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Modification of a Previously Published HPLC-UV Method for Metformin Determination, Validation and Application to Test Bioequivalence

Suhair Al-Nimry,[a*] Jaafar Al Tamimi,[b] Yousef Zaid,[c] Khaleda ALKhatib,[c] Claude Khalil,[d] and Marleine Akl [d] ✉

[a] Department of Pharmaceutical Technology, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan
[b] Clinical and Pharmacokinetics unit, ACDIMA Center for Bioequivalence and Pharmaceutical Studies, Amman, Jordan
[c] Bioanalytical unit, ACDIMA Center for Bioequivalence and Pharmaceutical Studies, Amman, Jordan
[d] Algorithm Pharmaceutical Manufacturers S.A.L., Beirut, Lebanon

ABSTRACT

The aims were to overcome the problem of peak deterioration caused by HCl used in a sample preparation and validate and apply the modified method to test the bioequivalence of Glystor (Algorithm S.A.L., Lebanon) and Glucophage® (Merck Santé S.A.S., France) tablets. Non-acidified samples were prepared by protein precipitation followed by washing with dichloromethane. The mobile phase consisted of acetonitrile and phosphate buffer (65:35) pH 7.5. A Hypersil silica column was used. The method was validated according to the FDA guidance and applied to test bioequivalence. No deterioration in the peak shape was observed. Only two columns were used during validation and analysis. Good separation of atenolol and metformin was achieved. Calibration curves (40-2000 ng/ml) were linear. Accuracy and precisions were within required limits. Absolute recoveries were >78.30%. Metformin was stable in stock solution and in plasma during processing and storage. Statistical analysis of pharmacokinetic parameters demonstrated bioequivalence. In conclusion, the modified method was functional and the two products were bioequivalent.

Keywords: Metformin; HPLC-UV; Human Plasma; Protein Precipitation; Bioequivalence.

INTRODUCTION

Metformin is an oral hypoglycemic drug used in the management of diabetes mellitus (non-insulin dependent or type II diabetes). It reduces blood glucose levels, not by affecting secretion of insulin but mainly by improving hepatic and tissue sensitivity [1]. The starting dose is usually 500 mg administered two times per day or 850 mg administered one time per day and it can be increased depending on a patient's response to a maximum of 2550 mg per day [2]. The absolute bioavailability is 50-60% of the administered dose because of incomplete gastrointestinal absorption. Most of the absorbed drug is excreted through kidneys with a plasma elimination half-life of about 2-6 hrs[1].

In order to test the bioequivalence of two products containing metformin, Glystor 850 mg and Glucophage® 850 mg tablets, one of the numerous high performance liquid chromatographic (HPLC) methods was selected. These methods were developed for the determination of metformin in human plasma either alone or simultaneously with other oral hypoglycemic drugs like glipizide[3], gliclazide[4], rosiglitazone[5], or sitagliptin[6]. The selection criteria were based: 1) on the sensitivity of the method which would allow the determination of the drug at low concentrations, 2) on the sample preparation technique which should be simple, rapid and efficient in removing endogenous interfering substances, and 3) on the retention times of the drug and the internal standard which should be reasonable [7].

After looking carefully at a large number of the published methods, it was found that most of them did not completely satisfy the aforementioned criteria and they suffered from a number of disadvantages, including lack of sensitivity, a limit of quantification (LLOQ) above 50

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✉ E-mail: ssnimry@just.edu.jo

ng/ml[3,8-11], long retention times[12-13], complicated and time-consuming sample preparation techniques including derivatisation[14] and complex extraction, ultrafiltration and a column switching system[10], liquid-liquid extraction followed by back extraction[1516], ion-pair solid phase extraction[17], or solid phase extraction[4]. However, the method developed by Cheng and Chou [18] seemed to satisfy the aforementioned criteria. The method had good sensitivity, protein precipitation was used for sample preparation and retention times for the drug, and the internal standards were reasonable (7.8 and 6.8 minutes, respectively). Therefore, it was selected and validation according to the Food and Drug Administration (FDA) guidance on a bioanalytical method validation, 2001[19] was started in the center.

Unfortunately, the peak shape began to deteriorate with time probably because of the rapid deterioration of the column packing material caused by HCl used for acidifying the plasma samples. This necessitated the use of a new column every two to three days which would greatly increase the cost of analysis since such a bioequivalence study generates a large number of samples.

Therefore, the objectives were to: modify the method to overcome the problem of rapid deterioration in peak shape, reduce the cost of analysis and end up with an efficient method that satisfies the aforementioned criteria, validate the modified method according to the FDA guidance on a bioanalytical method validation, 2001 [19], and apply it to test the bioequivalence of Glyostor 850 mg (test) and Glucophage® 850 mg (reference) tablets.

