Elimination of the Ultraviolet Spectrophotometric-Based Limitation on the Quantification of Metformin HCl in Acid-Stage Medium for a Comparative Dissolution Testing

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ABSTRACT

Metformin is an antidiabetic drug categorized in the mandatory list; thus, it must conduct an equivalence study, particularly in vitro, a comparative dissolution testing. This evaluation is carried out not only neutral but also acidic medium. However, it has low molar absorptivity in acid conditions; thus, it faces a big challenge in quantifying the drug released. Herein, this work aimed to develop an ultraviolet spectrophotometric assay for metformin HCl in comparative dissolution testing along with the acid stage medium.

Metformin HCl was incorporated into the immediate tablet formulation to mimic the analytical matrix system. The dissolution validation procedure was carried out by linearity, accuracy, precision, the limit of quantification, filter compatibility, and aliquot stability that fulfill the USP method for validation of dissolution procedure, particularly assay of the analyte.

The results revealed that metformin HCl had difficulty quantifying in acid pH due to the absence UV-band peak. The addition of pH-shifting agents promoted better performance for metformin quantification. Therefore, the method was successfully developed along with R^2, predicted R^2, accuracy, and precision of 0.9999, 0,9998, 100.57%, and 1.27%, respectively. In addition, it had the minimum placebo interference (0.47%) and was stable for long-term storage under determined conditions. Therefore, the validated method could be applied for quantifying metformin HCl, particularly acid-medium CDT.

Keywords: Metformin HCl; comparative dissolution testing; ultraviolet spectrophotometer; validation

ABSTRAK

Metformin merupakan obat antidiabetes yang diwajibkan untuk uji bioekuvalensi, sehingga memerlukan kajian ekuivalensi secara in-vitro. Pengujian disolusi terbanding dilakukan pada pH asam, dan metformin memiliki keterbatasan absorbsivitas molar yang rendah dan tanpa adanya puncak. Penelitian ini bertujuan untuk mengembangkan metode analisis metformin HCl berbasis spektrofotometer untuk kuantifikasi hasil uji disolusi terbanding metformin pada medium asam.

Metformin diinkorporasikan ke dalam formulasi tablet immediate release sebagai matriks proses analisis. Validasi dan pengembangan metode disolusi dilakukan berdasarkan parameter linearitas, akurasi, presisi, batas kuantifikasi, kompatibilitas penyaring, dan stabilitas larutan mengikuti prosedur untuk kuantifikasi analit hasil uji disolusi. Hasil menunjukkan bahwa metformin HCl memiliki keterbatasan dalam kuantifikasi di asam karena tidak terdapat peak pada puncak pita UV. Penambahan senyawa alkali untuk menggeser pH mampu meningkatkan kinerja kuantifikasi metformin HCl. Metode analisis telah dikembangkan dan divalidasi serta diperoleh parameter korefisien determinasi (R^2) 0,9999; *predicted* R^2 0,9998; akurasi 100,57%; dan presisi 1,27%. Interferensi placebo (formulasi tablet) mempengaruhi hasil analit sebesar 0,47% dan larutan sampel stabil dalam jangka waktu pengujian dan penyimpanan. Metode analisis ini dapat

diimplementasikan secara meluas untuk kuantifikasi metformin HCl pada pengujian disolusi di medium asam.

Kata Kunci: Metformin HCl; uji disolusi terbanding; spectrophotometer ultraviolet; validasi

1. INTRODUCTION

In Indonesia, the equivalency study has been mandatory for copy drug development since 2019, while the first regulation was issued in 2005 [1]. It is purposed to ensure the drug formulation fulfilled the safety and efficacy, particularly similarity to the innovator formulation. The manufacturer should obtain similarity in vitro and in-vivo through a bioequivalence study. In addition, the in-vitro testing is a guide for formulation development before the in-vivo testing is conducted [2,3]. Therefore, comparative dissolution testing (CDT) plays a fundamental role in the equivalency study, particularly for the mandatory list of bioequivalence studies. The critical performance of the CDT depends on the formulation to gain a similar profile to the innovator formulation [4]; however, quantifying the dissolved drug is the most crucial step. Therefore, the method to quantify is mandatory to be ensured to achieve valid results along with zero error tolerance [5].

Metformin HCl is a biguanide class of antidiabetic drug [6,7], and it has been the only one of the biguanide antidiabetic class included in the mandatory list for bioequivalence study from 2011 until now [8]. Therefore, pharmaceutical industries have a particular consideration on the formulation development to gain similarity in the in-vitro drug release and bioavailability [4,9,10]. Comparative dissolution testing has a standard procedure for dissolution testing, namely, three different pH mediums, i.e., 1.2, 4.5, and 6.8, respectively [1,11]. Although metformin is categorised as having high solubility and low permeability, the misled metformin dissolved promotes distinctive drug release profiles.

