DEVELOPMENT, COMPUTATIONAL STUDIES AND VALIDATION OF SPECTROPHOTOMETRIC METHOD OF METFORMIN HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Simple, fast, and sensitive spectrophotometric method was developed for the determination of metformin in pharmaceutical formulations. The spectrophotometric procedure was based on the oxidation of metformin with potassium permanganate in alkaline medium. The developed method was also validated according to International Conference on Harmonization guidelines parameters such as linearity, accuracy, precision, limit of detection and quantitation. Studies were conducted to investigate different parameters involved in color developments and optimized. The pure drug of metformin was extracted from a pharmaceutical dosage form. The extraction procedure was developed, and a possible reaction mechanism proposed in the manuscript. The IR spectrum of the extracted metformin was taken and compared with the simulated IR spectrum obtained from the density functional theory calculation. The vibrational assignment of the modes was done based on potential energy distribution.

Keywords: Spectrophotometer, Metformin, Method development, Validation, Tablets, Computational.

INTRODUCTION

Diabetes is due to high amount of glucose in the blood. It is coming with food into the body, increase the level. The insulin hormone provides energy to the glucose inside the cells. Without insulin, blood sugar can be more than the normal, called pre-diabetes because not enough to said diabetes. Then it was continued to type 2 diabetes, mainly affect the impaired insulin secretion and action, the main reason for metabolic disorder. According to the American Diabetes Association, people having diabetes should maintain 100 mg/dl low density lipoprotein (LDL) cholesterol to start treatment with statins.1

The well-known medication for diabetes does not depend on insulin is a biguanide hypoglycemic agent. For this purpose, metformin is the better option that improves and control glycemic activity. It has been observed that 40 to 100% of metformin is released in the small intestines within 3 hours of drug delivery, thus confirming that metformin exhibits the highest glucose absorption in intestinal and enhanced insulin sensitivity.2-4 It was also suggested as an anti-aging drug. Metformin hydrochloride is chemically known as 3-diaminomethylidene)-1,1-dimethylguanidine and hydrochloride salt of antihyperglycemic biguanide metformin (Figure 1). It can oxidize fatty acid and minimize the glucose level in the blood.5

Different analytical techniques including thin layer chromatography (TLC)6, high performance thin liquid chromatography7,8, capillary electrophoresis9, high performance liquid chromatography (HPLC)10-11, ion chromatography12 and liquid chromatography mass spectrophotometry (LC-MS)13 have been introduced to determine the concentration of metformin hydrochloride in pure and pharmaceutical preparations. Most of them required tedious pretreatment and laborious cleanup procedure before analysis. It is challenging for non-developed countries to follow the developed methods due to its operating cost and challenged for a simple procedure for the analysis.

Today UV-visible spectrophotometry has a great demand for the third world countries because of simple operating procedure and low cost with high sensitivity. According to the literature survey, few visible spectrophotometric methods were reported for the determination of metformin hydrochloride in bulk, pharmaceutical formulations, and biological fluids.14-19 The violet colored complex was determined after reaction with ninhydrin at 570 nm in tablets within the range 8–18 µg/ml.12 Coupling agent 1–naphthol was useful in basic medium20, as well as chromogenic agents like 2,4 dinitrophenol and picric acid were a better option for metformin in spiked human urine.21 Simple ultraviolet (UV) spectrophotometric methods were developed and validated for quantitation of metformin as single or in a binary mixture.22-26

The proposed method presents a validated spectrophotometric method for the quantitation of metformin hydrochloride in bulk and pharmaceutical formulations. The method was based on the reaction between metformin and potassium permanganate at room temperature in basic medium. The green colored complex was measured at 601 nm and determined metformin hydrochloride in pharmaceutical dosage forms. For identification, extracted was analyzed by IR spectroscopy, and the IR spectrum of the extract was compared with the simulated IR spectrum obtained from B3LYP/6-311G(d,p) computation. A reliable computational method that has been successfully used to predict the vibrational spectra of organic compounds.27-35

