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Application Note

ACQUITY UPLC with PDA Detection: Determining the Sensitivity Limits of Oxybutynin and Related Compounds

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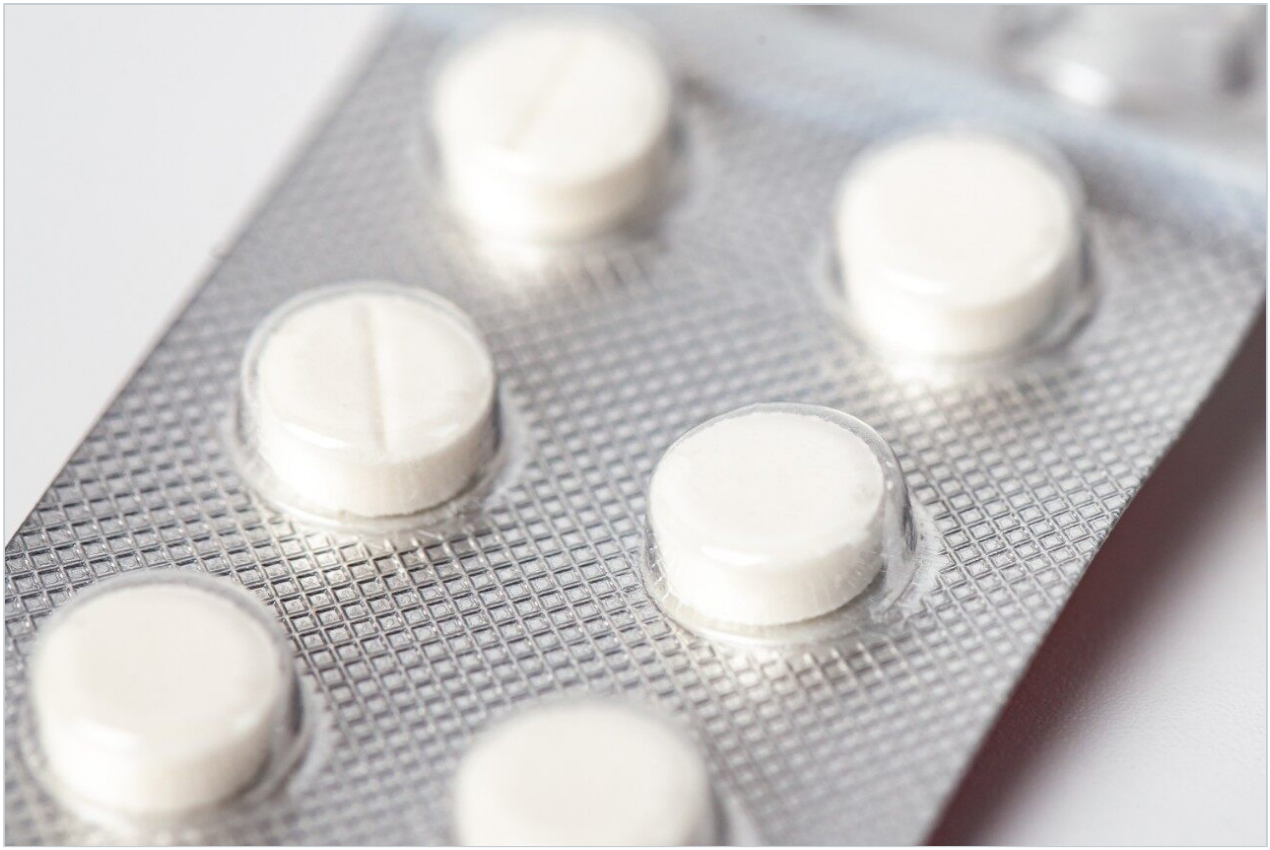


Abstract

The ACQUITY UPLC PDA combined with the improved resolution and speed of the ACQUITY UPLC System make it the ideal solution for quantitative impurity analysis.

