Bull. Pharm. Sci., Assiut University, Vol. 45, Issue 1, 2022, pp. 223-231.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

FACILE HPLC/UV METHOD FOR DETERMINATION OF METFORMIN AND 1-CYANOGUANIDINE USING NOVEL HALOGENATED STATIONARY PHASE

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Metformin is the most prescribed drug for diabetes. We aimed to develop a new HPLC method for determination of metformin and its main impurity 1-cyanoguanidine in raw material and its pharmaceutical tablets. The separation was achieved on pentabromobenzyl column using a mixture of 20% 10.0 mM phosphate buffer (pH 5.5) and 80% methanol as a mobile phase at a flow rate of 1.0 mL min^{-1} and UV detection at 227 nm. The analysis time was less than 4.0 minutes. The method was linear through concentration ranges of 3.0 -80 µg mL^{-1} and 0.5-10 µg mL^{-1} and detection limits of 0.73 µg mL^{-1} and 0.11 µg mL^{-1} for metformin and 1-cyanoguanidine, respectively. The method was successfully applied for determination of metformin in bulk and pharmaceutical dosage forms. The high sensitivity of the developed method allowed for determination of 1-cyanoguanidine below its specified limits in metformin raw material and tablets.

Keywords: *metformin, cyanoguanidine, HPLC, pentabromobenzyl.*

INTRODUCTION

Metformin (MET) is chemically described as 1,1-dimethylbiguanide hydrochloride (Figure 1). It is an orally administered and considered as the most prescribed drug in treatment of type-2 diabetes mellitus worldwide. It works through increasing insulin sensitivity and uptake of glucose by the cells and inhibits glucose production by the liver (gluconeogenesis)[1&2].

The British Pharmacopeia (BP) and the United State Pharmacopeia (USP) listed six impurities (impurity A-F) in MET monograph. HPLC assay of impurity A (1-cyanoguanidine; CGN; Figure 1) in MET raw material and tablets is described in both pharmacopeias. The limit of unwanted CGN is not more than 0.02% and 0.1% in raw material and tablets, respectively[3&4].

MET is assayed in BP, European Pharmacopeia (Ph. Eur.) and USP using non-aqueous titration[3-5]. Many other analytical methods for determination of MET in pharmaceutical and biological matrices, either alone or in combination with other drugs were also reported. Recent reported methods include spectroscopy[6-8], HPLC[9-11], HPTLC[12-14], LC/MS[15-19], capillary electrophoresis (CE)[20&21] and voltammetry[22-24]. Most of these previously published methods were focusing on the determination of MET regardless its main impurity CGN. Only very few methods considered CGN in their determination, and these methods include HPLC[25-27] and CE methods[28].

Received in 21/10/2021 & Accepted in 19/11/2021

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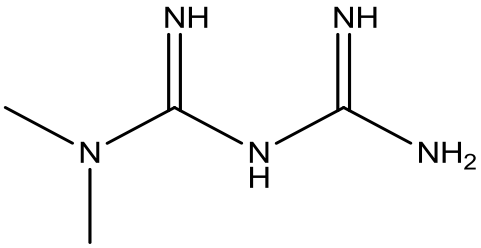
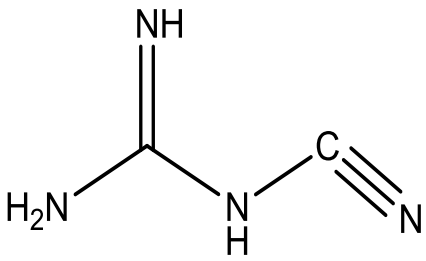


MET



CGN

Fig. 1: Chemical structures of MET and CGN

MET is highly hydrophilic compound, therefore, MET cannot adequately retain on the conventional reversed-phase C_{18} columns and eluted very rapidly[10]. Furthermore, MET strongly binds with polar normal-phase columns and consequently cannot be eluted easily[29]. Therefore, alternative chromatographic modes had been adopted for chromatographic analysis of MET including hydrophilic-interaction liquid chromatography (HILIC)[30&31], ion-exchange (IEC)[32] and ion-pair chromatography (IPC)[27&33].