EXPERIMENTAL SECTION

Chemicals and Reagents

The Atenolol reference standard was kindly donated by the Ram Pharmaceutical Industries Company. Metformin HCl reference standard was obtained from Dongbang Future Technique of Life Company (Korea). HPLC grade solvents were obtained from Tedia Company, Inc. (USA). Milli-Q water was used. Analytical grade potassium phosphate monobasic was obtained from Riedel de Hean (Germany). Analytical grade sodium hydroxide was purchased from Scharlau (Spain).

Standard Solutions

Stock solutions of 1.0 mg/ml metformin in Milli-Q water and 1.0 mg/ml atenolol in methanol were prepared. Working standards of 100 µg/ml metformin and 160 µg/ml atenolol were prepared from the respective stock solutions by diluting aliquots with water and methanol, respectively. The standard solutions of metformin were obtained by serial dilution of the working solutions with water.

Sample Extraction

An aliquot of 0.5 ml non-acidified plasma was spiked with 25 µl metformin standard solution (appropriate concentration) and 25 µl atenolol working solution. Then, proteins were precipitated with 750 µl acetonitrile; the mixture was vortex-mixed for 1.0 minute, and centrifuged at 4000 rpm at 4.0 °C for 5 minutes. The supernatant was decanted to another test tube, washed with 2.5 ml dichloromethane by vortex-mixing for 1.0 minute, and centrifuged at 4000 rpm at 4.0 °C for 5 minutes. A volume of 100 µl of the clear supernatant was transferred into autosampler vials and a volume of 25 µl was injected into the equilibrated HPLC-UV system.

Apparatus and Chromatographic Conditions

The analysis was performed using Waters 515 HPLC Pump and Waters 717 plus autosampler for mobile phase and sample delivery. A Waters 2487 UV detector was set at 235 nm. Data acquisition was performed using Empower 2 software. A Hypersil silica (100 mm × 4.6 mm ID) 5 µm column was used. The mobile phase consisted of acetonitrile and 0.05 M potassium phosphate monobasic (65:35, v/v) pH 7.5 at a flow rate of 0.3 ml/min (isocratic). The temperature of the autosampler chamber was 6 ± 3 ºC and the temperature of the column oven was 40 ºC.

Validation Procedure

The method was validated in accordance with the FDA guidance for the industry on bioanalytical method validation, 2001 [19].

Calibration and Linearity

A calibration curve consisting of 7 points corresponding to 7 concentrations of metformin (40, 100, 300, 500, 800, 1200 and 2000 ng/ml) was constructed daily during the validation by plotting peak area ratios (y) of metformin to atenolol versus metformin concentrations (x). A weighted linear regression model was used and the slope, intercept and correlation coefficient were obtained.

Intra- and Inter-Day Accuracy and Precision

Intra- and inter-day accuracy and precision were evaluated at three concentration levels (120, 1000 and 1600 ng/ml) by determining the metformin concentrations using 5 replicates of quality control samples (QCs) at each level daily for three days. The above concentrations were the low, medium and high range of the calibration curve. Each run included a blank plasma sample, a zero plasma sample spiked with the internal standard, calibration standards, and QC samples in 5 replicates.

The accuracy of the method was determined by comparing the assayed concentration with the nominal concentration. Precision was evaluated by determining the intra- and inter-day coefficients of variations (CV) at each concentration level.

Lower Limit of Quantitation

LLOQ was evaluated by determining the intra-day accuracy and precision of 5 plasma samples spiked with 40 ng/ml metformin and 8 µg/ml atenolol.

Recovery

Recoveries of metformin and atenolol were evaluated by comparing peak areas from human plasma spiked before and after the addition of the extracting solvent. The latter represents 100% recovery. The recovery of metformin was determined at three concentration levels (120, 1000 and 1600 ng/ml) (n=6) and the recovery of atenolol was determined at 8 µg/ml.

Specificity

The method was evaluated for specificity by comparing the signal of metformin obtained from the analysis of six blank plasma samples with that obtained from the analysis of plasma samples spiked with 40 ng/ml metformin (LLOQ) and 8 µg/ml atenolol.

Stability Studies

Short-term stability of metformin in plasma samples was evaluated at room temperature over 24 hours (longer than the time needed to process a batch). Long-term stability of metformin stored at -70 ± 15 ºC was evaluated after 10 weeks. Stored samples were thawed, allowed to reach room temperature and mixed well before analysis. Freeze and thaw stability was assessed after three freeze (-70 ± 15 ºC) and thaw (room temperature) cycles on three consecutive days. Autosampler stability of metformin in extracted samples (6 ± 3 ºC) was also evaluated over 48 hours (longer than the time needed to inject a batch).

All stability studies were performed using five replicates at each of the three concentration levels of metformin (120, 1000 and 1600 ng/ml). The mean (± standard deviation, SD) and CV (%) values were determined for the ratios of the five measurements. Metformin degradation was studied by comparing the results of the stability studies with those of the analysis of freshly spiked samples at the same concentration. The results were expressed as a mean variation.

