting point ranges from 223 to 226 ºC (da Trindade, Kogawa, & Salgado, 2018).Only a few methods, such as HPLC and GC, have been published for estimating metformin hydrochloride in pharmaceutical formulations and biological fluids (Zounr, Khuhawar, Khuhawar, Lanjwani, & Khuhawar, 2023) (Arayne, Sultana, & Zuberi, 2006).

HPLC methods were the most widely used for the analysis of metformin. The sensitive ion-pair HPLC methods were used for the quantification of metformin in plasma and the simultaneous determination of it with gliclazide and glipizide present in multicomponent dosage forms (Arayne et al., 2006). Liquid chromatography with electrospray ionization tandem mass (LC-ESI-MS/MS) spectrometric detection was used to analyze metformin in combination with glipizide in human plasma (Ding, Zhou, Ge, Zhi, & Ma, 2007). The official method for estimating the active ingredient in tablets is the UV spectrophotometric approach (Pharmacopoeia, 1996). Metformin was first synthesized in the 1920s (Thomas & Gregg, 2017; Werner & Bell, 1922), it was found to be beneficial in the 1940s and 1950s when used to treat an influenza infection, and it was found to drop glucose but not below physiologic values. Because other biguanide drugs have safety concerns, metformin fell out of favour until the 1990s. It was currently approved by the Food and Drug Administration (FDA) as a first-line treatment for type 2 diabetes, as well as on and off-label indications for diabetes prevention in prediabetes, polycystic ovarian syndrome, antipsychotic-associated weight gain, weight loss, gestational diabetes, and fertility enhancement (Control & Prevention, 2014; Finkelstein, Trogdon, Cohen, & Dietz, 2009).

The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) have recommended metformin as the first-line oral medication for the treatment of type 2 diabetes since 2009 (Nathan et al., 2009) after phenformin and buformin were withdrawn from the market in most countries due to their high risks of lactic acidosis (Nattrass & Alberti, 1978). Metformin has been shown to have long-term metabolic effects as well as lower cardiovascular risk. Metformin is becoming more widely recognized as a potential anticancer agent due to a lower cancer rate in diabetes people who take the medicine (Evans, Donnelly, Emslie-Smith, Alessi, & Morris, 2005). Recently, patients taking metformin were associated with a reduced risk of COVID-19-related mortality (Bramante et al., 2021; Crouse et al., 2021). In humans, oral absorption of metformin from immediate-release dose formulations is incomplete, with a population mean bioavailability of 55% (Graham, Punt, Arora, Day, & Doogue, 2011). Metformin's blood-glucose-lowering impact is attributed to numerous tissues, although its mechanism of action remains unknown after long-term therapeutic use. Reduced hepatic gluconeogenesis is the main mechanism of action mediated through the control of mitochondrial enzymes and antagonistic regulation of the glucagon signaling pathway (Madiraju et al., 2014). Metformin was found to improve the insulin sensitivity of muscle in rodents in vitro by boosting insulin receptor expression and activity, resulting in

higher insulin-dependent glucose uptake in cells (C. Bailey & Puah, 1986; Rossetti et al., 1990). The current method reported a new, simple, sensitive, precise, accurate, linear, and isocratic RP-HPLC method for the quantitative estimation of Metformin HCl.

MATERIALS AND METHODS

Chemicals and Reagents: Metformin HCl reference standard with a certified purity of 99% was purchased from Glentham Life Sciences (UK). HPLC grade acetonitrile was obtained from Carlo Erba (France). Potassium dihydrogen orthophosphate was obtained from El Nasr Pharma (Egypt), and water was obtained in-house by distillation. All other reagents and solvents used were of analytical grade.

Brand Selection and Sample Collection: This study is based on the comparison of different doses of metformin 500, 850, and 1000 mg tablet brands in the Libyan market that are available for consumer use. There are approximately twenty brands of metformin tablets in the Libyan pharma market. Among them, eight available brands were selected for the study of some physical and chemical parameters, and all brands were labelled by their trade names. Study samples were coded as shown in Table 1.

