[BROCHURE]

SUPPORTING THE CHALLENGE OF NITROSAMINE DRUG SUBSTANCE RELATED IMPURITIES ANALYSIS

Waters™

N-Nitrosamines are potentially mutagenic impurities that may pose a risk of cancer to humans when exposed over a prolonged period. Since 2018, global regulatory bodies have required pharmaceutical manufacturers to control and strive to mitigate the presence of nitrosamines in their products in order to protect patient safety and avoid regulatory recalls in the future.

The current major focus in N-Nitrosamines testing, and cause of many of the most recent drug recalls, is the formation of 'Nitrosamine Drug Substance Related Impurities' (NDSRIs). NDSRIs are formed when nitrosating agents interact directly with the active pharmaceutical ingredient (API) during manufacturing or in storage. Control of NDSRIs, through analytical testing, has presented unprecedented challenges due to their relatively high potential for formation and often very low level regulatory acceptable intake thresholds. There is a need for efficient development of bespoke assays that robustly support the quantification of NDSRIs in drug products to the threshold levels guided by Regulatory bodies.

Here we present a compendium of several examples of 'fit for purpose' assays developed for the quantification of a variety NDSRIs in relevant drug products using Waters™ quality Chemistry Products and LC-MS/MS Technologies.

Waters is committed to the ongoing support of all your Nitrosamine analysis needs and aiding robust quality method development, combining advanced separation products and analytical technologies with industry proven scientific expertise.

A Robust and Sensitive Instrument for Quantification of N-Nitroso-N-Des Methyl Diltiazem Impurity in Diltiazem Drug Product

INTRODUCTION

Diltiazem is a benzothiazepine derivative with antihypertensive and vasodilating properties. It is widely used for human consumption as a non-dihydropyridine calcium channel blocker. However, as per regulatory requirement, medicines which have possibility of forming Nitrosamine Drug Substance Related Impurities (NDSRIs) during manufacturing and/or storage, needs to be controlled. Due to the risk associated with such impurities, regulatory requirements are stringent which requires not only a highly specific analytical method but also a reproducible and sensitive detection technique.

SCOPE OF WORK

In the present study, the performance of method based upon LC-MS/MS, using an ACQUITY™ H-Class Plus UPLC™ System coupled with Xevo™ TQ-S Cronos tandem quadrupole mass spectrometer, is demonstrated for the determination of N-Nitroso-N-Des methyl Diltiazem impurity in Diltiazem drug product. The separation of impurity was achieved using Atlantis™ Premier BEH™ C_{18} AX, 100 mm X 2.1 mm, 2.5 µm Column. The developed method produced excellent sensitivity with S/N ratio >80 at 0.006 ppm level with respect to API and LOQ of 0.03 ppm. The observed spiked recovery was within 70 to 120% by adapting extraction approach.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR mode rapidly switches between MRM (MS/MS) mode and MS full scan acquisition which enables the analyst to monitor for matrix interferences, placebo, impurities, and degradants in a sample while accurately quantifying target compounds without losing sensitivity or performance. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI and eluting later. Diverting the API peak to waste, avoided the contamination of the mass spectrometer increasing the method robustness.

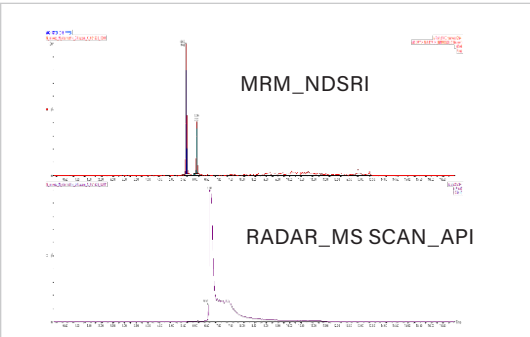


Figure 1. Chromatographic separation of N-Nitroso-N-Des methyl Diltiazem impurity and API by using RADAR scan.

Figure 2. Xevo TQ-S micro with ACQUITY UPLC H-Class Plus, Atlantis Premier BEH C_{18} AX 100 mm X 2.1 mm, 2.5 µm Column.

Limit Test	Limit/Range
Linearity	0.006 to 5.0 ppm
Method LOQ	0.03 ppm
Method LOD	0.006 ppm
Spiked recovery	70 to 120.0%

Table 1. Summary of N-Nitroso-N-Des methyl Diltiazem impurity method performance.

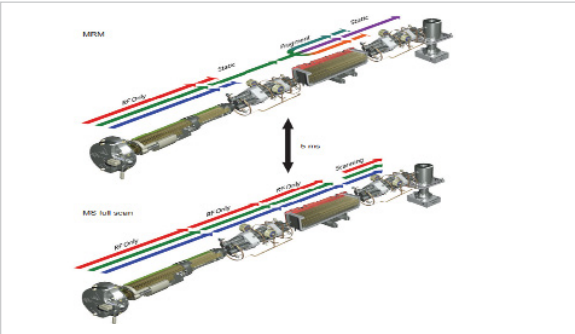


Figure 3. RADAR functionality.

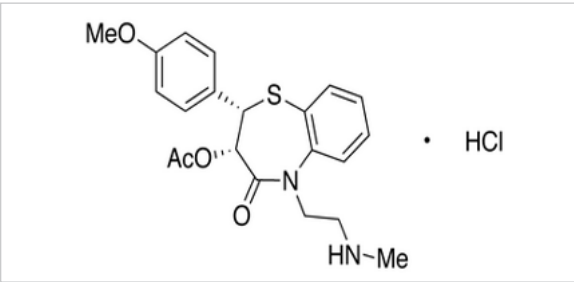


Figure 4. N-Nitroso-N-Des methyl Diltiazem.

