

Efficient Identification of Impurities in Nicotine API Using the SmartMS-Enabled ACQUITY RDa Detector

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Abstract

This application note demonstrates the efficient and easy to use workflow for the identification of Nicotine and its related impurities by using a novel compact benchtop SmartMS-enabled LC-MS system. The API (active pharmaceutical ingredient) and related impurities were identified and reported using the UNIFI application within the waters_connect Software platform. The full scan with fragmentation function on the ACQUITY RDa Detector generated fragment ion information, used for further confirmation of assignments. By ramping the fragmentation cone voltage additional structural information was generated.

Impurity profiling is a critical part of the drug development process. Identification of unknown impurities is a key factor to refining pharmaceutical drug potency and safety attributes. Providing a successful solution for the identification of impurities present in API is a complicated and challenging task. High resolution mass spectrometry (HRMS) is an analytical technique often employed to confidently identify the impurities present in API; however, it can require a high-level of experience and expertise to solve these analytical problems. The ACQUITY RDa Detector, with its automatic set up and calibration, allows accurate mass measurements to be obtained by scientists with a diverse range of analytical expertise. Thus, providing access to HRMS for non-expert users and empowering scientists with a far greater depth of analytical information.



Figure 1. ACQUITY RDa Detector.

Benefits

- Routine accurate mass measurements for impurity profiling
- A compact benchtop system with SmartMS technology
- Intuitive system health checks and dedicated end-to-end workflows
- Compliance-ready system ensuring data integrity

Introduction

Nicotine is a chiral alkaloid that is naturally produced and widely used recreationally as a stimulant and anxiolytic. As a pharmaceutical drug, it is used for smoking cessation to relieve withdrawal symptoms. Electronic cigarettes (e-cigarettes) and nicotine gums offer an alternative to the experience of smoking tobacco. The nicotine used is extracted from tobacco, and the purity of the extracted nicotine can vary depending upon manufacturer and grade (e.g., pharmaceutical). US Pharmacopeia (USP)-grade nicotine requires single impurities to be less than 0.5% (5 mg/g) and total impurities to be less than 1% (10 mg/g). Nicotine impurities are specified in the European Pharmacopoeia monograph 1452 as nicotine-N-oxides, cotinine, nornicotine, anatabine, myosmine, anabasine, and β -nicotyrine.

