Quantitative Method For The Determination Of Phenyl Hydrazine, A Potential Genotoxic Impurity Of Ondansetron Hydrochloride Using Tandem Mass Spectrometry

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ABSTRACT

To estimate the level of Phenylhydrazine, a potential genotoxic impurity in Ondansetron Hydrochloride API, a new simple, sensitive and accurate method was developed using Liquid Chromatographic Mass Spectrometry (LC-MS/MS). The chromatographic separation was achieved on Inert Sustain Swift C18, 5µ (150 x 4.6) mm column with gradient programme and elution was monitored by mass spectrometer in Multiple Reaction Monitoring mode using electrospray ionization. The LOD and LOQ values found to be 5 ppm and 15 ppm for the impurity with respect to the test concentration 2 mg/ml. The method was linear ($r^2>0.99$), precise (RSD<2%), accurate and well within acceptable ICH limits.

Keywords: Potential genotoxic impurities, Ondansetron Hydrochloride, LC-MS/MS, Multiple Reaction Monitoring (MRM), Threshold of Toxicological Concern (TTC)

INTRODUCTION

Synthesis of drug substances involves many reactive chemicals, solvents and reagents. Impurities are generated as by-products during the synthesis or can be formed due to subsequent degradation of the drug substance or drug products. For majority of these impurities, guidance for qualification and their control is provided in ICH Q3A and ICH Q3B [1-2]. There are certain impurities that are reactive to DNA and even when present at low levels have the ability to modify the DNA and as a consequence can cause cancer. These are called as potential genotoxic impurities (PGIs). ICH M7 guideline [3] published by International Conference on Harmonization highlights the requirement for assessment and control of PGTIs. The allowable levels of PGI's are determined by a staged toxicological threshold of concern (TTC) based on both the dose and duration of the intended clinical study. This allowable amount can be in low ppm range, which is much lower than the permissible levels of non-PGI impurities controlled under ICH Q3A guideline. This TTC value was estimated to be 1.5 μg/person/day. For example a drug dosed at 1g/day the genotoxic impurity level would be 1.5 ppm. [4-15].

Ondansetron Hydrochloride (Figure 1) is chemically named as 9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-2, 3, 4, 9-tetrahydro-1H-carbazol-4-one. It is 5-HT3 receptor antagonist used mainly as an antiemetic (to treat nausea and vomiting). The antiemetic activity of the drug is brought about through the inhibition of 5-HT3 receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract). It is also effective for treating gastroenteritis [16-17]. Phenyl hydrazine (Figure 2) is used during the synthesis of one of the intermediates of Ondansetron Hydrochloride drug substance. Hydrazine is a known genotoxic impurity and there have been many reported methods used to detect and quantify hydrazine using different analytical techniques [18-20]. Thus Phenyl hydrazine has been considered as a potential genotoxic impurities as per structural alert and must be controlled in Ondansetron Hydrochloride drug substance at low ppm level. The maximum daily dose of Ondansetron HCl is 24 mg and the TTC value for Ondansetron is 62.5 ppm (1.5/24*1000) justified.

Literature survey revealed that currently there is no method for the low level quantification of this potential genotoxic impurity Phenylhydrazine in Ondansetron Hydrochloride. The proposed method is a direct, sensitive and robust which involves MRM mode with electrospray ionization to achieve very low detection and quantification of potential genotoxic impurity Phenylhydrazine in Ondansetron Hydrochloride.
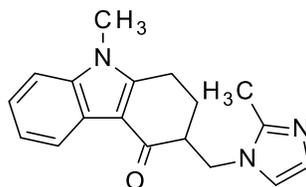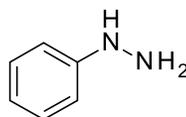
Figure 1: Ondansetron Hydrochloride

Figure 2: Phenyl Hydrazine

MATERIALS AND METHOD

LC-MS grade ammonium formate was purchased from Sigma-Aldrich. HPLC grade acetonitrile was purchased from JT Baker (Mumbai, India). Purified water collected through Milli-Q Plus water purification system (Millipore, USA). Ondansetron Hydrochloride and its potential genotoxic impurity (Phenyl hydrazine) were obtained from Natco Pharma Ltd, Natco Research Centre, and Hyderabad, India.

