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# Development and validation of an analytical method for metformin hydrochloride and its related compound (1-cyanoguanidine) in tablet formulations by HPLC-UV

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ABSTRACT

A simple, and stability-indicating liquid chromatographic method was developed and validated for the analysis of metformin hydrochloride and its related compound (1-cyanoguanidine) in tablet formulations. Liquid chromatography with a UV detector at a wavelength of 232 nm using a Nova-Pak silica column was employed in this study. Isocratic elution was employed using a mixture of ammonium dihydrogen phosphate buffer and methanol (21:79, v/v). This new method was validated in accordance with USP requirements for new methods for assay determination, which include accuracy, precision, specificity, linearity and range. The current method demonstrates good linearity over the range of 0.01–0.03 mg $mL^{-1}$ of metformin hydrochloride. The accuracy of the method is 100.4%. The precision of this method reflected by relative standard deviation of replicates is 0.30%. Validation of the same method for 1-cyanoguanidine determination was also performed according to USP requirements for quantitative determination of impurities which include accuracy, precision, linearity and range, selectivity, and limit of quantification (LOQ). Low LOQ of 1-cyanoguanidine using this method enables the detection and quantification of this impurity at low concentration.



## 1. Introduction

Metformin hydrochloride is an oral antidiabetic drug. Chemically it is N,N-dimethylimidodiacarbonimidic diamide [1]. Metformin hydrochloride is formulated as tablet dosage forms. A potential impurity of metformin hydrochloride, which is reported in USP and BP, is 1-cyanoganidine (metformin related A). A method of analysis of metformin hydrochloride and its impurity is therefore needed. USP and BP describe a nonaqueous titration as a method for metformin hydrochloride analysis, and another separate HPLC method for the analysis of its impurity (1-cyanoguanidine) [2,3]. In this respect, stability-indicating test method for the analysis of metformin hydrochloride and 1-cyanoguanidine is, therefore, needed.

Many HPLC methods were developed for the determination of metformin hydrochloride. Sahoo et al. have developed and validated a method for simultaneous determination of metformin hydrochloride and pioglitazone hydrochloride by RP-HPLC method in tablet formulations [1]. Deepti et al. have also developed a method for the determination of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulations [4]. Several other HPLC methods were also developed for the determination of metformin hydrochloride [5–14]. All of these methods, however, are not employed for the determination of metformin hydrochloride potential impurity (1-cyanoguanidine).

Shahid Ali et al. have developed and validated a method for simultaneous determination of metformin hydrochloride and its impurities in tablet formulations [15]. However, they used a HILIC (Hydrophilic Interaction Liquid Chromatography) technique which requires a special column, and expert analysts in this field which is not available in some laboratories. The objective of the current work is, therefore, to develop a simple RP-HPLC, stability-indicating method for analysis of metformin hydrochloride and 1-cyanoguanidine in tablet formulations. Validation of the current method was conducted for both determination of metformin hydrochloride in tablet formulations (assay), and for quantitative determination of its impurity (1-cyanoguanidine). Validation of the method for metformin hydrochloride analysis was performed according to the requirements of USP for assay determination which include accuracy, precision, specificity, linearity and range, while validation of the method for 1-cyanoguanidine was performed according to the requirements of USP for quantitative determination of impurities which include accuracy, precision, specificity, linearity and range, and LOQ.

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## 2. Experimental

### 2.1. Chemicals

Methanol HPLC grade is from J.T Baker (NJ, USA). Ammonium dihydrogen phosphate is from Merck (Darmstadt, Germany). Metformin hydrochloride, and 1-cyanoguanidine are from USP (Rockville, MD, USA).

### 2.2. Apparatus

HPLC system (Merck Hitachi Lachrome Elite HPLC system, Japan) with an L-2130 pump, an L-2200 autosampler, L-2300 column oven, and L-2490 UV detector was employed. The Ezochrom Elite software was employed. The chromatographic analysis was performed on a Nova-Pak silica (4 μm), (150 mm length, 3.9 mm inner diameter) (Waters Corporation, Milford, Massachusetts, USA). The column is kept at room temperature.