A Robust and Sensitive Instrument for Quantification Of N-Nitroso Labetalol Impurity in Labetalol Drug Product

INTRODUCTION

A recently published general chapter in USP-NF, General Chapter <1469> Nitrosamine Impurities, identifies possible sources in drug products, their components and their manufacturing process, along with the risks associated with each source. These nitrosamine drug substance-related impurities (NDSRIs) are a class of nitrosamines sharing structural similarity to the API. In some cases, the root cause of NDSRI formation has been attributed to nitrite impurities present in excipients at parts-per-million amounts. With so many potential sources, it is critical that manufacturers evaluate their products for risks. Ensuring the control of a product's impurities levels cannot simply be a last step in the manufacturing process; manufacturers must pursue quality in the lifecycle of a drug from beginning to end.

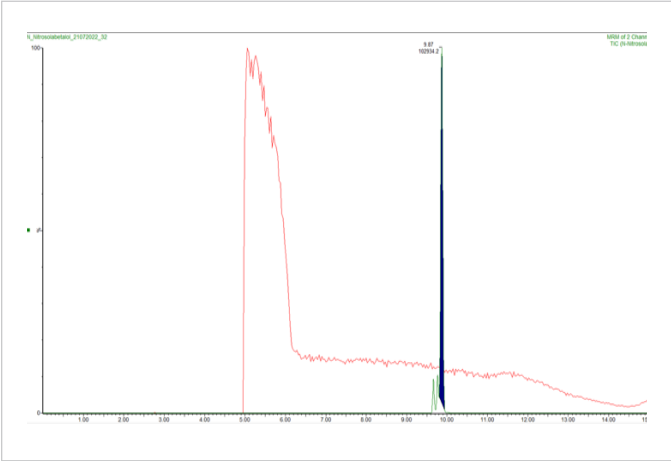


Figure 1. Chromatographic Separation of N-Nitroso labetalol Impurity and formulation by using RADAR scan.

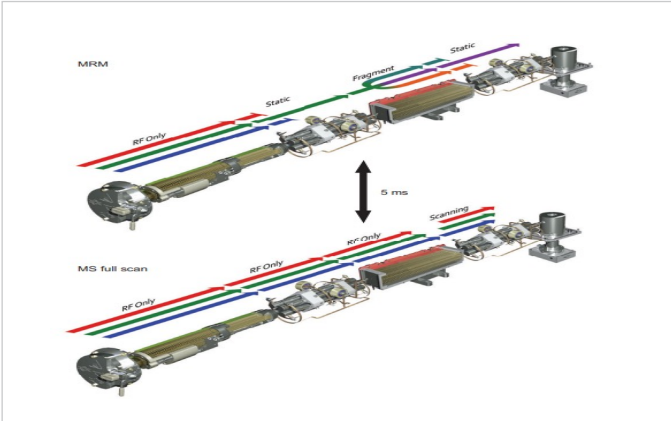


Figure 2. RADAR functionality.

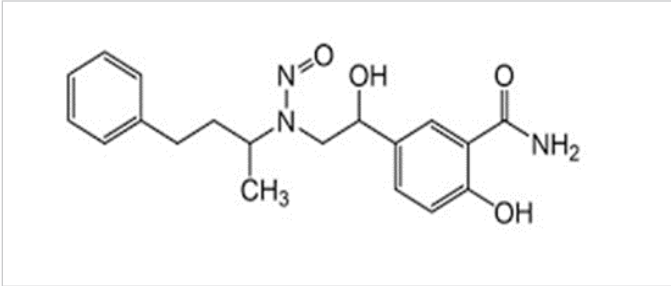


Figure 3. N-Nitroso labetalol.

SCOPE OF WORK

To overcome the analytical challenges of matrix effect and to improve the spiked recovery for N-Nitroso labetalol impurity quantification in drug product needs a suitable sample preparation technique and chromatographic conditions. Waters Xevo TQ-S Cronos coupled with ACQUITY UPLC H-Class plus and ACQUITY UPLC BEH C_{18} Column combination produced robust method for quantification of N-Nitroso labetalol impurity at method LOQ 0.03 ppm and the instrument shows excellent sensitivity with S/N ratio (>300) at 0.003 ppm level with respect to API. The observed spiked recovery was between 70 to 120 % by adapting extraction approach.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously without loss of sensitivity, a unique capability that can both simplify and accelerate development of robust methods. During method development, RADAR offers the ability to understand unexpected results due to matrix effects. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI and eluting before NDSRI. Diverting the API peak avoided the contamination of the mass spectrometer increasing the method robustness.

Figure 4. Xevo TQ-S Cronos with ACQUITY UPLC H-Class Plus , and ACQUITY UPLC BEH C_{18} Column.

Limit Test	Limit/Range
Linearity	0.003 to 1.5 ppm
Method LOQ	0.03 ppm
Method LOD	0.003 ppm
Spiked recovery	70 to 120.0%

Table 1. Summary for N-Nitroso labetalol impurity.

A Robust and Sensitive Instrument for Quantification of N-Nitroso Morpholine Impurity in Mycophenolate Mofetil Hcl Drug Product