Instrumentation:

The LC-MS/MS method development and validation was done using Waters Xevo TQ-S micro system including Acquity UPLC H-Class system connected to Mass detector equipped with electrospray ionization in positive mode.

LC-MS/MS chromatographic conditions:

The analysis was carried out using Inert Sustain Swift C18, 5μ (150 x 4.6) mm with a flow rate of 0.6 ml/min. The mobile phase used was a mixture of 5mM ammonium formate as mobile phase-A and Acetonitrile as mobile phase-B using a gradient programme of Time(mins)/%A: 0/75, 10/75, 12/20, 17/20, 18/75, 20/75. The column temperature was maintained at 35°C and the injection volume was 20μl. Mass spectrometer was operated in electrospray ionization with positive ion mode with a capillary voltage of 3 KV. The collision energy was set at 20V, cone voltage at 15V, the desolvation gas flow was 900 L/hr with a temperature of 450° C and source temperature at 150ºC. Under these conditions the potential genotoxic impurity in Ondansetron hydrochloride was quantified by MRM mode with the transition 109.2 > 92.0.

Preparation of standards and test sample solutions:

The standard stock solutions of Phenylhydrazine was prepared approximately at 125 ng/ml in diluent. The Ondansetron Hydrochloride test samples were typically prepared at 2 mg/ml in diluent.

RESULTS AND DISCUSSION:

Method development:

The aim of the present work was to develop a method that could successfully separate and quantify the potential genotoxic impurity Phenylhydrazine in Ondansetron Hydrochloride, different stationary phases and mobile phases were used and finally the desired chromatographic separation was achieved on Inert Sustain Swift C18, 5μ (150 x 4.6) mm column with a flow rate of 0.6 ml/min. The mobile phase used was a mixture of 5mM ammonium formate as mobile phase-A and acetonitrile as mobile phase-B using a gradient programme of Time(mins)/%A: 0/75, 10/75, 12/20, 17/20, 18/75, 20/75.

Method validation:

The method has been validated for the quantification of Phenylhydrazine in Ondansetron Hydrochloride to ensure that the performance characteristics of the method meet the requirements for its intended analytical applications. During the method validation the assessed parameters were specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, precision and accuracy.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ were calculated with signal to noise ratios of 3:1 & 10:1 respectively and by injecting a dilute solution having known concentrations of Phenylhydrazine and established the minimum level at which the Phenylhydrazine can be reliably detected. The LOD was 5 ppm and LOQ was 15 ppm obtained for the impurity.

System precision and system suitability:

The precision and system suitability was performed by injecting six replicates of the working standard solution of 62.5 ppm Phenylhydrazine with respect to the test sample concentration. The %RSD for the peak areas obtained was calculated. The data presented in Table 1 establishes system precision. Reference solution chromatogram for Phenylhydrazine is represented in Figure 3.

Table 1: **System suitability and system precision**

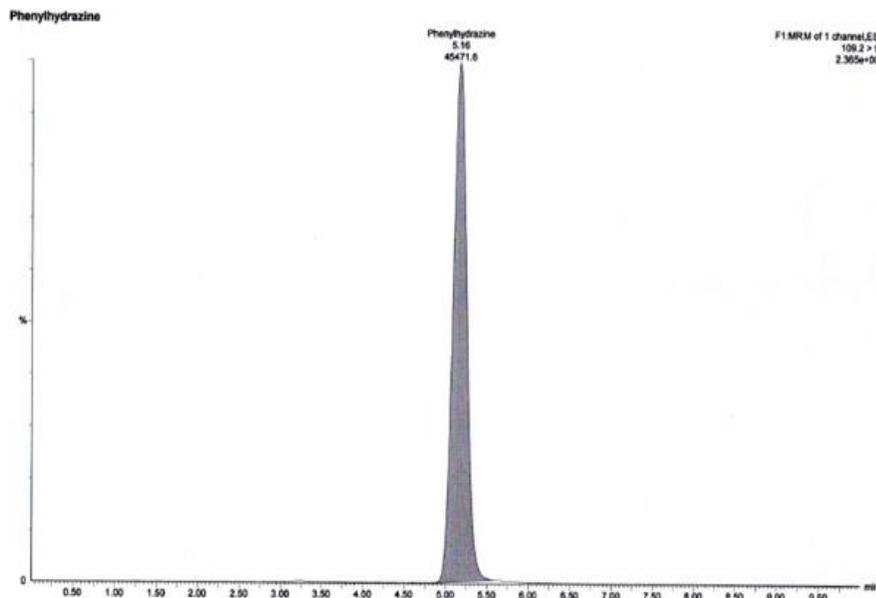| S.No	Peak area for Phenylhydrazine
1	45471.6
2	45448.5
3	45693.1
4	44645.1
5	44917.6
6	44275.8
Mean	45075.3
% RSD	1.2

