Waters™

Applikationsbericht

Exploiting the Orthogonality of the ACQUITY UPC2 System to Develop an Impurities Method for Ondansetron

Jennifer Simeone, Paula Hong, Patricia R. McConville

Waters Corporation

Abstract

This application note describes retention of small polar compounds using UPC^2 and simplified method development using ACQUITY QDa.

Supercritical fluid chromatography (SFC) is known to be orthogonal to RPLC, and uses reagents which are suitable for MS detection. In this study, eight impurities of ondansetron, including two polar impurities imidazole and 2-methyl imidazole, were easily retained and separated from the API using SFC coupled with UV and MS detection.

Benefits

- Retention of small polar compounds using UPC^2 and simplified method development using ACQUITY QDa.

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] Most methods developed to monitor API impurity levels are HPLC-UV based,[2] more specifically, reversed-phase LC methods. However, 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 techniques often require non-MS friendly mobile phases or involve tedious method development. Supercritical fluid chromatography (SFC) is known to be orthogonal to RPLC, and uses reagents which are suitable for MS detection. In this study, eight impurities of ondansetron, including two polar impurities imidazole and 2-methyl imidazole, were easily retained and separated from the API using SFC coupled with UV and MS detection.

Experimental

UPC2 conditions

System:	ACQUITY UPC^2 with a single Column Manager (CM-A)
Detection:	ACQUITY UPC^2 PDA Detector and ACQUITY QDa Mass Detector
Sample:	Ondansetron and Ondansetron Related Compounds A, C–G (Sigma Aldrich catalog numbers 1478582, 43924, 42243, 54318, 02739, 02736, 92318 respectively) and Ondansetron Related Compounds B and H (TLC Pharmaceutical Standards catalog numbers 0–038 and 0–039 respectively).
Column:	Waters ACQUITY UPC2 Torus DEA, 3.0 x 100 mm, 1.7 µm
Column temp.:	30 °C
Mobile phase A:	CO_2
Mobile phase B:	0.2% (v/v) NH_4OH in Methanol
Flow rate:	1.00 mL/min

Gradient

Time	%A	%B	Curve
Initial	1.00	95	–
3.5	1.00	85	6

Time	%A	%B	Curve
4.5	1.00	75	6
6.5	1.00	75	1
8.0	1.00	95	1

MS conditions

ABPR:	4000 psi
Injection volume:	2.0 µL
Wavelength:	212 nm – compensated 310–410 nm
Collection rate:	10 Hz
Needle wash:	Methanol
Seal wash:	Isopropanol

ACQUITY QDa with Isocratic Solvent Manager (ISM) using a dual splitter conditions

Ionization mode:	ESI+
Sampling frequency:	10 Hz
Probe temperature:	600 °C
Capillary voltage:	0.8 kV
Cone voltage:	5 V
Full scan:	50–650 *m/z*

Makeup flow solvent:	Isopropanol
Makeup flow rate:	0.300 mL/min
Data management:	MassLynx v4.1 SCN 925

Sample description

Ondansetron and Ondansetron Impurities A, C–G were purchased from Sigma Aldrich and Impurities B and H were purchased from TLC Pharmaceutical Standards. Samples were initially dissolved in methanol to yield solutions with a concentration of 1 mg/mL. Once dissolved, the stock solutions were diluted with isopropanol. Samples were vortexed and sonicated to ensure complete dissolution. The final concentration of the working standard was 10 µg/mL for all reference compounds.

Results and Discussion

Developing methods for impurity analysis is often a tedious and time consuming task for a variety of reasons. The most important requirement of a method is that all compounds being analyzed are separated from other compounds, as well as from background interferences. Because many impurities will share structural and chemical properties of the API, as well as other impurities, it can be quite difficult to find method conditions which provide adequate separation in a reasonable amount of time. The use of supercritical fluid chromatography is well known to have many advantages[3] and it is ideally suited for separation of structurally similar compounds, both chiral and achiral.[4] Additionally, due to the orthogonality or alternate selectivity of SFC when compared to RPLC, small polar compounds which are not well retained by RPLC are easily retained by SFC. To demonstrate the orthogonality of SFC to RPLC, a separation for ondansetron and impurities A–H under typical RPLC conditions (0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phase and a C_{18} column) is shown in Figure 1.