The stability of metformin and atenolol in a stock solution was studied after 6 hrs at room temperature and after storage at 2 – 8 °C for four days. Mean peak areas of the drug in the samples prepared from the stored stock solution were compared to those of the samples prepared from the fresh stock solution. The stability of the stock solutions was evaluated using triplicate injections and was expressed as stability %.

Application in Pharmacokinetics and Bioequivalence Studies

The modified method was used for the determination of the plasma drug concentration in 30 healthy volunteers, after administering a 850 mg tablet of metformin orally. The study protocol was approved by the Institutional Review Board (IRB) of the clinical site (Pharmaquestjo), the clinical investigator and the sponsor. A copy of the approved study protocol together with the approval letter forms were submitted to the Jordan Food and Drug Administration

(JFDA) before the start of the study. According to the declarations of Helsinki guidelines, 2000[20], signed informed consents were obtained. The ICH Guidelines, 1996 [21], for Good Clinical Practice were applied during clinical procedures. The study design was a single-dose, randomized, two-way crossover with a washout period of 1 week between the two treatments. After administering a single dose of Glystor 850 mg tablets orally, produced by Algorithm S.A.L., Lebanon as the test product, and Glucophage® 850 mg tablets, produced by Merck Santé S.A.S., France, as the reference product, according to the study protocol, blood samples (7.0 ml each) were collected in vacutainer tubes. Following centrifugation at 4000 rpm for 5 minutes, plasma was stored in polypropylene tubes at (-70 ± 15 ºC) until analysis.

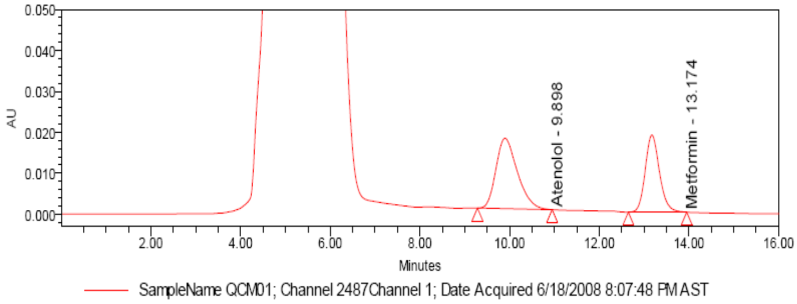
From the plasma concentration-time data of each individual and at each period, the pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, $C_{max}/AUC_{0\text{-}\infty}$, λ_Z and $T_{0.5}$ were determined or calculated applying non-compartmental analysis. These pharmacokinetic parameters were statistically analyzed by descriptive statistics, ANOVA test, Confidence Interval (CI) and Schuirmann's two one-sided t-test to test for the bioequivalence of the two products, according to FDA Guidance on Bioavailability and Bioequivalence studies for orally administered drug products, 2003 [22].

RESULTS AND DISCUSSION

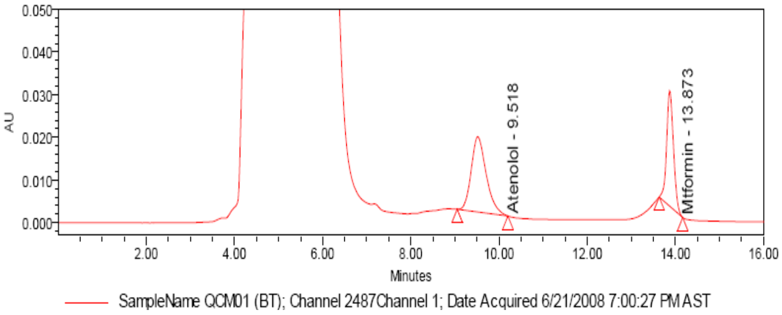
Chromatography and Specificity

Developing an assay for metformin is not an easy task because of its high polarity (partition coefficient is 0.01 [17]). This first makes its extraction from biological fluids using organic solvents difficult, even if it is converted totally to the non-ionized form using 1 M sodium hydroxide solution. Secondly, its retention on reversed phase columns is weak and it is eluted very rapidly, even if the organic content of the mobile phase is very low. To overcome the first, problem protein precipitation sometimes followed by washing with dichloromethane has been used for sample preparation[1,3,7-9,11-13,18,23-27]. However, it has been reported that the use of protein precipitation for sample preparation results in low sensitivity and that it is not efficient in removing interferences that result in peaks at the retention times of the drug and the internal standard[7,17]. To overcome the second problem, a more polar column (phenyl column) has been used; the retention time was increased but is still not optimal. The retention time has been further increased (more than 5 minutes) with the use of cyano and Si columns[17].