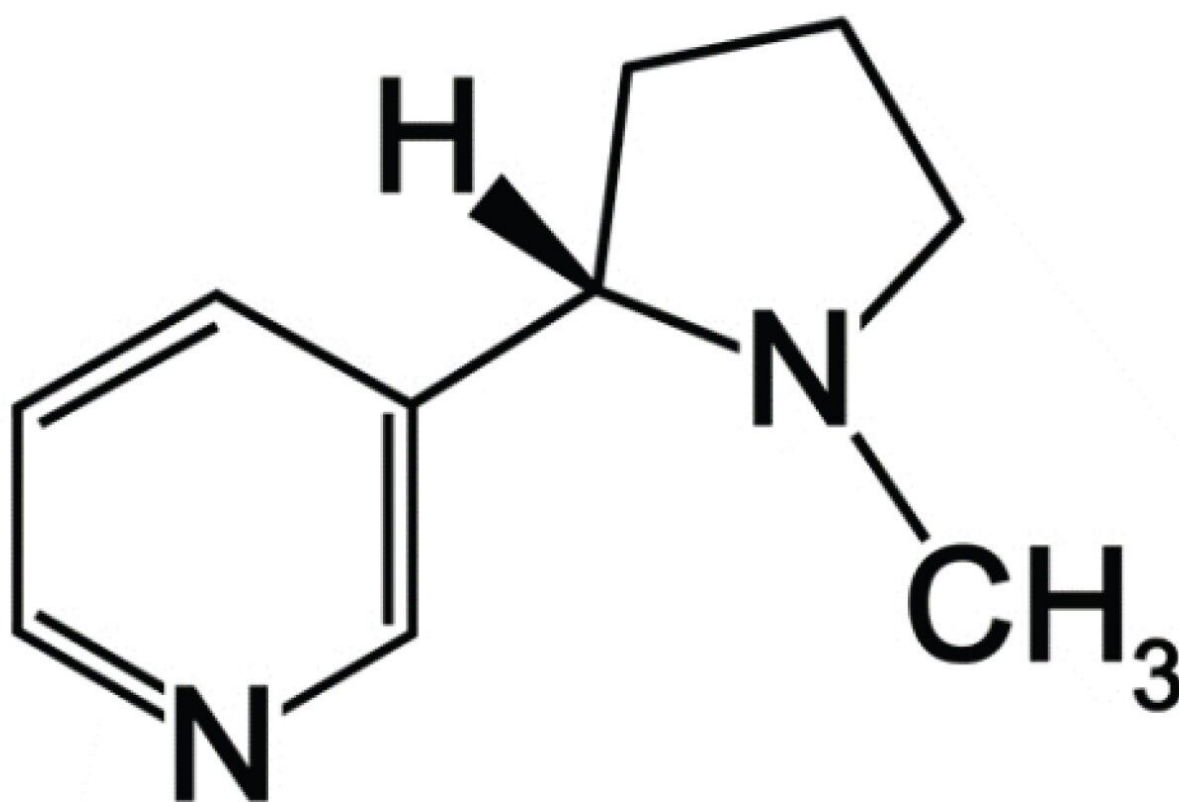


Figure 2. Nicotine.

There are many methods (GC, NPD, and MS) for the analysis of nicotine and related alkaloids.¹⁻⁴ The nicotine related compounds such as nicotine-N-oxide, is known to be thermally unstable at temperatures required for GC analysis. Some target compounds such as nornicotine can show severe issues with respect to carryover in the injector.^{1,2,4} These issues have been solved by using HPLC with UV detection, but it has limited

sensitivity and cannot differentiate potentially co-eluting compounds. So analytical techniques such as HRMS is required to overcome these issues. This technique usually requires experienced users to operate the instruments.

The ACQUITY RDa Detector with SmartMS capabilities is the right choice for the scientists who are non-expert users of HRMS to access accurate mass measurements and glean in-depth analytical information. Additionally, the waters_connect Software platform automatically acquires, processes, and reports results in a compliance-ready framework ensuring data integrity. Using the full scan with fragmentation function, we can simultaneously acquire both low and high energy spectra, generating fragment ion information for increased confidence in compound identification.

Experimental

The new ACQUITY RDa Detector, coupled to an ACQUITY UPLC I-Class PLUS System, was used for the impurity analysis of nicotine using USP monograph method with end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R (4.6 mm x 150 mm, 5 µm) as the stationary phase. The ACQUITY RDa Detector is a compact, benchtop time-of-flight (ToF) mass spectrometer that has a mass resolution of >10,000 FWHM for routine accurate mass measurements. The system can acquire both full scan and full scan with fragmentation data (a data independent acquisition mode). The UNIFI application is an easy to use and customizable platform that utilizes accurate mass, retention time and fragment ion information to quickly search a customizable application specific library to identify the compound. Figure 1 shows the ACQUITY RDa Detector.

Sample Description

A stock solution of impurity standard mix (100 ppm) was prepared in water. A 10 mg/mL sample solution of nicotine ditartrate dihydrate was prepared and spiked with an impurity standard mix using water as a diluent, with a final solution concentration at 100 ppb.