INTRODUCTION

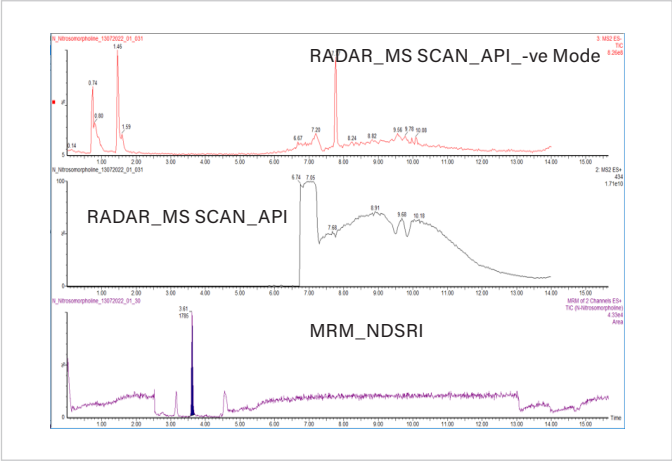
Mycophenolate is in a class of medications called immunosuppressive agents. It works by weakening the body's immune system so it will not attack and reject the transplanted organ. Mycophenolate is used with other medications to help prevent transplant organ rejection who have received kidney, heart, or liver transplants. Due to the risk of carcinogenic property N-Nitrosamine impurities are considered as concern for human consumption above acceptable limit. N-nitroso morpholine impurity is a related impurity of Mycophenolate Mofetil HCl. N-nitroso morpholine can be formed during manufacturing process, shelf-life storage period. The NDSRI is a concern due to its probable carcinogenic nature.

SCOPE OF WORK

Due to the similar properties of N-nitroso morpholine impurity and Mycophenolate there is a challenge of loss of recovery due close elution of the impurity and the drug substance. The combination of optimized chromatographic conditions, Waters Xevo TQ-S Cronos coupled with ACQUITY UPLC H-Class plus and ACQUITY UPLC BEH C_{18} Column produced robust method for quantification of N-nitroso morpholine impurity at method LOQ 0.03 ppm and the instrument shows excellent sensitivity with S/N ratio (>100) at 0.015 ppm level with respect to API. The observed spiked recovery was 70 to 120% by adapting extraction approach.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously without loss of sensitivity, a unique capability that can both simplify and accelerate development of robust methods. During method development, RADAR offers the ability to understand unexpected results due to matrix effects. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI and eluting later. Diverting the API peak avoided the contamination of the mass spectrometer increasing the method robustness.



Fugure 1. Chromatographic separation of N-nitroso morpholine Impurity and formulation sample by RADAR scan.

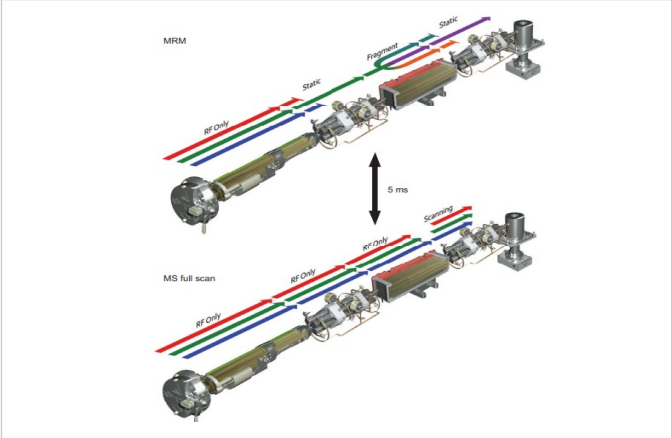


Figure 2. RADAR functionality.

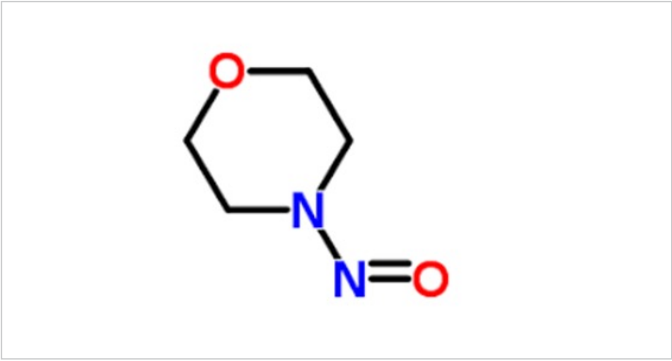


Figure 3. N-nitroso morpholine.

Figure 4. Xevo TQ-S Cronos with ACQUITY UPLC H-Class Plus.

Limit Test	Limit/Range
Linearity	0.015 to 0.75 ppm
Method LOQ	0.03 ppm
Method LOD	0.015 ppm
Spiked recovery	85%

Table 1. Summary for N-nitroso morpholine impurity.

A Robust and Sensitive Instrument for Quantification of N-Nitroso Propranolol Impurity in Propranolol Hydrochloride Drug Product

INTRODUCTION

Propranolol Hydrochloride is a synthetic beta-adrenergic receptor blocker with antianginal, antiarrhythmic, and antihypertensive properties. It is widely used as non-cardioselective beta-adrenergic antagonist. However, the selected drug products need to be screened for Nitrosamine Drug Substance Related Impurities (NDSRI). These impurities are structurally similar to APIs and can be generated during the manufacturing or storage period of the drug product. Consequently, there is a need of highly sensitive LC/MS/MS method for the quantification of N-Nitroso Propranolol in Propranolol API and tablets.

SCOPE OF WORK

The presented work overcomes the complications involved in NDSRI analysis and provides a complete analytical solution using Waters Xevo TQ-S Cronos coupled with ACQUITY UPLC H-Class Plus along with Symmetry™ C_8 Column. The developed method for quantification of N-Nitroso propranolol impurity produces reproducible results with LOQ of 0.01 ppm with respect to API. The instrument shows excellent sensitivity with S/N ratio >70 at 0.002 ppm level (LOD). The observed spiked recovery was within 70 to 120% by adapting extraction approach.