Introduction

Some of the most challenging methods for modern LC detectors are in performing quantitative impurity analysis. These methods entail the simultaneous analysis of a large amount of parent compound and low-level impurities, thus requiring a detector with low noise levels and a wide linear dynamic range. Combining these needs with the desire to perform the separations rapidly, the detector must also be able to perform at fast data acquisition rates to reproducibly quantitate narrow peaks while not significantly increasing noise levels. It is also desirable to collect spectral data with a narrow bandwidth to maintain high spectral quality for purity analysis and library matching.

The low noise characteristics and large linear range of the Waters ACQUITY UltraPerformance LC System equipped with an ACQUITY UPLC Photodiode Array (PDA) Detector allows for easy quantitation of low-level impurities and high levels of a parent compound, with fast run times and without sacrificing spectral integrity.

Waters ACQUITY UPLC System with the ACQUITY UPLC Photodiode Array Detector.

Experimental

Oxybutynin Method Conditions

LC system:	ACQUITY UPLC System and ACQUITY UPLC PDA Detector with Empower Software
Column:	ACQUITY UPLC BEH C_8, 2.1 mm x 50 mm, 1.7 µm
Detection:	Analytical flow cell 225 nm Normal time constant 10 pts/sec 1.2 nm bandwidth
Mobile phase:	65/35 10 mM Phosphate buffer (pH 7.0)/Acetonitrile

Flow rate:	0.800 mL/min
Temp.:	45 °C
Samples:	Oxybutynin and Oxybutynin Related Compounds B and C in 50/50 Water/Acetonitrile, 0.001% to 0.1% of 4.0 mg/mL standard
Injection volume:	4.8 µL characterized full loop
Strong wash:	200 µL 25/75 Water/Acetonitrile
Weak wash:	800 µL 75/25 Water/Acetonitrile

Results and Discussion

Sensitivity Limits of Low-Level Impurities

Sensitivity limits of low-level impurities are characterized by the limits of quantitation (LOQ) and detection (LOD). There are several methods for determining these parameters which are discussed in the USP Chapter 1225.[1] If the detection method has a noise signature, the noise level can be used to estimate LOQ and LOD. A signal-to-noise ratio of 10:1 is generally considered acceptable for estimating to LOQ and a signal-to-noise ratio of 3:1 is generally considered acceptable for estimating the LOD. To determine these values, signals of low-level samples are compared to blank injections. ASTM noise (average peak-to-peak noise) from the blank injection is used as the estimation of the noise levels for the theseparation. Other methods use the slope of thecalibration curve and the standard deviation of the response.

LOQ and LOD of Oxybutynin and Related Compounds

To establish the sensitivity limits of the ACQUITY UPLC with PDA detection, the LOQ and LOD of Oxybutynin and two Oxybutynin Related Compounds B (methyl ester of phenylcyclohexylglycolicacid) and C (4-(ethylmethylamino) but-2-ynyl (±)-2-cyclohexyl-2-hydroxy-2-phenylacetate) were determined. The chromatographic method (based upon the USP method) was scaled to the UPLC column dimensions and particle size. This method transfer step resulted in a run time reduction from 35 minutes to just 10 minutes,

with equivalent resolution (2.4 between oxybutynin and the major impurity). Signal-to-noise ratios were used to determine the LOQ and LOD by generating a calibration curve near the limits of detection. The concentrations of the standards were based upon the response of a 4.0 mg/mL oxybutynin standard at ~2.0 AU full scale (Figure 1), which was within the linear range of the detector.[2] The appropriate concentration range for the standards was established between 0.001% and 0.1% by mass of the main oxybutynin peak.