LC Conditions

LC system: ACQUITY UPLC I-Class PLUS

Detection: TUV@260nm

Vials: Total recovery vials

Column(s): XBridge Shield RP18, 4.6 x 150 mm, 5 µm

Column temp.: 30 °C

Sample temp.: 20 °C

Injection volume: 20 µL

Flow rate: 1.000 mL/min

Mobile phase A: 25 mL of 1 M acetic acid + 6 mL of ammonia solution, pH:10

Mobile phase B: Acetonitrile

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	1.000	100	0	6
3.00	1.000	100	0	6
3.01	1.000	95	5	6
28.0	1.000	74	26	6
32.0	1.000	60	40	6
37.0	1.000	100	0	6
40.0	1.000	100	0	6

MS Conditions

MS system: ACQUITY RDa Detector

Ionization mode: Full scan with fragmentation (pseudo-MS^E acquisition)

Acquisition range: 50–2000 *m/z*

Capillary voltage: 1.5 kV

Fragmentation cone voltage: 50–80 V

Cone voltage: 30 V

Polarity: Positive ion

Scan rate: 5 Hz

Desolvation temperature: 550 °C

Data Management

Informatics: waters_connect v1.9.12

Results and Discussion

The ACQUITY RDa Detector utilizes the accurate mass workflows for exact mass measurement required for identification and smart decision making. This work demonstrates the UNIFI Software application workflow for impurity analysis, which can also be extended to degradation studies, as shown in Figure 3.

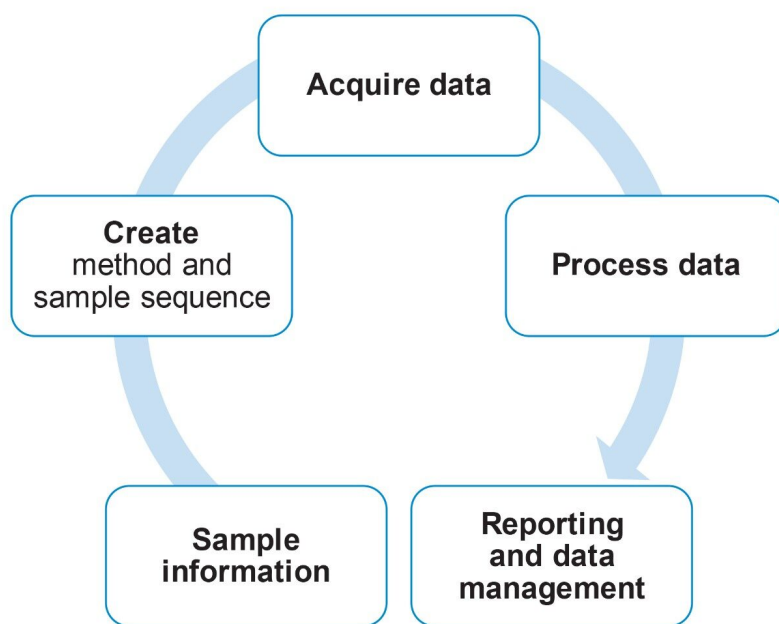


Figure 3. UNIFI application workflow for analysis.

As shown in Figure 4, UV acquisition was performed along with MS enabling a comparison between the two traces.