A pentabromobenzyl-bonded silica column known as Cosmosil PBr is a newly emerged halogenated stationary phase. It separates the analytes through dispersion force interactions which is useful for separation of structurally similar compounds, halogenated and polar compounds[34-36].

In this study, we aimed to develop a simple HPLC-UV method based on using PBr column as an alternative chromatographic approach for the determination of MET and its main impurity CGN in raw material and tablets with low cost, high sensitivity and short analysis time.

MATERIAL AND METHODS

Apparatus

The HPLC Dionex UltiMate 3000 (Thermo Scientific™, Dionex™, Sunnyvale, CA, USA) equipped with a (LPG-3400SD) quaternary pump, a (WPS3000TSL) autosampler, a (TCC-3000SD) column thermostat and a VWD-3000 variable wavelength detector was used for the separation and quantitation. Data processing and acquisition was carried out through Chromeleon 7 software. MET and CGN were analyzed using the Cosmosil PBr column (150 mm x 4.6 mm I.D., 5 µm particle size, Nacalai Tesque Co., Kyoto, Japan). pH-meter (Jenway 3510, UK) was used for measuring and adjusting pH values.

Materials and reagents

All chemicals used were of analytical grade and double distilled water was used during the whole experiments.

— Metformin pure sample (99%) was kindly supplied by Chemical Industries Development (CID) (Giza, Egypt).
— 1-Cyanoguanidine was purchased from Sigma Aldrich (St. Louis, MO, USA).
— Cidophage tablets labelled to contain 500.0 mg MET manufactured by Chemical Industries Development (CID) (Giza, Egypt) were obtained from local market.
— Methanol, ethanol, acetonitrile (ACN) (HPLC grade, > 99.9%), sodium dihydrogen phosphate, phosphoric acid (99.0%) and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Chromatographic conditions

The analysis was performed on a Cosmosil PBr (150 mm x 4.6 mm I.D., 5 µm particle size) column kept at 30 °C. The mobile phase was composed of methanol/phosphate buffer (10 mM, pH 5.5) (80:20 % v/v.), filtered through 0.45 µm membrane filter and sonicated for 30 minutes before use. The flow rate was adjusted at 1.0 mL/min and UV detection at 227 nm.

Preparation of stock solutions

Stock solution containing 1.0 mg mL^{-1} of each of MET and CGN were prepared in

methanol. Working solutions were prepared by appropriate dilution of stock solutions with the same solvent. The stock solutions were kept in the refrigerator at 4°C.

Construction of calibration graphs

Accurately measured aliquots of each of MET and CGN working solutions were transferred separately into a series of 10.0 mL volumetric flasks, completed to the volume with the mobile phase to obtain final concentrations of 3.0-80 and 0.5- 10 µg mL^{-1} of MET and CGN, respectively. 5 µl of each solution was injected in triplicate under the optimum chromatographic conditions. The peak area was plotted versus the concentration of each analyte in µg mL^{-1} to construct the calibration curves and the corresponding regression equations were derived.

Assay of pharmaceutical dosage forms

Twenty tablets were accurately weighted, finely ground and powdered. An amount of the powder equivalent to 10.0 mg was accurately transferred to 10.0 mL volumetric flask, sonicated with the mobile phase for 30.0 minutes and completed to the mark with the same diluent. The solution was centrifuged for 10.0 minutes and then an appropriate portion of the clear supernatant was further diluted with the same diluent for assay of MET in tables.

For analysis of CGN, the same procedures were followed but the final concentration of MET was adjusted to be 2.5 mg mL^{-1} and 0.5 mg mL^{-1} in MET raw material and MET tablets, respectively, in order to detect any trace amount of CGN.

RESULTS AND DISCUSSION

The developed assay permitted the separation of MET and its main impurity CGN in less than 4.0 minutes. The retention times for MET and CGN were 2.88 and 1.78, respectively. The new Cosmosil PBr column has achieved excellent separation and resolution of the studied polar analytes. Different factors that may affect the chromatographic behavior of MET and CGN were carefully studied and optimized to obtain optimum separation in reasonable time.

