Development and Validation of Stability-Indicating RP-HPLC Method for Simultaneous Determination of Metformin HCl and Glimepiride in Fixed-Dose Combination

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ABSTRACT: A simple reversed-phase high-performance liquid chromatography method was developed and validated for simultaneous determination of Metformin hydrochloride (MET) and Glimepiride (GLM) in combination and estimation of their principal degradation products. The separation was achieved using JASCO Finepak SIL (250 mm × 4.6 mm i.d. 5 µm) at ambient temperature. The optimized mobile phase composed of an aqueous phase (20 mM phosphate buffer, adjusted to pH 3.0) and an organic phase (methanol:acetonitrile; 62.5:37.5) in the ratio of 80:20. The flow rate was 1 mL/minute, and the analytes were detected at 230 nm. The developed method was validated for accuracy, precision, specificity, linearity, and sensitivity. The chromatographic analysis time was approximately six minutes with the complete resolution of MET (Rt = 2.75 minutes) and GLM (Rt = 5.87 minutes). The method exhibited good linearity over the range of 5–30 µg/mL for MET and 1–10 µg/mL for GLM. The drugs in combination were subjected to various stress degradation studies as per the International Conference Harmonization (ICH) guidelines. Results obtained from the stress degradation studies revealed that the developed method is applicable for stability studies.

KEYWORDS: Metformin HCl, Glimepiride, RP-HPLC method, stress degradation, fixed-dose combination

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Introduction

Metformin hydrochloride (MET) is chemically *N,N*-dimethylimidodicarbonimidic diamide hydrochloride (Fig. 1A) and extensively used as an antihyperglycemic agent to treat type 2 (noninsulin-dependent) diabetes mellitus. It reduces hepatic glucose production and improves insulin sensitivity by increasing peripheral glucose uptake.[1]

Glimepiride (GLM) is 1-[[*p*-[2-(3-ethyl-4-methyl-2-oxo-3-pyrrolinepyrroline-1-carboxamido) ethyl]-phenyl]-sulfonyl]-3-(*trans*-4-methylcyclohexyl) urea (Fig. 1B). It is a third-generation sulfonylurea used to reduce blood glucose levels by stimulating insulin secretions from the beta cells of the pancreas and is also known to increase peripheral insulin sensitivity, thereby decreasing insulin resistance.[2]

MET and GLM have been coformulated as a fixed-dose combination (FDC) and have been commonly employed for the management of type 2 diabetes. This combination has been proven to result in superior glycemic control compared to monotherapy of either drugs.[3] Furthermore, FDCs show better patient compliance and are cost-effective compared to administration of individual drugs.

Formulation of a combination of drugs in an individual dosage form calls for the need to develop a simultaneous analytical method for the routine and stability testing of the product. Pharmacopeial methods for the individual estimation of MET and GLM are available; however, no official methods are available for the analysis of this combination. Various liquid chromatography[4–6] methods and spectroscopy methods[7] are reported for the estimation of MET in combination with various drugs in pharmaceutical formulation. Literature reports are available citing various liquid chromatography-based methods for the simultaneous analysis of MET and GLM, which mostly require wavelength programming.[8] Although various methods are reported in the literature for estimation of MET and GLM, it was observed that MET response goes out of scale as in the FDC and the dosage of MET is much higher (500 mg or more) than that of GLM (2–8 mg). Simultaneous determination of MET and GLM remains difficult due to their different physicochemical properties, polarities, and wide difference in the label claim of the two drugs. Reversed-phase high-performance liquid chromatography (RP-HPLC) has been the most widely used

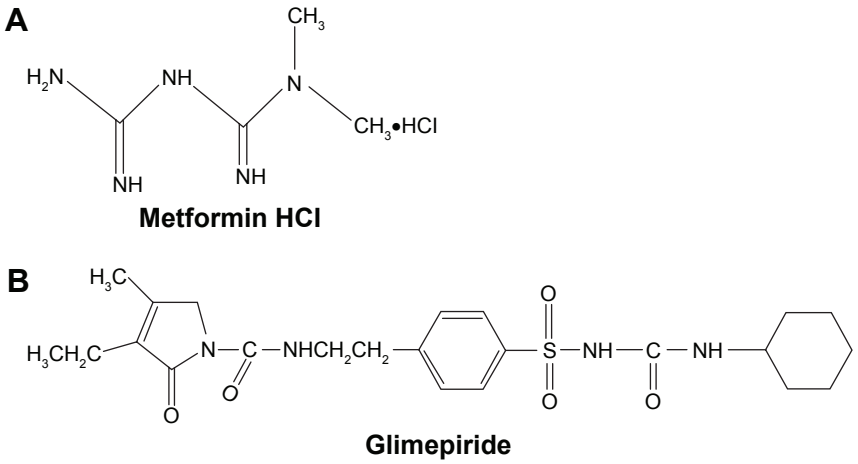

Figure 1. Chemical structure of (**A**) MET and (**B**) GLM.

