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Quantitative Method for the Determination of Potentially Mutagenic Impurities of Ondansetron Using UPC^2 Coupled with a Xevo TQ-S micro

Jennifer Simeone, Paula Hong, Patricia R. McConville

日本ウォーターズ株式会社

Abstract

This application note describes retention of small polar compounds using UPC^2 and the use of a high sensitivity MS for the detection and quantification of potential mutagenic impurities of ondansetron.

Benefits

Retention of small polar compounds using UPC^2 and the use of a high sensitivity MS for the detection and quantification of potential mutagenic impurities of ondansetron.

Introduction

There are many steps during the manufacturing process of an active pharmaceutical ingredient (API) where impurities can be introduced, whether as reagents, byproducts, intermediates, etc.[1] Some of these impurities may be mutagenic, or those that have the potential to interact with DNA and ultimately cause carcinogenicity. Methodologies associated with monitoring API purity levels are often HPLC-UV based,[2] which frequently do not provide the sensitivity levels needed to detect potentially mutagenic impurities (PMIs) at the levels required by regulatory agencies. Ondansetron is a pharmaceutical used in the prevention of nausea and vomiting, and contains one potential mutagenic impurity, 2-methyl imidazole, as well as a second impurity very closely related in structure, imidazole.

In addition, small polar compounds, such as imidazole and 2-methyl imidazole, are poorly retained under reversed-phase (RP) conditions. Alternate forms of chromatography, such as hydrophilic interaction chromatography (HILIC), or the use of ion-pairing reagents can be employed, but these often result in non-MS friendly mobile phases or require tedious method development. Supercritical fluid chromatography is known to be orthogonal to RPLC, and uses reagents which are suitable for MS detection. In this study, two polar impurities, imidazole and 2-methyl imidazole, were easily retained and separated from the API using SFC.

Experimental

Sample description

Ondansetron hydrochloride (HCl) and ondansetron impurities A, C, and D were purchased from the United

States Pharmacopeia. Impurity E (imidazole) and impurity F (2-methyl imidazole) were purchased from Sigma-Aldrich. Samples were initially dissolved in methanol to yield stock solutions with a concentration of 1 mg/mL, then further diluted to target concentrations using methanol. Samples were vortexed and sonicated to ensure complete dissolution. Calibrators and quality control (QC) samples were prepared in diluent containing 0.125 mg/mL API (ondansetron) in methanol at the following concentrations: calibrators at 15, 20, 25, 50, 75, 100, 125, 200, 300, and 500 ng/mL and QCs at 17.5, 95, and 350 ng/mL. This is equivalent to calibrators at 120, 160, 200, 400, 600, 800, 1000, 1600, 2400, and 4000 ppm and QCs at 140, 760, and 2800 ppm in reference to the API. Calibrators and QCs contained all impurities A, C–F at the described concentrations.

Method conditions

UPC^2 conditions

System:	ACQUITY UPC^2 with a single Column Manager
Detection:	Xevo TQ-S micro
Sample:	Ondansetron HCl and ondansetron Related Compounds A, C, and D (USP catalog numbers 1478582, 1478593, 1478618, and 1478629 respectively), imidazole and 2-methyl imidazole (Sigma Aldrich catalog numbers 02739 and 02736 respectively)
Column:	Torus 2-PIC, 1.7 μm, 3.0 x 100 mm
Column temp.:	30 °C
Mobile phase A:	CO$_2$
Mobile phase B:	0.2% (v/v) NH$_4$OH in methanol
Flow rate:	1 mL/min
ABPR:	2000 psi
Injection volume:	2 μL Weak needle wash/

UPC^2 conditions

seal wash: Isopropanol

Strong needle wash: Methanol

LC gradient:

Time	Flow	%A	%B	Curve
Initial	1.00	95	5	–
0.0	1.00	85	15	6
0.0	1.00	85	15	6
0.0	1.00	95	5	1

Xevo TQ-S micro with ISM for makeup flow conditions

Ionization mode: ESI+

Dwell time: 24 ms

Probe temp.:   650 °C

Capillary voltage: 0.4 kV

Cone voltage: 15 V

MRM conditions: Ondansetron – 294.1>170.1; CE = 6

                                                    Impurity A – 257.2>58; CE = 15

                                                    Impurity C – 200.1>144.1; CE = 25

                                                    Impurity D – 212.1>184.1; CE = 18

                                                    Impurity E – 69.1>42; CE = 15

                                                    Impurity F – 83.1>41; CE = 16

Makeup flow solvent:	0.2% (v/v) NH_4OH in methanol
Makeup flow rate:	0.5 mL/min
Data management:	MassLynx v4.1 SCN 909

