<https://doi.org/10.36293/sfj.2019.0020>

SPECTROPHOTOMETRIC DETERMINATION OF METFORMIN IN THREE SIMULATED PHYSIOLOGICAL pH

Nasir, I.^{1*}, Musa, A.², Abdullahi, M.I.¹ and Garba, M.²

¹Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

²Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria

Abstract

Three simple, accurate and reproducible spectrophotometric methods for the determination of metformin in simulated physiological fluids were developed and validated in this study. The methods were based on dissolution of standard metformin powder or extraction of the drug from tablet formulation using three dissolution media as solvents: 0.1 M HCl, simulated intestinal and blood pH of 6.8 and 7.4 phosphate buffers. Each resulting extract was filtered and scanned using ultraviolet visible (UV/Vis) spectrophotometer (Model SP 3000). The wavelength of maximum absorption (λ_{max}) of metformin in 0.1 M HCl, phosphate buffer pH 6.8 and phosphate buffers pH 7.4 were 205 nm, 235 nm and 232 nm respectively. Calibration curves for metformin within concentration ranges of 2.5 to 15.0 $\mu\text{g/ml}$ were prepared in each media. The precisions, accuracy (% relative error), % recoveries, limit of detections and limit of quantification's were determined according to ICH guidelines. The developed methods were then used to assay a sample of standard metformin powder and tablets; the results were compared with BP method for assay of metformin. The developed methods obeyed Beer's law within the calibration range with correlation coefficients of 0.9992, 0.9994 and 0.9990 respectively. The percentage content of metformin in the standard powder and the tablet brand assayed were within the BP range of 98.5 to 101.0 % and 95.0 to 105.0 % respectively. No statistically significant difference was observed between the proposed methods and the BP method ($p < 0.05$) analyzed with Graph Pad Prism 6 software using Paired Student's *t*-test. The developed methods can be used for quantitative determination of metformin in pure form and tablet dosage forms.

Keywords: Metformin, Buffers, Assay, Spectrophotometry

*Corresponding author e-mail: thenasir25@gmail.com; nasir.ibrahim@udusok.edu.ng

Introduction

Metformin (Figure 1), is a disubstituted biguanide antidiabetic agent. It is chemically named as N-1,1-Dimethyl biguanide hydrochloride. It is white or almost white crystals in appearance with melting point of

222-226°C and content range 98.5-101.0 % of the dried substance. It is freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone and in methylene chloride (BP, 2013).

Figure 1: Chemical structure of metformin

Metformin hydrochloride is slowly and incompletely absorbed from the gastrointestinal tract. The absolute bioavailability of a single 500 mg dose is reported to be about 50 to 60%, although this is reduced if taken with food. Once absorbed, protein binding in plasma is negligible; the drug is excreted unchanged in the urine. The plasma elimination half-life is reported to range from about 2 to 6 hours after oral doses, it is distributed into breast milk in small amounts (Sweetman, 2012). It is indicated for the treatment of type 2 diabetes as an adjunct to diet and exercise, either as a single oral agent or in combination with sulfonylureas, alpha-glucosidase inhibitors, or insulin. Its adverse effects are generally tolerable and self-limiting. Its therapeutic blood glucose-normalizing action is dependent on the presence of circulating insulin (Brunetti and Kalabalik, 2012).

UV-visible Spectrophotometric methods are commonly used in industrial and research laboratories because of their simplicity, accuracy, precision and low cost (Raza *et al.*, 2003; Raza *et al.*, 2005; Musa *et al.*, 2014; Nasir *et al.*, 2014). Some UV methods for determination of metformin are reported either by direct estimation of metformin using methanol as solvent (Rashmiet *et al.*, 2011) or utilizing the amino group in metformin and coupling it with some reagents like ninhydrin (Mubeen and Noor, 2009). However, these mentioned methods reported interference of tablet excipients with their respective determinations.

The biopharmaceutical classification system (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from solid oral dosage forms: dissolution, solubility, and intestinal permeability (Amidon *et al.*, 1995). A drug product is considered rapidly dissolving when 85 percent or more of the labelled amount of the drug substance dissolves within 30 minutes, using United States Pharmacopeia apparatus at 100 rpm or at 50 rpm or at 75 rpm when appropriately justified in a volume of 500 mL or less in each of the following media: 0.1 N HCl; a pH 4.5 buffer; and a pH 6.8 buffer without enzymes (USP, 2012).