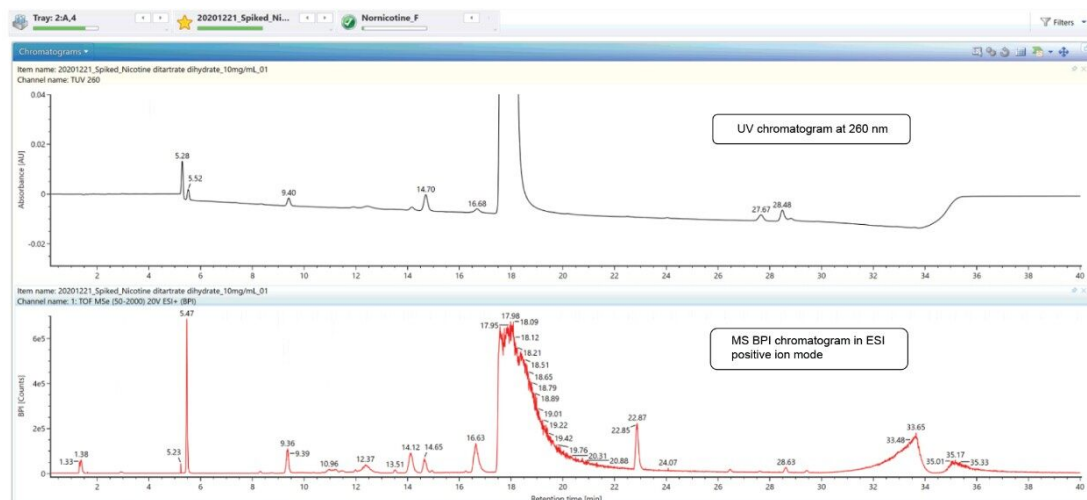


Figure 4. UV chromatogram at 260 nm vs MS BPI (base peak intensity) chromatogram in ESI positive ion mode of nicotine API spiked with the impurity mix.

Nicotine API sample (10 mg/mL) spiked with the impurity standard mix (100 ppb) was acquired using full scan with fragmentation function on the ACQUITY RDa Detector, where the low energy and high energy channel contains parent ion and fragment ion information, respectively (Figure 5).

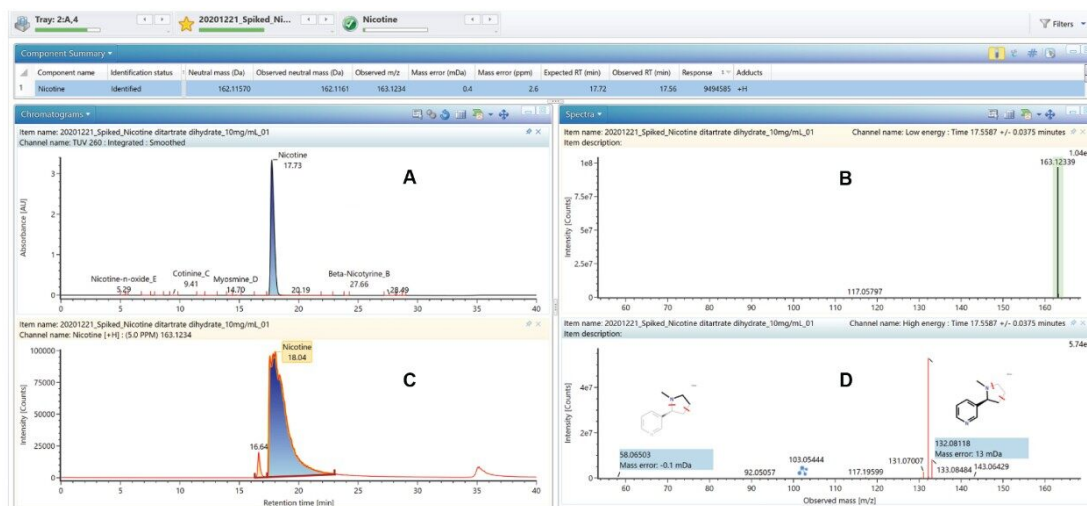


Figure 5. A-UV chromatogram, B-Low energy mass spectrum, C-MS XIC (extracted ion chromatogram) of nicotine, and D-High energy mass spectrum of nicotine.

Here in this application note we have demonstrated two different approaches; one using "Discovery Tool" and the other with "Transformations Tool" to identify an unknown impurity, as shown in Figure 6.