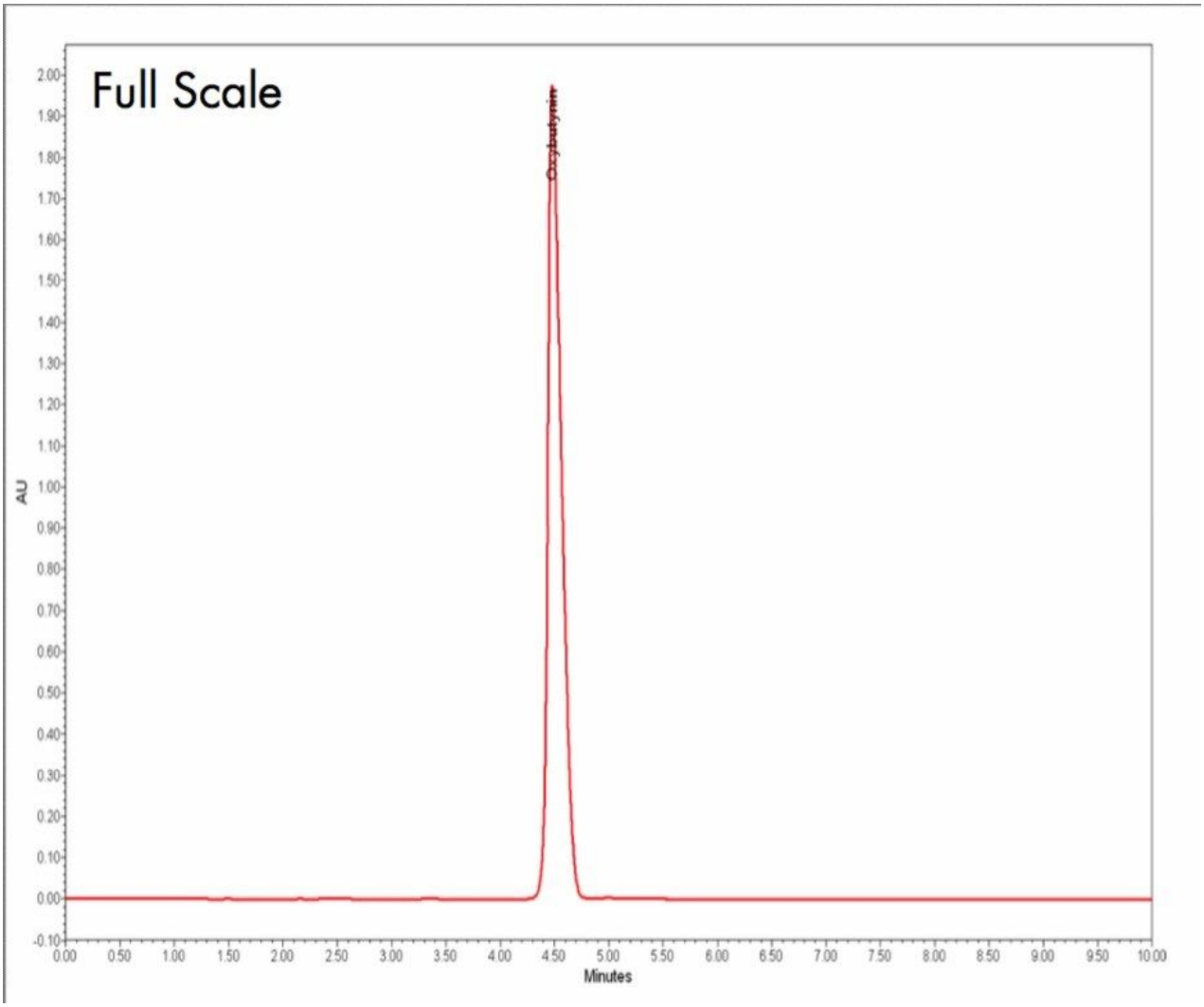


Figure 1. Oxybutynin sample at 4 mg/mL.

The chromatograms for the 0.005% and 0.01% standards, which are close to the LOQ and LOD, are shown in Figure 2. Standards were injected in triplicate. The calibration curves generated from these standards (Figure 3) were linear with R^2 values in excess of 0.999. Curves were based upon height in order to easily determine the LOQ and LOD (ratio to noise). The equation for these curves used to calculate the LOQ and LOD can be

found in Table 1. Blank injections were performed and the average peak-to-peak noise for these chromatograms was 27.5 µAU. From this, the LOQ and LOD were calculated for oxybutynin and its two related compounds, Table 1.