Results and Discussion

The USP monograph for ondansetron hydrochloride, which is an HPLC-UV method, includes identification and quantification of 5 related impurities, two of which are process impurities[3] E and F, or imidazole and 2-methyl imidazole respectively (Figure 1).
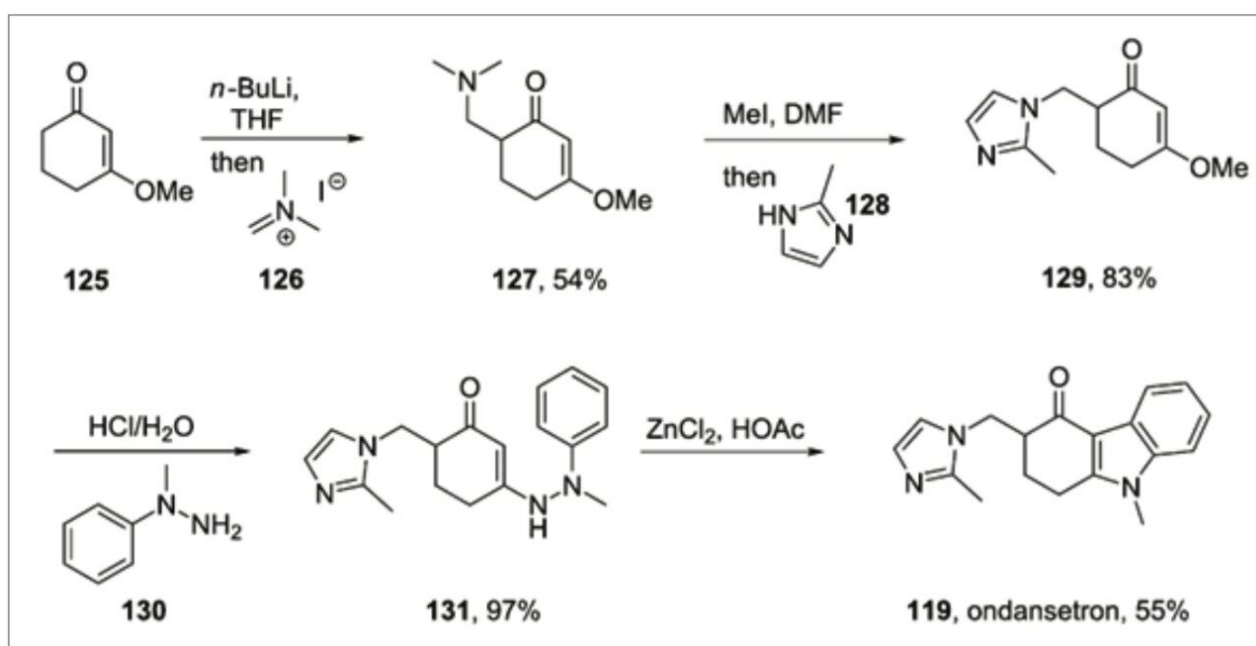

Figure 1. Synthetic pathway of ondansetron hydrochloride, where 2-methyl imidazole is introduced in the second step as a reagent. Imidazole is also a potential by-product of this synthetic pathway.

Studies done by the National Toxicology Program (NTP) on 2-methyl imidazole show exposure-related increases of micronucleated normochromatic erythrocytes in peripheral blood samples of male and female mice, which is an indicator of chromosomal damage. Additionally, the amount of damage increases with increasing duration of exposure.[4] In light of this information, 2-methyl imidazole can be considered to be a

potentially mutagenic impurity (PMI), and due to its close structure, for this study imidazole will also be considered as a PMI. Because of the dangers posed by PMIs, the maximum acceptable daily intake for PMIs is set to specific levels according to ICH M7 guidelines[5] (Table 1).

Duration of treatment	≤1 month	>1–12 months	>1–10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

Table 1. Daily allowable intake of potential mutagenic impurities based on ICH M7.