An analytical method is crucial for determining the amount of dissolved drug in the comparative dissolution testing [12]. A simple, robust, effective, and efficient method provides a better quantification method. Therefore, an ultraviolet (UV) spectrophotometer is the best choice due to its rapid analysis and cost-friendly, compared to high-performance liquid chromatography. In addition, metformin HCl can produce high sensitivity due to a high molar extinction [13]. However, it faces a problem in the acid medium due to less molar absorptivity. Bi-protonated structure promotes isolation of chromophore linkage [14]. High-performance liquid chromatography offers better selectivity due to the separation process [15].
Nevertheless, this method requires more time and is costly [16–18]. Interaction between metformin and ligan could be applied for better selectivity of the metformin analysis [19]. In addition, multivariate analysis could be used to improve the specificity [20]. On the other hand, metformin has high molar absorptivity. Hence, single drug formulation will be easy to analyse without modification due to less interference [13]. Herein, this present work purposed to develop a simple and fast analytical method for the CDT of metformin, particularly in the acid medium.

2. METODE PENELITIAN

2. 1. EQUIPMENT AND METHODS

A Thermo Genesys 10s spectrophotometer (ThermoScientific; Waltham, MA) was the main instrument in this work. In addition, a Biobase RC-06 dissolution tester was the principal instrument in the dissolution testing simulation.

Metformin HCl as a drug model was obtained from Sohan (India; Lot No. H09690922-23). Hydrochloric acid, potassium hydrogen phosphate, di-potassium hydrogen phosphate, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). The formulation excipients comprise microcrystalline cellulose (Avicel PH101, FMC Biopolymer), pregelatinised starch (Colorcon; West Point, PA), Kollidon CL (BASF; Ludwigshafen, Germany), magnesium stearate (Peter Greven, Germany), and Cab-O-Sil (Cabot; Shanghai, China).

2. 2. METHODS

Specificity and sensitivity assays

For pre-analytical treatment, 100.0 mg metformin HCl was accurately weighed, followed by dissolving in 100.0 mL HCl 0.1N and dilution until a 10 µg/mL concentration was achieved. The sample was scanned using a Thermo Genesys 10s spectrophotometer (Waltham, MA) from 300 to 200 nm along with a resolution of 1 nm, and this analysis was performed in triplicates. In addition, an HCl 0.1N was used as a blank solution. On the other hand, NaOH 0.1 N equal to the aliquot volume and doubled-volume were added before diluting with a phosphate buffer pH 6.8. The placebo interference towards metformin HCl response was calculated based on the percentage of placebo ($A_{placebo}$) and metformin HCl (A_{met}) responses considering the concentration of metformin HCl (C_{met}) and the proportional ratio according to the tablet formulation.

$$\text{Interference (\%)} = \frac{A_{placebo}}{A_{met}} \; x \; C_{met} \; x \; \frac{volume \; x \; dillution \; factor}{weighed \; sample} \; x \; 100\% \quad \text{..... Equation 1}$$

Calibration Model

The calibration model was constructed using the linear regression between concentration and absorbance. The metformin HCl was weighed and diluted accurately until the concentrations in the 1.0 -16.0 µg/mL range were achieved. Before the dilution process, NaOH 0.1N was added to the aliquot sample equal to the volume. The samples were scanned at the maximum wavelength of metformin HCl along with phosphate buffer pH 6.8 as blank in quintuplicates. Linear regression analysis was applied for model calibration fitting along with a confidence level of 95%. Several statistical parameters, namely coefficient determination (R^2), adjusted R^2, and residual of slope, were applied for model evaluation. In addition, the cross-validation model was also built to evaluate the calibration model along with a leave-one-out technique for calculating predicted R^2 and RMSECV.

Validation of Analytical Method for Dissolution Procedure

The validation of analytical methods parameters for the dissolution procedure consisted of placebo interference, filter compatibility, linearity, accuracy, precision, stability of the aliquot, and limit of detection and quantification. Those parameters are based on the United State Pharmacopeia 42 [21].

Placebo interference and filter compatibility assessments

The specificity/placebo interference was measured according to the aforementioned method. The linearity assessment was conducted according to the calibration model. The linearity was assessed according to the R^2 in the predetermined concentration range. Filter compatibility assay was also carried out. Shortly, 500 mg of metformin HCl and 250 mg of the formulation excipients were accurately weighed and dissolved in HCl 0.1N. The aliquot was withdrawn, and added NaOH 0.1 N in an equal volume, followed by dilution until the metformin concentration of 10 µg/mL was achieved. A 0.45 µm nylon membrane filter was utilised in the filter compatibility testing. The filtration was carried out in different sequences before the addition of NaOH 0.1N and in the final dilution. The percentage of the recovery was calculated according to the initial concentration of metformin HCl before the filtration process.