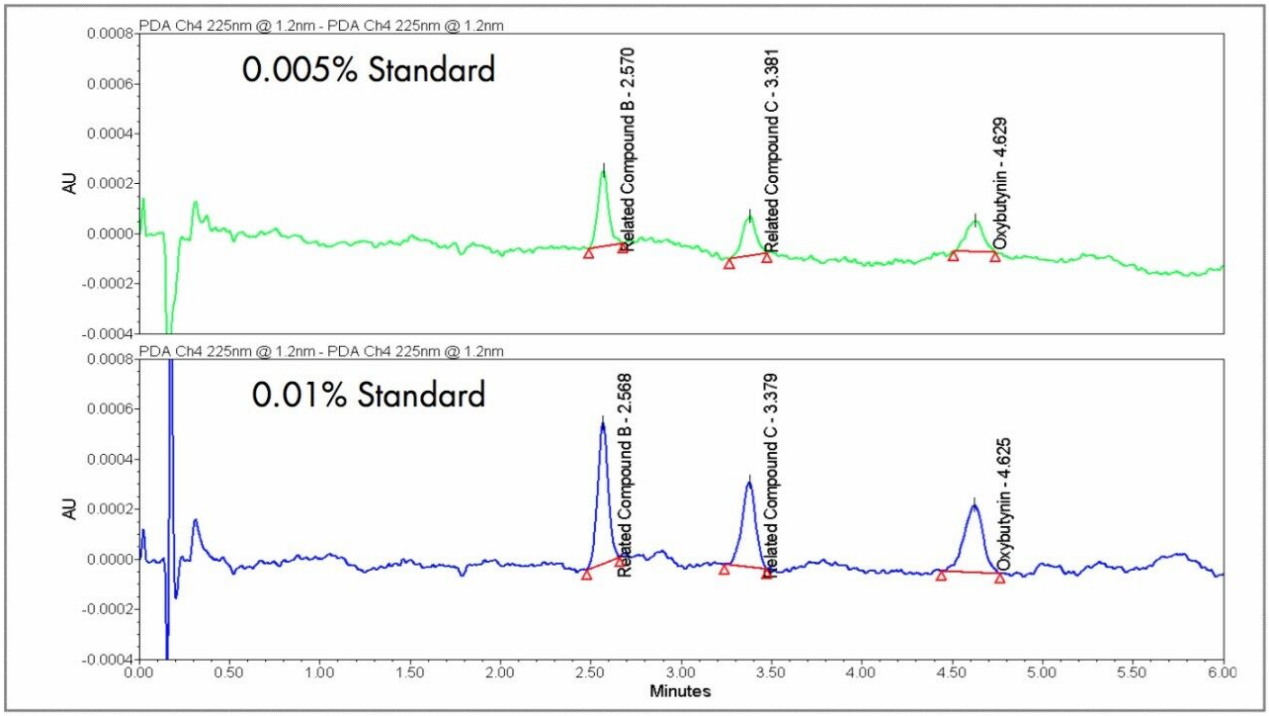


Figure 2. Oxybutynin and two related compounds spiked at levels of 0.005% and 0.01% of the concentration of the main oxybutynin peak at 4 mg/mL.