Method development and optimization

Choice of detection wavelength

To determine the optimum wavelength for the assay, the UV spectra of MET and CGN were investigated, MET and CGN were found to exhibit λ_{max} at 232 and 227 nm, respectively, as shown in Figure 2, UV detection at 227 nm was selected as the most suitable wavelength to obtain the highest possible sensitivity for the impurity CGN with adequate sensitivity for MET.

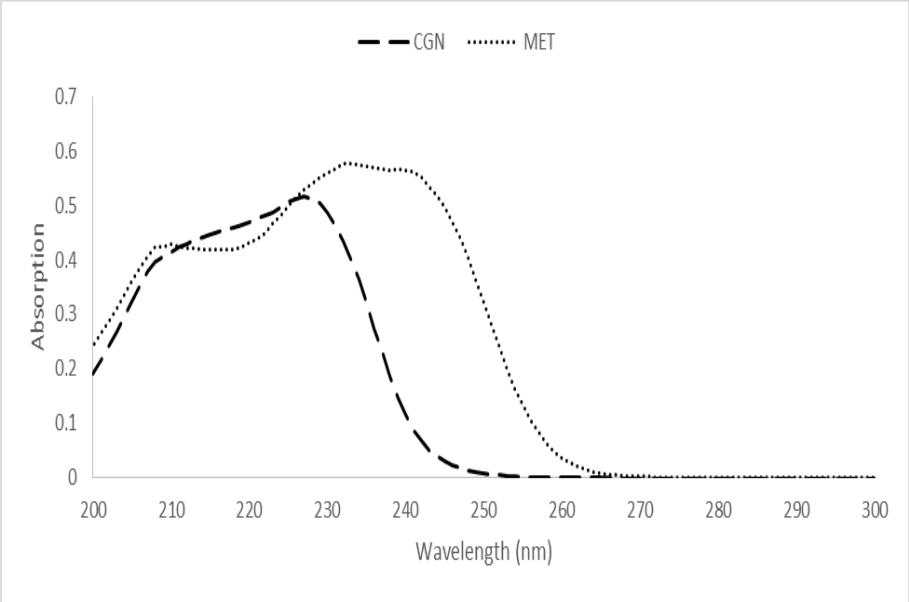


Fig. 2: Absorption spectra of MET and CGN (10.0 µg mL^{-1}) in methanol.

Mobile phase composition

Methanol, ethanol and acetonitrile in different ratios were investigated as the organic modifier, screening experiments revealed that the peak shapes are better upon using methanol or ethanol instead of acetonitrile, finally, methanol was selected as the organic modifier as it produces lower column backpressure than ethanol. Optimization experiments also revealed that, 80% was the optimum methanol content regarding geed separation and short analysis time.

Different buffers (acetate and phosphate buffer) at pH range of (3.5 and 6.0) were evaluated as an aqueous part of mobile phase. It was observed that, tailed broad peak of MET at pH lower than 5.0 was obtained, which may be attributed to the high ionization of the investigated drug at lower pH that cause high interaction with the stationary phase[25]. Optimum symmetric and sharp peaks with good resolution were obtained in the pH range of 5.0-6.0 in either of the studied buffers. Therefore, pH value of 5.5 of phosphate buffer was selected for further investigations.

Effect of flow rate and column temperature

The effect of flow rate of mobile phase on the separation of MET and CGN was investigated to select the optimum flow rate in term of good separation and minimal run time. Flow rate of 1.0 mL/min was suitable for the separation of analytes in short analysis time. Flow rates more than 1.0 mL/min caused high column backpressure. Similarly, different column temperatures were studied, and best result was attained at 30 °C.

Under the optimized conditions, good separation between MET and its main impurity CGN was obtained as shown in Figure 3 which displays a typical chromatogram for the separation of MET and CGN at retention time (t_R) of 2.88 and 1.78 minute for MET and CGN, respectively.

Method validation

Validation of the developed method was performed by following the international council for harmonization (ICH) guidelines[37]. Different validation characteristics were investigated as follows:

Linearity and range

Under the optimized experimental conditions, a linear relationship was established by plotting the peak area versus concentrations of each analyte as indicated by high correlation coefficients of 0.9996 and 0.9995 for MET and CNG, respectively, indicating good linearity of the proposed method. The analytical data of the calibration plots are summarized in Table 1.