Accuracy and precision

Accuracy and precision were carried out by an external calibration standard method and placebo simulation. Firstly, the metformin HCl was accurately weighed according to the proportion of tablet formulation, i.e., 80, 100, and 120% (400, 500, and 600 mg, respectively). Furthermore, the tablet formulation comprised Avicel PH101 (5%), Kollidon CL (3%), povidone K30 (30 mg, 3% (w/v) in 50% ethanol), magnesium stearate (0.8%), and Cab-O-Sil (0.2%). In addition, pregelatinised starch was added to achieve 750 mg in total amount. The mixture was dissolved in HCl 0.1N, followed by sonication in a water-bath sonicator for 15 min. The sample solution was filtered by a 0.45 µm nylon membrane filter, the aliquot was withdrawn, and NaOH 0.1 N in an equal volume ratio was added. Furthermore, the sample dilution was carried out using phosphate buffer pH 6.8 before quantification using a UV spectrophotometer at the maximum wavelength of metformin HCl. All concentrations were measured in triplicates. The accuracy was calculated according to the percentage of the observed concentration toward the actual concentration based on the dilution. Meanwhile, the relative standard deviation of all samples was denoted as precision.

Aliquot stability

The metformin stability was carried out based on the simulation of the dissolution process. Briefly, 500.0 mg of metformin HCl was accurately weighed and was added by 250 mg of the formation excipient. The mixture was dissolved in a 1000 mL volumetric flask using HCl 0.1N. Prior to the test, the metformin concentration was determined (C_0). The dissolution process simulation was performed using a Biobase RC-06 dissolution tester. The apparatus II (paddle) was utilised at 50 rpm, and the temperature was kept constant at 37±0.5 °C. Thereafter, the dissolution process simulation was carried out for one h, followed by aliquot withdrawal. The aliquot was stored in ambient conditions until the quantification was conducted. The aliquot was quantified at a predetermined time until 48 h after the dissolution process. Briefly, an aliquot sample was withdrawn and added by NaOH 0.1 in equal volume, followed by dilution. The sample was analysed using a spectrophotometer at the maximum wavelength of metformin HCl. The recovery of

the concentration at predetermined times (C_t) was determined according to the percentage of the initial concentration (C_0).

Limit of detection and quantification

The limit of detection (LoD) and quantification (LoQ) were calculated according to the residual slope and blank noise. The linearity calibration model and low concentration (below the concentration of the linearity calibration model) were utilised for the noise estimation based on the residual slope ($S_{y/x}$). In addition, the noise estimation from the blank measurement several times was also utilised for LoD and LoQ determination. The LoD and LoQ were calculated by Equations 2 and 3, respectively.

$$\text{LoD} = \frac{\mathbf{10}}{\mathbf{3}} \boldsymbol{x} \frac{\boldsymbol{S_{y/x}}}{\boldsymbol{slope}} \quad \text{..... Equation 2}$$

$$\text{LoQ} = \mathbf{10} \boldsymbol{x} \frac{\boldsymbol{S_{y/x}}}{\boldsymbol{slope}} \quad \text{..... Equation 3}$$

3. RESULTS AND DISCUSSION

The ultraviolet spectrophotometer is the preferable choice of the analytical instrument due to its cost-friendly, rapid analysis, and efficiency [13,17]. Therefore, this present work developed a spectrophotometric method to quantify the metformin HCl in a pH 1.2 medium for an aliquot comparative dissolution testing assay. According to the physicochemical properties of metformin HCl (Figure 1a), it has two pKa, pKa_1 3.1 and pKa_2 11.3. Hence, it produces different protonated forms depending on the pH. The pKa indicates that the metformin will be bi-protonated when dissolved in the acid stage of CDT. Figure 1b shows the mono-protonated metformin when the pH of analytical condition ranges from pKa1 (3.1) to pKa_2 (11.3). Meanwhile, the bi-protonated metformin will be observed when the pH of analytical condition below pKa1 (3.1) [14].