EXPERIMENTAL

Apparatus

All spectral runs were made on Jenway 6300 Spectrophotometer, and Cecil Spectrophotometer with 1 cm matched glass cells.
Materials and reagents

- Pharmaceutical preparations of metformin hydrochloride such as dialon (Julphar, Gulf Pharmaceutical Industries, UAE), Metfor (Tabuk Pharmaceutical Manufacturing Company, Saudi Arabia) and Glucare XR (Jazeera Pharmaceutical Industries, Saudi Arabia) were purchased from the local pharmacy.
- Potassium permanganate, KMnO₄ (Sigma Aldrich, USA) solution was prepared as 5.0×10⁻³ M in distilled water.
- Sodium hydroxide (Sigma Aldrich, USA) solution was prepared as 1.8 M in distilled water.
- Two tablets (500 mg/tablet) were ground into a powder with mortar and pestle, dissolved in 10 ml distilled water. Column chromatography was performed on glass columns packed with silica gel (Sisco Research Laboratories Pvt Ltd, India). The crude sample was applied to the column as a solution (dissolved in distilled water). Solvents with different polarities were used to elute the material as mobile phase system (Water: Methanol: Glacial acetic acid = 4.1: 5.9: 0.3 v/v/v), resulting in separation of compounds. When it reached at optimum length, removed and dried in an oven and collected the pure metformin as solid.

FTIR spectroscopy and computational methods

The IR spectrum of the extracted crystal of metformin was recorded from 400 to 4000 cm⁻¹ at a resolution of 4 cm⁻¹ and an average of 32 scans using a Thermo NICOLET 6700 FT-IR Spectrophotometer. The spectrum is compared with the simulated spectrum obtained from frequency calculations using the Density Functional Theory (DFT) method adopting Becke’s three-parameter exchange functional along with Lee-Yang-Parr ν correlation functional (B3LYP). The standard 6-311++G(d,p) basis set was used for all the atoms to carry out the calculation using the GAUSSIAN 09 program package.

Optimization of variables

Standard Stock Solution

100 mg of metformin hydrochloride was transferred into 500 ml volumetric flask & diluted with distilled water up to the mark (200 ppm). Further dilution was followed according to the requirement.

Effect of concentration of KMnO₄

The effect of the volume (0.1–1.4 ml) of KMnO₄ (5.0×10⁻³ M) on the colored complex was investigated, keeping constant NaOH (0.36M) and metformin (40 μg/ml) concentration. The highest absorbance at 601 nm was achieved with 0.8 ml KMnO₄. Therefore, 1.1 ml of KMnO₄ (5.0×10⁻³ M) was used as an optimum value for the experiment (Figure 2).

Effect of concentration of NaOH

The effect of the volume (0.2–3.0 ml) of NaOH (1.8M) was studied on the intensity coloured product, keeping constant KMnO₄ (1.1×10⁻⁴ M) and metformin (40 μg/ml) concentration. The maximum absorbance was found with 2.4 ml NaOH. Therefore, 2.7 ml of NaOH (1.8 M) was used throughout the experiment (Figure 3).

RESULTS AND DISCUSSION

The maximum absorption of potassium permanganate in basic medium exhibits at 530 nm. The absorption spectrum of metformin showed in the ultraviolet range, generally at 233 nm. However, the addition of KMnO₄ to metformin in alkaline medium shifted its highest absorption band to 601 nm. Because KMnO₄ is a strong oxidizing agent and formed manganite ion. The oxidation no of Mn changes from +7 (purple) to +6 (green) and the metformin converted to metformin N-oxide (Scheme 1). The colored product was measured after 5 minutes, and metformin can be determined in its pure solid form as well as in pharmaceutical formulations.

Scheme 1. The reaction mechanism for the formation of a green product.

