ThP 572

Highly sensitive LC-MS/MS method for the determination of NDMA, NDEA, NDIPA, NMBA, NEIPA and NDBA in Metformin drug substance

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1. Overview

Health Sciences Authority (HSA) of Singapore on 4th December 2019[1] recalled three out of 46 locally marketed Metformin medicines after detecting presence of NDMA "above the internationally acceptable level" and this subject came into mainstream. Subsequently, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have released regular updated into their investigations into the causes of medicine contamination.

Metformin (Figure 1) is the first-line drug control of high blood sugar levels in patients with type 2 diabetes, particularly in people who are overweight.

Here, a single LC-MS/MS method is developed for the quantitation of six nitrosamines namely NDMA, NDEA, NDIPA, NMBA, NEIPA and NDBA (Figure 2) in Metformin drug substance.

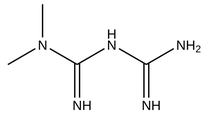

Figure 1. Metformin

2. Introduction

Nitrosamines, refer to any molecule containing the nitroso functional group. These molecules are of concern because nitrosamine impurities are probable human carcinogens. Although they are also present in some foods and drinking water supplies, their presence in medicines is nonetheless considered unacceptable.[2]

The formation of nitrosamines is generally only possible when secondary or tertiary amines react with nitrous acid. Nitrous acid itself is unstable but can be formed in situ from nitrites (-NO_2) under acidic condition.

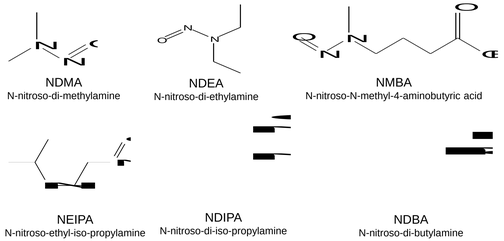

Figure 2. Structures of six nitrosamines

Metformin is an oral diabetes medicine that helps control blood sugar levels. Metformin is used together with diet and exercise to improve blood sugar control in adults with type 2 diabetes mellitus.

After regulatory agencies, found NDMA content in Metformin above the internationally acceptable level. It has created a need to have a single analytical method to quantitate not only NDMA but also these six nitrosamines in Metformin.

Taking advantage of the performance of the Shimadzu LCMS-8045 system with an APCI interface, a fast, sensitive method was developed,

3. Methods

3-1. Sample preparation

Preparation of standard stocks
Intermediate standard solution of 100 ng/mL was prepared in water using commercially available nitrosamine reference standards.

Linearity standards preparation
Linearity standards of mixed nitrosamines were prepared from 0.25 ng/mL to 100 ng/mL levels. Each level was spiked with two internal standards NDMA-D6 & NDEA-D10 to obtain a resultant concentration of 10 ng/mL.

Metformin drug sample preparation
Accurately weighed 100 mg of drug substance into a 2 mL of microcentrifuge tube. Dissolved completely in 1 mL of water. Filtered through 0.2 µm syringe filter in HPLC vial.

3-2. LC-MS/MS analysis

Six nitrosamines were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera X2 coupled with LCMS-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan (Figure 3).

LCMS-8045, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

All six nitrosamines are of mid polar compounds. They were easily ionized by Atmospheric Pressure Chemical Ionization (APCI) interface (Figure 4) in positive mode.

Figure 3. Nexera X2 with LCMS-8045 triple quadrupole mass spectrometer

Figure 4. APCI Probe

Table 1. Instrument parameters

UHPLC condition (Nexera X2 system)	
Column	Shim-pack GIST C18 (150 mm x 4.6 mm, 5 micron) **(P/N :227-30017-07)**
Mobile phase	A: 0.1% Formic acid in water; B: 0.1% Formic acid in methanol
Flow rate	0.7 mL/min
Gradient program (B %)	0-2 min →5(%); 2-6 min → 5-10(%); 6-12 min→10-90 (%); 12-15 min→90 (%);15-15.1 min →90-5(%);15.1-18 min →5 (%)
Injection vol.	30 uL
Column temperature	30 ^{0}C

Table 1. Instrument parameters (cont.)

MS Parameters (LCMS-8045)	
MS interface	APCI
Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 5 L/min
MS temperatures	Desolvation line- 200 ^{0}C; Heating block- 200 ^{0}C; Interface- 350 ^{0}C

4. Results

All six nitrosamine peaks are well separated from Metformin drug. Metformin drug peak is diverted to the waste by using divert valve to avoid the contamination of mass spectrometer (Figure 5).