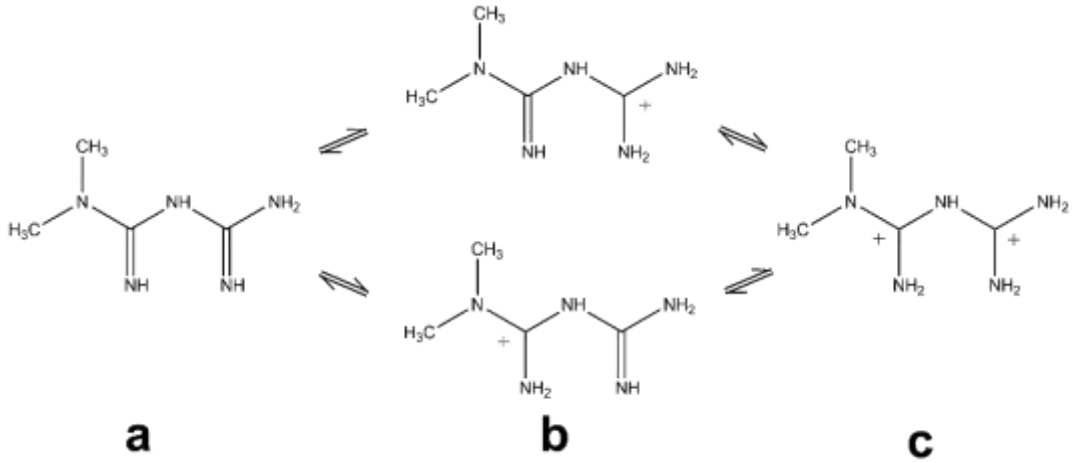

Figure 1. Molecular structure of Metformin HCl in the medium with pH > pKa_2 (a), pKa_1<pH<pKa_2 (b), and pH < pKa_1 (c)

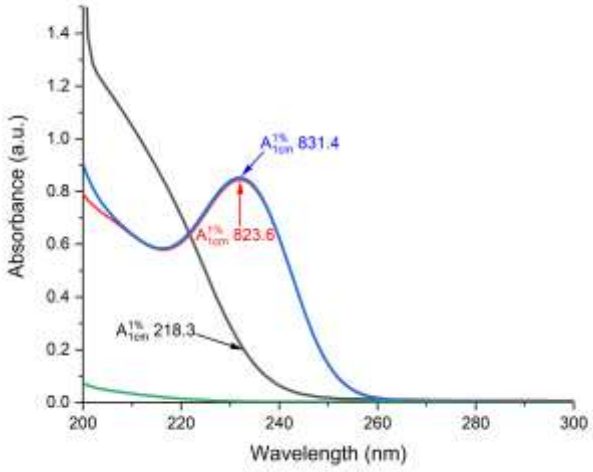

Figure 2. Ultraviolet band of metformin HCl in pH 1.2 (—), addition of sodium hydroxide 0.1N (1; — and 2×volume of aliquot; —), and the placebo excipients (—)

Figure 2 showed that the UV band of metformin in pH 1.2 had no specific peak due to the absence of chromophore structure (bi-protonated). A double-bound structure is required for the transition from π to π^* (HOMO-LUMO transition), which contributed to the presence of a peak [22]. The absorbance declined dramatically from 200 to 240 nm without any peak. It was not easy to quantify, particularly the linear correlation between the concentration and the response. Therefore, it should be altered to find a specific peak for better quantification. Metformin protonates in the acidic medium. Thus, the delocalisation of chromophore and reduction of auxochrome promotes the absence of peak [14]. Hence, pH shifting to achieve the unprotonated structure should be performed. Furthermore, the addition of NaOH 0.1 N in equal and double volume of aliquot was carried out. This treatment aimed to adjust the pH condition that contributed to the presence of chromophore. Integration of chromophore and auxochrome promotes greater intensity or molar absorptivity.

Figure 2 revealed the presence of a peak at 232 nm in both single or double volumes of NaOH. The molar absorptivity was not a significant *difference* ($p>0.05$). Consequently, the volume of NaOH 0.1N equal to the aliquot volume was applied for further evaluation. The sensitivity of the metformin increased nearly four times higher than that of the acidic condition. In addition, the higher sensitivity promoted a negligible effect of placebo interference. It was 0.47% and lower than that of the acidic condition (3.78%). The requirement of placebo interference for dissolution testing is less than 2 and 1% for excipients and blank interferences, respectively [21]. Therefore, the addition of NaOH in the dissolution aliquot met the requirement of the analytical procedure for dissolution assay.