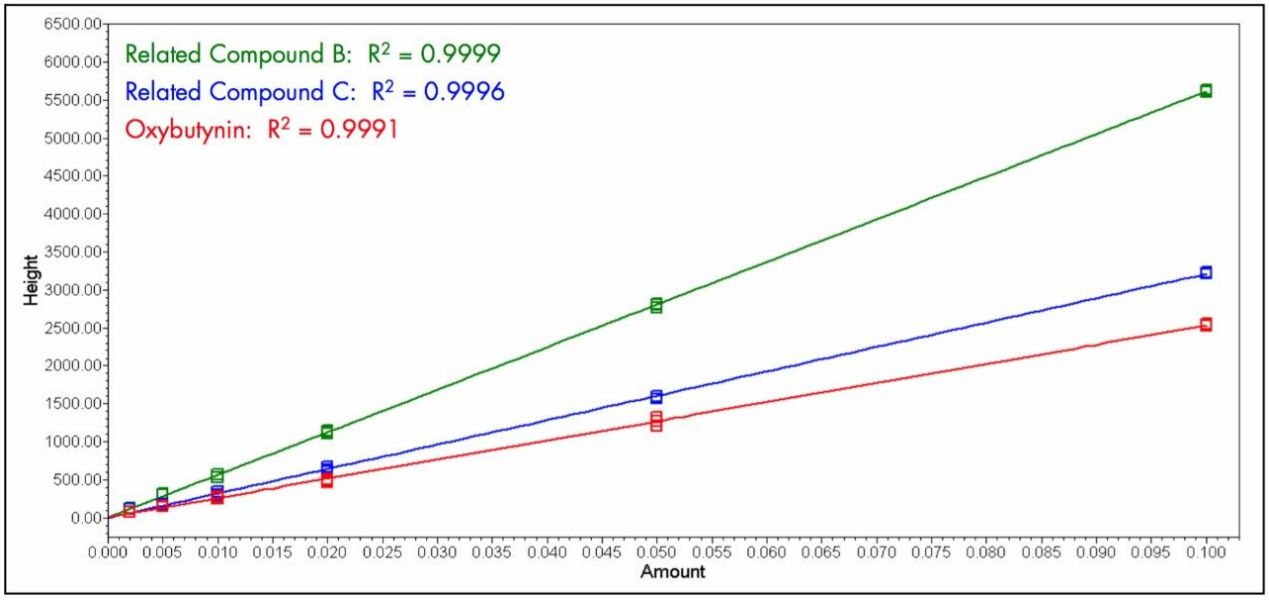


Figure 3. Calibration curves of oxybutynin and two related compounds near the limits of detection/quantitation.

Compound	*Equation of Calibration Curve*	*Average Peak-to Peak Noise (µAU)*	*Peak Height at LOD*	*LOD*	*Peak Height at LOQ*	*LOQ*
Oxybutynin	Y = 2.52 e+4 X + 10.3	27.5	82.5	0.0029%	275.0	0.0105%
Related Compound B	Y = 5.61 e+4 X – 0.8	27.5	82.5	0.0015%	275.0	0.0049%
Related Compound C	Y = 3.21 e+4 X + 3.4	27.5	82.5	0.0025%	275.0	0.0085%

Table 1. Limits of detection and quantitation for oxybutynin andtwo related compounds.

For impurity analysis, the USP recommends identification of all peaks at 0.1% of the parent compound for a drug product and 0.05% for a drug substance.[3] The LOQ for oxybutynin was determined to be 0.0105%, well below the minimum requirement, while the related compounds had quantitation limits of 0.0049% and 0.0085% for B and C, respectively. To verify these results, replicate injections were performed. Typical acceptance criteria for % RSD at the LOQ is 10%. For oxybutynin, replicate injections at LOQ had a % RSD of 3.1%. The corresponding % RSD values for related compounds B and C were 3.7% and 5.8%. Limits of detection were calculated using the same method and are listed in Table 1.

Spectral Integrity Near the LOQ AND LOD

Typically, a photodiode array detector is employed to gain spectral information for identification of unknowns, library matching and peak purity analysis. Therefore, it is important to maintain high spectral resolution to perform these functions. To minimize noise levels, some PDA/DAD detectors must increase bandwidth (the number of averaged diodes) or slit width (optical resolution) to improve limits of detection for low level impurities. However, increasing these parameters reduces spectral quality and can also negatively impact detector linearity. The highest quality spectra are obtained when using a narrow spectral bandwidth, typically 1–2 nm. By using a narrow bandwidth (1.2 nm), the highest resolution spectra for oxybutynin and its related compounds was achieved (Figure 4). Higher spectral resolution yield better library matching and peak purity results, especially for highly related compounds (Figure 5).