Depending on the treatment it is prescribed for, ondansetron may be taken for more than 30 (non-consecutive) days, thus the allowable genotoxic limit is 20 µg/day. The highest possible daily dosing for ondansetron is 48 mg/day,[6] which means that PMIs are allowable at 416 ppm, which is five times lower than the USP monograph limit of 0.2% (2000 ppm). For this reason the use of a higher sensitivity detector, specifically a tandem quadrupole MS, was employed for detection to provide detection levels much lower than allowable by a traditional UV method. Additionally, supercritical fluid chromatography is well suited for the retention of small polar compounds, such as imidazole and 2-methyl imidazole.[7] This example demonstrates the ability to use SFC-MS/MS for the detection of low levels of potential mutagenic impurities of ondansetron.

The threshold concentration for the two PMIs was determined to be 417 ppm in reference to the API, ondansetron, based on a maximum dosage of 48 mg/day and a PMI limit of 20 µg/day. This means that in 1 mg of API, there can be up to 417 ng of PMI (ng/mg = ppm). To mimic a true test sample, all calibrators and QCs were prepared in diluent containing 125 µg/mL of ondansetron. It was important to separate all impurity peaks from the main API peak to avoid any potential matrix effects, i.e. signal suppression or enhancement, due to the presence of API at such a high concentration, which can compete for ionization in the source. For this reason, it is beneficial to have the main peaks of interest elute prior to the main ondansetron peak. Additionally, because of the structural similarity of the API and impurities, there is a potential that in-source fragmentation can lead to erroneous identification or quantification of compounds. In source fragmentation occurs when a precursor compound is fragmented in the source and is then seen in Q1 at a different mass than expected. In this case, ondansetron (294 *m/z*) fragments in the source to form impurity D (212 *m/z*). Although the mass spectrometer cannot distinguish between the in-source fragmented ondansetron and native impurity D, the two compounds are separated chromatographically. The ondansetron peak in channel 212>184 is easily identified as ondansetron by retention time (Figure 2). Since ondansetron is not being quantified in this example, the in-source fragmentation is not problematic.
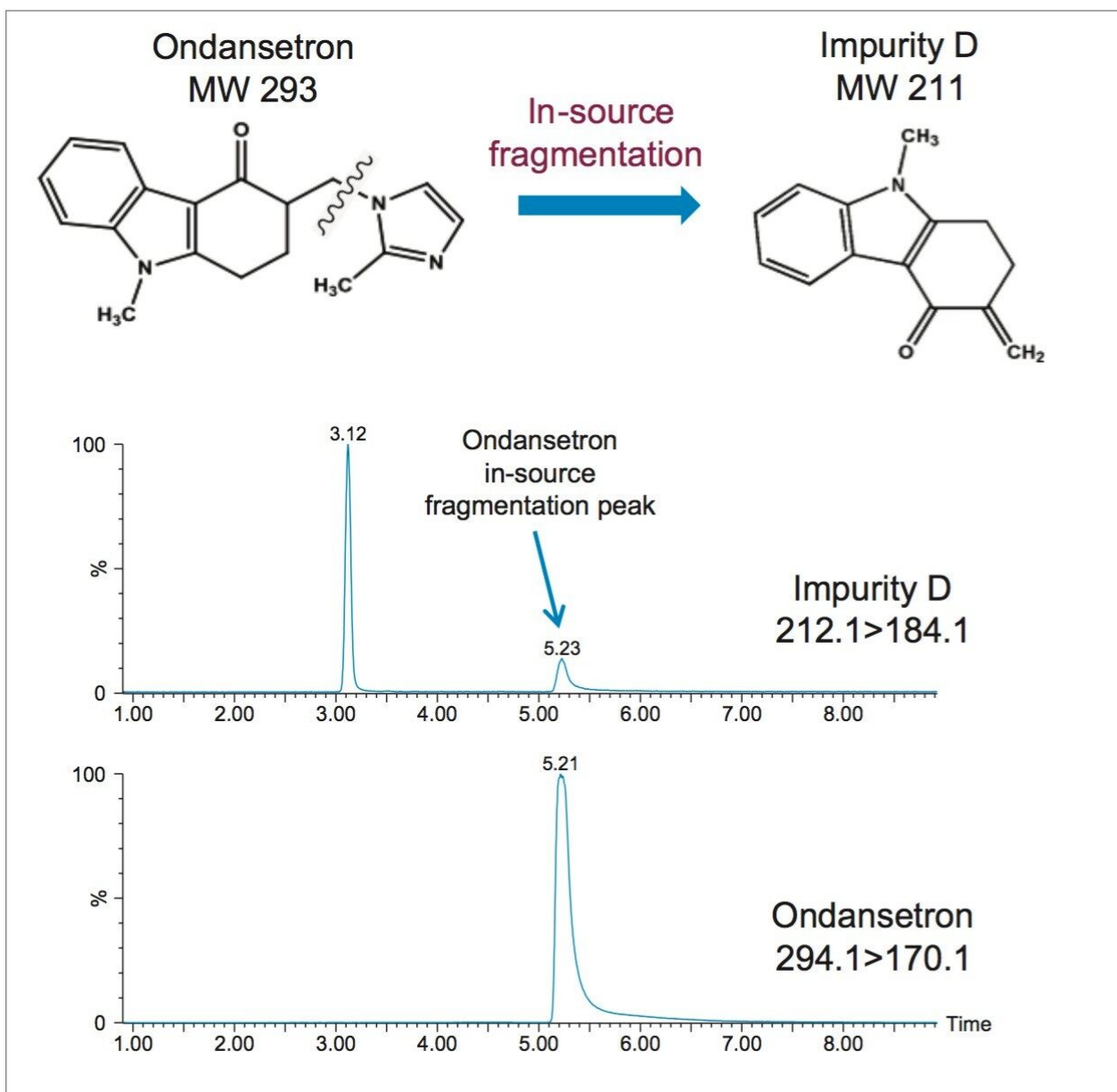
Figure 2. Ondansetron in-source fragmentation, which fragments to the same precursor mass as impurity D and thus shows up in the MRM channel. It can easily be identified by its retention time as ondansetron.