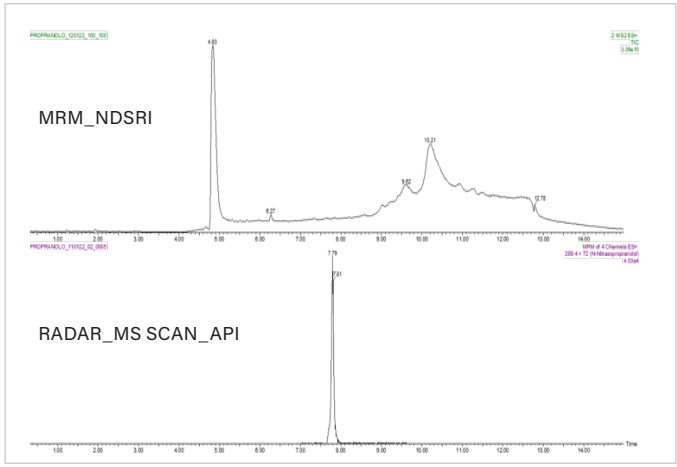


Figure 1. Chromatographic separation of N-Nitroso propranolol impurity and formulation by using RADAR scan.

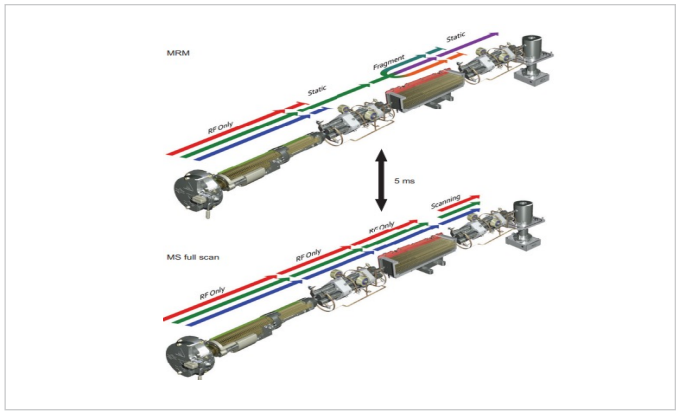


Figure 2. RADAR functionality.

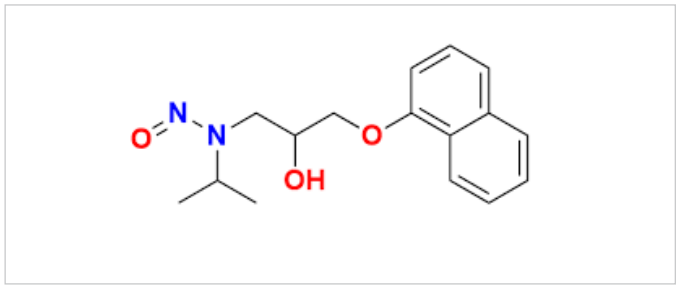


Figure 3. N-Nitroso propranolol.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously, a unique capability that can both simplify and accelerate development of robust methods. During method development, RADAR offers the ability to understand unexpected results due to matrix effects. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI and eluting later. Diverting the API peak avoided the contamination of the mass spectrometer increasing the method robustness.

Figure 4. Xevo TQ-S Cronos with ACQUITY UPLC H-Class Plus, and Symmetry C_8 Column.

Limit Test	Limit/Range
Linearity	0.002 to 2.0 ppm
Method LOQ	0.01 ppm
Method LOD	0.003 ppm
Spiked recovery	70 to 120.0%

Table 1. Summary for N-Nitroso propranolol impurity method performance.

A Robust and Sensitive Instrument for Quantification of N-Nitroso Nortriptyline Impurity in Amitriptyline Drug Product

INTRODUCTION

Recently, FDA has received additional reports of certain types of nitrosamine impurities that formed in several drug products. These nitrosamine drug substance-related impurities (NDSRIs) are a class of nitrosamines sharing structural similarity to the API.

Amitriptyline is from a group of medicines called tricyclic antidepressants. They are thought to work by increasing a chemical called serotonin in your brain. Recently there have been references that N- Nitroso nortriptyline impurity can be formed in Nortriptyline tablets due to presence of a tertiary Amino group which can interact with a nitrite group to form the NDSRI of Amitriptyline.

SCOPE OF WORK

To overcome the analytical challenges of matrix effect and to improve the spiked recovery for N-Nitroso Nortriptyline impurity quantification in drug product needs a suitable sample preparation technique and chromatographic conditions. Waters Xevo TQ-S Cronos coupled with ACQUITY UPLC H-Class plus and ACQUITY UPLC BEH C_{18} Column combination produced robust method for quantification N-Nitroso Nortriptyline impurity at method LOQ 0.03 ppm and the instrument showed excellent sensitivity with S/N ratio (>200) at 0.015 PPM level with respect to API. The observed spiked recovery was between 70 to 120% by adapting selective extraction approach. The method demonstrates linear results in the concentration range of 0.002 to 2.0 ppm.

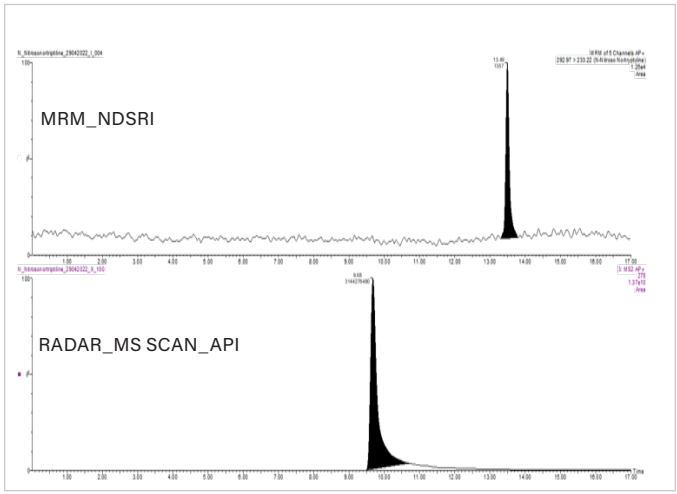


Figure 1. Chromatographic Separation of N-Nitroso Nortriptyline impurity and Amitriptyline DP by using RADAR scan.