method for pharmaceutical analysis, as it ensures accurate quantification of drugs without interference from any of the excipients that are normally present in pharmaceutical dosage forms. RP-HPLC method is capable of producing accurate, specific, and reproducible results. Hence, there is a need to develop an RP-HPLC method for the estimation of MET and GLM in bulk and its formulations with a simple mobile phase and nontedious sample preparation steps with improved sensitivity and a short chromatographic run time with effective resolution of both drugs.

Force degradation testing plays an important role in the development of a stability-indicating analytical method, as it helps to determine the degradation pathways and degradation products of the Active pharmaceutical ingredients (APIs) that could form during storage and facilitate formulation development, manufacturing, and packaging.[9,10] Degradation products or compounds are formed due to change in the active ingredient as a result of processing or storage (eg, oxidation and hydrolysis) or reaction of the active ingredient with an excipient or container.[11,12] It is recommended that force degradation studies should be performed to evaluate the effect of temperature, humidity, photostability, oxidation, and hydrolytic degradation.

Hence, the objectives of the current research paper were to develop and validate an RP-HPLC method for the simultaneous estimation of MET and GLM in bulk and in formulations as per the ICH guidelines Q2 (R1)[13] and to perform stress studies as per the ICH guidelines Q1A (R2).[14] The guidelines dictate that the analytical methods should be validated and stress studies should be carried out to determine the inherent stability of drug and to support the suitability of the proposed analytical procedures.

Experimental

Chemicals and reagents. MET was purchased from Sohan Healthcare Pvt. Ltd., and GLM was obtained as a gift sample from Ajanta Pharma. Acetonitrile, methanol, and orthophosphoric acid were of HPLC grade supplied by Merck Ltd. Potassium dihydrogen orthophosphate was purchased from S. D. Fine Chemicals. All other chemicals used were of analytical grade unless otherwise indicated. Milli-Q water was used for preparation of mobile phase.

Instrumentation. Chromatography was performed using Agilent 1200 series HPLC system, G1311A Quaternary pump connected with G1314B variable wavelength detector and G1328B manual injector. The data acquisition was performed by the Agilent Chemstation software (version B.04.03).

Chromatographic condition. Separation was achieved on JASCO C_{18} column (JASCO Finepak SIL C18T-5; 4.6 × 250 mm; 5 µm particle size) with a constant flow rate of 1.0 mL/minute. The isocratic mobile phase consisted of an organic phase (80%) along 20 mM phosphate buffer, with pH adjusted to 3.0 (20%). The organic phase comprised methanol:acetonitrile in the ratio of 62.5:37.5. The mobile phase was filtered through a 0.22-µm membrane and degassed prior to use. The injection volume was 20 µL for standards and samples. All analyses were done at ambient temperature. The samples were analyzed at different wavelengths, but finally, 230 nm was selected for analysis because at this wavelength, sharp and clearly resolved peaks for both drugs were observed.

Preparation of stock and standard solutions. Stock solutions of MET and GLM were prepared separately by dissolving accurately weighed 10 mg of each drug in 100 mL of methanol to obtain a stock solution of an individual drug of 100 µg/mL. These stock solutions were further diluted with mobile phase as appropriate to obtain the working standard solution of 5–30 µg/mL and 1–10 µg/mL of MET and GLM, respectively.

Method validation. The optimized chromatographic method was validated according to the procedures described in the ICH guidelines Q2 (R1) for the validation of analytical method.[13]

Linearity. To establish the linearity of the analytical method, a series of dilutions with mobile phase were prepared in order to obtain the mixture of MET and GLM ranging from 5 to 30 µg/mL and 1 to 10 µg/mL, respectively. All the

solutions were filtered through a 0.22-µm membrane filter. The final solutions were injected in triplicate, keeping the injection volume constant (20 µL). Calibration curve was plotted between the mean peak area and the respective concentration. The correlation coefficient and slope were determined from the calibration curve.

Accuracy. Accuracy was evaluated by addition of three known concentrations of the drug to the standard solution, and the spiked solutions were analyzed under optimized conditions in six replicates. The recovery of added drug was determined by calculating the preanalyzed drug concentration and correlating with the concentration of a spiked drug.