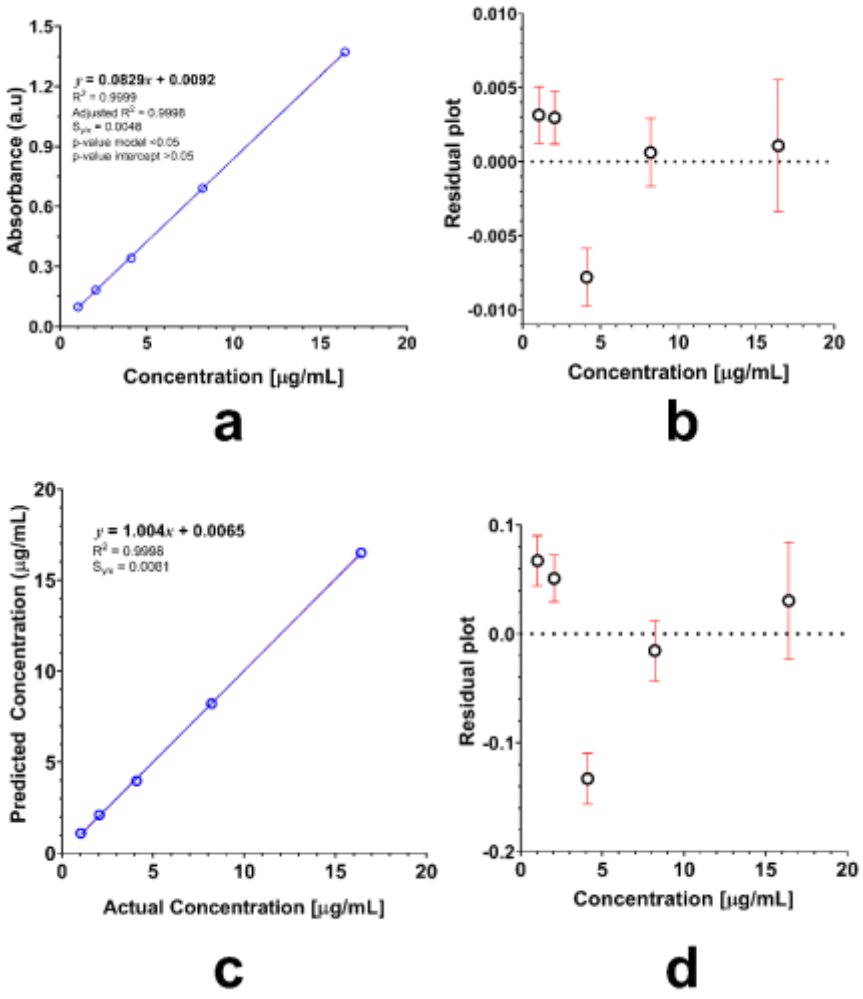

Figure 3. Calibration model of metformin HCl in phosphate buffer pH 6.8 after NaOH 0.1 N addition (a), the residual plot of calibration model (b), the actual-predicted plot (c), and the residual plot of the actual-predicted plot (d). — (linear regression calibration model); ····· (95% confidence interval line); mean±SD, n=3

For quantification purposes, the calibration model was constructed (Figure 3a). There was a linear correlation between concentration and absorbance. Limited error was found around less than 0.01%. Statistical analysis reported that there was a significant model ($p<0.05$) along with an insignificant intercept ($p>0.05$). It proved that the model adequate for quantification [20]. The dissolution procedure assay recommended that the R^2 be more than 0.98 and the intercept equal to zero [21]. The residual plot (Figure 3b) showed that the replication data was normally distributed along with relatively low residual. The leave-one-out technique was applied for validation, and the predicted and actual concentration based on the cross-validation is presented in Figure 3c. The results indicated 100.4% accuracy, and the error was only 0.65%. The residual plot (Figure 3d) showed a maximum

residual value of 0.2%. According to the statistical evaluation, the model was adequate for quantification and had fewer interference/errors.

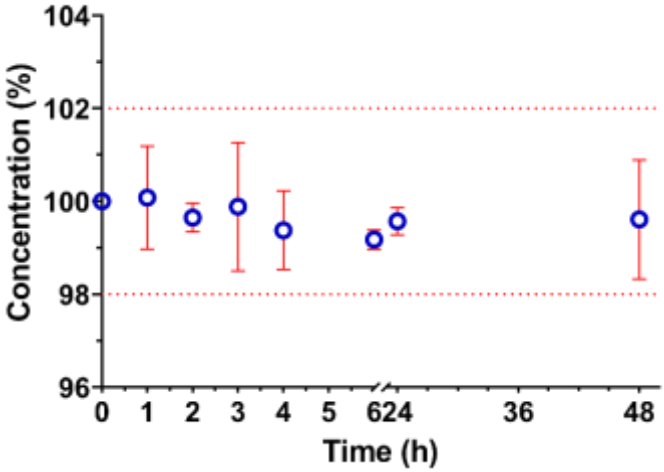

Figure 4. Concentration stability of metformin HCl under dissolution simulation and sample storage in ambient condition mean±SD, n=6

Metformin HCl's stability in the dissolution simulation process was carried out. It is presented in Figure 4. The simulation mimicked the actual stability of the dissolution [23], namely the dissolution process and storage of the aliquot until the analytical procedure was completed. Unstable metformin HCl was indicated by the concentration deviation of more than 2%. The concentration assay proved that the mean and standard deviation were acceptable during dissolution testing and storage. Generally, the degradation pathway can be easily predicted by the molecular structure. In addition, hydrolysis, particularly in acidic conditions, plays a fundamental role in stability during dissolution testing. Meanwhile, another pathway, e.g., oxidation and photolysis, should also be considered for solution stability in dissolution testing [24].