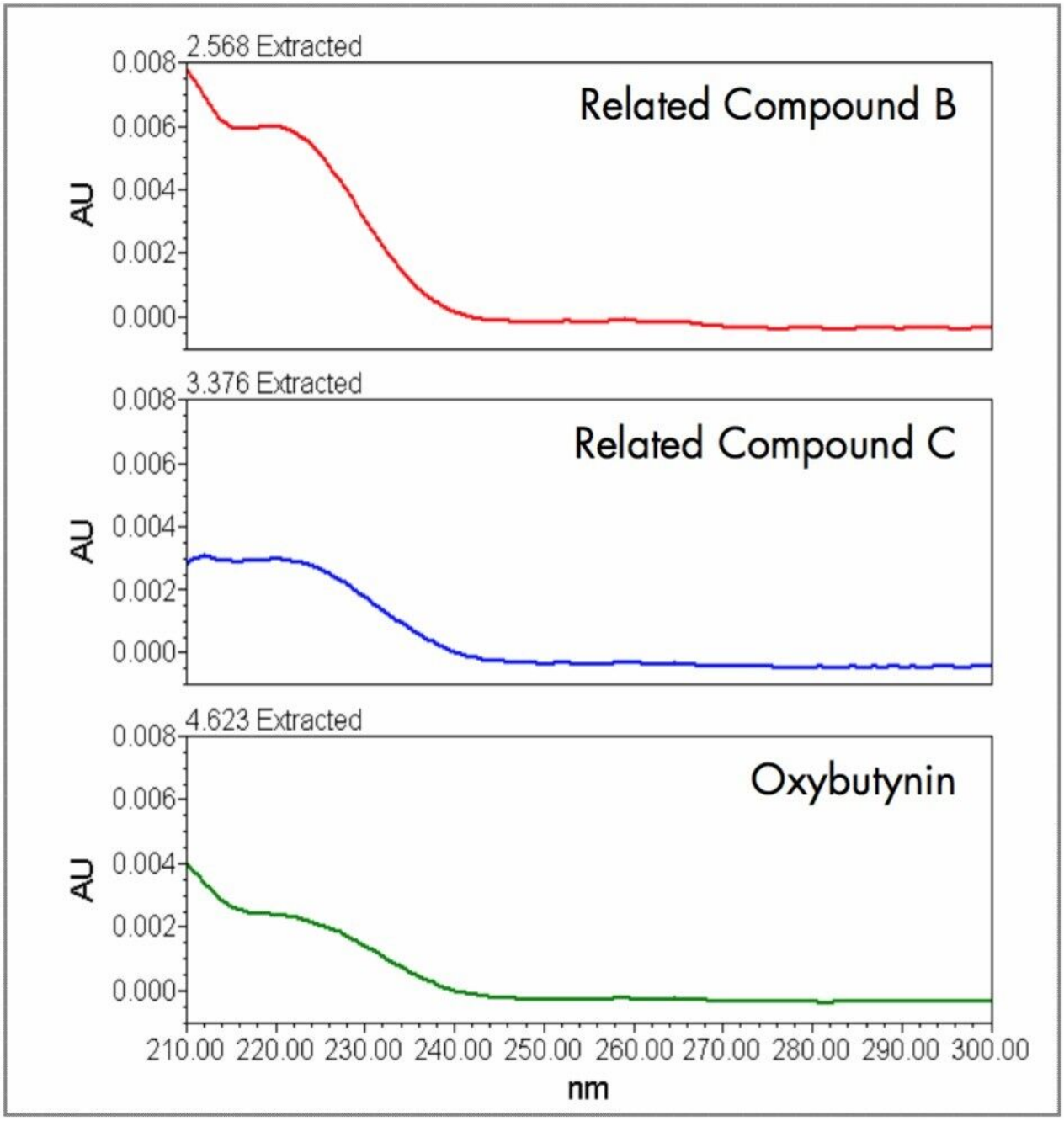