Table: (1). Metformin hydrochloride (MTF HCL) tablets available in the Libyan market

MTF HCl Brand Names	Sample Codes		
	500 mg	850 mg	1000 mg
Mylan France	MTF 1-1	MTF 1-2	MTF 1-3
Bristol	MTF 2-1	MTF 2-2	MTF 2-3
Dialon XR UAE	MTF 3-1	MTF 3-2	MTF 3-3
Glyformin Cyprus	MTF 4-1	-	-
Metforal Italy	-	MTF5-2	-
Glucophage Turkey	-	MTF 6-2	-
Diabitos Tunisia	-	-	MTF 7-3
Glucophage France	-	-	MTF 8-3

Analytical Methods

Melting Point Determination: The melting point of Metformin was determined by using Stuart Equipment SMP 10 capillary melting point apparatus.

Thin Layer Chromatography Materials: Thin-Layer Chromatography was carried out on aluminum-backed (Silica gel 60 F 254, Merck, Germany) by using TLC Nanomate 4 device, and spots were visualized using ultraviolet light (254 nm). The eluting solvent was according to BP. Its solvent system was prepared to be used in chromatographic controls of compounds which were Glacial Acetic Acid: Butanol: Water (10: 40: 50).

Development of TLC Plate: Dragging conditions: The solvent system was poured into the TLC chamber and remained for 24 hours to reach saturation, then filled with the development solvent to a depth of no greater than 0.5 cm.

Different brands of metformin HCl and the standard material were dissolved in water and then applied to thin-layer chromatography (TLC) plates which were dragged for 15 cm at room temperature.

HPLC Instrumentation: HPLC operating conditions used a Mobile phase consisting of 0.1M Potassium dihydrogen orthophosphate (pH 5.5), 52% acetonitrile, and 48% aqueous phase. The flow rate was 1.0 mL min^{-1} with an injection volume of 20 µL. Three replicate injections of each calibration standard were performed. Data analysis was carried out using Thermo Electron Corporation software (ChromQuest).

Validation of HPLC method: Validation was acceptable for its intended purpose, as defined in the International Conference on Harmonization (ICH) guidelines (Harmonization 1994). Analytical validation was assessed the accuracy, linearity, precision, LOD, and LOQ.

The precision (RSD) was expressed with respect to variation in the expected drug concentrations. The accuracy was determined by measuring a known amount of standard material under different conditions. After validation, the developed method was applied to a pharmaceutical dosage form containing Metformin HCl.

Preparation of Standard Solution: Calibration standard was prepared as 10.0 mg of the metformin HCl weighed accurately into a 100 ml volumetric flask and diluted to 100 µg mL-1 with the mobile phase. Concentrations of 4.0, 6.0, 8.0, 10.0, and 12.0 µg mL^{-1} were used for the construction of the standard r curve.

Preparation of Sample Solution: Twenty tablets were randomly selected from each brand, weighted and crushed. An accurately weighed amount of powder equivalent to 10.0 mg of metformin HCL was transferred into a 100 mL volumetric flask, shaken with 70 mL of mobile phase for 10 min, and then diluted to 100 mL with the same solution, then filtered and diluted to 8.0 µg mL^{-1}. The sample was analysed using HPLC, three injections were run on each brand, and the area of metformin-HCl peaks was quantified with the equation of calibration series of metformin standard to get the amount of metformin in the percentage present in each brand.

Statistical Analysis: Data were analyzed using Minitab version 20 software. Statistical differences were tested using one-way ANOVA. Differences were considered significant at p values ≤ 0.05, and LD analysis was then performed to determine whether there were statistically significant differences at p values ≤ 0.05 between the concentrations of the different samples.

RESULTS AND DISCUSSION

Identification of Metformin HCl in Formulated Tablet by TLC: Because the detected structure of the active ingredient has a chromophore, all the spots of Metformin HCl in commercial brands and standards appeared clearly under UV lights. Thus, the distance traveled by the Metformin HCl substance from the origin (where the compound was applied onto the TLC plate) was divided by the distance traveled by the solvent from the origin to obtain the Rf value shown in Table 2.