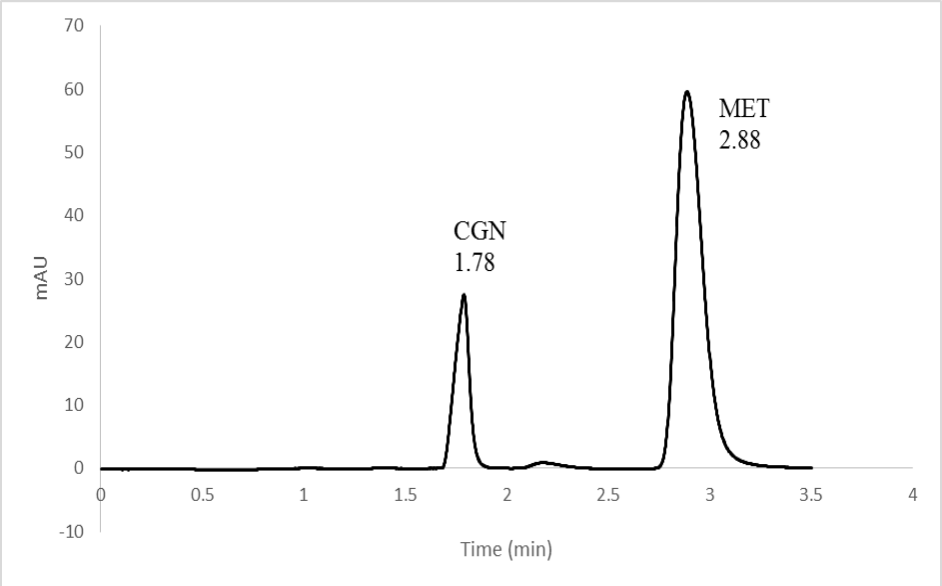


Fig. 3: Representative chromatogram showing good separation of MET (60.0 µg mL^{-1}) and CGN (6.0 µg mL^{-1}).

Table 1: Analytical performance date for the determination of MET and CGN by the proposed HPLC method.

Parameter	MET	CGN
Range (µg mL^{-1})	3.0 – 80.0	0.5 – 10.0
LOD (µg mL^{-1})	0.73	0.11
LOQ (µg mL^{-1})	2.22	0.33
Correlation coefficient (r)	0.9996	0.9995
Determination coefficient (r^2)	0.9994	0.9992
Slope	0.149	0.307
Intercept	0.065	0.436
S.D of intercept	0.033	0.010

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values of MET and CGN were determined according to ICH Q2 (R1) recommendations.

$$LOD = 3.3\ Sa/b$$

$$LOQ = 10\ Sa/b$$

Where, Sa is the standard deviation of the intercept and b is the slope of the calibration plot. The results are shown in Table 1. The LOD values were 0.73 and 0.11 µg mL^{-1} for MET and CGN, respectively, indicating high sensitivity of the proposed HPLC method.

Accuracy and precision

The accuracy and precision of the developed method were studied at three concentration levels for MET and CGN using three replicates for each concentration through the same day for intra-day precision and three consecutive days for inter-day precision (Table 2). The obtained RSD values were less than 2% indicate high precision of the proposed method.

Table 2: Intra-day and inter-day precision and accuracy of the developed HPLC assay.

Conc. Taken (µg mL^{-1})	Intra-day	Inter-day
MET	% Recovery ± RSD	% Recovery ± RSD
5	101.66 ± 1.07	99.73 ± 0.72
40	99.44 ± 1.09	99.36 ± 1.03
75	101.83 ± 0.91	101.31 ± 0.63
CGN	% Recovery ± RSD	% Recovery ± RSD
1	101.22 ± 0.81	100.50 ± 1.06
4	98.62 ± 1.24	99.57 ± 1.31
8	99.71 ± 0.41	99.11 ± 1.05

Robustness

Robustness of the developed method was assessed by studying the influence of small deliberate changes in the experimental conditions on system suitability parameters. The method was found to be robust to minor changes in chromatographic conditions including methanol concentration (± 1% v/v), flow rate (± 0.1 mL min^{-1}) and pH (± 0.5).

