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A Validated Ultra Performance Liquid Chromatography Method for Quantification of Metformin in Human Plasma

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ABSTRACT

Metformin is oral hypoglycemic or blood sugar-lowering drug which is used for the first- line drug in the treatment of diabetes mellitus type 2. This study presented the validated of Ultra Performance Liquid Chromatography UltraViolet (UPLC-UV) method for the determination of metformin in human plasma. Metformin levels were measured using UPLC with UV detector and liquid-liquid extraction method. Separation was carried out on a Acquity UPLC HSS T3 100mm × 2.1mm i.d. column (1.8µm partikel size) using gradient elution of acetonitrile and phosphate buffer 0.02 M (0.6 mL/min) as mobile phase at 30°C. The analyte was monitored at 236 nm. No endogenous substances were found to interfere with the peaks of drug and internal standard. The value of percent deviation and the coefficient variation obtained respectively less than the percentage set in the FDA guidelines. The linearity factor values were more than 0.997 and LOD was 0.01µg/mL. UPLC with UV detector is able to analyze metformin in a short time with good precision and accuracy which is useful for bioequivalence and bioavailability studies.

Key word: metformin, human sample, plasma, UPLC, validated

INTRODUCTION

Metformin (MTF) is a biguanide oral anti-hyperglycemic agent widely used in the management of type-2 diabetes mellitus (T2D) [1,2]. Metformin has been used as the first choice of T2D treatment guidelines. It is relatively inexpensive and leads to less weight gain [3,4]. It reduces blood glucose levels, by improving hepatic and peripheral tissue sensitivity to insulin without affecting the secretion of insulin [5]. There is substantial inter-individual variability in response to MTF, leading to research on MTF pharmacokinetics to predict variations in response as well as aid the tailoring of MTF therapy [6].

Metformin analysis of several kinds of samples especialy human body fluids is widely developed using numerous chromatographic methods over years. The extraction process to remove other endogenous disorders from the sample matrix is usually the first step for

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analysis. Precipitation proteins are widely used for MTF analysis, although simple and fast but not specific and less effective in removing other analytical disorders. Pairing ion liquid-liquid extraction [7–10] and solid liquid extraction [11] have also been developed to overcome the polarity of MTF. Ultrafiltration with the cation exchange column has also been reported but its sensitivity is low [12]. The method of chromatographic gas with the addition of a derivatitation agent was quite time-consuming and complicated [9].

Liquid-liquid extraction becomes an option beside due to simple enough to do, it is also effective for removing the intruder analyte from the sample matrix. The highly polar character of MTF gives challenge in the extraction process and its retention capability in the column. The use of Ultra Performance Liquid Chromatography (UPLC) as an analytical tool is expected to provide a sensitive analysis with faster analysis time and economically can reduce reagent consumption. The method is therefore considered to be sensitive enough to be appropriate for pharmacokinetic studies of MTF.

EXPERIMENT

Chemicals and instrumentation

Metformin-hydrochloride (97% purity) were bought by Sigma-Aldrich. Acetonitrile (UPLC grade), hexane, 1-butanol, sodium hydroxide, ammonium acetate, acetic acid, and phosphoric acid were obtained from Merck Millipore. The Milli-Q water provided by the Pharmacokinetic lab (Prepared by using Merck Milli-Q Integral System Water Purification). The blank human plasma was supplied by Local Blood Center-Bandung.

The chromatography system consists of UPLC with a quartanery pump system, autosampler with variable injection valve and Mass Spectrometry detector all from Waters. Data processing for chromatogram and instrumentation system used Empower 3 software. The separation was carried out by Acquity UPLC HSS T3 column 100 mm × 2.1 mm i.d., particle size 1.8 µm (Waters). The mobile phase consisted of acetonitrile-potassium dihydrogen phosphate 0.02 M pH 3.23 with gradient change. The flow rate of the mobile phase through the analysis column is 0.6 mL/min and temperature set at 30°C. The analyte was monitored at 236 nm.