Eliminating undissolved materials or drugs in dissolution testing is crucial for achieving reliable results. Insoluble material can interfere with absorbance and dramatically increase the response, but it is a fake-positive effect. On the other hand, the undissolved drug promotes further dissolution in the sampling chamber; thus, the drug concentration will rise significantly [21,25]. Therefore, filtration is required for this process. In addition, centrifugation is not recommended for dissolution samples due to continuing undissolved drug dissolution process [21]. This work simulated the aliquot filtering process after sampling or in the final solution. The data are presented in Table 1. It indicated no interaction between the filter and metformin HCl because no concentration reduction was observed. However, the sequence of the filtering process affected the recovery. The filtration after sampling was preferable to that of the final solution due to higher accuracy and precision.

Table 1. Filter compatibility assessment filter under different filtration steps

Filtration was carried out after dilution							
No Sample	Concentration (µg/mL)						Recovery (%)
	Before filtration			After filtration			
	Rep 1	Rep 2	Mean	Rep 1	Rep 2	Mean	
1	10.24	10.35	10.29	11.30	11.37	11.34	110.14
2	10.14	10.15	10.15	10.43	10.44	10.44	102.85
3	10.13	10.12	10.12	10.91	10.89	10.90	107.68
Recovery (%)							106.89
RSD							3.47
Filtration was carried out before dilution							
No Sample	Concentration (µg/mL)						Recovery (%)
	Before filtration			After filtration			
	Rep 1	Rep 2	Mean	Rep 1	Rep 2	Mean	
1	10.05	10.12	10.08	10.15	10.17	10.16	100.78
2	10.20	10.21	10.21	10.12	10.14	10.13	99.23
3	10.05	10.08	10.06	10.31	10.25	10.28	102.16
Recovery (%)							100.72
RSD							1.45

Accuracy and precision were also validated in this method. The results are presented in Table 2. Those evaluations were conducted at 80-120% of the actual concentration. The data showed no significant difference between those levels ($p>0.05$). The total recovery was 100.57%, along with an RSD of 1.29%. The results provided valid and reliable data for quantification in the range of 98-102% and less than 2%, respectively [21,26]. The excellent accuracy and precision data were supported by the placebo interference data. There was no significant impact on the placebo for quantification of metformin. Hence, the results met the accuracy and precision criteria.

Table 2. Precision and accuracy data of metformin HCl using external validation model

Proportion (%)	Actual Concentration (µg/mL)	Observed Concentration (µg/mL)	Recovery (%)	Recovery Mean (%)	RSD (%)
80	8.175	8.149 8.270 8.197	99.68 101.15 100.27	100.37	0.74
100	10.219	10.434 10.332 10.488	102.10 101.10 102.63	101.95	0.76
120	12.263	12.152 12.122 12.291	99.10 98.85 100.23	99.39	0.74
Recovery (%) Precision (%)			100.57 1.29		

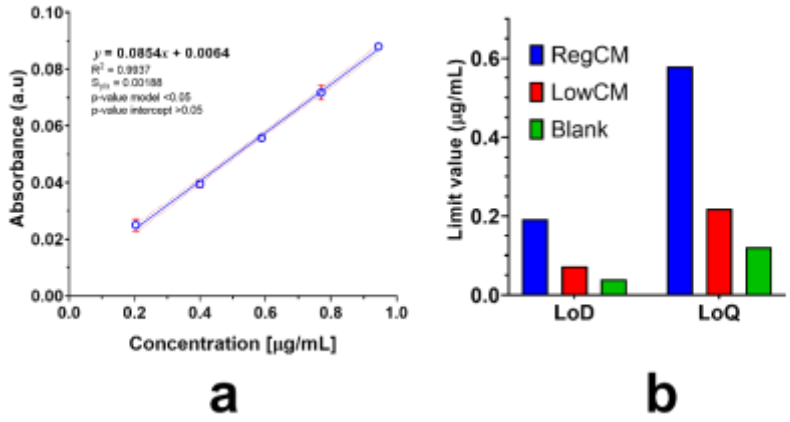

Figure 5. Calibration model of below the lowest concentration in regular calibration model (a) and limit of detection (LoD) and quantification (LoQ) under different methods (b). RegCM, regular calibration model; LowCM, lower of regular calibration model, and blank deviation, — (linear regression calibration model), ····· (95% confidence interval line); mean±SD, n=3

The dissolution assay is categorised as a quantitative assay, which only requires the LoQ. Meanwhile, for several reasons, for instance, the standard addition method should be applied if the response is too low in the range between LoD and LoQ or around LoQ. However, the determination of LoQ and LoD was confusing due to the noise source. Generally, noise is calculated according to the residual slope in the regular calibration model. Some researchers reported that the calibration model should be in a low concentration of regular calibration [27]. It is presented in Figure 5a. The data showed there was a nearly similar molar absorptivity between the regular calibration model ($A_{1\,cm}^{1\%}$ 829) and below its concentration ($A_{1\,cm}^{1\%}$ 854). In addition, the standard deviation of the blank could be applied as the noise. Therefore, in this work, we applied the different noise sources to calculate the LoD and LoQ. The results (Figure 5b) showed that the regular calibration model had the highest noise level; thus, the LoD and LoQ were the greatest. Although, the blank was considered the lowest LoD and LoQ levels. It was affected by the residual value of the calibration model. The residual error assumes the noise; meanwhile, the actual noise was the interference from blank or placebo. Therefore, the noise is attributed to the instrument or measurement mistakes leading to the inaccurate calculation [28]. According to the data, the actual noise was applied for analytical method validation, i.e., 0.04 and 0.121 µg/mL for LoD and LoQ, respectively.