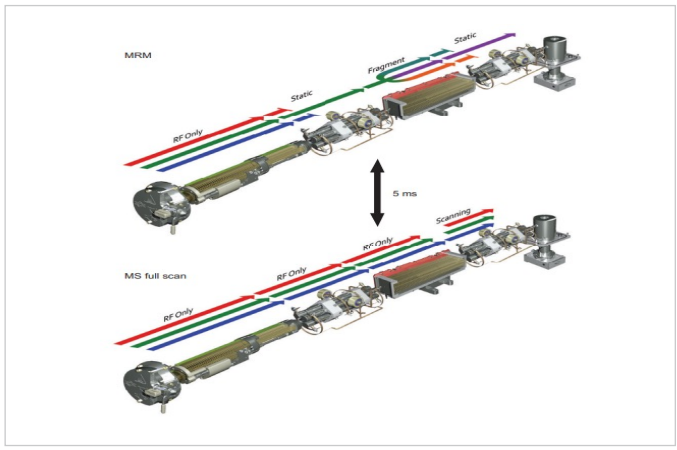


Figure 2. RADAR functionality.

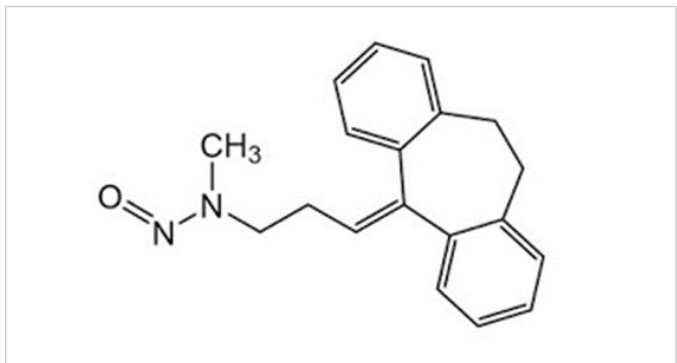


Figure 3. N-Nitroso Nortriptyline.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously without compromising sensitivity, a unique capability that can both simplify and accelerate development of robust methods. During method development, RADAR offers the ability to understand unexpected results due to matrix effects. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI which is eluting later. Diverting the API peak avoided the contamination of the mass spectrometer increasing the method robustness.

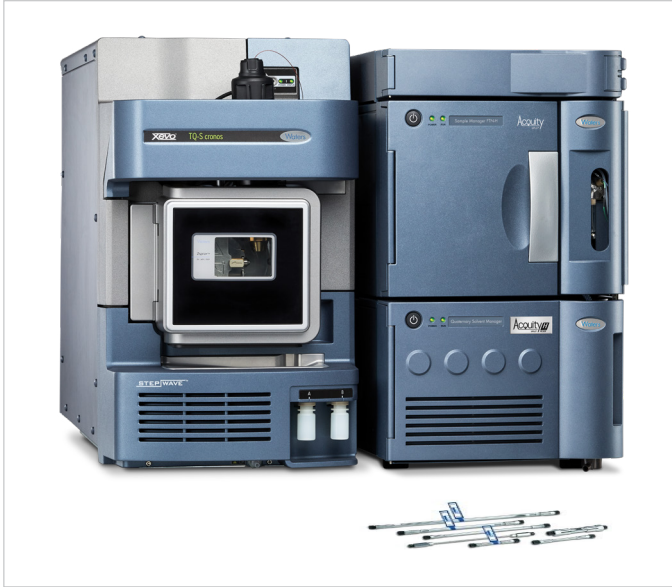


Figure 4. Xevo TQ-S Cronos with ACQUITY UPLC H-Class Plus , and Symmetry C_8 Column.

Limit Test	Limit/Range
Linearity	0.002 to 2.0 ppm
Method LOQ	0.03 ppm
Method LOD	0.01 ppm
Spiked recovery	70 to 120.0%

Table 1. mary for N-Nitroso Nortriptyline impurity.

A Robust and Sensitive Instrument for Quantification of N-Nitroso Argatroban Impurity at 0.3 PPM in Argatroban Drug Product

INTRODUCTION

Recently, FDA has received additional reports of certain types of nitrosamine impurities that formed in several drug products. These nitrosamine drug substance-related impurities (NDSRIs) are a class of nitrosamines sharing structural similarity to the API. NDSRIs can be generated during manufacturing or the shelf-life storage period of the drug product. In many cases, the root cause of NDSRI formation has been attributed to nitrite impurities present in excipients at trace amounts. Nitrite impurities have been observed in a range of commonly used excipients which may lead to the formation of NDSRIs in certain drug products.