Procedure reaction

Stock solutions of MTF (500 µg/mL) were made in methanol. Each solution then aliquot as much as 1 mL into the microtubes and stored in 4˚C conditions. Stock solutions were further diluted with plasma order to obtain standard solution which the concentration ranging from 0.01-5.0 µg/mL. For internal quality control solution also diluted with plasma in three concentrations i.e. 0.25; 1.5; and 4.0 µg/mL.

A total of 100 µL plasma samples was inserted into conical glass tubes. The 40 µL of NaOH 4 M was added into each conical glass tube. The 1-butanol/hexane (50:50 v/v) as extraction agent was added to each tubes as much as 1.5 mL. Each sample in the conical glass tube was votexed and centrifuged for 1 and 5 minutes at 1600 rpm and 1910 g respectively. The top layer of sample was remove as much as 1 mL to microtube, and it was vortexed for 1 minute at 1600 rpm after added by 200 µL phosporic acid 0.1% into each sample. The sample was centrifuged for 5 minutes at 1950 g, and it was pipetted as much as 150 µL from thebottom layer inside the microtube into the UPLC vial. The volume of injection through the UPLC column was setting at 10 µ.

RESULT AND DISCUSSION

Results

The developed UPLC method was optimized for the analysis of metformin in human plasma. The method was validated for selectivity, linearity, limit of quantification, accuracy, precision and recovery as per the international guidelines. Also, an analysis method should be evaluated and tested to ensure that the method is capable to generate objective and valid data, in other words that the method must be validated first. Some parameters tested at the time of validation relate with the ability of other substances interfering the identification or quantitative analysis of the analyte target [13].

Validation involves five concentrations in its implementation, i.e. highest limit of quantification (HLOQ) or standard 1, lowest limit of quantification (LLOQ) or standard 6 in the calibration curve and three solutions of the internal quality control (QC), high (QC H), medium (QC M), and low concentration (QC L). The within-day assay validation data was shown in Table 1.

Table 1. Results of Validation Assay

Theoritical concentration (µg/ml)	Respond of MTF	Calculated concentration (µg/ml)	Statistic parameters
HLOQ 5.04	56051	5.892	Mean 5.549 µg/mL % Var 4.3 Accuracy 110.1%
	52793	5.361	
	62716	5.574	
	63323	5.374	
	61315	5.546	
LLOQ 0.01	166	0.011	Mean 0.011 µg/mL %Var 5.6 Accuracy 113.3%
	147	0.012	
	163	0.012	
	162	0.011	
	164	0.011	
QC H 4.032	55255	4.664	Mean 4.256 µg/mL %Var 6.7 Accuracy 112.2%
	50633	4.449	
	49676	4.545	
	38434	4.121	
	46825	4.851	
QC M 1.512	17312	1.47	Mean 1.491 µg/mL %Var 3.8 Accuracy 98.6%
	19174	1.592	
	16924	1.449	
	16677	1.471	
	17146	1.475	
QC L 0.252	2833	0.262	Mean 0.251 µg/mL %Var 4.3 Accuracy 99.7%
	3165	0.248	
	3012	0.237	
	3159	0.246	
	2816	0.263	

Acceptance criteria for accuracy and precision the parameter is if the accuracy value should be within 15% except at LLOQ, where it should not more than 20%. The deviation of the mean from the result values of 5 times repetition toward each its true value concentration serves as measure of the accuracy and the precision value. The results showsvalue of within-day accuracy and precision for metformin in this measurement is 98.6-113.3% and coefficient

variation below 7%. These results meet the requirements of the acceptance criteria for accuracy and precision parameter of an analysis method.

The selectivity test can be used to determine the ability of the analytical method to differentiate and quantitate the analyte from the presence of interference in the matrix. The test was performed by extracting together six blank matrices (in this study human plasma) along with the smallest standard solution (LLOQ). The calculations of the ratio between the actual peak height of analyte and interference were chosen. The height peak value of the interference peak does not allow greater than 20% from analyte at the lowest concentration, whereas for the internal standard should not be greater than 5% [13]. Results of the test showed that there was no interference peak at the analyte and internal standard retention time, the retention times obtained for metformin and buformin in plasma were 0.870 and 2.267 respectively (Figure 1).