Precision. The precision of the analytical method was studied by analyzing six replicates over three concentrations of both MET (5, 15, and 30 µg/mL) and GLM (1, 4, and 10 µg/mL) at two levels (intra- and interday). The results were interpreted as percent relative standard deviation (%RSD) values for the areas of each of them.

Limit of detection and limit of quantification. The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentrations of both the drugs, as described earlier. The LOD was defined as the concentration that yields a signal-to-noise ratio of 3:1, while the LOQ was calculated to be the lowest concentration that could be measured with a signal-to-noise ratio of 10:1. The LOD and LOQ were calculated by measuring the standard deviations of the response and slope.

Robustness and ruggedness. The robustness evaluation was carried out by making small changes in various conditions, such as a composition of mobile phase, pH of buffer, and flow rate in the optimized method, while the ruggedness of the method was determined by comparing the intra- and interday precision results for MET and GLM. This was performed on different days with different analysts. Both robustness and ruggedness were assessed based on the significant change in the peak areas (%RSD) of MET and GLM when compared to optimized conditions.

Solution stability. To determine the solution stability, all active sample solutions were spiked and the spiked sample solutions (20 µg/mL) were capped tightly and kept at room temperature for 12 hours. The contents of MET and GLM were determined at 1-, 2-, 4-, 6-, and 12-hour intervals by following the procedure, as described earlier.

Forced degradation studies. Forced degradation studies were performed on MET and GLM to prove stability, indicating a property of the method. The stress conditions employed for degradation study include acid hydrolysis (0.1 N HCl), base hydrolysis (0.1 N NaOH), oxidation (3% H_2O_2), and thermal and photolytic degradation.

Acid and base degradation. Acid and base degradation studies were carried out by taking 1 mL aliquot each of MET and GLM from the sample stock solution in a 10-mL standard volumetric flask and mixing with 0.1 N HCl. The flask was kept in a controlled temperature bath at 80°C ± 2°C for a period of four hours. Similarly, forced degradation in basic media was performed using 0.1 N NaOH instead of 0.1 N HCl. Both the samples were neutralized, and the final solution was injected in triplicate under optimized chromatographic conditions.

Oxidative degradation. Oxidative degradation was performed by transferring 1 mL aliquot of sample solution of an individual drug into a 10-mL standard volumetric flask and mixing with 1 mL of 3% v/v of hydrogen peroxide. The flask was kept in a controlled temperature bath at 80°C ± 2°C for a period of four hours. The final solution was injected in triplicate under optimized chromatographic conditions.

Thermal degradation. For thermal stress, 1 mL aliquot each of a sample solution of MET and GLM was transferred to a 10-mL standard volumetric flask and then placed in a controlled temperature oven and heated at 80°C ± 2°C for a period of four hours. This solution was further diluted with mobile phase, and the final solution was injected in triplicate to obtain the chromatogram.

Photolytic degradation. Photolytic degradation was conducted by transferring 1 mL aliquot each of a sample solution of MET and GLM into a 10-mL standard volumetric flask and exposing it to direct sunlight for a period of four hours. This solution was further diluted with mobile phase and injected in triplicates under an optimized chromatographic condition.

Results and Discussion

The present study indicates the suitability of a reversed-phase column procedure for the simultaneous determination of MET and GLM in a combined dosage form with a wide difference in the label claim. The proposed HPLC method required fewer reagents and materials; moreover, it was simple and less time-consuming. The complete run time for analyzing both the drugs was less than six minutes with a clear resolution. The chromatogram results of MET and GLM were shown in (Fig. 2a and b) with a retention time of 2.753 minutes and 5.877 minutes, respectively. The tailing factor for the peak was less than 2 for both the drugs. This method could be used as a quality control test in pharmaceutical industries. Initially, chromatographic conditions were optimized by making a change in mobile phase composition, pH, and buffers used in the mobile phase.

Optimization of mobile phase composition was performed based on the resolution between the drugs, symmetric factor, and the number of theoretical plates. Initial trials with different ratios of methanol and phosphate buffer were experimented to optimize the mobile phase. Only methanol (75%) as organic content in mobile phase resulted in broad and distorted peak of MET. In addition, it was observed that retention time for GLM was around 10 minutes, while for MET, it was around two minutes. The poor retention of MET may be due to its high polarity and strong basic nature. On the basis of this observation, acetonitrile was added in the increasing ratio in the organic content of mobile phase. Finally, the mobile phase comprising