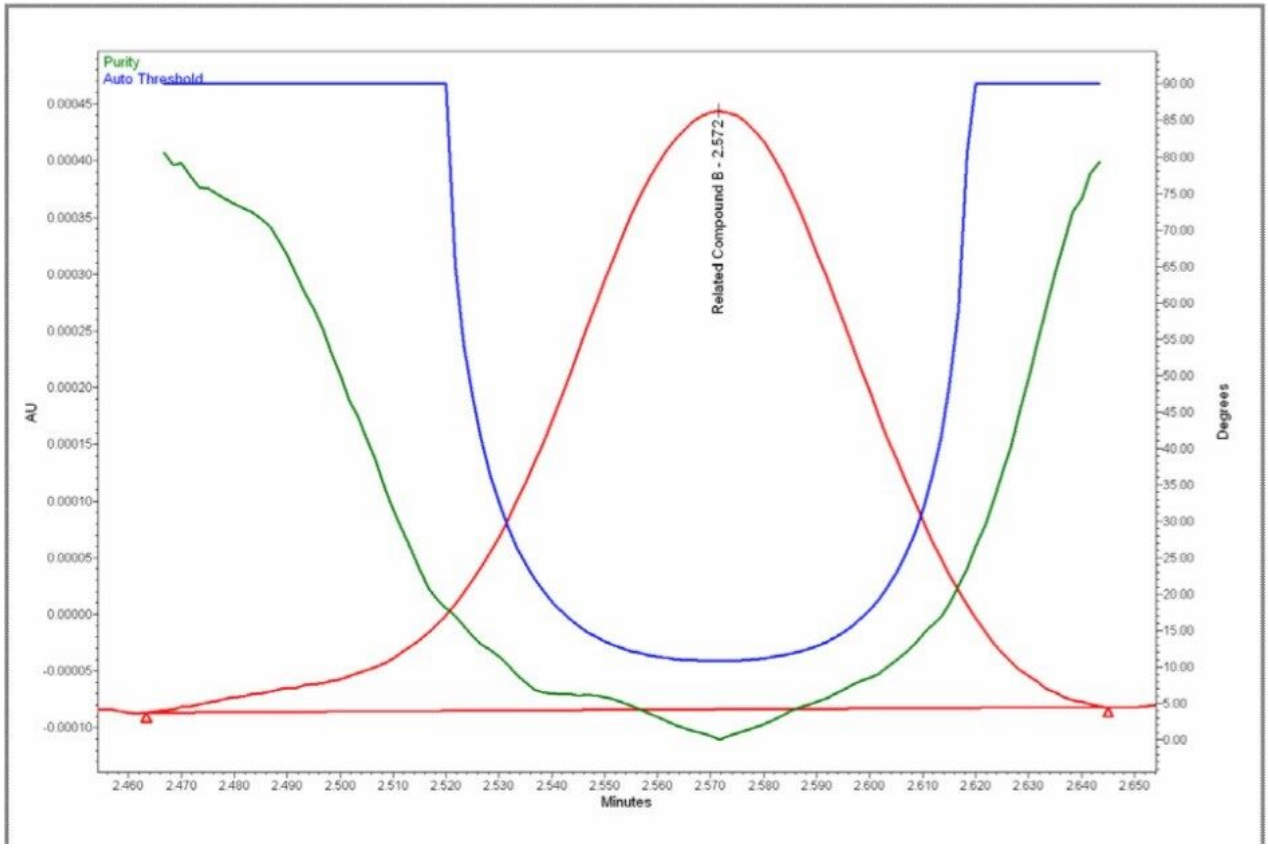