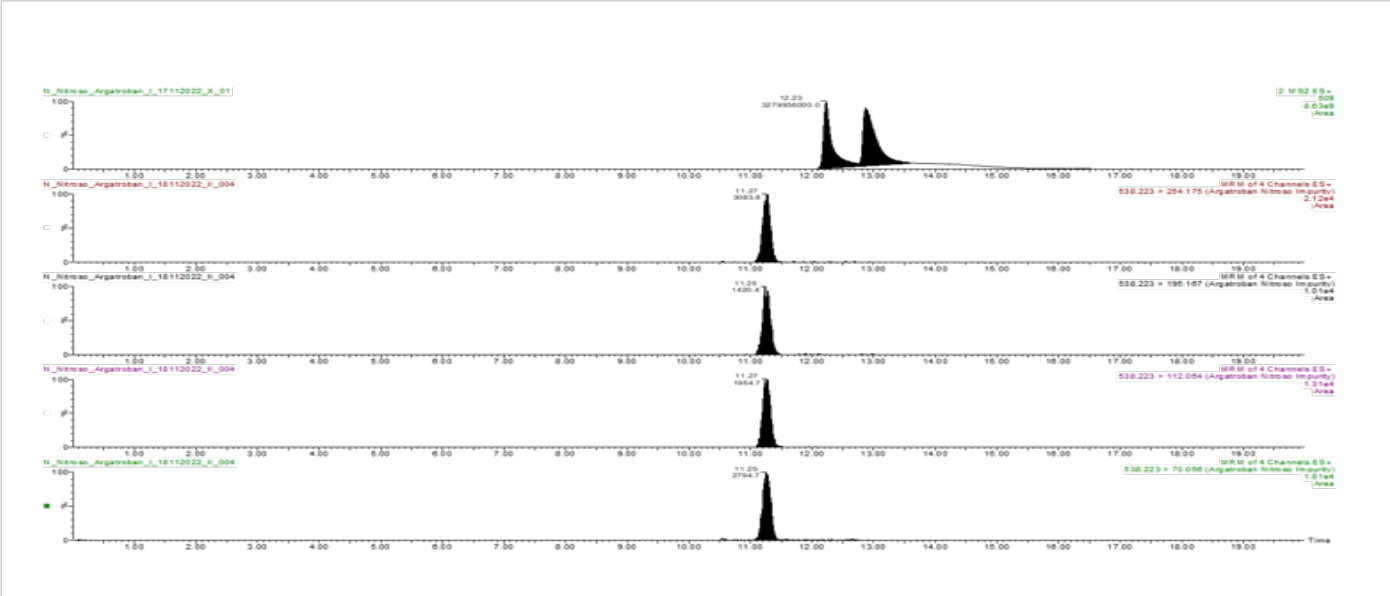


Figure 1. Chromatographic Separation of N-Nitroso Argatroban Impurity and formulation by using RADAR scan.

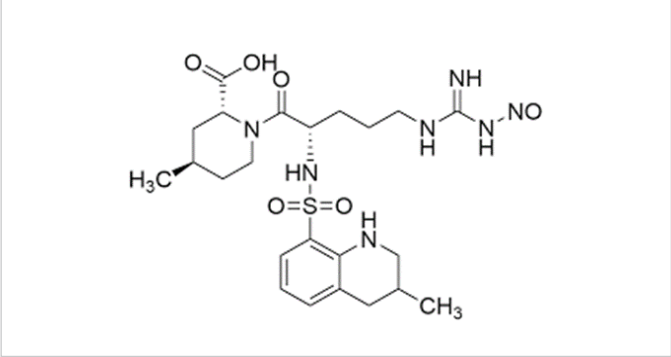


Figure 2. N-Nitroso Argatroban.

SCOPE OF WORK

To overcome the analytical challenges of matrix effect and to improve the spiked recovery for N-Nitroso argatroban impurity in drug product needs a suitable sample preparation technique and chromatographic conditions. Waters Xevo TQ-S Cronos coupled with ACQUITY UPLC H-Class plus and ACQUITY UPLC HSS T3 Column combination produced robust method for quantification N-Nitroso argatroban impurity method performance at LOQ of 0.3 ppm, the instrument showed excellent sensitivity with S/N ratio (>1000) at 0.05 ppm level with respect to API. The observed spiked recovery was between 70 to 120% by adapting extraction approach with minimal sample concentration.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously without compromising sensitivity, a unique capability that can both simplify and accelerate development of robust methods. During method development, RADAR offers the ability to understand unexpected results due to matrix effects. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI and eluting later. Diverting the API peak avoided the contamination of the mass spectrometer increasing the method robustness.

Figure 3. Xevo TQ-S Cronos with ACQUITY UPLC H-Class Plus, and ACQUITY UPLC HSS T3 Column.

Limit Test	Limit/Range
Linearity	0.05 to 50 ppm
Method LOQ	0.3 ppm
Method LOD	<0.005 ppm
Spiked recovery	94%

Table 1. Summary for N-Nitroso Nortriptyline impurity.

A Robust and Sensitive Instrument for Quantification of N-Nitroso Diethanolamine Impurity in Cholestyramine Drug Product

INTRODUCTION

Cholestyramine is used along with a proper diet to lower cholesterol in the blood. Lowering cholesterol helps decrease the risk for strokes and heart attacks. N-Nitroso diethanolamine impurity is a specific N-Nitroso impurity for cholestyramine. During Fourth Nitrosamine Implementation Oversight Group (NIOG) meeting of EMA held during Nov 2022, discussion was happened mainly on concerns and adaptions on N-Nitroso drug substance(NDSRI's). NDSRI's are a class of nitrosamines, sharing structural similarity to the Drug substance. These impurities are generated during manufacturing process, shelf-life storage period, due to nitrite residues present in excipients formulations. Nitrite residues have been observed in a range of commonly used excipients and may lead to the formation of NDSRIs in certain drug products.

SCOPE OF WORK

Similar chemical properties of the drug substance, the N-Nitroso impurity posed a challenge of low spiked recovery for LCMS method. Adoption of the sample preparation by extracting impurity from Drug Product produced good recovery between 70 to 80%. Optimized chromatographic conditions on Waters Xevo TQ-S Cronos coupled with ACQUITY UPLC H-Class plus and Symmetry C_8 Column combination produced robust method for quantification N-Nitroso diethanolamine impurity at method LOQ 0.09 ppm and the instrument shows excellent sensitivity with S/N ratio (>10) at 0.006ppm level with respect to API.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously without compromising on sensitivity, a unique capability that can both simplify and accelerate development of robust methods. During method development, RADAR offers the ability to understand unexpected results due to matrix effects. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI and eluting later. Diverting the API peak avoided the contamination of the mass spectrometer increasing the method robustness.