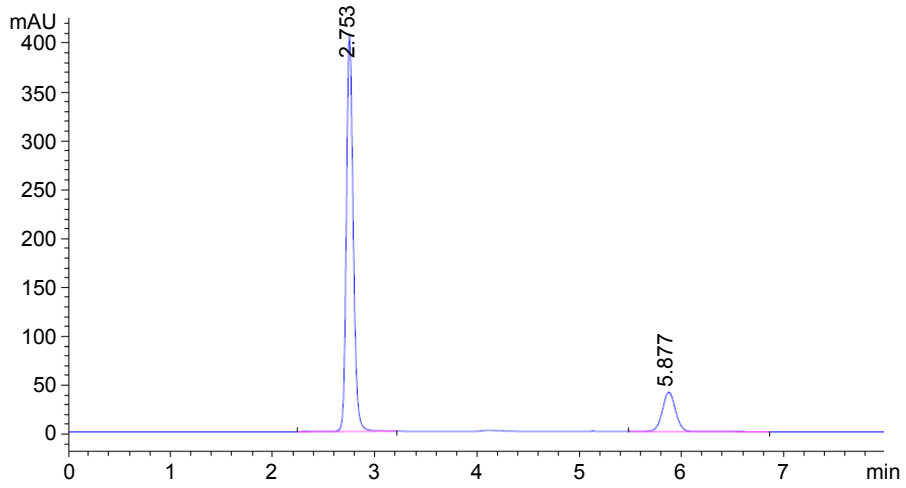

Figure 2. Chromatogram of (a) MET and (b) GLM at λ_{max} 230 nm.

80% organic content (methanol:acetonitrile, 62.5:37.5) and 20% aqueous content (potassium phosphate buffer, pH 3) was selected on the basis of peak shape and resolution. For simultaneous detection of both drugs, various wavelengths from 228 nm to 235 nm were scanned, considering the λ_{max} of the drugs (λ_{max} of MET was 235 and λ_{max} of GLM was 228). At 235 nm, MET was found to show very high absorbance; in contrast, GLM showed poor absorbance. While at 228 nm, an exact opposite of this phenomenon was observed. Finally, 230 nm was selected as the wavelength for analysis owing to optimum absorbance showed by both GLM and MET at this wavelength. Flow rate of 1 mL/minute and total run time of six minutes were selected for further studies after several preliminary investigatory chromatographic runs. The developed method was validated, as described below, for the following parameters: system suitability, linearity, LOD, LOQ, ruggedness, robustness and recovery-precision.

Linearity. Linearity was evaluated by analyzing the standard solution containing both MET and GLM at six different concentrations.[13] The response was found to be linear over the range of 5–30 µg/mL for MET and 1–10 µg/mL for GLM. The peak area response with respect to a concentration of each drug was subjected to regression analysis for calculating the calibration equations and correlation coefficients. Linear calibration graph was obtained with correlation coefficient of the regression equation greater than 0.999 in all cases, and the results are summarized in Table 1. The data provide conclusive evidence of linearity between concentration of each drug and instrumental response.

System suitability. System suitability test is an integral part of chromatographic method development, and it is used to verify that the resolution and reproducibility of the system is adequate for the analysis to be performed. The resolution, retention time, theoretical plate value, and symmetry were calculated for the combination of two APIs, and the results are reported in Table 2. The suitability results of the chromatographic system are found to be within the acceptance criteria of the CDER guidance document.[15]

Accuracy. The accuracy of the analytical method is defined as the similarity of the results obtained by this method to the true value.[16] The accuracy was determined by measuring the recovery of MET and GLM at three different levels (low, medium, and high). Known amounts of each drug were added to the placebo. The results of recovery studies are shown in Table 3. The results listed in Table 3 specify that the method enables highly accurate simultaneous determination of both drugs.

Precision. The precision of the analytical method is defined as the degree of that similarity.[16] The proposed method was evaluated by studying the precision as %RSD.

Table 1. Linear regression least square fit data for HPLC assay of two drugs.

DRUG	METFORMIN HCl	GLIMEPIRIDE
Linear dynamic range (µg/mL)	5–30	1–10
Slope (m)	91.90	58.85
Intercept (b)	34.94	3.36
Correlation coefficient (r)	0.999	0.999

Table 2. System suitability parameters.

PARAMETERS	METFORMIN HCl	GLIMEPIRIDE
Retention time (Rt, min)	2.8	5.8
No. of theoretical plates (N)	8201	10620
Resolution	–	16.94
Symmetry	0.76	0.88

Table 3. Accuracy/recovery studies.