Figure 4. Spectra of oxybutynin and related compounds B and C at 0.1%.


Purity
Auto Threshold
Related Compound B - 2.572
AU
Degrees
Minutes
0.00045
0.00040
0.00035
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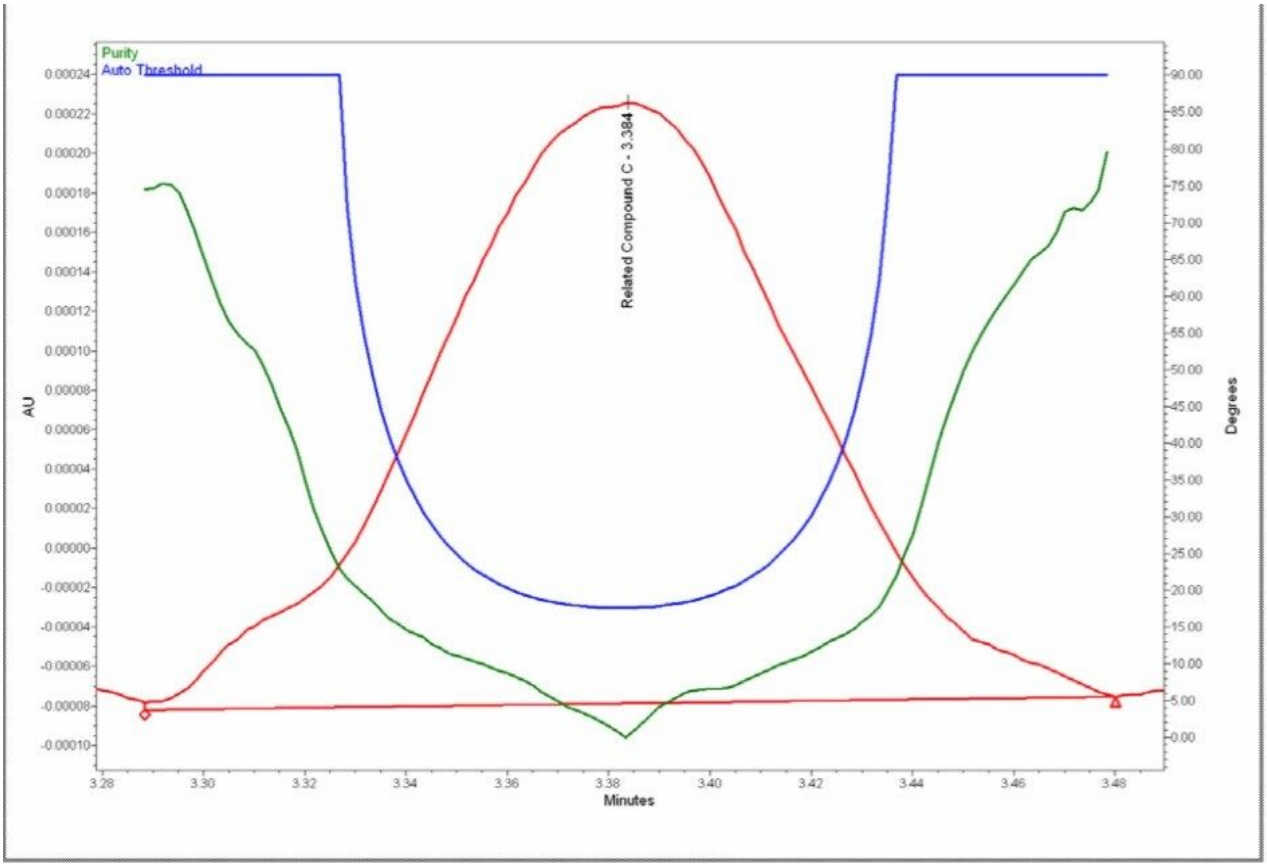

Figure 5. Peak purity analysis for oxybutynin related compounds B and C at 0.01%.

Conclusion

The low noise characteristics and wide linear dynamic range of the ACQUITY UPLC PDA combined with the improved resolution and speed of the ACQUITY UPLC System make it the ideal solution for quantitative impurity analysis. Limits of quantitation below 0.005% and limits of detection as low as 0.0015% are achievable without sacrificing spectral information.

References

1. General chapter <1225>: Validation of compendialmethods. United States Pharmacopeia(USP) XXIII, National Formulary, XVIII. 1995:1710–1612.
2. Jenkins T. Demonstrating superior linearity: The ACQUITY UPLC Photodiode Array Detector. 2006 March; Waters Application Note 720001593EN < https://www.waters.com/webassets/cms/library/docs/720001593en.pdf> .
3. Monographs: Oxybutynin Chloride. USP XXIII, NF XVIII. 1995.
4. General chapter <621>: Chromatography – System Suitability. USP XXIII, NF XVIII. 1995.

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