### 2.3. Standard solutions preparation

Ammonium dihydrogen phosphate buffer was prepared by dissolving 1.15 g ammonium dihydrogen phosphate in 1000 mL of water for HPLC (0.01 mole $L^{-1}$, pH 5.0).

Stock standard solution of metformin hydrochloride was prepared by dissolving 100 mg of metformin hydrochloride in 100 mL of methanol to obtain a solution having a known concentration of 1.0 mg $mL^{-1}$. Nominal standard solution was prepared by diluting 2 mL of Stock Standard Solution to 100 mL mobile phase to obtain a solution having a known concentration of 0.02 mg $mL^{-1}$ metformin hydrochloride.

Standard solution of 1-cyanoguanidine was prepared by dissolving 10 mg in 100 mL of methanol, and diluting 1 mL of this solution to 100 mL mobile phase to obtain a solution having a known concentration of 0.001 mg $mL^{-1}$.

Resolution solution of metformin hydrochloride and 1-cyanoguanidine was prepared by dissolving 100 mg of metformin hydrochloride and 20 mg of 1-cyanoguanidine in 100 mL methanol, and diluting 1 mL of this solution to 100 mL of mobile phase.

Samples of formulated metformin hydrochloride (tablets) were prepared by dissolving a quantity of the powdered tablet equivalent to 100 mg of metformin hydrochloride in 100 mL mobile phase to get a high concentration of metformin hydrochloride (1 mg $mL^{-1}$) in order to detect 1-cyanoguanidine or any other impurity which may present in the sample of metformin hydrochloride tablets.

## 3. Results and discussion

### 3.1. Method development

Nova Pak silica column was tested using a mixture of ammonium dihydrogen phosphate buffer/methanol mixture as a mobile phase. Regarding the mobile phase composition, first I have tested potassium dihydrogen phosphate buffer/methanol mixture as well as sodium dihydrogen phosphate buffer/methanol mixture (different volume fractions). The use of these different combinations of mobile phase, however, gives broad peaks with poor resolution between metformin hydrochloride and 1-cyanoguanidine. However, a mixture of ammonium dihydrogen phosphate buffer (0.01 mol $L^{-1}$, pH 5.0) with methanol gives sharp peaks of both metformin hydrochloride and 1-cyanoguanidine. To optimize the separation, different volume fractions of methanol and this buffer were tested, and optimum separation was obtained using 79% methanol/21% buffer (v/v) at a flow rate of 1.0 mL $min^{-1}$. UV detection was performed at 232 nm, and injection volume was 20 μL.

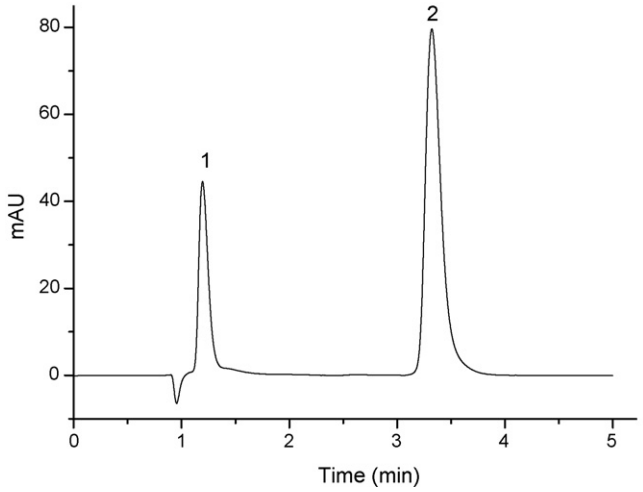


**Fig. 1.** Chromatogram of 1-cyanoguanidine (0.002 mg $mL^{-1}$) (1) and metformin hydrochloride (0.01 mg $mL^{-1}$) (2). Mobile phase: Ammonium dihydrogen phosphate buffer, methanol (21:79, v/v), flow rate 1.0 mL $min^{-1}$, injection volume 20 μL. Column: Nova-Pak silica, 4 μm, 15 cm length, 3.9 mm inner diameter, UV detection: 232 nm.