DRUG	RECOVERY LEVEL	AMOUNT OF DRUG ADDED (µg/mL)	AMOUNT OF DRUG RECOVERED (µg/mL)	% RECOVERY (MEAN ± SD)	% R.S.D
Metformin HCl	80%	12	12.01	99.92	0.52
	100%	15	15.07	100.60	0.74
	120%	18	17.98	99.91	0.31
Glimepiride	80%	4	4.05	101.25	0.49
	100%	5	5.03	101.40	0.90
	120%	6	5.98	100.33	0.86

The results of intra-and interday precision are listed in Table 4. The %RSD was found to be less than 2 for all the drugs, which indicate that the method is precise.

LOQ and LOD. The LOQ and the LOD were calculated according to the ICH guidelines Q2 (R1),[13] using the following equations:

$$LOQ = 10\ Sa/b \text{ and } LOD = 3.3\ Sa/b$$

where Sa is the standard deviation of the intercept of the regression lines and b is the slope of the calibration curve.

The results are shown in Table 5. LOD values for MET and GLM were found to be 0.73 µg/mL and 0.24 µg/mL, respectively. Similarly, LOQ values for MET and GLM were calculated and were observed to be 2.21 µg/mL and 0.74 µg/mL, respectively. This indicates that the sensitivity of the method is adequate.

Robustness and ruggedness. Robustness is the measure of the performance of a method when small, deliberate changes are made to the specified method parameters. The intention of robustness is to identify critical parameters for the successful implementation of the method. Data were acquired and calculated for %RSD. The results of ruggedness and robustness (Table 6) were found to be within the acceptable limits, which indicate that the method is highly robust.

Solution stability. The stability of the analytical solutions of the method was studied by analyzing the standard and sample solutions initially and at different time intervals. The %RSD of MET and GLM concentration during solution stability experiments was within 1%. There was no significant change observed for the chromatograms of standard solution and the experimental solution at different time intervals. Furthermore, the absence of degradation peaks confirmed that sample is stable in solvent used under the condition investigated.

Forced degradation studies. The chromatograms of the samples of MET and GLM subjected to various forced degradation conditions showed well-separated peaks of the actives and the degradation products at different retention times. However, in some conditions, the actives did not show separate peaks of the degradation products, rather a decrease in height and area of the peak was observed. The peaks of the degradation products were identified and compared with that of the standard solution and were found to be well resolved from the peaks of the actives (Figs. 3–7). The degradation studies revealed that the sample of MET and GLM in combination was more stable against oxidation, photolytic studies, and thermal studies than acid and base degradation. It was observed that MET is more susceptible toward alkaline condition than acid condition, while GLM degraded more in acidic condition.

Acid degradation. Acid degradation studies showed the presence of two additional peaks at 3.5 and 3.7 minutes, but only a small change in peak area and a decrease in peak high

Table 4. Precision studies.

DRUG	AMOUNT OF DRUG ADDED (µg/mL)	REPEATABILITY		INTERMEDIATE PRECISION	
		AMOUNT OF DRUG FOUND (µg/mL)	% R.S.D	AMOUNT OF DRUG FOUND (µg/mL)	% R.S.D
Metformin HCl	10	10.01	1.93	9.96	1.02
	15	15.20	1.06	15.22	0.53
	20	19.94	0.62	19.99	0.46
Glimepiride	4	4.05	0.38	3.98	1.05
	6	5.98	0.77	6.05	0.67
	8	8.14	0.86	8.06	0.80

Table 5. LOD and LOQ.

PARAMETERS	METFORMIN HCl	GLIMEPIRIDE
Limit of Detection (µg/mL)	0.73	0.24
Limit of Quantification (µg/mL)	2.21	0.74

were observed in the case of MET and GLM. This extra peak represents the formation of degradation products. However, acid degradation is lesser as compared to alkali. The chromatogram for acidic degradation of MET and GLM showed degradation of approximately 15.24% and 29.00%, respectively (Fig. 3).

Base degradation. The chromatogram for basic degradation of the actives showed degradation of approximately 36.70% and 1.21% for MET and GLM, respectively (Fig. 4).

Oxidative degradation. MET and GLM were evinced at 10.78% and 35.42%, respectively, in response to oxidation degradation, and one additional peak at 3.7 minutes for MET was observed (Fig. 5).

Photo degradation. Photo degradation resulted in degradation of 4.85% and 8.19% for MET and GLM, respectively (Fig. 6).

Heat degradation. The chromatograms of MET and GLM showed 2.22% and 1.99% degradation, respectively (Fig. 7).