Vibrational IR spectrum Analysis

The calculated vibrational wavenumber along with the experimental data, are presented in Table 1. The DFT calculation often overestimates the vibrational wavenumber and as such the overestimated vibrational wavenumbers obtained from B3LYP calculations were scaled by a factor of 0.9668. The assignment of vibrational modes obtained from the Potential Energy Distribution using the VEDA4 program is presented in the last column of Table 1. Metformin contains two imines (C=N–H), primary amine, secondary amine and tertiary amine functional groups. The primary amine shows two NH bands in the range of 3500–3300 cm⁻¹ corresponding to NH symmetric and asymmetric stretching while the secondary amine shows a band at around 3000 cm⁻¹ corresponding to NH stretching. The NH stretching mode is usually observed around 3500 to 3300 cm⁻¹. In this study, the vibrational modes (ν₁ and ν₈) are attributed to the NH stretching. These modes are pure NH stretching with at least 97% contributions to PED. The vibrational ν₇ and ν₉ are attributed to NH asymmetric and symmetric stretching of the primary amine group of metformin. The NH vibrational mode of the secondary amine of metformin is observed at 3372 cm⁻¹ and the modes (ν₁ and ν₈) with 100% contributions to PED are attributed to the imine group. The vibrational modes (ν₁₀ and ν₁₁) are attributed to the CH stretching of the methyl groups of metformin. The C=N stretching modes of metformin was observed at 1731 cm⁻¹ which is typical of guanidine moiety. Guanidines show strong absorption due C=N stretching at around 1685-1580 cm⁻¹. The detailed descriptions of other vibrational modes are provided in the last column of Table 1. The vibrational infrared spectrum of the extracted metformin along with the simulated spectrum, are presented in Figure 4. The IR vibrational wavenumbers and their corresponding intensity are used to simulate the IR absorption spectrum of metformin. The simulated spectrum conforms with the experimental spectrum, and the figures illustrate the spectral characteristics of the metformin molecules as provided in Table 1.
Table 1. Experimental and calculated vibrational wavenumbers (cm\(^{-1}\)) of metformin.