A drug product is considered very rapidly dissolving when 85 percent or more of the labelled amount of the drug substance dissolves within 15 minutes using the mentioned conditions (BP, 2013). In addition, some solid oral dosage forms are categorized as having rapid or very rapid dissolution under which metformin belongs. Within this framework, when certain criteria are met, the BCS can be used as a drug development tool to help sponsors and applicants justify requests for biowaivers (Yuet *et al.*, 2002). A Biowaiver means that relative bioavailability and/or bioequivalence tests are not required for the registration of a medicine by the regulatory authority, when a suitable *in vitro* assay can replace it (Butler and Dressman, 2010). The main reason for biowaiver based on the BCS is that in some situations, *in vitro* assays are as good as *in vivo* tests to determine the bioequivalence of oral solid dosage forms, and, sometimes, better in terms of direct evaluation of product performance.

Moreover, biowaiver eliminates unnecessary exposure of healthy subjects to in vivo studies, reduces the burden of evaluating petitions for registration requiring bioequivalence studies, and provides economic relief, maintaining the quality standard of dispensed medicines to public health and thus ensuring therapeutic equivalence (Cook *et al.*, 2010). An estimated 66 to 76 million dollars can be saved each year in costs of clinical studies if biowaiver is granted to all applications for new drugs containing drug class I. If this is extended to class III compounds, additional savings from 62 to 71 million dollars can be performed (Cook *et al.*, 2010). These show that dissolution, especially in each of the stated three media, plays an important role in biowaiver studies. Also, there is lack of methods for analysis of metformin in the stated simulated physiological fluids that could be used for biowaiver studies to the best of our literature search.

This research work is aimed at developing and validating a rapid, simple, accurate, economical and reproducible UV spectrophotometric method for the determination of metformin in pure form and tablet dosage form in three simulated physiological fluids: 0.1 M HCl, simulated intestinal and blood pH of 6.8 and 7.4 phosphate buffers. The developed methods were validated using ICH guidelines for validation of analytical methods (ICH, 2006) and the assay results were compared with the British Pharmacopoeia method (BP, 2013).

Materials and Methods

Equipment and reagents

Standard metformin powder was obtained from Sigma Aldrich (Germany). A tablet brand of metformin (500 mg) with batch No: LV1511039 was purchased from a registered pharmacy premises. Potassium dihydrogen

orthophosphate was BDH-Laboratory reagents. All weighing were carried out using DENVER INSTRUMENT APX-200 digital weighing balance. A double scanning UV/VIS spectrophotometer (Model SP3000) was used to monitor the drug content.

Preparation of dissolution media

Three dissolution media were used, simulated gastric juice (containing 0.1M hydrochloric acid), intestinal pH (phosphate buffer pH 6.8) and blood pH (phosphate buffer pH 7.4). Both media were prepared using official methods (BP, 2013).

Preparation of standard stock solutions

Stock solutions of metformin were prepared by accurately weighing and dissolving 5 mg of metformin in 50 mL of each of the media to obtain a concentration of 100 µg/mL.

Determination of λ_{max}

A portion (1 mL) of each stock solution of metformin was taken and diluted to 10 mL with the same medium. The resulting solution in each case was scanned at wavelength ranges of 200 to 600 nm on spectrophotometer and the wavelength of maximum absorption determined (Figures 2, 3 and 4).

Preparation of calibration curve

From each stock solution, different aliquots in the range 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 mL were transferred into series of 10 mL volumetric flasks and the volume made up to the mark with the same medium to obtain solutions with concentrations 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 µg/mL. The absorbance of each solution was then determined at the respective λ_{max} of metformin in each of the three media. The absorbances obtained were then plotted against their corresponding concentrations (Figures 5, 6 and 7).

Figure 2: Metformin spectrum in 0.1 N HCl showing λ_{max} at 255 nm

Figure 3: Metformin spectrum in medium pH 6.8 showing λ_{max} at 235 nm

Figure 4: Metformin spectrum in medium pH 6.8 showing λ_{max} at 232 nm

Application of the proposed methods

Twenty (20) tablets were randomly selected from the metformin tablet brand, ground into a fine powder using mortar and the resulting powder was weighed. A quantity of the powder, equivalent to 50 mg metformin, was weighed and transferred separately into three different calibrated volumetric flasks (100 mL) labelled A, B and C containing about half their volumes with 0.1M hydrochloric acid, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 respectively. The volume of each flask was made up to the mark with the medium and shaken for 10 minutes. The solutions were filtered through a Whatman Filter paper no. 41. A portion (1 mL) of each filtrate was transferred into a volumetric flask (10 mL) and made up to the mark with the medium to obtain a solution of concentration 50 µg/mL in each case. The absorbance of each of the resulting solutions was determined at the respective λ max of each medium.