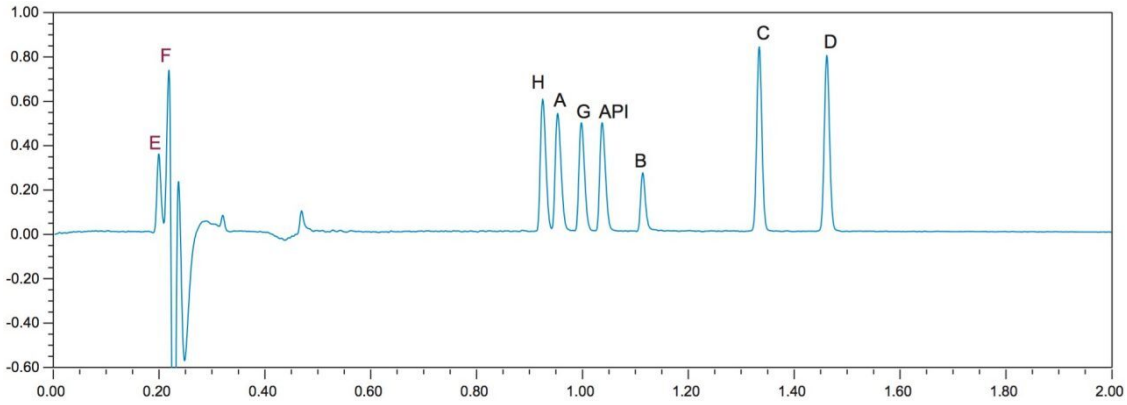

Figure 1. Example separation of ondansetron and impurities A–H using traditional RPLC conditions of 0.1% Formic Acid in Water/ACN and a C_{18} column. Impurities E and F are not retained under these conditions.

Note that impurities E and F (imidazole and 2-methyl imidazole respectively) are not retained under these conditions and elute in the void volume. This is undesirable for a number of reasons, including the inability to accurately integrate and quantify the co-eluting peaks. It may be possible to develop a method in which impurities E and F are better retained, however it would likely require screening various mobile phase additives, column chemistries, column temperatures, etc- all of which would consume time and resources. Alternatively, a different form of chromatography which is better suited for analysis of small polar compounds could be employed. Specifically, supercritical fluid chromatography can be used to provide alternate selectivity when compared to RPLC. A separation for ondansetron and impurities A–H was developed using compressed CO_2 and methanol modified with 0.2% ammonium hydroxide as the co-solvent. The final method conditions provided baseline separation for all 9 compounds (Figure 2) as well as retention of impurities E and F.