4. CONCLUSION

Herein, the analytical method for quantification of metformin in the acid stage of comparative dissolution testing has been successfully developed and validated. The calibration model was validated in the range of 1.03 – 16.43 µg/mL, along with accuracy and precision of 100.57% and 1.29%, respectively. In addition, the metformin HCl and nylon membrane filter had no adsorption phenomenon, and the solution was stable during the dissolution process and storage for 48 hours. This

method was useful for quantifying the metformin HCl in the acid-stage medium in the compendia dissolution or comparative dissolution testing along with valid and reliable drug release data.

5. ACKNOWLEDGEMENT

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6. REFERENCES

[1] BPOM. Peraturan Badan Pengawas Obat dan Makanan Nomor 11 Tahun 2022 tentang Tata Laksana Uji Bioekivalensi 2022.
[2] Chen M-L, Blume H, Beuerle G, Mehta M, Potthast H, Brandt A, et al. Summary report of second EUFEPS/AAPS conference on global harmonization in bioequivalence. European Journal of Pharmaceutical Sciences. 2019;127:24–8. https://doi.org/10.1016/j.ejps.2018.10.019.
[3] Mehta M, Blume H, Beuerle G, Tampal N, Schug B, Potthast H, et al. The global bioequivalence harmonisation initiative: Report of EUFEPS/AAPS third conference. European Journal of Pharmaceutical Sciences. 2020;151:105383. https://doi.org/10.1016/j.ejps.2020.105383.
[4] Xie F, Ji S, Cheng Z. In vitro dissolution similarity factor (f2) and in vivo bioequivalence criteria, how and when do they match? Using a BCS class II drug as a simulation example. European Journal of Pharmaceutical Sciences. 2015;66:163–72. https://doi.org/10.1016/j.ejps.2014.10.002.
[5] Poluyanov AM, Kochug A, Mitrofanova LS, Nikitin ID, Vergasov OYu, Shohin IE. Development, Validation and Approbation Analytical Method for the Quantitative Determination of Taurine by HPLC-UV Method in the Test of Comparative Dissolution Kinetics. Drug Development and Registration. 2023;12:62–72. https://doi.org/10.33380/2305-2066-2023-12-2-62-72.
[6] Chapler MR, Ray SD. Biguanides. Ref. Module Biomed. Sci., Elsevier; 2023. https://doi.org/10.1016/B978-0-12-824315-2.00201-3.
[7] Surbakti C, Ginting PAB, Abadi H, Duha Y. Uji Aktivitas Antidiabetes Ekstrak Etanol Daun Cengkeh (Syzygium aromaticum) Terhadap Tikus Putih Jantan dan Gambaran Histologi Pankreas. Jurnal Farmasi Indonesia. 2022;19:161–8. https://doi.org/10.31001/jfi.v19i1.1375.
[8] BPOM. Peraturan Badan Pengawas Obat dan Makanan Nomor 65 Tahun 2022 tentang Daftar Obat Generik Tertentu Wajib Uji Bioekuivalensi 2022.
[9] Alsulais HM, Alqaisum AK, Aldoulah AA, Mohsin AA, Ghonaim HM. Comparative Study of Brand and Generics Ciprofloxacin Tablets Available in the Saudi Market. Dissolution Technologies. 2023;30:GC12–8. https://doi.org/10.14227/DT300223PGC12.
[10] Renukuntla J, Palakurthi SS, Bolla PK, Clark BA, Boddu SHS, Manda P, et al. Advances in in-vitro bioequivalence testing methods for complex ophthalmic generic products. International Journal of Pharmaceutics. 2022;627:122209. https://doi.org/10.1016/j.ijpharm.2022.122209.