In the selected method, developed by Cheng and Chou, the sample preparation technique involved the addition of 50 µl of 1M HCl with the internal standard and the precipitating agent to 0.5 ml of human plasma samples. Unfortunately, after approximately 75 injections (Day 3 of the validation), the peak shape began to deteriorate, probably because of the rapid deterioration of the column packing material (silica) caused by HCL (Figure 1). The parameters that were set for peak integration (when to start and when to end the integration) did not result in the proper integration of the peak (Figure 1 B). The integration of the required peak was inconsistent from one chromatogram to another and it needed manual adjustment.



(a)



(b)

Figure 1: HPLC-UV chromatograms showing deterioration in peak shape with time when HCl was used in sample preparation. (a) A sample spiked with 1000 ng/ml metformin (Mid QC) injected on day 1 (b) A sample spiked with 1000 ng/ml metformin (Mid QC) injected on day 3 on the same column. All samples were spiked with the 8.00 µg/ml atenolol as the internal standard.

In an attempt to solve the problem, the column was washed overnight using the mobile phase and re-used for the analysis of plasma samples but this did not solve the problem. The peak shape began to deteriorate after a few injections (Figure 2). This necessitated the use of a new column for the analysis every two to three days. This would greatly increase the cost of analysis since such a bioequivalence study generates a large number of samples.

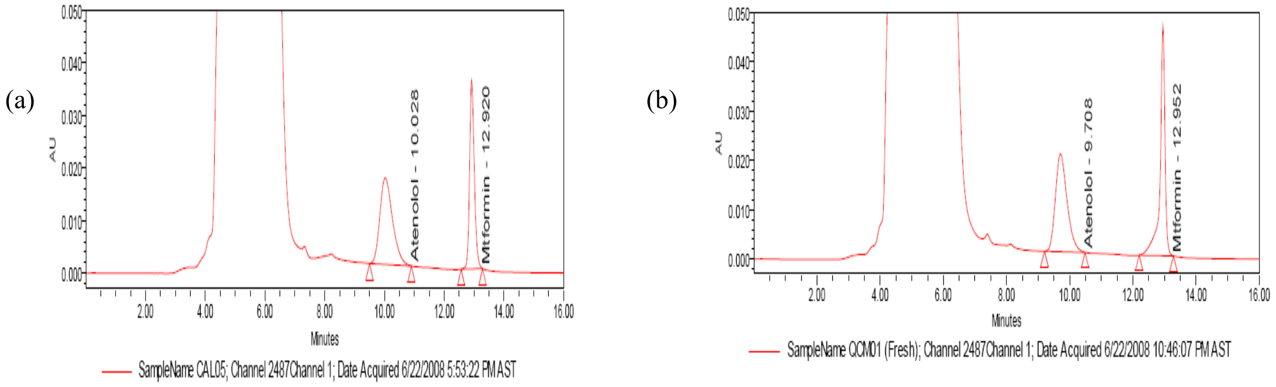
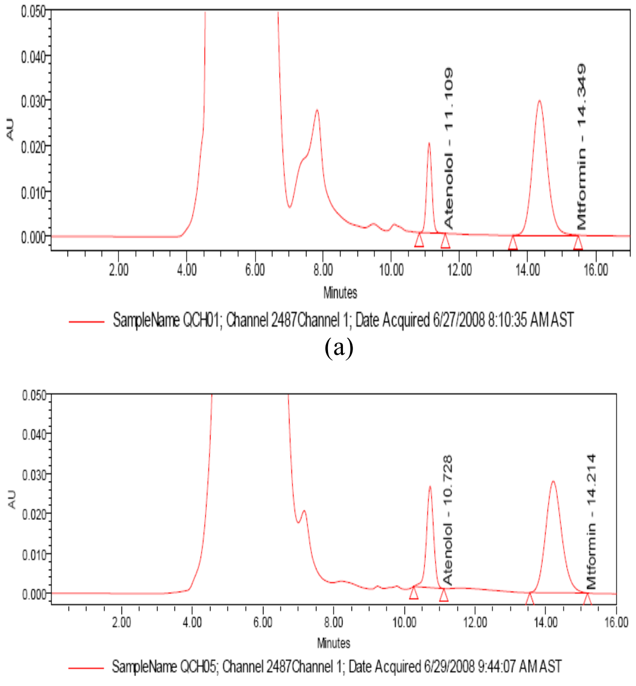


Figure 2: HPLC-UV chromatograms showing deterioration in peak shape with time when HCl was used in sample preparation after washing the column with the mobile phase overnight. (a) A sample spiked with 800 ng/ml metformin (Cal 5) (b) A sample spiked with 1000 ng/ml metformin (Mid QC) injected after approximately 5 hrs from sample (a). All samples were spiked with the 8.00 µg/ml atenolol as the internal standard.

In order to solve this problem, the addition of HCL to plasma samples during the preparation was eliminated, and the HPLC conditions (mobile phase, column length and flow rate) were optimized. With the improved extraction procedure, no deterioration in the peak shape with time was observed and the analytical column exhibited sufficient stability (Figure 3). Just two columns were used during the whole study, one column for the validation (1200 sample) and another column for the routine analysis (1100 sample), thus the cost of analysis was significantly reduced. This is considered as an improvement and a great advantage in the method compared to the original one.