Table: (2). Rf values of standard and tested brands

No.	Brand code of MTF tables	Rf Values
0	Standard	0.88
1	MTF1-1	0.81
2	MTF-2-1	0.85
3	MTF-3-1	0.88
4	MTF4-1	0.85
5	MTF-1-2	0.85
6	MTF2-2	0.85
7	MTF3-2	0.84
8	MTF5-2	0.85
9	MTF6-2	0.88
11	MTF1-3	0.88
12	MTF2-3	0.85
13	MTF3-3	0.88
14	MTF7-3	0.84
15	MTF8-3	0.88

Development and Validation HPLC Method: A suitable analytical method was developed after evaluating the major and critical separation parameters of chromatography. Isocratic elution was carried out using the HPLC method, and the first parameter to be changed was the pH of the mobile phase buffer which increased from 3.5 to 5.5. The organic modifier was also increased to 52% using pH 5.5 with the aqueous component. The result is shown in Figure 1. The retention time was reduced to 2.9 min. Thus, this eluting time was satisfactory and showed that there was no interference of excipients. Therefore, this showed that the method used was selective for metformin HCl analysis. Utilizing a UV–vis spectrophotometer, the absorbance maxima of metformin HCl were determined to be 236 nm.

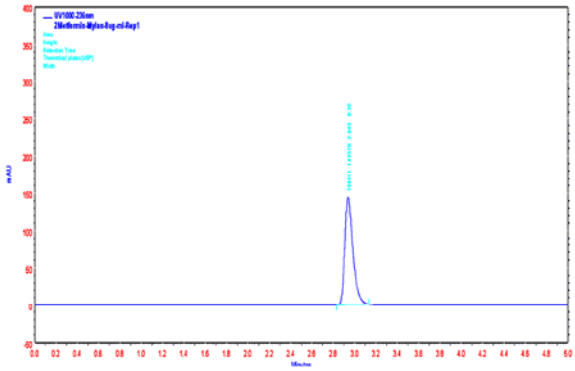

Figure: (1). Representative chromatogram of the solution containing Metformin HCl standard (15 µgmL-1) obtained using the Isocratic HPLC method (mobile Phase: Potassium dihydrogen orthophosphate buffer (0.1M, pH 5.5): Acetonitrile)

The optimized method for analyzing metformin HCl was validated and the reproducibility was tested. This involved making up a fresh solution of calibration standards that contained metformin HCl in the mobile phase and then running it three times on the HPLC system(Khreit, Elfowiris, Aljali, & Abduljalil, 2021). The linearity of metformin HCl was established by preparing a series of concentrations with metformin HCl standard solutions ranging from 4.0 µg mL^{-1} to 12.0 mg mL^{-1}. The calibration standards demonstrated a linear response (r 2 = 0.999 -1.0). The linear equation was extrapolated, and the correlation coefficient, slope, and intercept were for the calibration curve.

Repeatability of the system (interday precision) was checked by injecting the different concentrations of standard solution on the same day, and the relative standard deviation (RSD) of the chromatographic method was less than 2 % within a day, which complies with the BP requirements (RSD = 0. 1-0.57 %, n=3). The limits of detection and the limit of quantification for metformin HCl were determined to be 2.70 and 8.20 µg mL^{-1}, respectively.

Table 3 shows also some other validation parameters that were been calculated from the calibration series chromatograms, including retention time, capacity factor, as well as symmetry factor, which showed metformin HCl standard solution eluted with a slight peak tailing (A_s =1.21).

Table (3): Summary of validation data for the quantification of Metformin HCl using Brownlee Bio C18 column (250x4.6 mm and 5 µm particle size); mobile phase: 0.1M Buffer: Acetonitrile;
Detector wavelength: 236 nm.

	Metformin HCl
t_R (min) ($t0$ = 2.2 min)	2.9 min.
Capacity factor (k′)	0.32
Symmetry factor (A_s)	1.21
LOD (µg mL^{-1})	2.70 µg mL^{-1}
LOQ (µg mL^{-1})	8.20 µg mL^{-1}
Co-efficient of regression (r2)	0.999 (101079x+3779.4)
Precision (% RSD) (n= 3)	
4.0 µg mL^{-1}	0.37
6.0 µg mL^{-1}	0.38
8.0 µg mL^{-1}	0.57
10.0 µg mL^{-1}	0.39
12.0 µg mL^{-1}	0.10

The results obtained in the evaluation of robustness showed that a small variation in the composition of the mobile phase, its pH variations, flow rate during chromatography, and column temperature, has an insignificant impact on metformin HCl chromatogram.

Application of HPLC Method for Determination of Metformin HCl in Commercial Products : Once the final method had been developed and validated, the fourteen metformin HCl samples of eight brands were tested using this method to identify the metformin HCl compound in tablet samples. The samples were powdered and then dissolved in the mobile phase as previously described and analyzed by HPLC based on the HPLC method described above. The injection of each sample was repeated three times, and chromatograms were recorded. Representative chromatograms for the fourteen samples are shown in Figure 2. This result indicates that the peak of the analyte was pure, and this confirmed the specificity of the method.