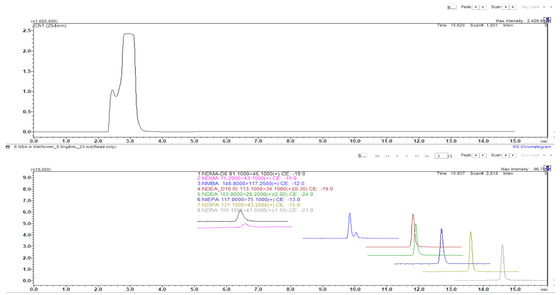

Figure 5. Comparison between UV Chromatogram of Metformin (254nm) and mass chromatograms of NDMA, NDEA, NMBA, NDIPA, NEIPA and NDBA

Calibration curves were plot by using internal standard method and regression coefficient >0.99 over the linearity range were observed. LOD, LOQ and range are given in Table 2. Absolute concentration is mentioned in 'ng/mL' unit and with respect to sample concentration is mentioned in 'ppm' unit. Recovery study was performed by spiking mix standard 0.005 ppm in the Metformin sample and it was found to be well within the criteria.

Table 2. LOD and LOQ levels of six nitrosamines

Description	Unit	NDMA	NDEA	NMBA	NDIPA	NEIPA	NDBA
LOD	ng/mL	0.25	0.10	0.10	0.10	0.10	0.10
	ppm	0.0025	0.0010	0.0010	0.0010	0.0010	0.0010
LOQ	ng/mL	0.50	0.25	0.25	0.25	0.25	0.25
	ppm	0.0050	0.0025	0.0025	0.0025	0.0025	0.0025
Range	ng/mL	0.50-100	0.25-100	0.25-100	0.25-100	0.25-100	0.25-100
	ppm	0.0050-1.0	0.0025-1.0	0.0025-1.0	0.0025-1.0	0.0025-1.0	0.0025-1.0

4-1. Case study of Metformin (False Positive)

One of the Metformin samples showed positive for the NDMA during the evaluation. This peak in the sample showed response for both the transitions of the NDMA with the similar ion ratio as that of the NDMA standard, however retention time was slight different than that of standard (Figures 6A & 6B). For further confirmation, this sample was subjected to Q-TOF analysis.

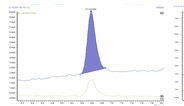

Figure 6A. NDMA standard (Ion ratio-25)

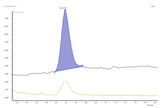

Figure 6B. Sample (Ion ratio-23)

The NDMA standard and suspected Metformin sample were analysed on LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer system, Shimadzu, Japan. The LCMS-9030 enhances the most important features of Q-TOF instrumentation; mass accuracy, sensitivity, and speed to address qualitative and quantitative challenges with genuine confidence and ease.

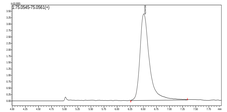

Figure 7A. Mass chromatogram of NDMA

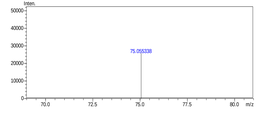

Figure 7B. High resolution mass spectrum of NDMA peak

The NDMA molecule has a monoisotopic mass of 74.048012 Da. Hence, total theoretical mass of protonated NDMA is $[M+H]^+$ 74.048012+1.007276 = 75.055288 Da (Figures 7A & 7B). The observed m/z of NDMA on LCMS-9030 system is 75.055338 Da with the mass accuracy of **0.6 ppm**.

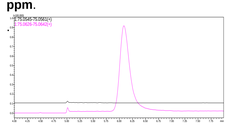

Figure 8A. Overlay Extracted mass spectra of interference peak & NDMA

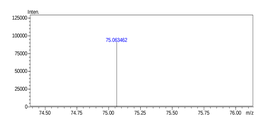

Figure 8B. High resolution mass spectrum of interference peak

The m/z of peak from Metformin sample is 75.063462 Da (Figure 8B) with the mass error of **109 ppm** .Secondly, this sample peak is showing no response at the mass of NDMA (Figure 8A), thus, it confirmed that this sample is negative to the NDMA.

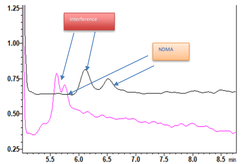

Figure 9. Comparison of NDMA mass chromatograms at different gradient programs

Furthermore, gradient trials were taken to separate this sample peak from the NDMA peak. The sample got separated from the NDMA peak at the optimized gradient program (Figure 9).

This case study helps us to understand the scenario wherein false positive results on the triple quadrupole system can be further investigated and confirmed on the high-resolution mass spectrometer such as Q-TOF system. Therefore, high resolution mass spectrometer become an inevitable tool to identify the false positive/suspected samples.

5. Conclusion

- A highly sensitive LC-MS/MS method for the determination of NDMA, NDEA, NDIPA, NMBA, NEIPA and NDBA in Metformin drug substance is developed.
- Triple quadrupole mass spectrometer LCMS-8045 is a very good tool for the high sensitive quantitative analysis which can be used for regular QC screening. In addition to that high resolution mass spectrometer LCMS-9030 plays an imperative role to identify and confirm the false positive results.

6. References

[1] HSA Press Release "HSA Recalls Three out of 46 Metformin Medicines", December, 2019.

[2] Tabrez et al, Journal of Advances in Pharmacy Practices, Volume 2, Issue 1,48-57, 2020.

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