(a)

(b)

Figure 3: HPLC-UV chromatograms showing no deterioration in peak shape with time with the modified method. (a) A sample spiked with 1600 ng/ml metformin (QCH) injected on day 1 (b) A sample spiked with 1600 ng/ml metformin (QCH) injected on day 3 on the same column. All samples were spiked with the 8.00 µg/ml atenolol as the internal standard.

The modified method had a good recovery and it was not compromised by the elimination of HCL. Metformin is a weak basic drug with pKa values of 2.8 and 11.5 for the two biguanide groups [28,29]. The first biguanide group was 97.54 % ionized only at pH 1.2. Therefore, most of the references [23,30-32] ignore this biguanide group and report one pKa (11.5 or 12.4) for metformin. The 2nd biguanide group is 99.99% ionized at pH less 8.5 (less than pKa by 3 units). Therefore, further a reduction in the pH caused by the addition of 50 μl of 1M HCl will not affect the ionization of this biguanide group. As a result, the solubility of metformin is not greatly affected by the pH of the solutions usually encountered. It is greater than 100 mg/ml in Milli-Q water, 0.1N HCl, pH 4.5, pH 6.8 and pH 9.5 phosphate buffers. Additionally, the highest dose strength of a metformin tablet (1000 mg) is soluble in 250 ml aqueous media over the range 1–7.5 [31]. Thus, the recovery of the drug is not expected to decrease due to the elimination of HCl and this was evident from the results. The mean absolute recovery from the plasma samples after extraction in the modified method was equal or slightly higher (78.30%) than that in the method developed by Cheng and Chou (76%).

The modified method was specific; a good separation of atenolol and metformin without any interfering endogenous peaks was achieved. Retention times of atenolol and metformin were 11.267 minute and 14.535 minute, respectively.

For a more specific description of both methods, capacity factors of the drug and the internal standard were calculated using the following equation:

$$k' = (t_R - t_M)/ t_M$$

where k' is the capacity factor, t_R is the retention time (time between injection and elution), t_M is t_R of the unretained species (i.e. how long it took for the solution injected to travel all the way through the length of the column).

For the modified method, the capacity factors of atenolol and metformin were 1.00 and 1.64 respectively, whereas for the method developed by Cheng and Chou, the capacity factors of atenolol and metformin were 0.637 and 0.877, respectively. Thus, the obtained capacity factors for the modified method were closer to the value recommended by the FDA ($k' > 2$) [33] and the peaks were better resolved from each other and from the void volume.

Representative chromatograms of a blank plasma sample, a zero plasma sample, a plasma sample spiked with 40 ng/ml metformin (LLOQ), a plasma sample spiked with 120 ng/ml metformin (Low QC), a plasma sample spiked 1000 ng/ml with metformin (Mid QC), and a plasma sample spiked with 1600 ng/ml metformin (High QC) are shown in Figure 4. All samples except for the blank plasma sample were spiked with 8.00 µg/ml atenolol as the internal standard.