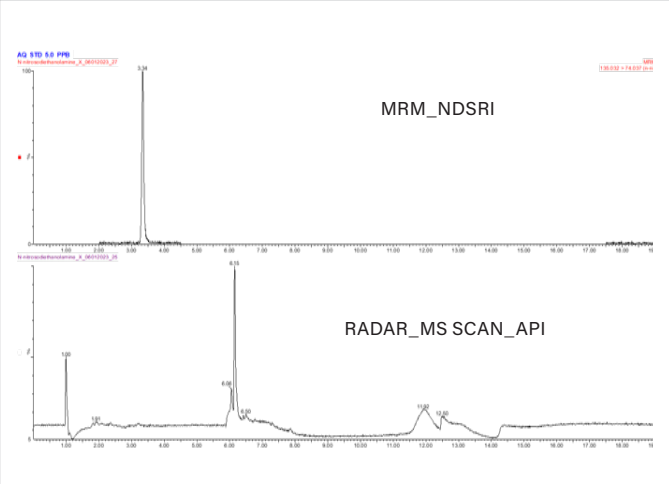


Figure 1. Chromatographic Separation of for N-Nitroso diethanolamine impurity and formulation by using RADAR scan.

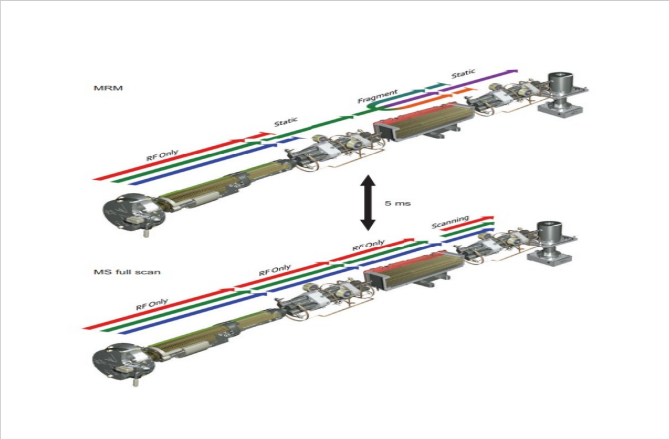


Figure 2. RADAR functionality.

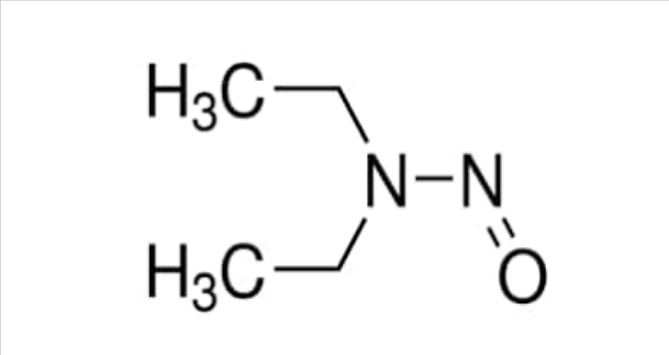


Figure 3. N-Nitroso diethanolamine.

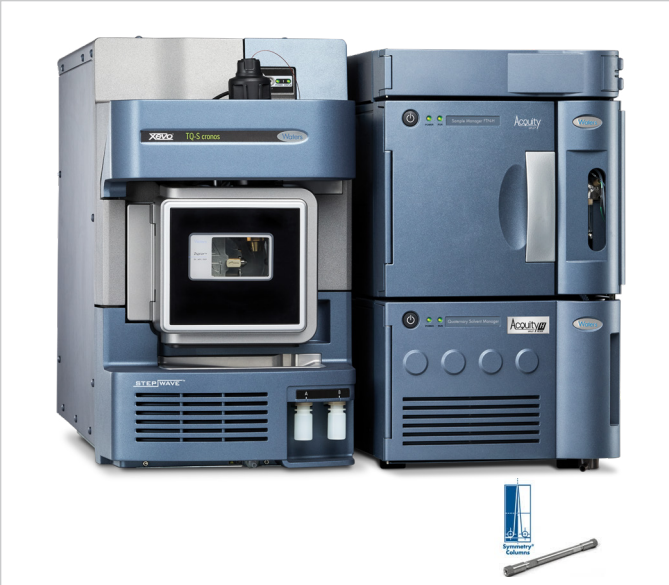


Figure 4. Xevo TQ-S Cronos with ACQUITY UPLC H-Class Plus , and Symmetry C_8 Column.

Limit Test	Limit/Range
Linearity	0.006 to 33.0 ppm
Method LOQ	0.09 ppm
Method LOD	0.006 ppm
Spiked recovery	70 to 80.0%

Table 1. Summary for N-Nitroso diethanolamine impurity.

A Robust and Sensitive Instrument for Quantification of N-Nitroso Nortriptyline Impurity in Amitriptyline Drug Product

INTRODUCTION

Recently, the FDA has received additional reports of certain types of nitrosamine impurities that formed in several drug products. These nitrosamine drug substance-related impurities (NDSRIs) are a class of nitrosamines sharing structural similarity to the API. NDSRIs can be generated during manufacturing or during the shelf-life storage period of the drug product. In some cases, the root cause of NDSRI formation has been attributed to nitrite impurities present in excipients at parts-per-million amounts. Nitrite residues have been observed in a range of commonly used excipients and may lead to the formation of NDSRIs in certain drug products.

SCOPE OF WORK

To overcome the analytical challenges of matrix effect and to improve the spiked recovery for N-Nitroso Nortriptyline impurity quantification in drug product needs a suitable sample preparation technique and chromatographic conditions. Waters Xevo TQ-S Cronos coupled with ACQUITY UPLC H-Class plus and ACQUITY UPLC BEH C_{18} Column combination produced robust method for quantification N-Nitroso Nortriptyline impurity at method LOQ 0.03ppm and the instrument shows excellent sensitivity with S/N ratio (>200) at 0.015ppm level with respect to API. The observed spiked recovery was 70 to 120% by adapting extraction approach.