It was also necessary to screen the ondansetron HCl reference standard for the presence of any trace amounts of impurities since the USP reference standard was certified with a purity of 99.8%. Injection of API only shows the presence of Impurity D, however it was determined to be below the lower limit of quantitation of 120 ppm and thus would not impact quantification.

General method requirements included the ability to accurately and precisely detect 2-methyl imidazole and imidazole at concentrations of 417 ppm or greater. The calibration curve spanned the range of 120 –

4000 ppm, and the lower limit of quantitation showed a signal to noise of 119 for 2-methly imidazole and 8.5 for imidazole (Figure 3).
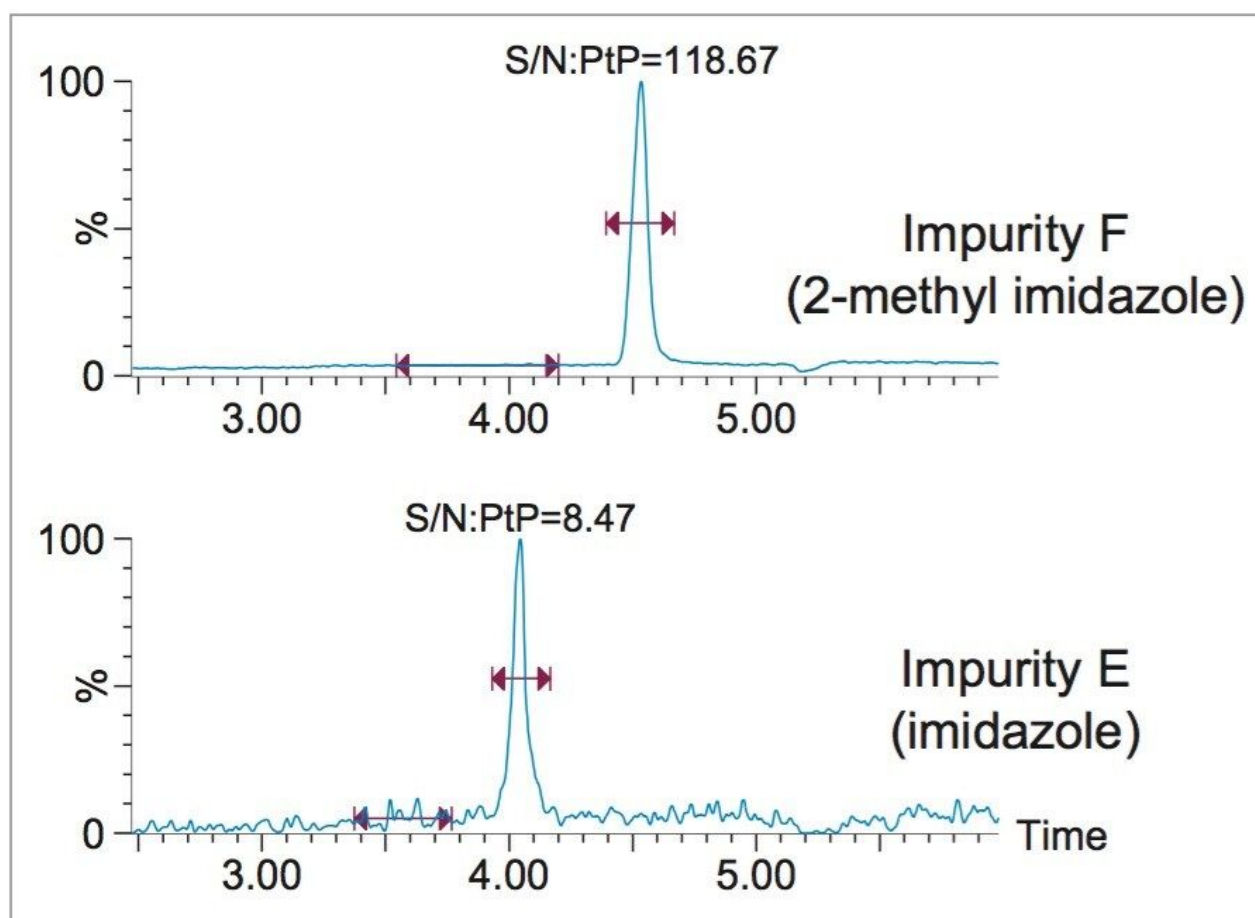