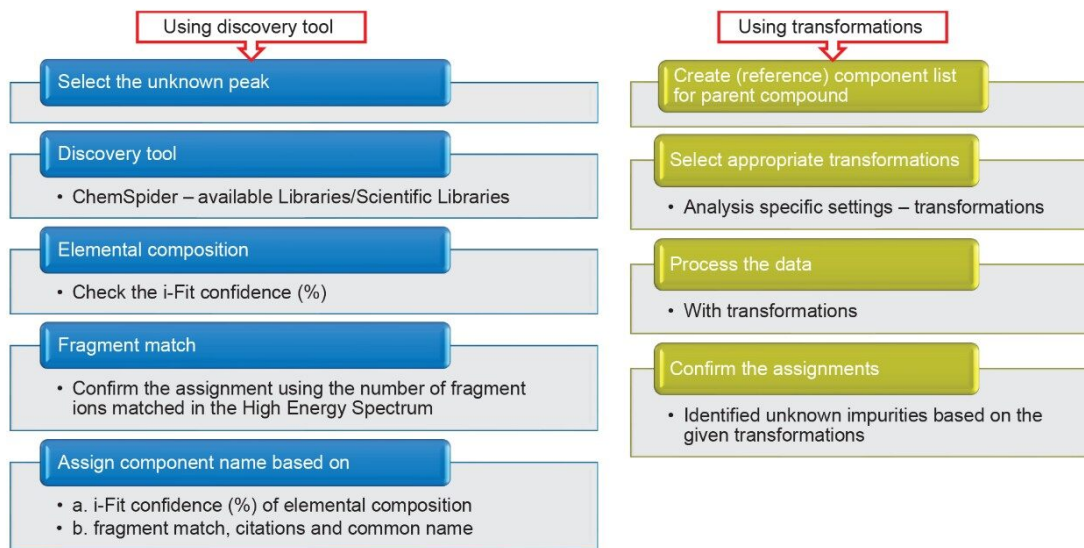


Figure 6. Discovery tool approach and Transformations tool approach for the identification of unknown impurities.

The integrated discovery tool allows the user to interrogate unidentified peaks and quickly perform a structural database ChemSpider search for putative identifications of an unknown compound. By using discovery tool, we can identify the nicotine peak at RT of 17.57 minutes as shown in Figure 7.

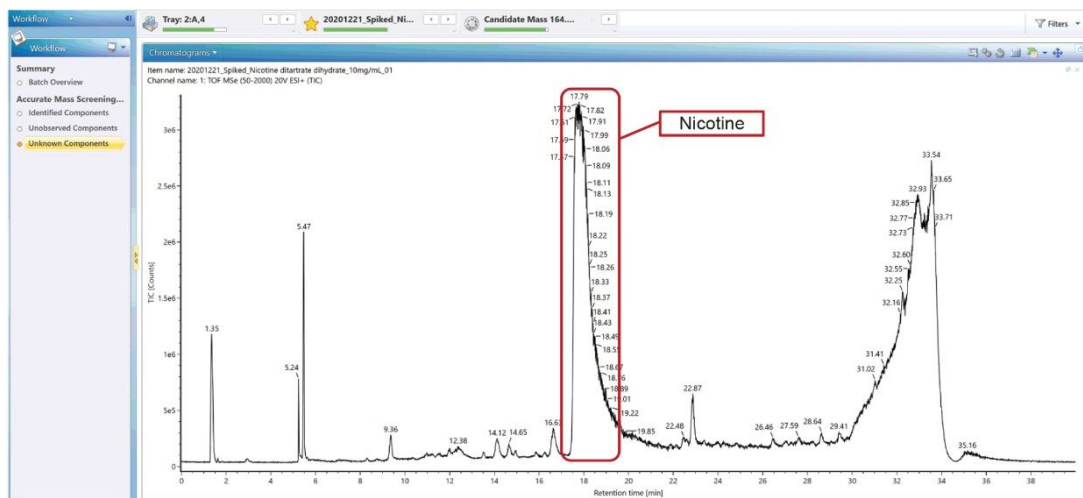


Figure 7. Nicotine peak at 17.57 minutes.

