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Identification, Isolation and Characterization of unknown Impurity in Metformin hydrochloride

[1]Rajan V. Rele.*, [2]Sandip. P. Patil.
Central research laboratory,D.G. Ruparel College, Matunga, Mumbai 400016.
Corresponding author: [1]Rajan V. Rele

ABSTRACT: An unknown impurity in the tablet of metformin hydrochloride was observed by reversed phase gradient high pressure liquid chromatography at 35.5 min. This enriched impurity was isolated, and Characterized with mass and NMR spectral study. Based on spectral data the impurity was identified as N, N-Dimethylamidino.

Keywords: Mass spectrometry, Metformin HCl, NMR, Preparative HPLC, Unknown impurity.

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I. INTRODUCTION

Metformin hydrochloride is chemically known as 1,1-Dimethylbiguanide hydrochloride. Metformin hydrochloride is a biguanide hypoglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus not responding to dietary modification. Metformin improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose. Metformin Hydrochloride is the hydrochloride salt of the biguanide metformin with antihyperglycemic and potential antineoplastic activities. Metformin hydrochloride is a Food and Drug Administration approved drug [1]. Impurity profile of drug products is critical to its safety assessment and manufacturing process. The present study provides the isolation and characterization of unknown impurity appearing the ICH limit in the drug product. The said impurity was isolated using preparative HPLC and characterized by using NMR and MS spectral data. Literature survey reveals that HPLC [2-15], LC-MS [16], TLC [17] and UV spectrometry [18-19] methods for the determination of metformin HCl. The present study provides the isolation and characterization of an unknown impurity appearing above the ICH limit in the final API samples. The said impurity was isolated by preparative HPLC and characterizes by using NMR and mass spectral data.

1.1 Chemical and reagents

Reference standard of metformin HCl was obtained from reputed firm with certificate of analysis. Tri-fluro-acetic acid was used of analytical grade and the HPLC grade water was used from Merck.

II. ANALYTICAL METHODOLOGY

2.0High performance liquid chromatography:

2.1 chromatographic conditions:

The chromatographic separation was performed on Shimadzu LC2010C system equipped with separation module and DAD detector. The chromatogram was recorded and peak quantified by mean of PC based LC solution software. The analytical condition used were, Inertsil ODS 3V, 250 X 4.6 mm, 5µ column with flow rate 1.0 ml/min. The detector wavelength was set at 218 nm and injection volume 5 µl. A SHIMADZU analytical balance was used.

2.1.1. Preparation of mobile phase:

For gradient system, the mobile phase A was a mixture of buffer and acetonitrile (94:06 % v/v). The mobile phase B was methanol. The buffer was a mixture of 1.36g potassium dihydrogen phosphate and 1.2g pentane sulphonic acid sodium salt anhydrous in 1000 ml water adjusted pH 3.0 with dilute orthophosphoric acid. The mixture of acetonitrile and water in the ratio of 20:80% v/v was used as diluent. The gradient programme is shown in Table 1.

Table 1: gradient HPLC method

Time minutes	**Mobile phase A (%)**	**Mobile phase B (%)**
0	100	0
50	100	0
51	40	60
65	40	60
66	100	0
85	100	0

2.0 Preparative High performance liquid chromatography:

2.1 chromatographic conditions:

The observed unknown impurity was isolated from metformin hydrochloride sample by using preparative HPLC method. The LC 2010 preparative HPLC system was used for isolation. For isolation phenyl hexyl 250 X 21.2 mm, 10µm column was used. The flow rate was set at 10.0 ml/min and detector wavelength was set at 218nm. The column temperature kept at 25°c. The diluent was used as water and injection volume was maintained at 3 ml.

2.1.1. Preperation of mobile phase:

The mobile was a mixture of 0.1% tri-fluro-acetic acid in water and acetonitrile in the ratio of 98:2% v/v. Water used as diluent.

III. ANALYTICAL METHODOLOGY

1. Sample preparation:

For isolation, concentration of 50mg/ml sample was used. Total 20 runs were performed using 2 ml in each loading on to the preparative HPLC column.

2. Isolation of impurity:

The major peak isolated individually. All isolated fractions having maximum purity were combined and reanalyzed on analytical HPLC. The combined fractions were concentrated under high vacuum using Buchi rotavapor R-124. The concentrated fractions were then lyophilized using freeze dryer and lyophilized sample was used for identification purpose after a final integrity check on analytical HPLC.