After this optimization, this method was employed for the separation of metformin hydrochloride and 1-cyanoguanidine. A good separation with adequate resolution was obtained, see Fig. 1.

### 3.2. Method validation

#### 3.2.1. Validation of metformin hydrochloride method of analysis

After method development, validation of the current test method for metformin hydrochloride was performed in accordance with USP requirements for assay determination (Category-I: Analytical methods for quantification of active ingredients in finished pharmaceutical products) which include accuracy, precision, specificity, linearity and range.

*3.2.1.1. Linearity and range.* To evaluate the linearity of this method, standard solutions covering the range between 50% and 150% of the nominal standard concentration (0.02 mg $mL^{-1}$) were prepared by diluting specific volume of the stock standard to get several concentrations (0.01, 0.015, 0.02, 0.025, 0.03 mg $mL^{-1}$). The linearity between peak area and the concentration was examined. Results have shown that the method is linear over the specified range with $R^2$ of 0.999. This correlation coefficient is comparable to that obtained using other methods listed in the references, e.g. 0.9975 (for the method in Ref. [1]) and 0.999 (for the method in Ref. [8]).

*3.2.1.2. Accuracy.* Accuracy of the method was studied by preparing the placebo of the drug formulation according to the formulation procedure. To the required quantity of placebo, a known quantity of metformin hydrochloride with the same proportion as in the drug formulation was added to get three concentrations (0.01, 0.02 (nominal concentration), and 0.03 mg $mL^{-1}$). Results have shown that the mean recovery of metformin hydrochloride is within 100 ± 2.0%,

**Table 1**
% recovery of metformin hydrochloride in tablet formulation at three concentration levels.

| RSD for three replicates | % recovery | Metformin hydrochloride Concentration (mg $mL^{-1}$) |
|---|---|---|
| 0.3% | 101.0 | 0.01 |
| 0.9% | 100.3 | 0.02 |
| 0.7% | 99.8 | 0.03 |

**Table 2**
Chromatographic parameters of metformin hydrochloride and 1-cyanoguanidine in Fig. 1.

| Analyte | Resolution | Asymmetry | Theoretical plates | Relative retention time |
|---|---|---|---|---|
| 1-Cyanoguanidine | 10.1 | 1.26 | 1600 | 0.36 |
| Metformin hydrochloride | | 1.31 | 2500 | 1.00 |

and the RSD is lower than 1.0%, see Table 1. Accuracy of the current method is comparable to the accuracy of other methods listed in the references.

*3.2.1.3. Precision.* The precision of this method was evaluated by calculating the RSD of the peak areas of six replicate injections of the nominal standard solution, which was found to be 0.3%. Furthermore, the RSD of the peak areas for the recovery data analyzed in accuracy study for each level was calculated, and it was found to be less than 1.0% for each level, see Table 1. These results show that the current method for metformin hydrochloride analysis is repeatable.

*3.2.1.4. Specificity (stability-indicating evaluation).* Specificity of the current method was demonstrated by good separation of metformin hydrochloride and 1-cyanoguanidine with adequate resolution, see Fig. 1. Table 2 shows the chromatographic parameters of the separated peaks in Fig. 1. Also, matrix components e.g. excipients do not interfere with metformin hydrochloride or with 1-cyanoguanidine peaks.

*3.2.2. Validation of 1-cyanoguanidine method of analysis*

Validation of the method for 1-cyanoguanidine analysis was performed according to USP requirements for quantitative determination of impurities (Category II) which include accuracy, precision, specificity, linearity and range, and LOQ.

*3.2.2.1. Limit of detection and limit of quantification.* The Limit of detection (LOD) and Limit of quantification (LOQ) of 1-cyanoguanidine using this method was determined by diluting the standard solution of 1-cyanoguanidine several times to obtain different concentrations. LOD was selected to be the concentration that gives a signal to noise ratio of 3–10, while LOQ was selected to be the concentration that gives a signal to noise ratio of 10–20.