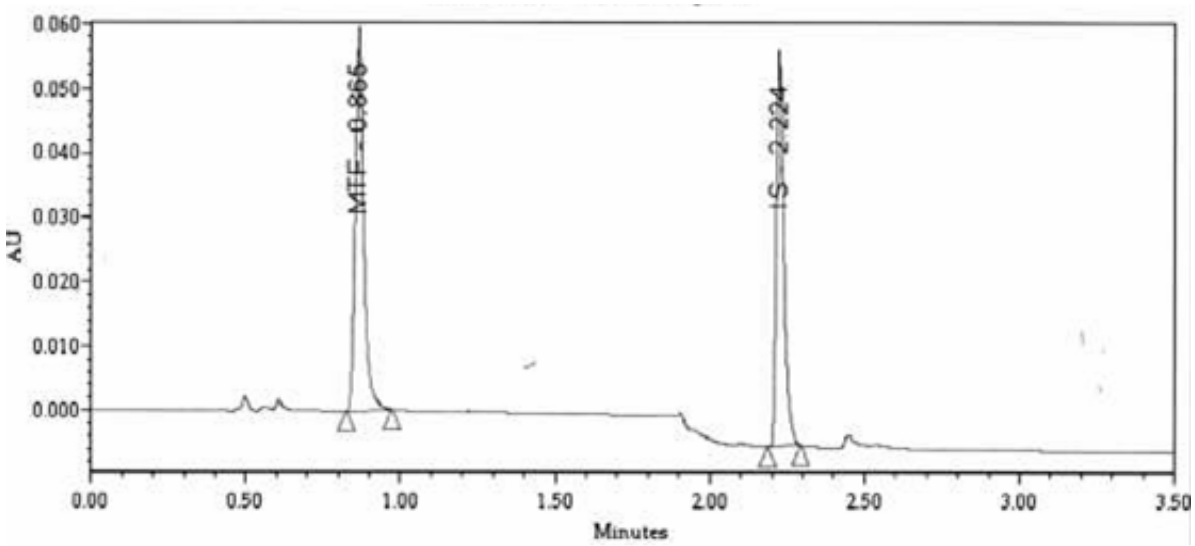

Figure 1. A typical chromatogram of metformin analyte and standard internal

Linearity is a parameter that describes a linear relationship between detector response and the concentration of analyte in the range of concentration at calibration curve. The linearity is indicated by the value of the correlative coefficient that its enormity must be close to one. From the standard curve of the test results were obtained that correlative coefficient value greater than 0.997. In this method the highest concentration value in the working range is 5 µg/mL and the lowest limit at the range is 0.01 µg/mL. The lowest limit value of the working range is called limit of detection, the smaller LOD value means the more sensitive of the analysis instrument that used.

The recovery of an analyte is a comparison value between the detector response obtained from the amount of analyte that added and then extracted from the sample matrix, compared to the detector response for the correct concentration of the standard. The recovery value should not be 100%, but the level of its recovery (both of analyte and internal standard) should be consistent (for all tested concentrations). From the calculation of its recovery value for this method obtained that the recovery value was as much as 62,7% which liquid-liquid extraction were choosen to withdraw the analyte from human blood plasma.

Discussion

New validated method for analysis metformin had been conducted by Ashutosh [14] using the HPLC Waters Alliance 2695 instrument equipped with 2487 Detector with X-Terra C18 column (4.6 × 150 mm, 3.5 µm). The sample is injected with an automatic injector. A total of 20 µL samples were injected prior to precipitation protein extraction. The input and output operations of the chromatography system use the Waters Empower Software. The selected flow rate is 1.0 mL per minute. Detection was performed at 235 nm at 25 °C and a retention time of 8.0 min. The concentration used is quite large from the range of 300 to 700 mg/L with the linearity shown R2 = 0.997, indicating a good R2 value because it is almost close to the value 1 [11]. However, the smallest standard value is very large 300 ppm so that if the concentration of sample is found in below that smallest standard value, it will not be quantified. Recent study more efficient because we just need 5 µL of samples if we compare in this journal needs 20 µL to be injected.