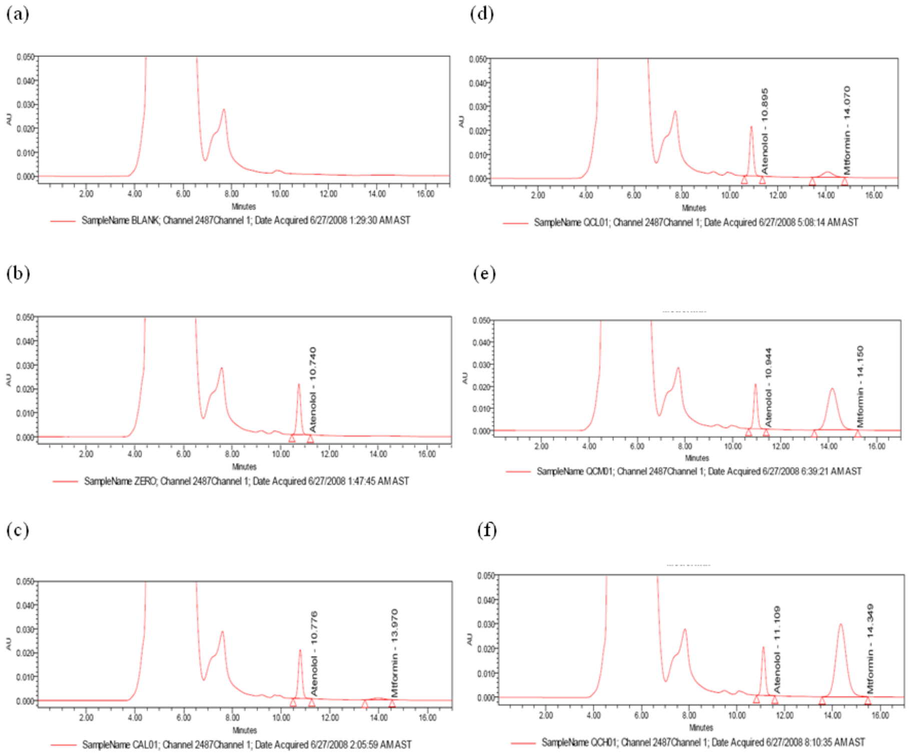


Figure 4: HPLC-UV chromatograms obtained for (a) Blank plasma sample (b) Zero plasma sample (c) Sample spiked with 40 ng/ml metformin (LLOQ) (d) Sample spiked with 120 ng/ml metformin (Low QC) (e) Sample spiked with 1000 ng/ml metformin (Mid QC) (f) Sample spiked with 1600 ng/ml metformin (High QC). Samples (b)-(f) were spiked with the 8.00 µg/ml atenolol as the internal standard.

Validation

The results indicated that the method met the acceptance criteria as indicated below:

Linearity

Linear correlation between the peak area ratios of metformin/atenolol versus known concentrations of metformin in plasma in the range of 40-2000 ng/ml was observed. Mean coefficients of correlation (R^2) (± SD) of the curves prepared on different days (n = 6) were 0.9975 ± 7.68×10^{-4}. The precision around the mean values of the concentrations did not exceed 15% CV.

Precision and Accuracy

Intra- and inter-day accuracy and precision, evaluated by analyzing QC samples (120, 1000 and 1600 ng/ml) are given in Table 1.

Table 1: Intra- and inter-day precision and accuracy of metformin in human plasma.

Quality control (n=5)	Conc. (ng/ml)	Intra-day assay			Inter-day assay		
		Mean	Precision (CV, %)	Accuracy (%)	Mean	Precision (CV, %)	Accuracy (%)
LLOQ	40	41.638	2.48	104.095	-	-	-
QC-low	120	121.304	11.74	101.09	121.862	7.46	101.55
QC-mid	1000	979.187	8.02	97.92	1005.329	5.34	100.53
QC-high	1600	1532.304	2.75	95.77	1542.713	5.02	96.42

As shown in Table 1, the intra- and inter-day precision did not exceed 15% of the CV, and in terms of accuracy, the mean value of the assayed concentration was within 15% of the actual value.

Recovery

The mean absolute recovery from the plasma samples after extraction was greater than 78.30% as shown in Table 2.

Table 2: Absolute recovery of the analytical method.

Quality control (n=6)	Absolute recovery (metformin)		Absolute recovery (atenolol)	
	Recovery	Precision (CV, %)	Recovery	Precision (CV, %)
QC-low	88.10	9.03	-	-
QC-mid	78.30	3.23	86.96	5.54
QC-high	81.60	5.39	-	-

Thus, the recovery was not compromised by the elimination of the HCl addition during sample preparation. It was consistent, precise and reproducible.

Limit of Quantitation

FDA guidance on bioavailability and bioequivalence studies for orally administered drug products, 2003 [22] recommends that samples are collected for four half-lives of the drug. The mean peak plasma concentration was about 1700 ng/ml and the mean value of the elimination half life was 3.5 hr. Accordingly, after a time period corresponding to four metformin half-lives, plasma concentrations were expected to be around 100 ng/ml. The LLOQ of the method (40 ng/ml) was much lower than the expected concentration, and was adequate for the bioequivalence study.

The obtained signal-to-noise ratio at the retention time of metformin was at least five times greater than any interference in blanks at the retention time of metformin at the LLOQ (40 ng/ml). The mean concentration and the intra-day precision and accuracy are shown in Table 1.

The precision did not exceed 20% of the CV and in terms of accuracy, the mean value of the assayed concentration was within 20% of the actual value.

Stability Studies

Stability of metformin in human plasma was studied at three different concentrations (120, 1000 and 1600 ng/ml) under different conditions and the results were compared to those obtained for freshly prepared samples. The results are shown in Tables 3, 4, 5 and 6.

Table 3: Results of short term (bench top) stability study

Quality control (n=5)	Conc. (ng/ml)	Experimental concentration in plasma (ng/ml)						Variation of the mean (%)
		Fresh prepared			After 24 hrs at the bench top			
		Mean	SD	Precision (CV, %)	Mean	SD	Precision (CV, %)	
QC-low	120	128.847	6.020	4.67	116.549	4.653	3.99	9.54
QC-mid	1000	968.359	38.244	3.95	947.767	28.707	3.03	2.13
QC-high	1600	1622.097	70.806	4.37	1574.819	39.666	2.52	2.91