The method has proven specificity as the peaks of degraded products are well separated from the peaks of MET and GLM. MET was found to be most susceptible to degradation under alkaline more than any stress conditions, while GLM was found to be most susceptible to oxidative degradation followed by an acidic condition. The oxidative stress condition had the significant impact on GLM; however, no distinct degradation product was formed, which can be confirmed by the absence of an additional peak in the chromatogram. MET and GLM were almost stable in photolytic and thermal stress conditions and showed a minimum degradation.

Conclusion

The proposed RP-HPLC method is applicable for the simultaneous separation and determination of MET and GLM. It has distinct advantages over other existing methods with respect to sensitivity, time saving, and minimum detection limits. All the analytes were well resolved and separated in a short chromatographic run time. The developed method is a stability-indicating method, which relies on the use of simple working procedure and can be easily used in routine analysis of pharmaceutical dosage form and stability samples of MET and GLM, with excellent accuracy, precision, selectivity, and reproducibility.

Author Contributions

Conceived and designed the experiments: PNV. Analyzed the data: PNV. Wrote the first draft of the manuscript: PNV. Contributed to the writing of the manuscript: PDA. Agree with manuscript results and conclusions: PNV and PDA. Jointly developed the structure and arguments for the paper: PNV and PDA. Made critical revisions and approved final version: PDA. Both authors reviewed and approved of the final manuscript.

Table 6. Robustness and ruggedness.

PARAMETER	MODIFICATION/ LEVEL	RETENTION TIME (MIN)		ASYMMETRY	
		MET	GLM	MET	GLM
Flow rate (mL/min)	0.8	2.87	5.98	0.51	1.21
	1.0	2.83	5.81	0.76	0.88
	1.2	2.79	5.62	1.11	1.23
Wavelength (nm)	228	2.82	5.77	1.27	0.92
	230	2.83	5.81	0.76	0.88
	232	2.84	5.69	0.98	1.03
Analyst	Analyst 1	2.82	5.98	0.77	0.87
	Analyst 2	2.86	5.68	1.14	1.49
	Analyst 3	2.83	5.81	0.97	1.17
pH	2.8	2.88	5.84	0.89	1.37
	3.0	2.83	5.81	0.76	0.88
	3.2	2.81	5.89	1.11	1.24
Organic content (%)	75	2.91	5.95	0.98	1.42
	80	2.83	5.81	0.76	0.88
	85	2.78	5.80	1.53	1.09

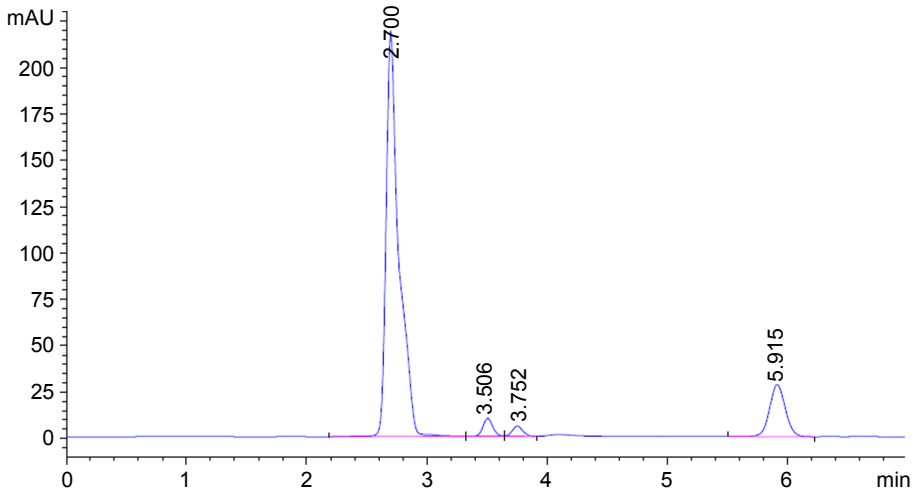

Figure 3. Chromatogram of acid (0.1 N HCl heated for four hours at 80°C) treated MET and GLM.