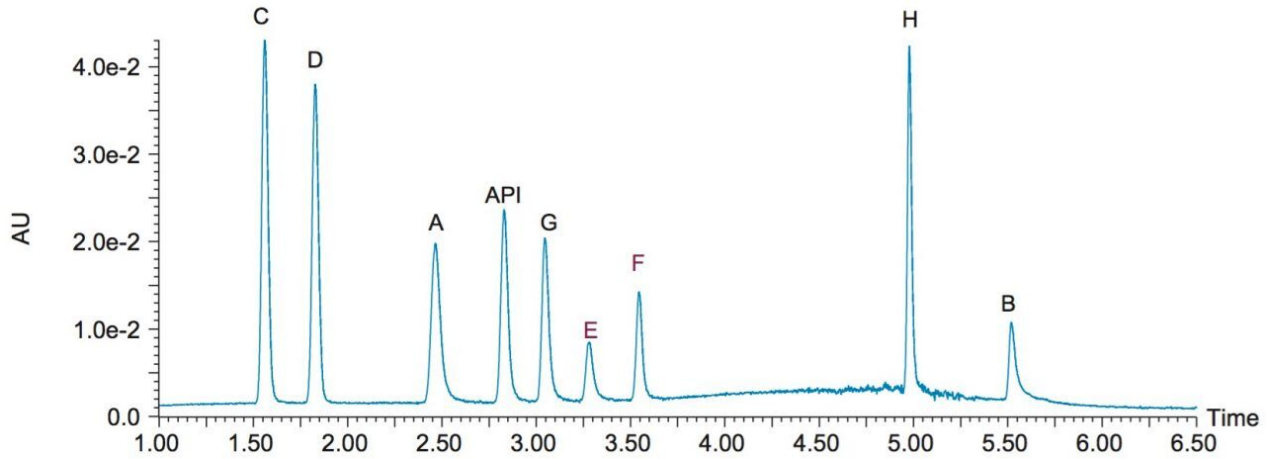

Figure 2. Separation of ondansetron (API) and impurities A–H using ACQUITY UPC² with UV detection.

The most notable difference when comparing the RPLC and SFC chromatograms from Figures 1 and 2 is the retention of impurities E and F in the UPC² separation, with k' values of over 5. There is a clear selectivity difference between the two separations. For example, in RPLC impurities C and D are the two latest eluting compounds (Figure 1), whereas in the UPC² separation they are the first two compounds eluted from the column. These changes in retention and selectivity are a consequence of the previously stated orthogonality of the two chromatographic methods. Additionally, because no ion-pairing reagents were required, it was possible to acquire MS data in addition to UV. The addition of mass detection made peak identification and peak tracking easier during method development. The ACQUITY QDa was used to collect full scan data over the range of 50–650 *m/z*. The total ion chromatogram (TIC) can be viewed in Masslynx with automatic peak annotation based on the base peak mass (Figure 3). The automated mass annotation made it simple to identify all peaks without having to run separate standard solutions of each compound during method development.