| No. | Expt. | Cal  | Scaled\(^a\) | Intensity\(^b\) | Assignment\(^c\) (PED ≥ 10%) |
|-----|-------|------|-------------|--------------|-----------------------------|
| v\(_1\) | -     | 3651 | 3530        | 26.72        | νNH(99)                     |
| v\(_2\) | 3395  | 3615 | 3495        | 20.88        | νNH(98)                     |
| v\(_3\) | 3372  | 3548 | 3430        | 23.32        | νNH(97)                     |
| v\(_4\) | 3333  | 3524 | 3407        | 14.41        | νNH(100)                    |
| v\(_5\) | 3294  | 3517 | 3400        | 4.41         | νNH(100)                    |
| v\(_6\) | 3171  | 3146 | 3042        | 7.39         | νCH(89)                     |
| v\(_7\) | -     | 3137 | 3033        | 17.48        | νCH(99)                     |
| v\(_8\) | -     | 3075 | 2973        | 36.00        | νCH(93)                     |
| v\(_9\) | -     | 3069 | 2967        | 29.50        | νCH(84)                     |
| v\(_{10}\) | 2931  | 3001 | 2901        | 62.91        | νCH(91)                     |
| v\(_{11}\) | 2970  | 2980 | 2881        | 62.57        | νCH(90)                     |
| v\(_{12}\) | 1636  | 1731 | 1674        | 384.50       | νCN(67)                     |
| v\(_{13}\) | 1620  | 1677 | 1621        | 215.87       | νCN(63) + δHCN(12)          |
| v\(_{14}\) | 1574  | 1626 | 1572        | 35.78        | δHINH(74)                   |
| v\(_{15}\) | -     | 1528 | 1477        | 99.65        | δHCH(63) + τHCNC(11)        |
| v\(_{16}\) | -     | 1506 | 1456        | 120.85       | δHCN(10) + δHCH(50) + τHCNC(10) |
| v\(_{17}\) | -     | 1500 | 1450        | 330.24       | νCN(18) + δHCN(25) + δHCH(16) |
| v\(_{18}\) | 1473  | 1495 | 1445        | 19.82        | δHCH(60) + τHCNC(13)        |
| v\(_{19}\) | -     | 1480 | 1431        | 37.37        | δHCH(56) + τHCNC(14)        |
| v\(_{20}\) | 1420  | 1474 | 1425        | 15.95        | δHCH(63)                     |
| v\(_{21}\) | 1404  | 1445 | 1397        | 2.10         | δHCH(82)                     |
| v\(_{22}\) | -     | 1423 | 1376        | 28.19        | νCN(29) + δHNC(24)          |
| v\(_{23}\) | -     | 1340 | 1296        | 115.78       | νCN(18) + δHNC(33)          |
| v\(_{24}\) | 1291  | 1276 | 1234        | 87.18        | νCN(14) + δHNC(18) + τHCNC(10) |
| v\(_{25}\) | -     | 1233 | 1192        | 13.47        | νCN(39) + δHNC(10) + τHCNC(16) |
| v\(_{26}\) | -     | 1176 | 1137        | 44.46        | δHNC(68)                     |
| v\(_{27}\) | -     | 1171 | 1132        | 13.68        | δHCH(18) + τHCNC(60)        |
| v\(_{28}\) | -     | 1131 | 1093        | 161.75       | νCN(48) + δHNC(36)          |
| v\(_{29}\) | -     | 1120 | 1083        | 3.64         | δHCH(13) + τHCNC(61)        |
| v\(_{30}\) | 1057  | 1097 | 1060        | 127.26       | νCN(33) + δHCN(32)          |
| v\(_{31}\) | 1049  | 1073 | 1038        | 14.25        | νCN(20) + δHCH(13) + τHCNC(42) |
| v\(_{32}\) | 1035  | 1001 | 1001        | 44.76        | νCN(55) + τHCNC(11)         |
| v\(_{33}\) | 933   | 949  | 917         | 12.76        | νCN(49)                      |
| v\(_{34}\) | -     | 875  | 846         | 10.94        | τHNCN(83)                    |
| v\(_{35}\) | -     | 864  | 835         | 22.60        | νCN(54) + δNCN(12)          |
| v\(_{36}\) | 802   | 800  | 774         | 43.28        | τHNCN(82)                    |
| v\(_{37}\) | 733   | 746  | 721         | 97.50        | τHNCN(65)                    |
| v\(_{38}\) | -     | 727  | 702         | 13.13        | τHNCN(53)                    |
| v\(_{39}\) | 648   | 643  | 622         | 16.71        | νCN(16) + δNCN(33)          |
| v\(_{40}\) | -     | 612  | 592         | 178.34       | δHNH(10) + τHCNC(64)        |
| v\(_{41}\) | 540   | 577  | 558         | 25.80        | δNCN(64)                     |
| v\(_{42}\) | 470   | 488  | 472         | 85.34        | τHNCN(70)                    |

\(^a\)Scaled IR vibrational wavenumbers, cm\(^{-1}\) (scaled with 0.9668).

\(^b\)Calculated infrared intensities in km mol\(^{-1}\).

\(^c\)ν is stretching, δ is bending, and τ is torsion.
Method validation

According to the Food and Drug Administration (FDA), the developed method needs to validate as per USFDA regulations or followed the International Conference on Harmonization (ICH) guidelines. The parameters to validate a method are specificity/selectivity, linearity, linear range, accuracy, precision, limit of detection, limit of quantitation, ruggedness, and robustness. Validation is not only necessary for regulatory purposes, but it has a great demand for long-term analysis. The ICH guidelines discussed calculation and data interpretation for the present analysis and explained the importance of previous data for the future analysis.\(^{51-53}\)

The concentration of metformin was linear in the range of 0.4–8.5 μg/ml. The linear regression equation and optical parameters for quantification of metformin were tabulated in Table 2. The intraday precision (within-day precision) was studied with corresponding concentration 2, 4 and 6 μg/ml through replicate analysis (n=5) for metformin. Similarly, the interday precision was executed each day for consecutive five days with identical concentration as applied with within-day precision for the pure sample. The percentage recovery and relative standard deviation (RSD) were within the limit of 98–102 % and ± 2% respectively. The percentage recovery and RSD values were 98–100.13, 0.298–0.722 and 99–100.27, 0.364–1.237, respectively, for intraday and interday precision (Table 3). Intraday and interday precision were continued with pharmaceutical dosage form with concentration 2, 5 and 8 μg/ml. The % recovery and RSD values were in the range of 98.4–100.0, 0.210–1.159 and 98.2–100.6, 0.381–1.980 respectively for within-day and between-day precision in pharmaceutical formulations (Table 4). The results were satisfactory and showed its high accuracy for the proposed developed method.