The proposed methods were compared with an official method for assay of metformin by assaying the same tablet brand using official method (BP, 2013).

Statistical analysis

The results obtained were analysed using student t-test with GraphPad Prism 6 software. P values less than 0.05 were considered to be statistically significant.

Results and Discussion

The wavelengths of maximum absorption as

well as calibration parameters of metformin in each of the three media are shown in Table 1. It was observed that the calibration curves of metformin in all the three media obeyed the Beer-Lambert's Law. The correlation coefficient (r) values were indicative of the very good linear relationship between absorbance (A) and concentration (C in % w/v) in the regression equations.

The results of the validation parameters for the proposed methods are shown in Table 2. The low % RSD (≤ 1.9) for all the three methods shows the precision of the methods. With a good technique and a reliable methodology, the precision should be < 15 % CV (Harvey, 2000). This shows that the precision of the proposed method is satisfactory. The accuracy of the proposed methods expressed as the measure of percentage relative error or % Er (≤ 2.9) were within the range (1 – 5 %) for moderately accurately procedure (Harvey, 2000). The average percentage recovery for the methods were found to be 99.5, 98.8 and 99.3 % respectively showing that the three methods have good recoveries especially when compared with 99.44, 99.5 and 99.09 % reported by Sulakshana *et al.* (2015) methods for estimation of metformin in 0.1 N HCl, 0.01 N HCl and NaOH respectively. The detection limits (≤ 0.58 µg/mL) and quantitation limits (≤ 1.76 µg/mL) were calculated for the three proposed methods are satisfactory. This shows that the method's sensitivity is good for the analysis of the drug.

Figure 5: Calibration curve of metformin in 0.1 M HCl

Figure 6: Calibration curve of metformin in phosphate buffer pH 6.8

Figure 7: Calibration curve of metformin in phosphate buffer pH 7.4

Table 1: Calibration curve parameters of the developed methods

Parameter	0.1M HCl (pH 1.2)	pH 6.8	pH 7.4
$\lambda_{\max}$ (nm)	205	235	232
Linearity range ($\mu\text{g/ml}$)	2.50 – 15.0	2.50 – 12.5	2.50 – 10.0
Correlation coefficient (r)	0.9992	0.9994	0.9990
Regression equation	$A = Cy + x$	$A = Cy - x$	$A = Cy + x$
Slope (y)	0.0524	0.1415	0.1561
Intercept (x)	0.012	0.02	0.0465

Table 2: Validation parameters of the developed methods

Parameter	0.1M HCl (pH 1.2)	pH 6.8	pH 7.4
Precision (% RSD)	1.8	1.6	1.9
Accuracy $\pm$ SD(% Er)	2.3 ± 0.10	2.9 ± 0.16	2.0 ± 0.32
Recovery $\pm$ SD (%)	99.5 ± 0.50	98.8 ± 0.35	99.3 ± 0.18
Detectionlimit ($\mu\text{g/mL}$)	0.53	0.47	0.58
Quantitationlimit ($\mu\text{g/mL}$)	1.61	1.43	1.76

RSD = relative standard deviation; Er = relative error

The assay results revealed the content of metformin in both the pure powder and tablet sample and were within range (98.5 to 101.0 % and 95.0 to 105.0 %) specified by the BP (Table 3). No statistically significant difference ($p < 0.05$) was observed between

the percentage content of metformin quantified by developed methods and that by official method (BP, 2013). Thus, the proposed methods can be used in place of the official method

Table 3: Comparison of assay results of the proposed methods and BP method

Dissolution Media	% content of metformin $\pm$ SD	
	Developed methods	BP Method
0.1M HCl (pH 1.2)	102.97 $\pm$ 0.12	102.60 $\pm$ 0.10
Phosphate buffer (pH 6.8)	102.75 $\pm$ 0.07	102.60 $\pm$ 0.10
Phosphate buffer (pH 7.4)	102.57 $\pm$ 0.21	102.60 $\pm$ 0.10

BP range (95 – 105 %), n = 3

Conclusion and Recommendation

From the results obtained, it can be concluded that the developed methods for determination of metformin in the present study are simple, rapid, precise, accurate, sensitive, reproducible and cost effective. All the validation parameters fall within the

acceptable limit for analytical method, hence the proposed methods can successfully be applied for quantitative estimation of metformin in pure and tablet dosage forms and for routine quality control and bioequivalence studies of the drug.