Figure 3: **Reference solution (62.5 ppm) chromatogram**

Linearity:

Linearity of the method was checked by preparing the solutions at 6 concentration levels from LOQ to 150% of specification limit (15, 31.25, 50, 62.5, 75.0, 93.75 ppm). Results obtained are shown in Tables 2 and Table 3. The mean responses recorded for Phenylhydrazine were plotted against concentration. The correlation coefficient of linear regression was found to be greater than 0.99, indicating good linearity. Corresponding linearity graph is shown in Figure 6a and overlaid chromatogram is represented in the Figure 6b.
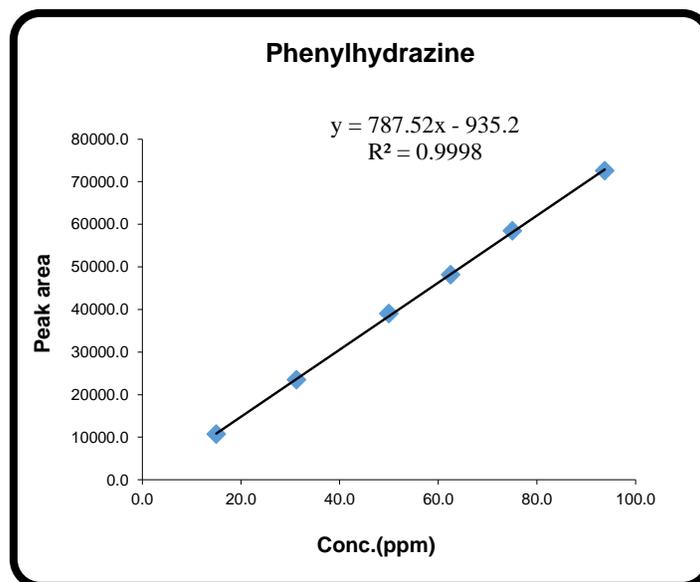

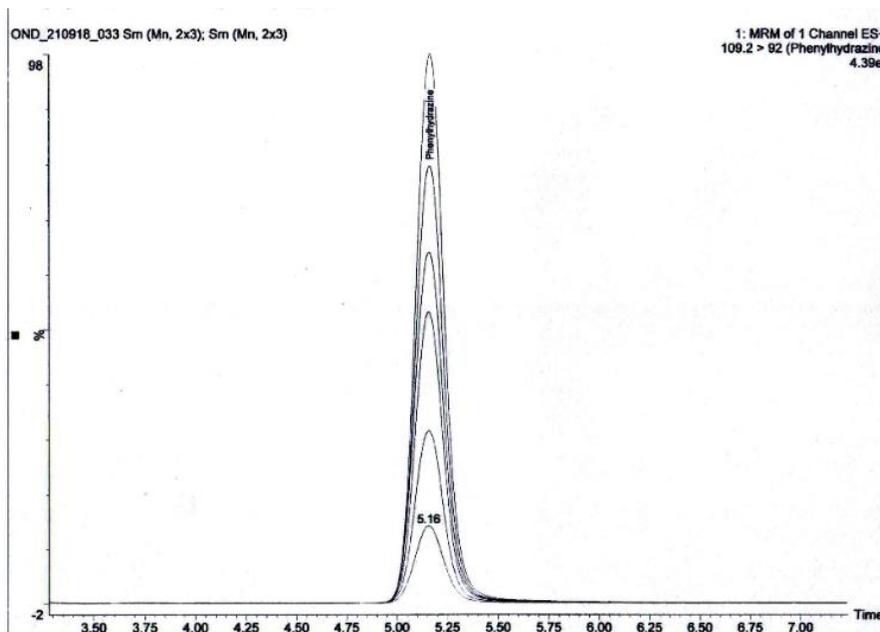**Figure 6a: Linearity graph for Phenylhydrazine**