3. Mass Analysis

Mass spectra of unknown impurity were carried out on Thermo-Finnigan mass spectrometer equipped with Electro-spray ionization source. Instrument control and data were performed with the help of Mass Lynx 4.1software. The samples were infused into the ion source chamber with a T- junction delivering approximately 1/3 of the flow to the mass spectrometer. The ion source temperature was 300^0C and the ESI needle voltage was set at 3.0 kV. Nitrogen gas was used as the drying gas which was maintained at a flow rate of 10 mL / min. The collision energy was set between 5.0 and 10.0 to maximize the ion current in the spectra. The spray voltage was 3.0 kV. The temperature of the capillary was 300 oC. Ions were generated in the collision cell wherein Argon was used as the collision gas.

4. NMR Spectroscopy

NMR experiments were performed on Bruker Avance II plus 300 MHz NMR spectrometer with deuterated solvent DMSO-d6 as a diluent at ambient temperature. The chemical shift values were reported on the δ scale in ppm. The peak appearing at δ 2.08 ppm and 3.160-3.171 ppm corresponds to traces of acetonitrile and methanol solvents respectively.

IV. RESULT AND CONCLUSION

1.0 Results:

The present investigation describes the isolation and characterization of the unknown impurity as observed at **2.7 RRT** during the HPLC analysis. The representative HPLC chromatogram of metformin hydrochloride and unknown impurity is shown in fig. 1 and 2 respectively.

Figure 1: representative HPLC chromatogram of metformin hydrochloride

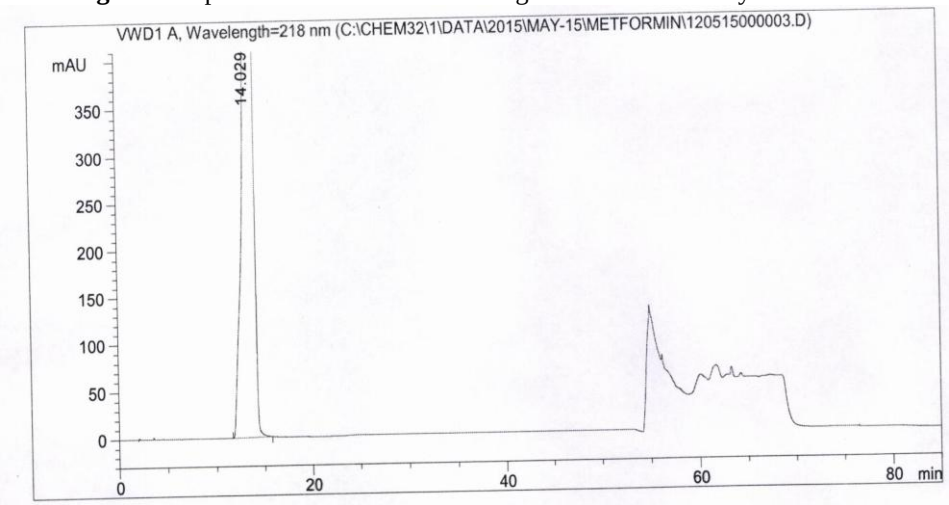
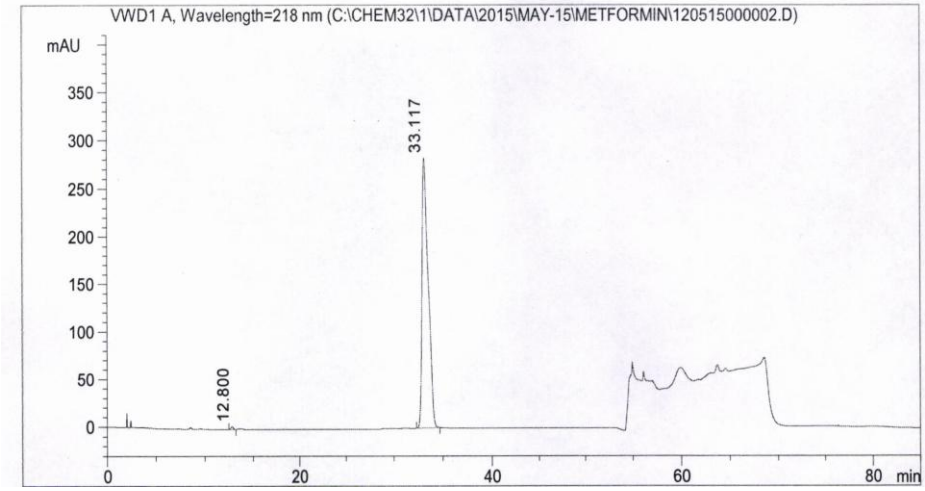
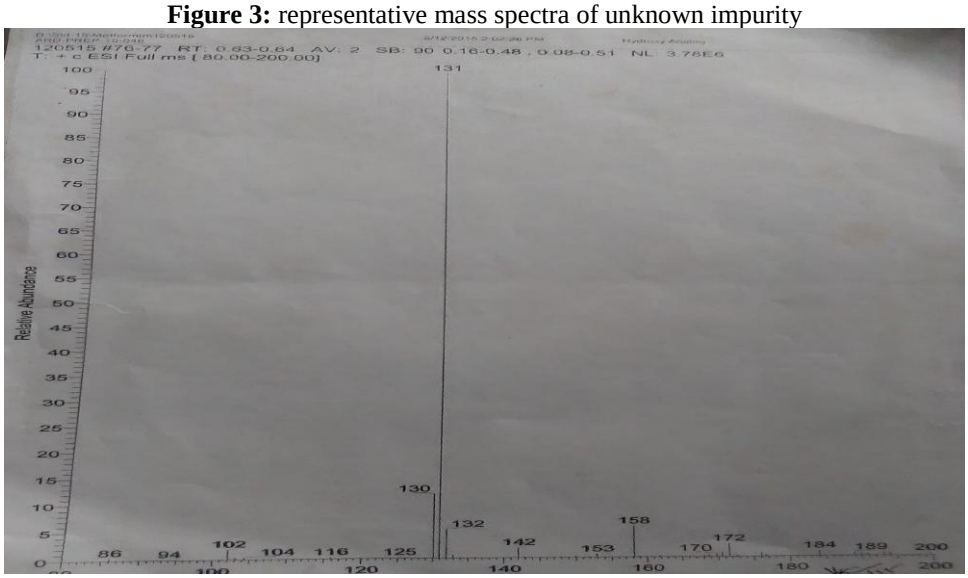