Table 2. Summary of optical and regression characteristics of the proposed method.

| Parameters                  | Metformin hydrochloride |
|-----------------------------|-------------------------|
| Linear dynamic range (μg/ml)| 0.4–8.5                 |
| Regression equation         | Y=0.05X + 0.003         |
| Correlation coefficient (r²)| 0.9999                  |
| SD of the calibration curve (Sx) | 0.002345               |
| Slope of calibration curve (b)| 0.0.05            |
| LOD (μg/ml)                 | 0.16                    |
| LOQ (μg/ml)                 | 0.47                    |

Table 3. Summary of accuracy and precision results of the proposed method.

| Proposed methods | Amount (μg/ml) | % Recovery | % RSD\(^{a}\) | SAE\(^{b}\) | CL\(^{c}\) |
|------------------|---------------|------------|---------------|------------|-----------|
| Taken            | Found ± SD\(^{a}\) |           |               |            |           |
| Intraday         | 2             | 1.96 ± 0.014  | 98.0      | 0.722     | 0.0063   | 0.0176   |
|                  | 4             | 3.99 ± 0.023  | 99.7      | 0.572     | 0.0102   | 0.0283   |
|                  | 6             | 6.01 ± 0.018  | 100.13    | 0.298     | 0.008    | 0.022    |
| Interday         | 2             | 1.98 ± 0.025  | 99.0      | 1.237     | 0.011    | 0.030    |
|                  | 4             | 3.99 ± 0.023  | 99.7      | 0.572     | 0.0102   | 0.0283   |
|                  | 6             | 6.02 ± 0.022  | 100.27    | 0.364     | 0.01     | 0.028    |

Mean for five independent analyses. \(^{a}\)SD, standard deviation, RSD, relative standard deviation; \(^{b}\)SAE, standard analytical error; \(^{c}\)C.L., confidence limit at 95 % confidence level and 4 degrees of freedom (t=2.776).

Table 4. Summary of accuracy and precision results of the proposed method in pharmaceutical formulations.

| Proposed methods | Amount (μg/ml) | % Recovery | % RSD\(^{a}\) | SAE\(^{b}\) | CL\(^{c}\) |
|------------------|---------------|------------|---------------|------------|-----------|
| Taken            | Found ± SD\(^{a}\) |           |               |            |           |
| Intraday Dialon  | 2             | 1.96 ± 0.023  | 98.4      | 1.159     | 0.0102   | 0.0283   |
|                  | 5             | 4.96 ± 0.032  | 99.2      | 0.638     | 0.0141   | 0.0393   |
|                  | 8             | 7.976 ± 0.017 | 99.7     | 0.210     | 0.0075   | 0.0208   |
| Interday Dialon  | 2             | 1.998 ± 0.022 | 99.4     | 1.147     | 0.0102   | 0.0283   |
|                  | 5             | 4.96 ± 0.039  | 99.28    | 0.775     | 0.0172   | 0.0478   |
|                  | 8             | 8.00 ± 0.0378 | 100.0    | 0.468     | 0.0167   | 0.0465   |
| Glucose XR       |               |            |              |           |           |
|                  | 2             | 1.976 ± 0.016 | 98.8     | 0.847     | 0.0074   | 0.0207   |
|                  | 5             | 5.008 ± 0.042 | 99.7    | 0.828     | 0.0185   | 0.0515   |
|                  | 8             | 7.976 ± 0.046 | 99.7    | 0.572     | 0.0204   | 0.0566   |
| Interday Dialon  | 2             | 2.012 ± 0.030 | 100.6   | 1.508     | 0.0136   | 0.0377   |
|                  | 5             | 4.968 ± 0.039 | 99.36   | 0.785     | 0.0174   | 0.0484   |
|                  | 8             | 7.968 ± 0.030 | 99.6    | 0.381     | 0.0136   | 0.0377   |
| Metformin XR     |               |            |              |           |           |
|                  | 2             | 2.020 ± 0.040 | 101.0   | 1.980     | 0.0179   | 0.0497   |
|                  | 5             | 4.992 ± 0.046 | 99.84   | 0.922     | 0.0206   | 0.0572   |
|                  | 8             | 7.960 ± 0.047 | 99.50   | 0.589     | 0.021    | 0.058    |
| Glucose XR       |               |            |              |           |           |
|                  | 2             | 1.964 ± 0.026 | 98.20   | 1.328     | 0.0117   | 0.0324   |
|                  | 5             | 4.996 ± 0.052 | 99.92   | 1.036     | 0.0232   | 0.0643   |
|                  | 8             | 7.972 ± 0.059 | 99.65   | 0.744     | 0.0265   | 0.0737   |