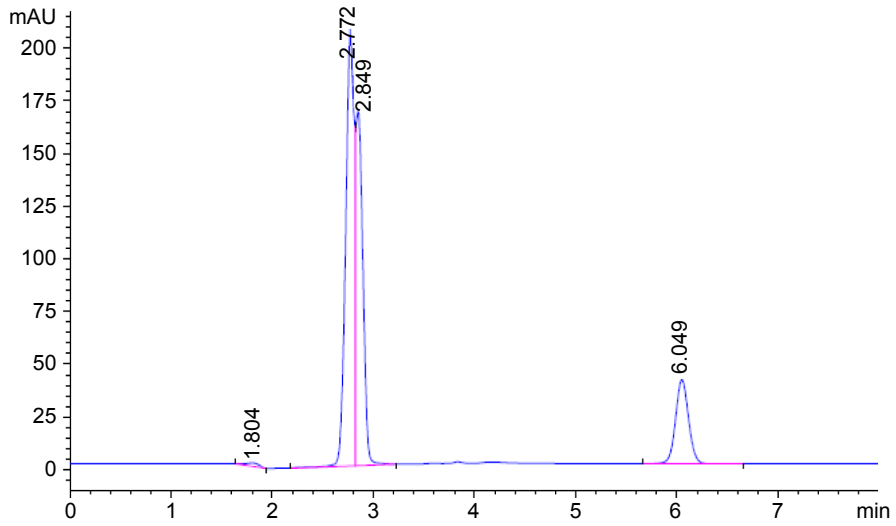

Figure 4. Chromatogram of base (0.1 N NaOH heated for four hours at 80°C) treated MET and GLM.

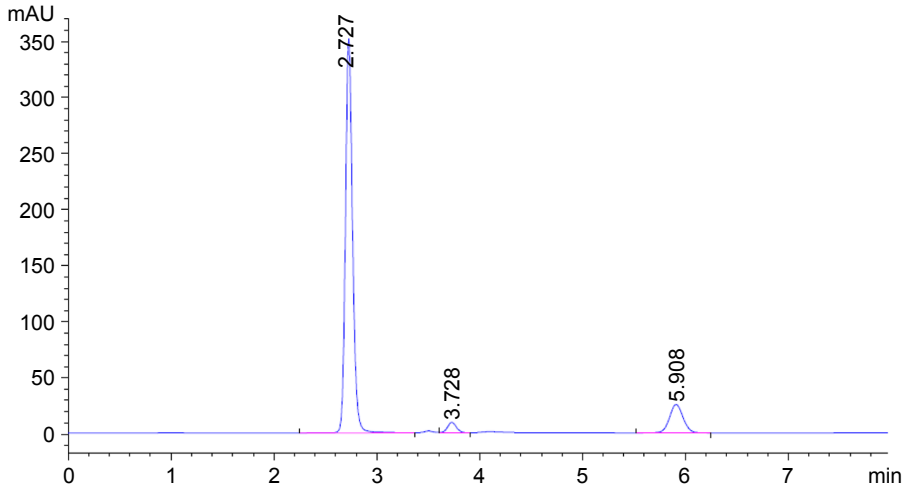

Figure 5. Chromatogram of H_2O_2 (3% v/v)-treated MET and GLM.

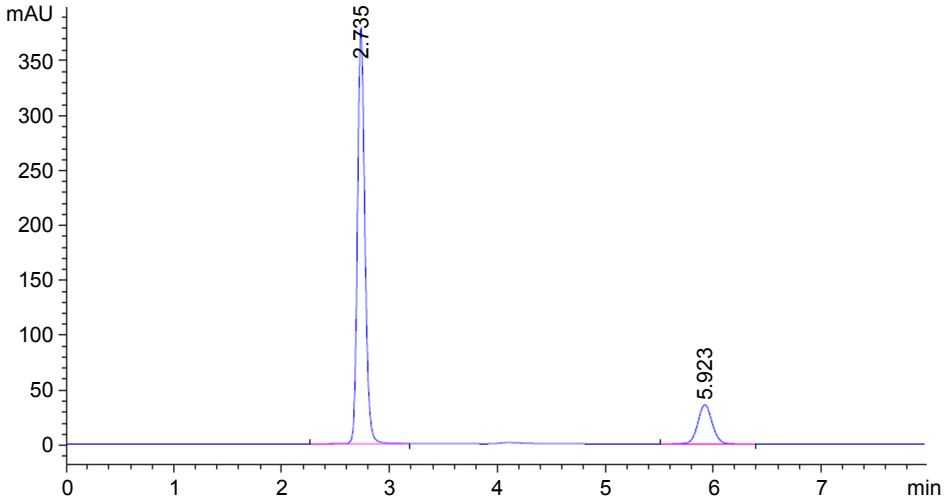

Figure 6. Chromatogram of photochemical degradation of MET and GLM.