Figure 6b: Linearity overlaid chromatogram

Table 2: Linearity for Phenylhydrazine

Level	Concentration (ppm)	Peak area
LOQ	15.00	10717.4
50%	31.25	23480.8
80%	50.00	38999.8
100%	62.50	48103.6
120%	75.00	58443.7
150%	93.75	72554.7

Table 3: Regression Parameters Summary

Component	Slope	Intercept	Correlation coefficient (R)	R^2
Phenylhydrazine	787.5152	-938.2028	0.9999	0.9998

Accuracy:

Accuracy of the method was evaluated by using Ondansetron Hydrochloride spiked with the Phenylhydrazine at LOQ and at specification level (62.5 ppm). Each concentration level was prepared in triplicates. The percentage recovery results obtained for the Phenylhydrazine are listed in Table 4. A representative spiked chromatogram is shown in Figure 7.

Table 4: Summary of Recovery study for Phenyl hydrazine

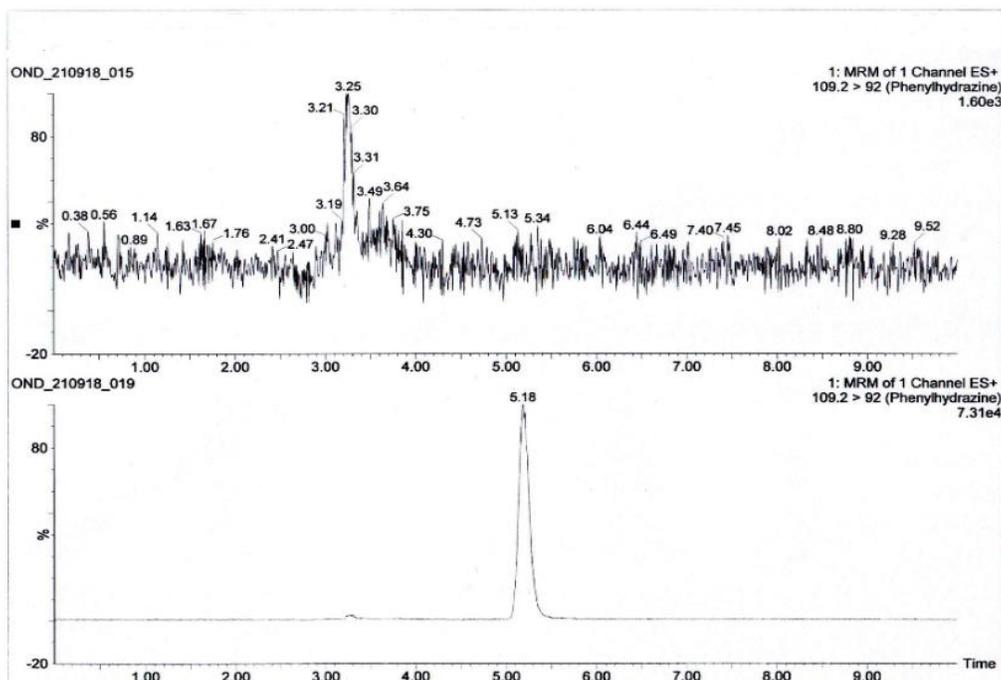| Test+ Spiked *(n=3)*	*Amount added (ppm)*	*Amount found (ppm)*	*%Recovery*
Test + LOQ spiked	15.09	14.13	93.6
Test + 100% spiked	62.87	70.59	112.3

Figure 7: Representative spiked chromatogram

CONCLUSION:

The proposed LC-MS/MS method is simple, sensitive and accurate to quantify potential genotoxic impurity Phenylhydrazine at ppm level present in Ondansetron Hydrochloride. The validated parameters are well within the limits and this method is found suitable for routine quality control test of Ondansetron Hydrochloride.

ACKNOWLEDGEMENT:

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