Figure 2: representative HPLC chromatogram of unknown impurity



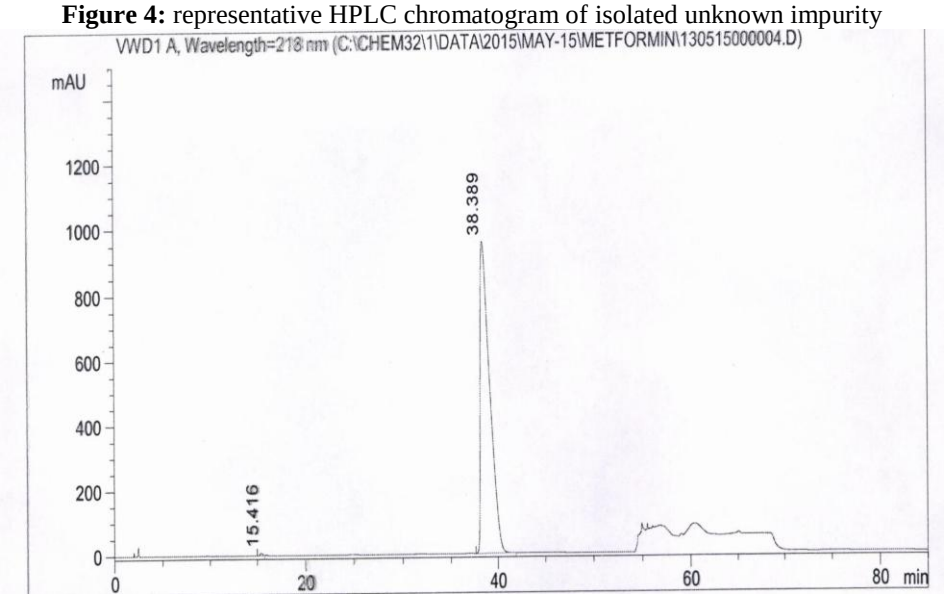
The mass spectra followed by MS of the observed unknown impurity showed major molecular ion at 131 indicating a molecular weight of 130 for the impurity. The mass spectra of unknown impurity are shown in fig 3.