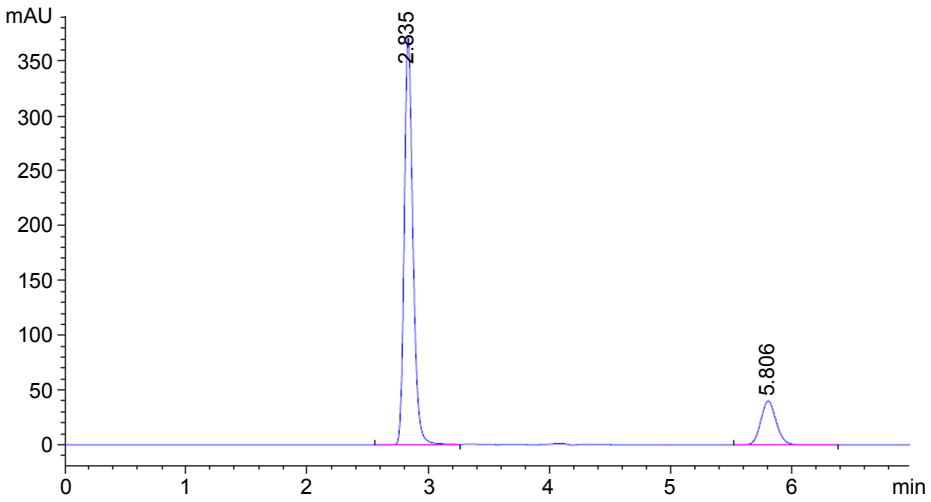

Figure 7. Chromatogram of heat degradation product of MET and GLM.

REFERENCES

1. Sujana K, Swathi Rani G, Bhanu Prasad M, Reddy MS. Simultaneous estimation of Pioglitazone hydrochloride and Metformin hydrochloride using UV Spectroscopic method. *J Biomed Sci Res*. 2010;2(2):110–115.
2. Mohd AB, Sanka K, Gullapelly R, Diwan PV, Shastri N. Development and validation of RP-HPLC method for glimepiride and its application for a novel self-nanoemulsifying powder (SNEP) formulation analysis and dissolution study. *J Anal Sci Technol*. 2014;5:27.
3. Charpentier G, Fleury F, Kabir M, Vaur L, Halimi, S. Improved glycaemic control by addition of glimepiride to metformin monotherapy in Type 2 diabetic patients. *Diabet Med*. 2001;18:828–834.
4. Gandhimathi M, Anandakumar K, Cheriyan A, Ravi TK. Simultaneous estimation of Metformin and gliclazide in tablet using reverse phase high performance liquid chromatography. *Indian J Pharm Sci*. 2003;65(5):530–534.
5. Vasudevan M, Ravi J, Ravishankar S, Suresh B. ION-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms. *J Pharm Biomed Anal*. 2001;25(1):77–84.
6. Yardimci C, Ozaltin N. Simultaneous determination of rosiglitazone and metformin in pharmaceutical preparations by LC. *Chromatographia*. 2007;66:589–593.
7. Jain AK, Agrawal RK. Simultaneous estimation of Gliclazide and Metformin hydrochloride in combined dosage forms. *Indian J Pharm Sci*. 2002;64(1):88–91.
8. Lad NR, Bhoir SI, Bhoir IC, Sundaresan M. Concurrent assay of metformin and glimepiride in tablets using RP-HPLC with wavelength programming. *Indian J Pharm Sci*. 2003;65(6):650–653.
9. Kats M. Forced degradation studies: regulatory considerations and implementation. *Bio Pharm Int* 2005. Available at: http://www.biopharminternational.com/forced-degradation-studies-regulatory-considerations-and-implementation
10. John WD. Stability indicating assay: LC troubleshooting. *LCGC North Am*. 2002; 20(4):346–349.
11. Aubry A-F, Tattersall P, Ruan J. Development of stability indicating methods. In: Huynh KB, ed. *Handbook of Stability Testing in Pharmaceutical Development*. New York, NY: Springer; 2009:139–161.
12. Monika B, Singh S. Development of validated stability-indicating assay methods—critical review. *J Pharm Biomed Anal*. 2002;28(6):1011–1040.
13. International Conference Harmonization (ICH). Topic Q2 (R1) Validation of Analytical Procedure: Test and Methodology. Geneva: IFPMA; 2005. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
14. International Conference Harmonization (ICH). Topic Q1A (R2) Stability Testing of New Drug Substances and Products. Geneva: IFPMA; 2003. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2__Guideline.pdf
15. Center for Drug Evaluation and Research, U.S. Food and Drug Administration. Reviewer Guidance, Validation of Chromatographic Methods. Rockville, MD: FDA; 1994. Available at: http://www.fda.gov/downloads/Drugs/./Guidances/UCM134409.pdf
16. Miller JN, Miller JC. *Statistics and Chemometrics for Analytical Chemistry*. 5 ed. Harlow, England: Pearson Education Limited; 2005:256.