Mean for five independent analyses. \(^{a}\)SD, standard deviation, RSD, relative standard deviation; \(^{b}\)SAE, standard analytical error; \(^{c}\)C.L., confidence limit at 95 % confidence level and 4 degrees of freedom (t=2.776).

The proposed method was used for estimating the metformin from the tablet after spiking with 50, 100 and 150% of additional pure drug, respectively. The results are reported in Table 5. It can be seen from Table 5 that the recovery and RSD values were in the ranges 98.27–100.40 and 0.334–1.35% respectively. The selectivity of the proposed method was evaluated by incorporating magnesium stearate, microcrystalline cellulose, polydextrose, titanium dioxide into standard pure drug solutions. It was observed that the excipients did not interfere with the proposed method.
Table 5. Summary of data for the determination in pharmaceutical preparations by the standard addition method.

| Proposed methods | Amount (µg/ml) | % Recovery | % RSD | SAEx |
|------------------|---------------|------------|-------|-------|
|                  | Taken  | Added | Found ± SD* |       |       |
| Dialon           | 2 1    | 2.948 ± 0.036 | 98.27 | 1.23  | 0.016 |
|                  | 2 2    | 3.968 ± 0.045 | 99.20 | 1.05  | 0.019 |
|                  | 2 3    | 4.964 ± 0.030 | 98.28 | 0.59  | 0.013 |
| Metform          | 2 1    | 2.960 ± 0.040 | 98.67 | 1.350 | 0.018 |
|                  | 2 2    | 3.988 ± 0.023 | 98.70 | 0.572 | 0.010 |
|                  | 2 3    | 4.976 ± 0.033 | 99.52 | 0.660 | 0.015 |
| Glucare XR       | 2 1    | 3.012 ± 0.011 | 100.40| 0.360 | 0.005 |
|                  | 2 2    | 3.992 ± 0.023 | 99.80 | 0.571 | 0.010 |
|                  | 2 3    | 5.004 ± 0.017 | 100.08| 0.334 | 0.008 |

Mean for five independent analyses; *SD, standard deviation, RSD, relative standard deviation; xSAE, standard analytical error.

CONCLUSION

Spectrophotometric method was applied for the routine quality control analysis of metformin in pharmaceutical formulations. The quantification of metformin in the literature shows that higher instrumental methods require more pretreatment as well as separation procedure. However, the conventional method like the extractive spectrophotometric method for determination metformin is effective as well are tedious, time-consuming, but the problem it requires a large amount of sample and reagents with possible contamination and losses of the analyte. Therefore, the present method can be a better alternate for quantification of metformin in pure form and pharmaceutical formulation. The extracted metformin was confirmed using the IR spectroscopy, and the spectrum shows vibrational modes that are in agreement with the simulated IR spectrum. The vibrational modes were reliably assigned based on potential energy distribution.

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