The discovery tool takes the accurate mass for the peak measured at 17.57 minutes, proposes the elemental

formulae and searches in the selected ChemSpider libraries for a possible match. In this case, twelve database matches were found within the tolerances used. In addition, the discovery tool performs in-silico fragmentation on the proposed database match (using the .mol file) to yield theoretical fragment ions and compares these with the acquired fragment ion spectrum. The first candidate found is nicotine with the suggest fragment ion assignments matching the high energy spectrum as shown in Figure 8.

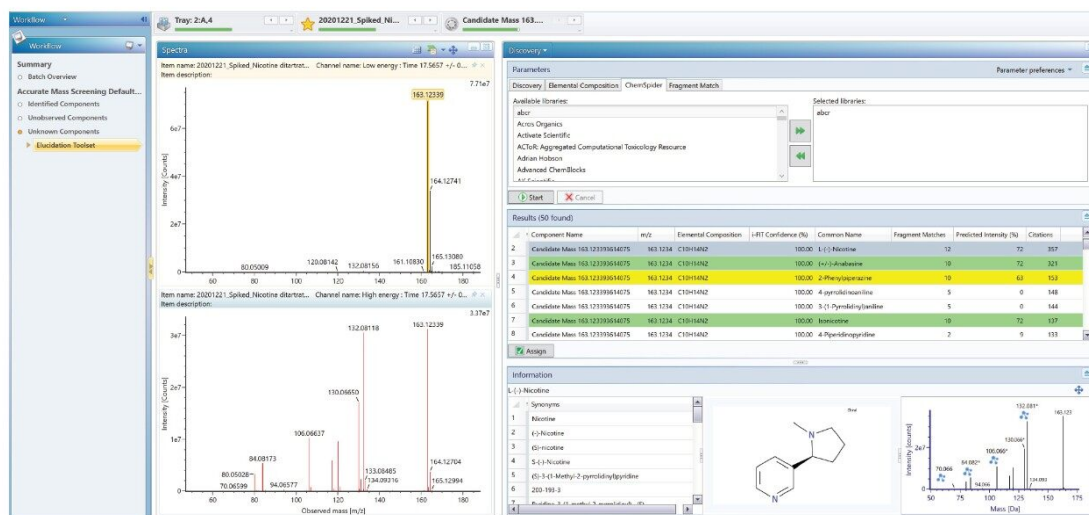


Figure 8. Identification of nicotine by using Discovery tool with the help of ChemSpider search functionality.

Once identified using the discovery tool, the unknown impurities can be entered into a scientific library and used for the routine analysis. Figure 9 demonstrates the identification of nicotine and its related impurities through the scientific library.

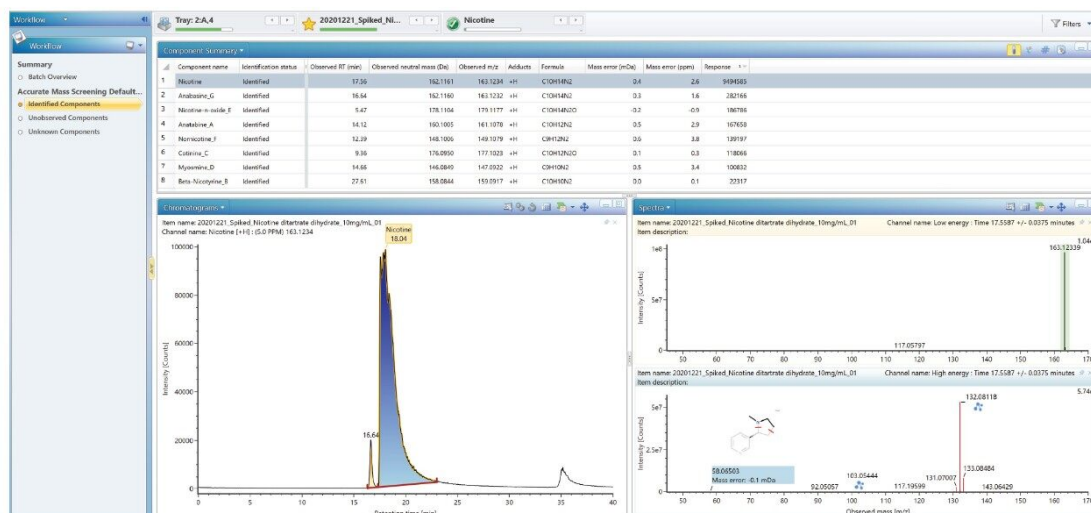


Figure 9. Identification of nicotine and its related impurities by using scientific library.