Figure 3: representative mass spectra of unknown impurity



Unknown impurity was isolated by preparative HPLC. All impurity fraction collected from preparative HPLC was concentrated and after lypophilization about 40 mg of unknown impurity was obtained. This impurity was injected in HPLC method for checking its integrity and was found to be more than 98% pure. For details refer HPLC chromatogram of the isolated Impurity as depicted in Fig 4.

Figure 4: representative HPLC chromatogram of isolated unknown impurity

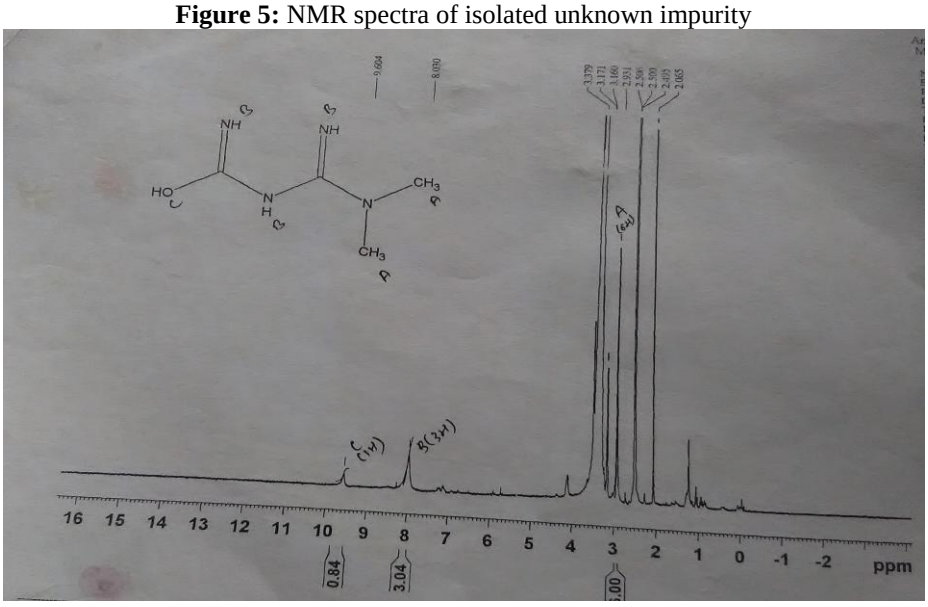


1.1 Structural elucidation of unknown impurity

The High Resolution Mass Spectral data of the unknown impurity showed similar pattern as obtained during LC-MS experiments that is it showed a major molecular $[M+H]^+$ ion at m/z 131 [(calculated 130 for $C_4H_{10}N_4O$). Besides it showed two daughter ions at m/z 132 and 158.

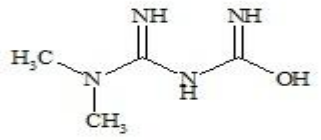
1H NMR data for the impurity was studied and concluded. In the 1H NMR of the impurity signal corresponding to 6 protons of methyl group [CH_3] at δ 2.931 probably aliphatic methyl group [-CH_3]. The other 3 protons are observed at δ 8.030 ppm with broad singlet. The 1 proton with broad singlet is observed at δ 9.604 ppm. This indicated that probably the impurity structure was having a different group. The NMR spectra of unknown impurity are shown in fig no 5.

Figure 5: NMR spectra of isolated unknown impurity



Thus from all the mass and NMR spectral data the impurity was found to N, N-Dimethylamidino. Urea also known as hydroxyl analog2 impurity. The structure so identified also justified the mass fragmentation pattern. The structure of hydroxyl analog2 impurity is shown in fig no. 6

Fig no, 6: structure of unknown impurity (hydroxyl analog2 impurity)



1.2 Source of impurity

The tablets of metformin hydrochloride was kept at 50°c and 75% relative humidity for 3 months, the hydroxyl analog2 (RRT 2.7) impurity was enriched. At the time of stability study the impurity was enriched.

V. CONCLUSION

The unknown impurity observed at ~2.7 RRT during HPLC analysis of some of the in house manufactured metformin hydrochloride tablets was isolated using preparative HPLC and subsequently characterized by spectroscopic techniques namely NMR and mass spectrometry. The impurity was found to be N, N-Dimethylamidino Urea also known as hydroxyl analog2 impurity which is increased during stability analysis. This isolated impurity will be used for analysis related substances test of metformin hydrochloride. Hence this method is strongly recommended for isolation of hydroxyl analo2 impurity.

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