References

- Amidon, G. L., Lennernäs, H., Shah, V. P., and Crison, J. R. 1995. A Theoretical Basis for a Biopharmaceutics Drug Classification: The Correlation of In Vitro Drug Product Dissolution and In Vivo Bioavailability. *Journal of Pharmaceutical Research* 12, 413-420.
- British Pharmacopoeia. 2013. Vol II. Printed for Her Majesty's Stationary office at University Press, Cambridge, Appendix VII. Electronic version.
- Brunetti, L. and Kalabalik, J. 2012. Management of Type-2 Diabetes Mellitus in Adults: Focus on Individualizing non Insulin Therapies. *Journal of Pharmacy and Therapeutics* 37(12), 687-696.
- Butler, J. M. and Dressman, J. B. 2010. The Develop Ability Classification System: Application of Biopharmaceutics Concepts to Formulation Development. *Journal of Pharmaceutical Sciences* 99(12), 4940-4954.
- Cook, J. A., Davit, B. M. and Polli, J. E. 2010. Impact of Biopharmaceutics Classification System-Based Biowaivers. *Journal of Molecular Pharmacy* 7(5), 1539-1544.
- Havey, D. 2000. *Modern analytical Chemistry*. The McGraw-Hill company, Inc. pp. 39
- International Conference on Harmonization. 2006. "Q29(R1): Text on *Validation of Analytical Procedures*", Federal register CPMP/ICH/381/95.
- Mubeen, G. and Noor, K. 2009. Spectrophotometric Method for Analysis of Metformin Hydrochloride. *Indian Journal of Pharmaceutical Sciences* 71, 100-104.
- Musa, A., Nasir, I. and Garba, M. 2014. A Novel Spectrophotometric Method for Determination of Lamivudine in Pure and Tablet Dosage Form by Formation of a Coloured Hydrazone Using 2,4-dinitrophenyl Hydrazine. *African Journal of Pharmaceutical Research and Development* 6(1), 1-5.
- Nasir, I., Musa, A. and Garba, M. 2014. Spectrophotometric Method for Determination of Lamivudine from Methanolic Extract of Pure and Tablet Dosage Form. *Nigerian Journal of Pharmaceutical Sciences* 13(2), 28-34.
- Rashmi, R., Sarangi, V., Badawy, S. S. and Ibrahim, M. M. 2011. Simultaneous UV-spectrophotometric Estimation of Glipizide and Metformin in Bulk and Its Dosage Form, *International Journal of Pharmaceutical and Biological Archives* 2(4), 1137-1145.

- Raza, A., Ansari, T. M. and Rehman, A. 2003. Spectrophotometric Determination of Tranexamic Acid in Pharmaceutical Bulk and Dosage Forms. *Analytical Sciences* 21(9), 11-33.
- Raza, A., Ansari, T. M. and Rehman, A. J. 2005. Spectrophotometric Determination of Lisinopril in Pure and Pharmaceutical Formulation. *Journal of the Chinese Chemical Society* 52(5), 1055-1059.
- Sulakshana, S., Sankar, R. V., Roa, N. R., Harithanadin, Y., Dastagiri, R. and Pushpalatha, E. 2015. Spectrophotometric Method Development and Comparative Study of Metformin HCl in API and Solid Dosage Form Using UV-spectroscopy. *International Journal of Pharmacy and Biological Sciences* 5(2), 323-330.
- Sweetman, S. C. 2012. *Martindale. The complete drug reference* (Eds) 35th edition: The Royal pharmaceutical society of Great Britain, London. Electronic version.
- The United States Pharmacopeia and National Formulary USP. 2012. *The United States Pharmacopeial Convention, Inc.*: Rockville, MD; 35-NF 30.
- Yu, L. X., Amidon, G. L., Polli, J. E., Zhao, H., Mehta, M. U. and Conner, D. P. 2002. Biopharmaceutics classification system: The scientific basis for biowaiver extensions. *Journal of Pharmaceutical Research* 19(7), 921-925.