Table 4: Results of long term stability study

Quality control (n=5)	Conc. (ng/ml)	Experimental concentration in plasma (ng/ml)						Variation of the mean (%)
		Fresh prepared			After 10 weeks at -70±15°C			
		Mean	SD	Precision (CV, %)	Mean	SD	Precision (CV, %)	
QC-low	120	128.847	6.020	4.67	124.598	11.656	9.35	3.30
QC-mid	1000	968.359	38.244	3.95	1023.018	21.752	2.13	-5.64
QC-high	1600	1622.097	70.806	4.37	1673.064	53.915	3.22	-3.14

Table 5: Results of freeze and thaw stability study

Quality control (n=5)	Conc. (ng/ml)	Experimental concentration in plasma (ng/ml)						Variation of the mean (%)
		Fresh prepared			After three freeze and thaw cycles			
		Mean	SD	Precision (CV, %)	Mean	SD	Precision (CV, %)	
QC-low	120	115.959	2.253	1.94	122.043	2.247	1.84	-5.25
QC-mid	1000	923.888	15.255	1.65	1006.271	57.527	5.72	-8.92
QC-high	1600	1524.577	40.678	2.67	1555.512	77.590	4.99	-2.03

Table 6: Results of autosampler stability study

Quality control (n=5)	Conc. (ng/ml)	Experimental concentration in plasma (ng/ml)						Variation of the mean (%)
		Fresh prepared			After 48 hrs 6±3°C			
		Mean	SD	Precision (CV, %)	Mean	SD	Precision (CV, %)	
QC-low	120	113.751	1.703	1.50	116.228	2.456	2.11	-2.20
QC-mid	1000	936.917	10.103	1.08	947.338	14.758	1.56	-1.12
QC-high	1600	1517.033	18.636	1.23	1541.911	14.400	0.93	-1.65

Results in Tables 3, 4, 5, and 6 indicate that metformin was stable in the plasma during the sample collection, storage and processing and after undergoing three freeze and thaw cycles (the mean variation is not more than 9.54%).

The results of the stability evaluations of metformin in stock solutions are shown in Table 7 and those of atenolol in stock solution are shown in Table 8.

Table 7: Results of metformin stock solution stability.

Peak Area										
Fresh prepared			After 6hrs at RT				After 4 days at 2-8°C			
Mean	SD	Precision (CV%)	Mean	SD	Precision (CV%)	% Stability	Mean	SD	Precision (CV%)	% Stability
1188662	12534	1.05	1177750	10843	0.92	99.08	1138767	13914	1.22	95.80

Table 8: Results of atenolol stock solution stability.

Peak Area										
Fresh prepared			After 6hrs at RT				After 4 days at 2-8°C			
Mean	SD	Precision (CV%)	Mean	SD	Precision (CV%)	% Stability	Mean	SD	Precision (CV%)	% Stability
175873	16727	0.95	1707450	19907	1.17	97.09	1712458	15441	0.9	97.38

Stock solutions of metformin and atenolol were stable at room temperature for 6 hrs and at 2 – 8 °C for 4 days (Stability % > 95.80 %).

Application to Pharmacokinetic and Bioequivalence Studies

The mean plasma concentration–time curve of metformin, after administering 850 mg orally to 30 healthy volunteers is shown in Figure 5.

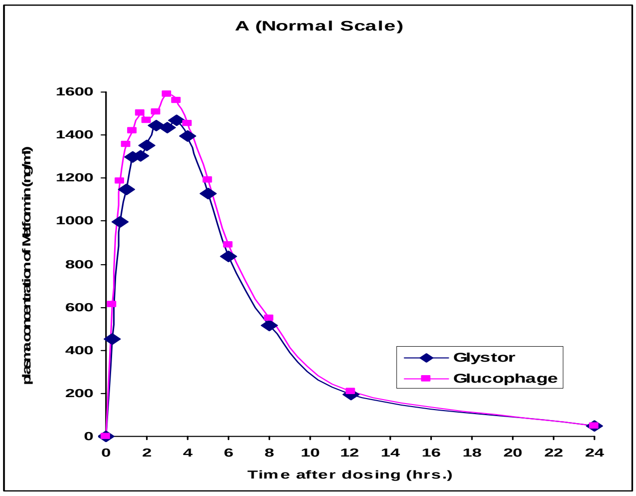


Figure 5: Mean plasma concentrations of metformin (ng/ml) versus time (hr) profile for 30 volunteers after administration of Glystor 850 mg Tablets and Glucophage® 850 mg Tablets.

Pharmacokinetic parameters of metformin HCl (mean ± SD) after administering a single dose of 850 mg of the two formulations orally to human volunteers are shown in Table 9.