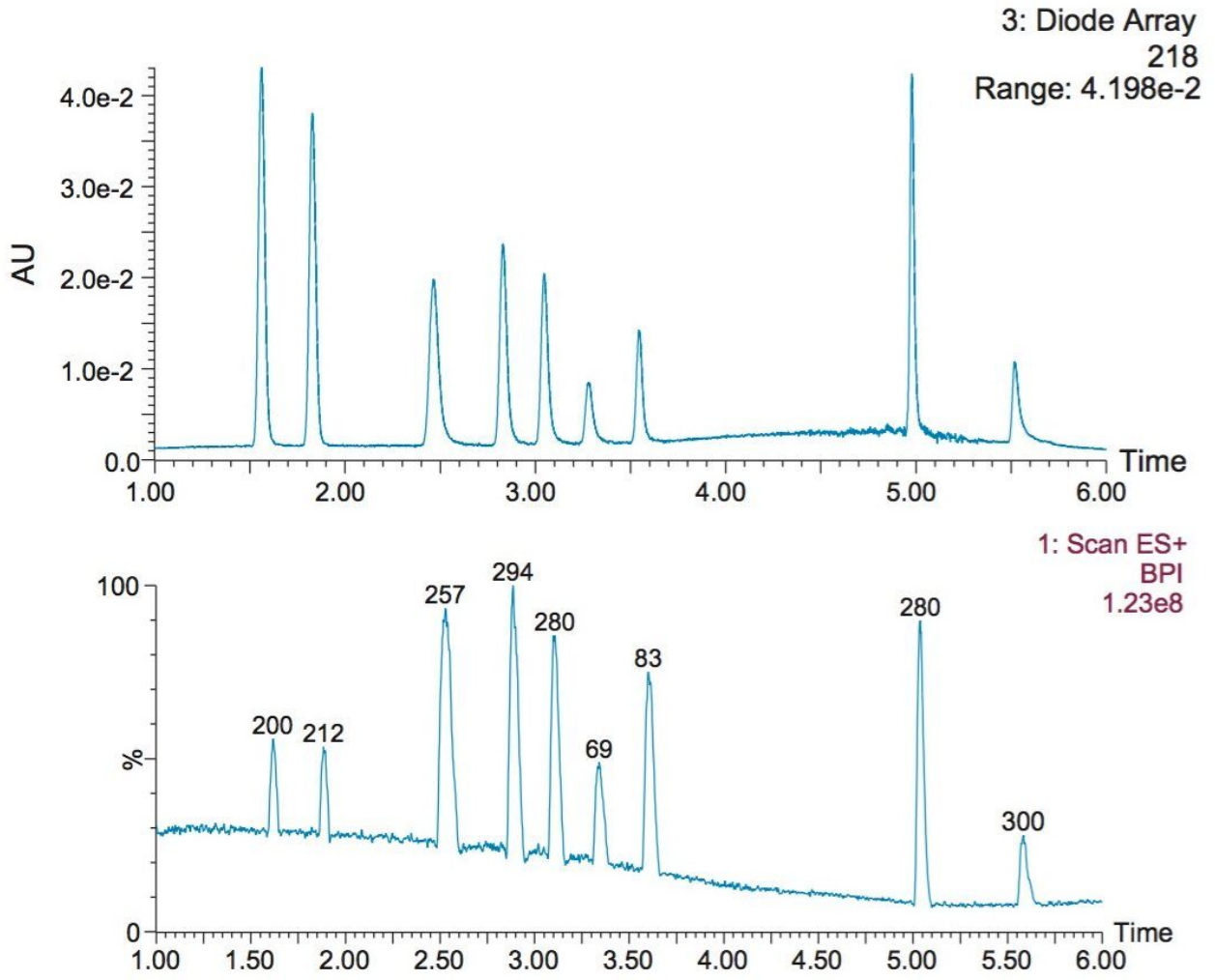

Figure 3. Acquired UV channel 218 nm (top) and ACQUITY QDa acquired full scan data (bottom) labeled with peak masses to aid in identification. m/z of 200, 212, 257, 294, 280, 69, 83, 280, and 300 refer to impurities C, D, A, Ondansetron, G, E, F, H, and B respectively.

Conclusion

Many methods developed to monitor API impurity levels are reversed-phase methods which can be problematic for small polar compounds. By taking advantage of the orthogonality of supercritical fluid chromatography, it was possible to develop an impurity method for ondansetron which includes retention of imidazole and 2-methyl imidazole. Additionally, the use of MS compatible mobile phases meant that mass data could be collected in addition to UV and used for easy peak tracking and identification during method

development.

References

1. Reddy, A.V.B., Jaafar, J., Umar, K., Majid, Z.A., Aris, A. B., Talib, J., Madhavi, G., *J. Sep. Sci.* 2015, 38, 764–779.
2. Official Monographs, USP 37 NF32 S2. United States Pharmacopeia and National Formulary (USP 37-NF 32 S2) Baltimore, MD: *United Book Press, Inc.*; 2014. p. 4968.
3. Grand-Guillarme Perrenoud, A., Veuthey, J-L., Guillarme, D., *J. Chromatogr. A,* 2014, 1339, 174–184.
4. Simeone, J. et al, Enantiomeric Separation of Warfarin and Propranolol and their Associated Hydroxylated Metabolites using UPC^2-MS/MS. Technology Brief 720005187EN. Oct 2014.

Featured Products

MassLynx MS Software <https://www.waters.com/waters/en_US/MassLynx-MS-Software/nav.htm?locale=101&cid=513662>

ACQUITY UPC2 System <https://www.waters.com/waters/en_US/ACQUITY-UPC2-System/nav.htm?locale=101&cid=134658367>

ACQUITY QDa Mass Detector <https://www.waters.com/waters/en_US/ACQUITY-QDa-Mass-Detector-for-Chromatographic-Analysis/nav.htm?locale=101&cid=134761404>

720005732, June 2016