Alternatively, the discovery tool can be used to find the possible identity of the API and the transformation tool used to identify impurities. Here, impurities related to API including oxidations, reductions and dealkylations were successfully identified using the inbuilt transformation tool within the UNIFI application. The suspected impurities are shown within the component summary, where all peaks are listed that match the m/z of predicted impurities (Figure 10). The chromatogram and spectral view are also displayed, with the automatic fragment ion assignment in the high energy spectrum annotated with the blue icon (Figure 10). Incorporating the impurity analysis workflow, shown on the left-hand side of Figure 10, is an efficient and effective approach to identifying unknown impurities that are related to API.

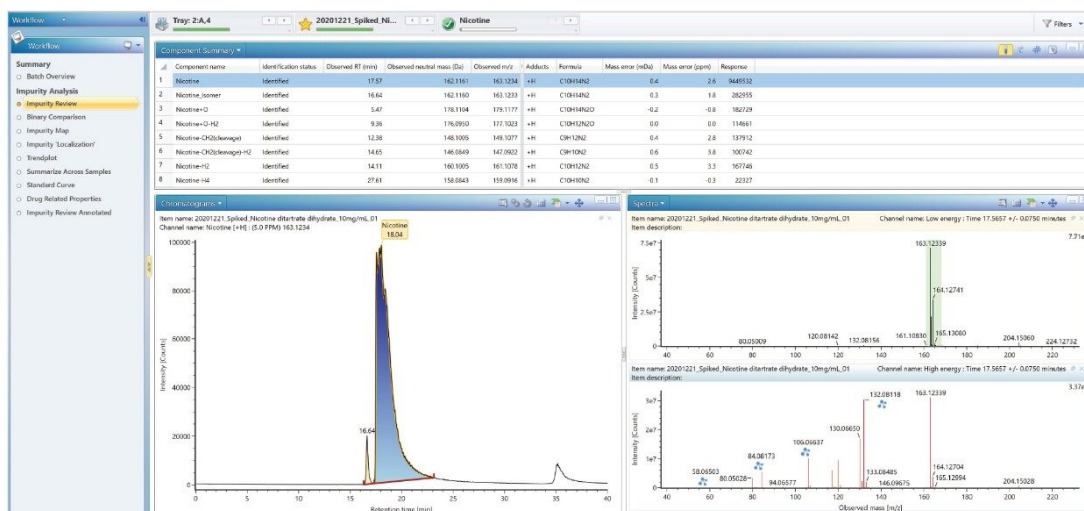


Figure 10. Identification of related impurities of Nicotine API by using transformations tool and incorporating the impurity analysis UNIFI workflow.

All related substances of nicotine ditartrate dihydrate were identified using ACQUITY RDa Detector with waters_connect Software platform.

Conclusion

Nicotine and its related impurities were successfully identified using the ACQUITY RDa Detector, ACQUITY UPLC I-Class PLUS System with TUV and waters_connect Software platform. Through the incorporation of accurate mass workflows scientists are able to monitor the levels of APIs and their related impurities. This allows them to evaluate and control these types of sample sets, thus establishing specification limits within the pharmaceutical industry. The waters_connect Software platform with UNIFI application was used to automatically identify nicotine and its related impurities with a high degree of confidence based on mass measurement and fragment ion assignment.

The Waters ACQUITY RDa Detector with routine workflows supports the chemist in identification and characterization of impurities, allowing them to make informed and impactful decisions.

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