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✉ E-mail: ssnimry@just.edu.jo

Table 9: Pharmacokinetic parameters of metformin HCl (mean ± SD) after oral administration of single doses of 850 mg of two formulations to human volunteers

Pk parameter	Glyostor	Glucophage®
AUC_{0-t} (ng.hr/ml)	10501.1±2978.8	11431.9±3245.3
C_{max} (ng/ml)	1646.4±467.0	1772.1±509.2
T_{max} (hr)	2.37±0.98	2.60±0.99
$C_{max}/AUC_{0-\infty}$	0.1497±0.0217	0.1495±0.0256
λ_Z	0.2154±0.0571	0.2158±0.0625
$T_{0.5}$	3.47±1.00	3.52±1.13

Statistical comparison applying the ANOVA test between the two products for these pharmacokinetic parameters revealed insignificant differences at 5% significance level (≢ = 0.05) ($P \geq 0.05$). The 90% CI of the ratio (Test/Reference) of the Ln-transformed C_{max} and AUC was within 80.00-125.00 (Data not shown).

The area under the plasma concentration-time curve from 0 to 36 hrs (the time of the last sample) (AUC_{0-36}) and from 0 hr extrapolated to infinity ($AUC_{0-\infty}$) were calculated and the ratio AUC_{0-36} /$AUC_{0-\infty}$ was higher than 85% for all volunteers (data not shown), as recommended by the FDA guidance on bioavailability and bioequivalence studies for orally administered drug products, 2003 [22]. Accordingly, Glyostor 850 mg tablets and Glucophage® 850 mg tablets were concluded to be bioequivalent.

CONCLUSION

The modified method met the aforementioned criteria and was functional within the laboratory; Metformin was determined at a low concentration (40 ng/ml) and the sensitivity of the modified method was adequate for the bioequivalence study. The sample preparation technique (protein precipitation) was simple, rapid and efficient in eliminating interferences from endogenous substances contrary to what has been reported [7,17].

The main advantages of the modified method as compared to the original method, and possibly to other methods that use HCl during sample preparation, were 1) better resolution of the drug and the internal standard from each other and from the void volume as evident from the calculated capacity factors, and 2) the significant reduction in the cost of analysis achieved by enhancing the column stability. No deterioration in the peak shape was observed with time and a single column was used for the analysis of more than 1,200 samples.

The method was validated and met the acceptance criteria of the FDA guidance. It was successfully applied to test the bioequivalence of Glystor 850 mg and Glucophage® 850 mg tablets and they were concluded to be bioequivalent.

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تحديث طريقة منشورة سابقا لتحليل المتفورمن بإستخدام السوائل عالية الأداء مقرونة بكشف فوق بنفسجي وتقييمها وإستخدامها في دراسة تكافؤ حيوي.

سهير النمري[1*] وجعفر التميمي[2] ويوسف زايد[3] وخالدة الخطيب[3] وكلود خليل[4] ومرلين عقل[4]

[1] قسم الصيدلة التكنولوجية، كلية الصيدلة، جامعة العلوم والتكنولوجيا الأردنية، إربد، الأردن
[2] وحدة الدراسات السريرية و دراسات حركية الدواء، مركز أكديما للتكافؤ الحيوي والدراسات الصيدلانية، عمان، الأردن
[3] وحدة التحاليل الحيوية، مركز أكديما للتكافؤ الحيوي والدراسات الصيدلانية، عمان، الأردن
[4] شركة الغوريثم للصناعات الصيدلانية س.أ.ل، بيروت، لبنان

ملخص

هدف البحث إلى التغلب على مشكلة تدهور شكل القمة الناتج عن استخدام حمض الهيدروكلوريك في تحضير العينة وتقييم طريقة التحليل واستخدامها لدراسة التكافؤ الحيوي لمستحضري الجلايوستور والجلايوفيج. تم تحضير العينات دون إضافة الحمض عن طريق ترسيب البروتينات والغسل باستخدام ثنائي كلوريد الميثان. تكون الطور والمتحرك من الأسيتونتريل ومحلول الفوسفات الراصد (درجة حموضة 7.5). تم استخدام عمود فصل كروماتوغرافي من نوع الهابرسل سيليكا. ثم تقييم طريقة التحليل حسب الخطوط الإسترشادية لمنظمة الغذاء والدواء الأمريكية واستخدامها لدراسة التكافؤ الحيوي. وبينت النتائج عدم حدوث تدهور في شكل القمة. وتم إستخدام فقط عمودين فصل كروماتوغرافي خلال الدراسة. أظهرت النتائج فصلا جيّدا بين المتفورمن والأتينولول. كانت منحنيات المعايرة خطية في مجال التراكيز المستعملة. وكانت الدقة والتباين ضمن الحدود المطلوبة. تجاوز معدل إسترداد الدواء 78.3%. وأظهر الدواء ثباتا في المحاليل المركزة وفي البلازما طوال فترة الحفظ والتحليل. وأظهر التحليل الإحصائي للنتائج تكافؤ المستحضرين. ويمكن الاستنتاج أن طريقة التحليل المعدلة فاعلة وأن المستحضرين كانا متكافئين حيوياً.

الكلمات الدالة: متفورمين، كروماتوغرافيا السوائل عالية الأداء مقرونة بكشف فوق بنفسجي، بلازما بشرية، ترسيب البروتينات، تكافؤ حيوي.

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