Figure 3. Example chromatograms for the lower limit of quantitation (120 ppm) for impurity F (2-methyl imidazole) and impurity E (imidazole).

To assess method accuracy and precision, all calibrators were injected in replicates of three, and QCs in replicates of 6. Below are the R^2 values for the calibration curves and the mean and RSD values for three QC concentration levels.

Calibrator results		
	Fit	R^2
Impurity A	Linear; Log, Log	0.999
Impurity C	Linear; 1/x	0.996
Impurity D	Linear; No weighting	0.998
Impurity E (imidazole)	Linear; Log, Log	0.996
Impurity F (2-methyl imidazole)	Linear; Log, Log	0.998

Quality control results						
	Mean			RSD		
	140 ppm	760 ppm	2800 ppm	140 ppm	760 ppm	2800 ppm
Impurity A	131	749	2718	1.8	1.3	1.8
Impurity C	164	819	2761	4.2	6.9	3.9
Impurity D	139	760	2766	10.1	8.8	3.9
Impurity E (imidazole)	125	669	2879	2.8	1.5	0.9
Impurity F (2-methyl imidazole)	135	811	2663	1.6	0.7	0.5

Table 2. Experimental results for ondansetron impurities A, C–F in the presence of 125 µg/mL API (ondansetron).

The ICH guidelines for analytical method validation8 state that a linear fit is required, however mathematical transformation prior to regression may be necessary in order to obtain a linear relationship. In this case, a linear log-log fit was required for impurities A, E, and F.

The method developed for the quantification of PMIs of ondansetron met the general requirements of an accurate and reproducible method. Any possibility for matrix effects or negative effects due to in-source fragmentation was eliminated by adequate separation of the peaks of interest from the main API peak. Additionally, the lower limit of quantitation of 120 ppm was well below the concentration required (416 ppm), and all calibrator and QC data showed good accuracy and precision values. For the two impurities of interest, E and F, the RSDs for QCs at three concentration levels ranged from 0.7 to 3.2 percent. These exceptionally low values highlight the precision and robustness of the developed method.

Conclusion

Many methods developed to monitor API impurity levels are reversed-phase HPLC based methods which may not provide the level of detection required to monitor potential mutagenic impurities. Additionally, reversed-phase LC can be problematic for small polar compounds. By taking advantage of the orthogonality of supercritical fluid chromatography, and coupling it with a high sensitivity tandem quadrupole mass spectrometer, it was possible to develop a reliable and robust method for the analysis of

potential PMIs of ondansetron.

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