[11] Matsui K, Nakamichi K, Nakatani M, Yoshida H, Yamashita S, Yokota S. Lowly-buffered biorelevant dissolution testing is not necessarily biopredictive of human bioequivalence study outcome: Relationship between dissolution and pharmacokinetics. International Journal of Pharmaceutics. 2023;631:122531. https://doi.org/10.1016/j.ijpharm.2022.122531.
[12] Sano AY, Lourenço FR. Measurement uncertainty arising from sampling and analytical steps of dissolution test of prednisone tablets. Journal of Pharmaceutical and Biomedical Analysis. 2023;234:115501. https://doi.org/10.1016/j.jpba.2023.115501.
[13] Azis SBA, Syafika N, Qonita HA, Mahmud TRA, Abizart A, Permana AD. Application of validated spectrophotometric method to quantify metformin in the development of glucose-responsive microparticles loaded dissolving microneedles. Microchemical Journal. 2022;183:108051. https://doi.org/10.1016/j.microc.2022.108051.
[14] Hernández B, Pflüger F, Kruglik SG, Cohen R, Ghomi M. Protonation–deprotonation and structural dynamics of antidiabetic drug metformin. Journal of Pharmaceutical and Biomedical Analysis. 2015;114:42–8. https://doi.org/10.1016/j.jpba.2015.04.041.
[15] Maritha V. HPLC Method Development and Validation for Simultaneous Assay of Metamizole, Thiamine and Pyridoxine in Tablet. Jurnal Farmasi Indonesia. 2016;13:126–32. https://doi.org/10.31001/jfi.v13i2.281.
[16] Kannaiah KP, Sugumaran A. Environmental benign AQbD based estimation of ketoconazole and beclomethasone by RP-HPLC and multi-analytical UV spectrophotometric method. Microchemical Journal. 2022;172:106968. https://doi.org/10.1016/j.microc.2021.106968.
[17] Kaur M, Mittal SK, Chawla R. Simultaneous estimation of tramadol and piroxicam by UV spectrophotometer and RP-HPLC. Materials Today: Proceedings. 2022;48:1735–9. https://doi.org/10.1016/j.matpr.2021.10.041.
[18] Tarigan RE, Lutvia L, Sitorus RMT. Development and Validation of Area Under Curve Spechtrophotometry Method For Binary Mixture Of Paracetamol and Ibuprofen In Tablet Dosage Form. Jurnal Farmasi Indonesia. 2022;19:246–52. https://doi.org/10.31001/jfi.v19i2.1388.
[19] Mubeen G, Noor K. Spectrophotometric Method for Analysis of Metformin Hydrochloride. Indian Journal of Pharmaceutical Sciences. 2009;71:100–2. https://doi.org/10.4103/0250-474X.51947.
[20] Choiri S, Ainurofiq A. Spectrophotometric analysis of desloratadine multicomponent crystal formulation: Comparison of conventional methods and chemometric analysis. Molekul. 2020;15:1–8. https://doi.org/10.20884/1.jm.2020.15.1.560.
[21] USP. <1092>The dissolution procedure: Development and Validation. United Stated Pharmacopoeia 42-Natl. Formul. 37, Rockville, Maryland, USA: The United States Pharmacopeial Convention; 2019, p. 7178–97.
[22] Lawrence M, Paulraj EI, Rajesh P. Spectroscopic characterization, electronic transitions and pharmacodynamic analysis of 1-Phenyl-1,3-butanedione: An

effective agent for antipsychotic activity. Chemical Physics Impact. 2023;6:100226. https://doi.org/10.1016/j.chphi.2023.100226.
[23] Kuncahyo I. Effect of Mg Stearat, Talc or Their Mixtures To The Disintegration Time and Dissolution of Prednisone Interactive Mixtures Tablet. Jurnal Farmasi Indonesia. 2009;6:9–20. https://doi.org/10.31001/jfi.v6i1.9.
[24] Sonntag E, Kolář J, Djukaj S, Lehocký R, Štěpánek F. Accelerated reactive dissolution model of drug release from long-acting injectable formulations. European Journal of Pharmaceutics and Biopharmaceutics. 2023;189:122–32. https://doi.org/10.1016/j.ejpb.2023.06.003.
[25] Horkovics-Kovats S. Disintegration Rate and Properties of Active Pharmaceutical Ingredient Particles as Determined from the Dissolution Time Profile of a Pharmaceutical Formulation: An Inverse Problem. Journal of Pharmaceutical Sciences. 2014;103:456–64. https://doi.org/10.1002/jps.23767.
[26] Wardani GA, Erlinasari W, Tuslinah L. Pengaruh Konsentrasi dan Waktu Perendaman Ampas Tebu terhadap Kadar Akrilamida pada Minyak Goreng Bekas. Jurnal Farmasi Indonesia. 2022;19:133–47. https://doi.org/10.31001/jfi.v19i1.1335.
[27] Uhrovčík J. Strategy for determination of LOD and LOQ values – Some basic aspects. Talanta 2014;119:178–80. https://doi.org/10.1016/j.talanta.2013.10.061.
[28] Hidayah SN, Hastuti AAMB. Comprehensive estimation of measurement uncertainty in determination of antioxidant activity in natural product by 2,2'-diphenil-1-picrylhydrazyl (DPPH) radical scavenging assay. Chemical Papers. 2023;77:4579–87. https://doi.org/10.1007/s11696-023-02808-1.