Results showed that LOD and LOQ for 1-cyanoguanidine using this method is 0.0005 and 0.001 mg mL$^{-1}$, respectively. This low LOD and LOQ permit the detection of 1-cyanoguanidine at low concentrations. The working concentration of 1-cyanoguanidine was chosen to be 0.001 mg mL$^{-1}$ (same as LOQ) so that it can be detected and quantitated in formulated metformin hydrochloride tablets at low concentration levels.

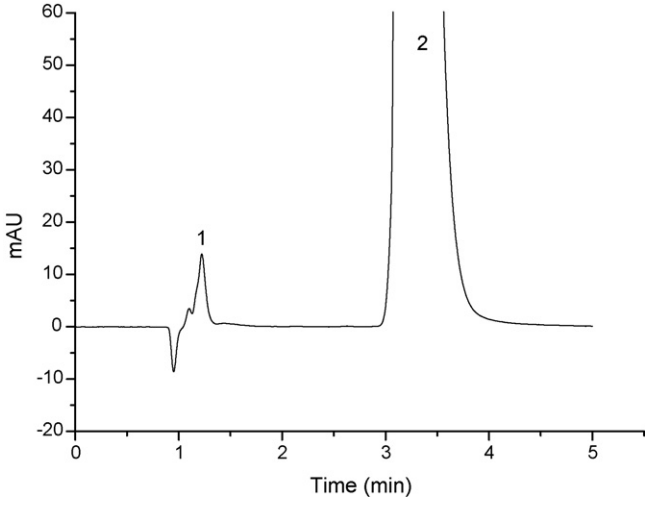


**Fig. 2.** Chromatogram of 1-cyanoguanidine (0.001 mg mL$^{-1}$) (1) and metformin hydrochloride (1.0 mg mL$^{-1}$) (2). For other experimental conditions, see Fig. 1.

*3.2.2.2. Linearity and range.* Linearity of the current method was established using five concentrations: 50%, 75%, 100%, 125%, and 150% of the working concentration (0.001 mg mL$^{-1}$) of 1-cyanoguanidine. Results have shown that this method is linear over the range of 50–150% with $R^2$ of 0.997.

*3.2.2.3. Accuracy.* Accuracy of the method for 1-cyanoguanidine analysis was demonstrated by spiking samples of metformin hydrochloride tablets with known amounts of 1-cyanoguanidine. Accordingly, three solutions were prepared for this study having a concentration of 1.0 mg mL$^{-1}$ of metformin hydrochloride and three different concentrations of 1-cyanoguanidine: 0.0010 mg mL$^{-1}$ (100%), 0.0005 mg mL$^{-1}$ (50%), and 0.0015 mg mL$^{-1}$ (150%). Low concentration of 1-cyanoguanidine relative to metformin hydrochloride was employed to check if this low concentration of 1-cyanoguanidine can be recovered in the presence of high concentration of metformin hydrochloride. It was found that the average recovery of 1-cyanoguanidine for the three levels is 99.5% with a relative standard deviation of 0.85%. The chromatogram (Fig. 2) of 1-cyanoguanidine (0.001 mg mL$^{-1}$) and metformin hydrochloride (1.0 mg mL$^{-1}$) shows that this impurity can be recovered at this low concentration.

*3.2.2.4. Precision.* Precision of the method for 1-cyanoguanidine analysis was demonstrated by analyzing 6 replicates of the working concentration of 1-cyanoguanidine (0.001 mg mL$^{-1}$) and calculating the RSD for the peak responses (Area). Results have shown that the RSD for these six replicates is 0.52%.

*3.2.2.5. Specificity.* Specificity of the current method for 1-cyanoguanidine analysis was demonstrated by separation of 1-cyanoguanidine from metformin hydrochloride with adequate resolution; see Fig. 1 and Table 2.

## 4. Conclusion

A simple, accurate and precise stability-indicating HPLC analytical method was developed and validated for the analysis of metformin hydrochloride in tablet formulations. The current method has the ability to separate metformin hydrochloride from its related compound (1-cyanoguanidine). Low LOD and LOQ for 1-cyanoguanidine using this method enables the detection and quantification of this impurity at low concentration.

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