Another validated method using UPLC had been done by Mohammed [15] using Waters UPLC Xevo TQD (Milford, Massachusetts, USA) using the 1.38 µm 2.1 × 50 mm HSS T3 Acquity UPLC column at 40° C. In previous study of selectivity was found that there is no endogenous peaks which can interfere the quantification of metformin and internal standard. It is known that use of UPLC is preferred because of fewer eluent use, fewer samples, shorter retention times so that analysis can be performed effectively and efficiently. Our study has the smallest concentration (0.010 mg/L) than previous study (300 mg/L) so that the non-quantifiable concentration can be obtained.

CONCLUSION

The utility of Ultra Performance Liquid Chromatography (UPLC) with UV detector as an analytical instrument is provided to give a rapid sensitive analysis and economically can reduce reagents consumption. Validated analysis method of metformin in human plasma gives good precision, simple and accurate which is useful for bioequivalency/bioavailability studies.

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REFERENCES

[1] O'Neil, M.; Heckelman, P.; Koch, C.; Roman, K., The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 4th ed., **2012,** Station, W., Ed.; Merck & Co. , Inc: New Jersey.
[2] Nielsen, F.; Christensen, M. M. H.; Brasen, K., *Ther. Drug Monit.,* **2013**, 36 (2), 211–217.
[3] Research, D. P. P., *Diabetes Care,* **2012**, 35, 731–737.
[4] American Diabetes Association. Standards of Medical Care in Diabetes - 2017. *Diabetes Care,* **2017**, 40 (Supplement 1), S33–S43.
[5] Chhetri, H. P.; Thapa, P.; Van Schepdael, A. *Saudi Pharm. J.,* **2014**, 22, 483-487.
[6] Santoro, A. B.; Stage, T. B.; Struchiner, C. J.; Christensen, M. M. H.; Brosen, K.; Suarez-Kurtz, G., *Br. J. Clin. Pharmacol.,* **2016**, 82, 1002–1010.
[7] Amini, H.; Ahmadiani, A.; Gazerani, P., *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2005**, 824 (1–2), 319–322.
[8] Muadifah, A.; Sulistyarti, H.; Prasetyawan, S., *J. Pure Appl. Chem. Res.* **2017**, 6 (3),

196–206.
[9] Gabr, R. Q.; Padwal, R. S.; Brocks, D. R., *J. Pharm. Pharm. Sci.* **2010**, 13 (4), 486–494.
[10] Ranetti, M.-C.; Ionescu, M.; Hinescu, L.; Ionică, E.; Anuţa, V.; Ranetti, A. E.; Stecoza, C. E.; Mircioiu, C., *Farmacia,* **2009**, 57 (6), 728–735.
[11] Tahara, K.; Yonemoto, A.; Yoshiyama, Y.; Nakamura, T.; Aizawa, M.; Fujita, Y.; Nishikawa, T., *Biomed. Chromatog,*. **2006**, 20, 1200-1205.
[12] Vesterqvist, O.; Nabbie, F.; Swanson, B., *J. Chromatogr. B Biomed. Appl.,* **1998**, 716, 299-304.
[13] FDA. *Guidance for Industry: Bioanalytical Method Validation,*; **2018.** US FDA: Rockville.
[14] Ashutosh, K.; Manidipa, D.; Seshagiri, R.; Gowri, S., *J. Chromatogr. Sep. Tech.* **2015**, 6 (6), 293.
[15] Mohammed, S. A. E.; El-Dardiri, M.; Elhabak, M. A.; Abu Zaid, K. I., *Eur. J. Chem.***2015**, 6 (2), 178–182.