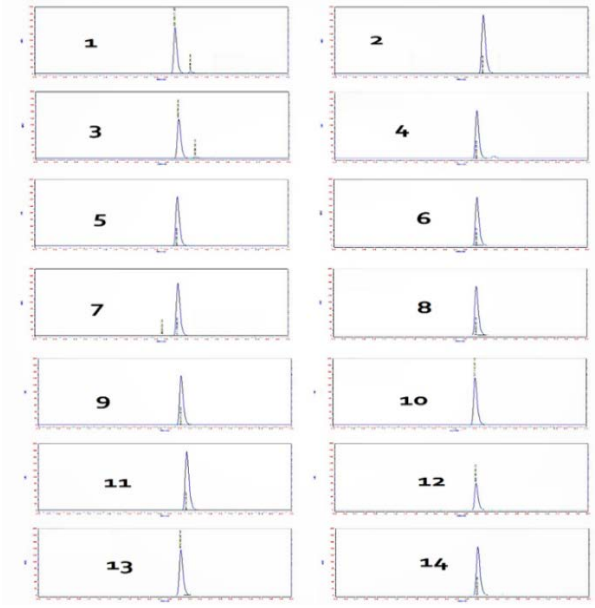

Figure: (2). Representative chromatogram for tested tablets of different brands of metformin (1) Mylan 500mg (2) Bristol500mg (3) Dialon xr 500 mg (4) Glyformin 500mg (5) Metforal 850mg (6) Dialon 850mg (7) Glucophage 850 (8) Bristol 850 (9) Mylan 850mg (10) Mylan 1000mg (11) Bristol 1000mg (12) Dialon 1000mg (13) Diabitos 1000m **(**14) Glucophage 1000mg

The regression equation of linearity was used to calculate the concentration of extracted tablet samples. The state of the content of metformin HCl in powder assayed from the different brand samples is shown in Table 4.

Table: (4). Assay result of the amount of metformin HCl obtained from HPLC analysis.

Sample Code	State of Content (±SD)		
	500 mg	850 mg	1000 mg
MTF 1	95.56 ± 0.019	95.5 ±0.32	87.60± 0.081
MTF 2	91.14 ±0.29	97.31 ± 1.7	95.49± 0.074
MTF 3	88.97 ±2.5	92.72± 0.27	83.55± 0.24
MTF 4	73.51 ±0.12		
MTF 5		1.75±96.69	
MTF 6		105.45±0.79	
MTF 7			87.60±0.86
MTF 8			83.55±0.29

Metformin HCl was studied for its active pharmaceutical ingredient percentage at three concentrations (500 mg, 850 mg, and 1000 mg) manufactured by different commercial brand companies (State of Content). These samples were collected from various pharmacies in El-Beida, and an HPLC instrument was utilized to analyze them.

Table 3 shows that some formulations containing 500, 850, and 1000 mg metformin HCl per tablet complied with BP specifications for metformin HCl content (95% - 105% of the labelled content(, with the majority of them lying outside of the BP 2012 specification range. In the first series of different brands of metformin tablets 500 mg, MTF 1-1 only compiled with the BP specifications and showed a significant difference from the other brands tested. However, MTF 2-1, MTF 3-1, and MTF 4-1 did not comply with BP specifications. In the second metformin 850 mg series with different brand companies. The results showed a significant difference between the MTF 6-2 and the other brands tested. However, MTF 1-2, MTF 2-2, and MTF1 complied with BP specifications. In the last group of metformin series with 1000 mg analysis of different companies, MTF 1-3, MTF3-3, MTF 7-3, and MTF 8-3 did not comply with BP specification except for MTF 2-3, which was within the BP specification range.

CONCLUSION

This study investigated different commercial brands of metformin HCl collected from different pharmaceutical markets in El-Beida under wild environmental conditions, and their state of content was evaluated using the HPLC method. The presented, developed, and validated method was rapid, economic, simple, accurate, sensitive, robust, specific, and linear. This optimized isocratic method can be employed as an absolute qualification and quantification method for routine analysis of metformin tables, either alone or in combination products.

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