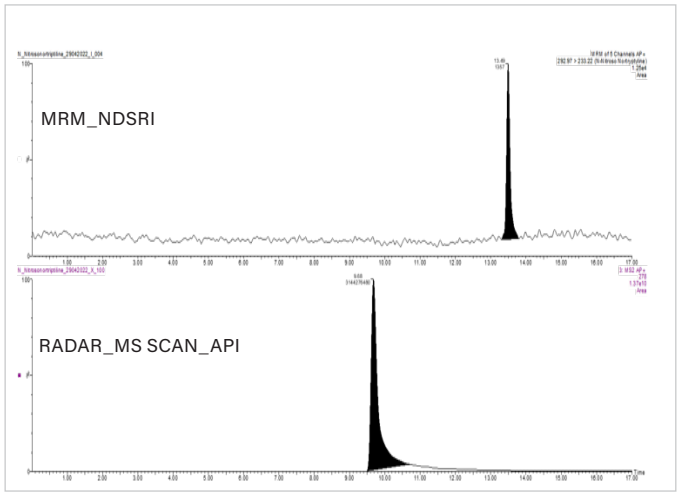


Figure 1. Chromatographic Separation of N-Nitroso Nortriptyline impurity and Amitriptyline DP by using RADAR scan.

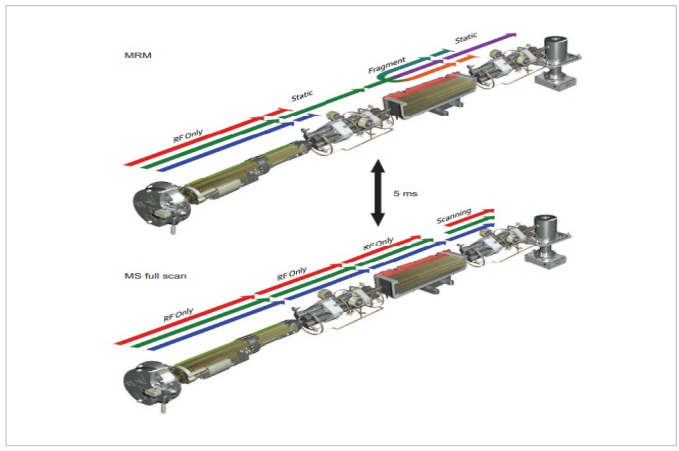


Figure 2. RADAR functionality.

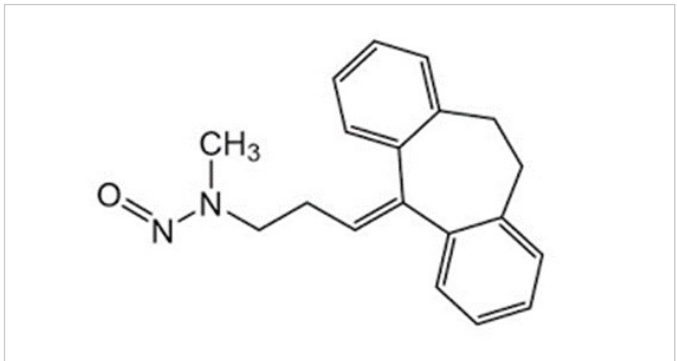


Figure 3. N-Nitroso Nortriptyline.

RADAR SCAN: UNDERSTANDING SAMPLE COMPLEXITY, INTELLIGENT METHOD DEVELOPMENT, AND UNDERSTANDING MATRIX EFFECTS

RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously without compromising sensitivity, a unique capability that can both simplify and accelerate development of robust methods. During method development, RADAR offers the ability to understand unexpected results due to matrix effects. Figure 1 shows a RADAR scan investigation results where API is clearly separated from the NDSRI which is eluting later. Diverting the API peak avoided the contamination of the mass spectrometer increasing the method robustness.

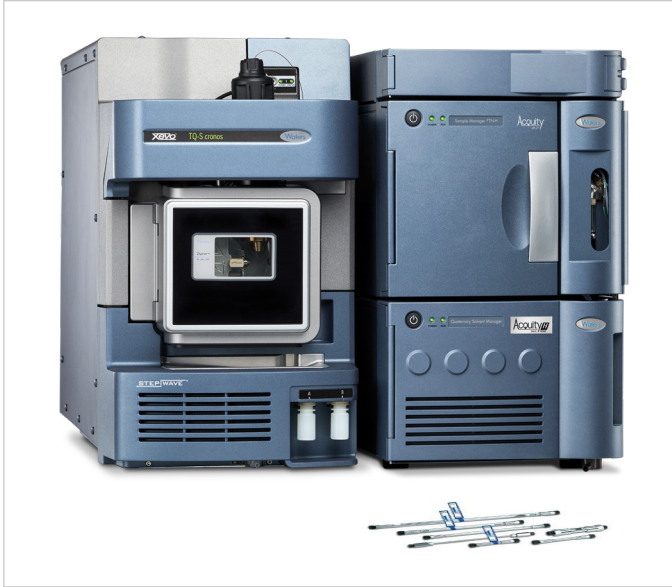


Figure 4. Xevo TQ-S Cronos with ACQUITY UPLC H-Class Plus , and Symmetry C_8 Column.

Limit Test	Limit/Range
Linearity	0.002 to 2.0 ppm
Method LOQ	0.03 ppm
Method LOD	0.01 ppm
Spiked recovery	70 to 120.0%

Table 1. mary for N-Nitroso Nortriptyline impurity.

Learn more about Waters Analytical solution for trace-level mutagenic and genotoxic impurity analysis:
waters.com/NitrosamineAnalysis

For your local sales office, please visit waters.com/contact

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
waters.com

Waters™