System suitability test

According to ICH recommendations[37], number of theoretical plates *(N)*, resolution *(R_s)*, capacity *(K')* and selectivity *(α)* factors were measured as the parameters for system suitability testing. Table 3 shows the system suitability parameters under the optimum chromatographic conditions.

Table 3: System suitability data for the developed HPLC assay.

Parameter	CGN	MET
Retention time	1.78	2.88
Capacity factor *(K')*	0.27	1.05
Number of theoretical plates *(N)*	2814	2211
Symmetry factor	0.90	1.42
Resolution *(R_s)*	2.66	
Selectivity *(α)*	3.88	

Method applications

Application of the proposed method to quality control of MET commercial tablets

The proposed method was successfully applied for the determination of MET in its commercial tablet formulations without

interference from co-existing excipients, the results are shown in Table 4. The good percentage recoveries and small RSD values indicate suitability of the developed method for routine analysis of MET in commercial tablets. The obtained results were statistically compared with those obtained by the reference method using student's *t* test and variance ratio *F* test and the results indicate that there was no significant difference between the proposed method and reference method as shown in Table 4.

Table 4: Assay results for the determination of MET in tablet formulation by the proposed and reference methods.

Dosage form	% Recovery* ± SD	
	Proposed method	**Reference method** [3]
Cidophage tablets® (500mg MET/tablet)	99.76 ± 1.19	100.14 ± 1.53
t-test	0.68 (2.78)**	
F-test	1.65 (6.39)**	

*mean of five determinations

** The value of the tabulated *t* and *F* at *p*= 0.05

Application of the proposed method for determination of CGN in MET bulk and tablet dosage form

CGN was successfully determined in MEW raw material and tablet formulations by the proposed HPLC method, the results are presented in Table 5. The obtained results indicate the applicability of the proposed method for its use in QC laboratories.

Table 5: Assay results for the determination of CGN in MET drug substance and dosage form by the proposed HPLC method.

Preparation	Added CGN µg mL^{-1}	% Recovery* ± RSD
MET raw material	0	Not detected
	2	101.96 ± 0.84
	4	101.97 ± 0.54
	8	98.81 ± 1.63
MET tablets	0	Not detected
	2	98.63 ± 1.84
	4	98.23 ± 1.21
	8	100.99 ± 1.14

*mean of three determination

Conclusion

A simple, sensitive and rapid HPLC method was developed for the determination of MET and CGN using pentabromobenzyl column. The method was validated according to ICH guidelines in terms of linearity, precision, accuracy, limit of detection, limit of quantitation and robustness. The method was successfully applied for determination of MET and CGN in raw material and tablet formulation. The short analysis time and simple analytical procedure for the developed method allow its use for cost-effective and routine analysis in quality control laboratories.

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Bull. Pharm. Sci., Assiut University, Vol. 45, Issue 1, 2022, pp. 223-231.

نشـرة العـلوم الصيدليـــــة
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طريقة سهلة باستخدام كروماتوغرافيا السائل ذات الأداء العالي مع الكشف الفوق بنفسجي لتعيين الميتفورمين و ١-سيانوجوانيدين باستخدام طور ثابت هالوجيني جديد

على عبد الحكيم[١*] – علياء شعلان[٢] – محمد حماد[١] – مها أبو العلمين[٢*]

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يهدف البحث لاستحداث طريقة جديدة لقياس تركيز مركب السيانوجوانيدين، أحد شوائب دواء الميتفورمين المستخدم لعلاج مرض السكر، يمكن أيضا للطريقة تعيين تركيز الميتفورمين، الطريقة تعتمد على فصل المركبين بتقنية كروماتوجرافيا السائل عالي الأداء باستخدام عمود Pentabromobenzyl، وطور متحرك مكون من مزيج من الميثانول ٨٠%، وحمض الفسفور المخفف ١٠ ملي مول ٢٠% ح/ح، وقياس المركبات عند طول موجي ٢٢٧ ن.م. عند تركيزات تتراوح من ٠,٥ إلى ١٠ و٣ إلى ٨٠ ميكروجرام/مل لكل من السيانوجوانيدين والميتفورمين على التوالي. وقد تم تطبيق الطريقة المقترحة بنجاح على دواء الميتفورمين في صورته النقية والمستحضرات الصيدلية.