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Application Note

Extending High Performance Liquid Chromatography Method Development Capabilities by Adding Single Quadrupole Mass Spectrometry Detection

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Abstract

This application note describes the peak tracking capabilities of the Waters 3100 MS Detector during the method development screening of a series of tricyclic antidepressants (TCAs).

Introduction

Traditionally, High Performance Liquid Chromatography (HPLC) method development can be a long, arduous process. However, by applying an automated systematic screening approach to method development, the time necessary to produce a method that meets the acceptance criteria can be significantly reduced.[1] Assessing the variables that will have the most significant effect on chromatographic selectivity – such as column bonded phase, pH, and organic modifier – yields a higher success rate for achieving the necessary chromatographic resolution. During the screening process, peak tracking can be challenging and, therefore, it is important to utilize a detection technique that provides additional information about the peaks as they elute. Many method development strategies incorporate photodiode array (PDA) detection; however, co-elution can make it difficult for this method to adequately track all the peaks of interest. Additionally, when components are spectrally similar, even the best spectral analysis algorithms may not be able to definitively track the peaks; this is even more critical when mobile phase contributions impact the UV spectra. By adding mass spectrometric (MS) detection to the method development system, peaks can be tracked by mass and UV spectra to increase the success of correctly identifying all the peaks of interest.

This application note describes the peak tracking capabilities of the Waters 3100 MS Detector during the method development screening of a series of tricyclic antidepressants (TCAs). This family of compounds is very spectrally similar in the UV spectra but can easily be tracked by MS. The screening process was performed on a Waters Alliance HPLC System with a three-column switching valve, utilizing a Waters 2998 PDA Detector and the 3100 MS Detector (Figure 1). This system provides the means to automatically screen three different columns at two different pHs with two different organic modifiers. This method development scheme with definitive peak tracking produced a method with excellent resolution fit for further optimization. The MS methods were developed with IntelliStart Technology, a simple, easy-to-use software that automatically tunes, develops, and writes the MS method for the compounds of interest.

Figure 1. Alliance HPLC System comprised of the e2695 XC Separations Module and the 3100 Mass Detector.

Experimental

A mix of tricyclic antidepressants was prepared at 10 ng/µL. The individual components were doxepin, nordoxepin, nortriptyline, imipramine, trimipramine, and desipramine. The HPLC system was setup as follows:

LC Conditions

LC system:	Alliance e2695 XC Separations Module with 3-position column switching valve 2998 PDA Detector 3100 MS Detector
Software:	Empower 2 Software
Columns:	SunFire C_{18} 4.6 mm x 50 mm, 3.5 µm XBridge C_{18} 4.6 mm x 50 mm, 3.5 µm Atlantis T3 C_{18} 4.6 mm x 50 mm, 3.0 µm

Injection volume:	10 µL
Temperature:	40 °C
Flow rate:	1.5 mL/min
Mobile phase A:	10 mM ammonium formate buffer pH 3.0
Mobile phase B:	10 mM ammonium bicarbonate buffer pH 10.0
Mobile phase C:	Acetonitrile
Mobile phase D:	Methanol
Gradient:	5% to 95% Organic over 10 min
Needlewash solvent:	70/15/15 acetonitrile/isopropanol/water
PDA conditions:	Wavelength range: 210 nm to 400 nm at 1.2 nm bandwidth Detection wavelength: 250 nm Data rate: 5 Hz Time constant: 0.4 s (Normal)

MS Conditions

Ionization mode:	ESI+
Cone voltage:	35.0 V
Capillary voltage:	3.8 kV
Source temperature:	150 °C

Desolvation temperature:	400 °C
Desolvation gas flow:	800 L/hr
Cone gas flow:	50 L/hr
SIR masses [M+H]:	264.4 Da (nortriptyline) 266.4 Da (nordoxepin) 267.4 Da (desipramine) 280.4 Da (doxepin) 281.4 Da (imipramine) 295.5 Da (trimipramine
Scan range:	50 Da to 500 Da
Scan rate:	5000 Da/s

Results and Discussion

Peak tracking during method development can be accomplished by comparing spectra from a PDA detector or injection of individual standards. The latter of these methods is very time-consuming and is only used when the first method does not yield definitive results. When multiple peaks begin to co-elute, using UV spectra for peak tracking becomes difficult, if not impossible. Peak apex spectra may become distorted, making identification difficult, particularly for spectrally similar compounds. In addition to issues with co-elution, mobile phase can have a significant impact on spectral features. Switching the mobile phase pH (buffer) and organic modifier can significantly impact spectral definition, making it difficult for spectral contrast theory to assign any spectra differences to the compound and not to the spectral changes of the mobile phase. Because of this, it is often necessary to inject individual pure standards for each change in column, pH, and organic modifier. This process results in a very large number of injections and a very large amount of data to process. However, the components' mass is unaffected by co-elution or mobile phase composition and will therefore yield definitive peak tracking. Historically, MS has been viewed as a much more difficult detection

method to optimize. Although MS provided more information about the peaks of interest, its complexity did not make it the first choice for routine method development. However, with the IntelliStart Interface (Figure 2), the instrument can be automatically tuned on the compound of interest; an SIR (single ion recording) method is automatically developed; and the method is written to the chromatographic data system.

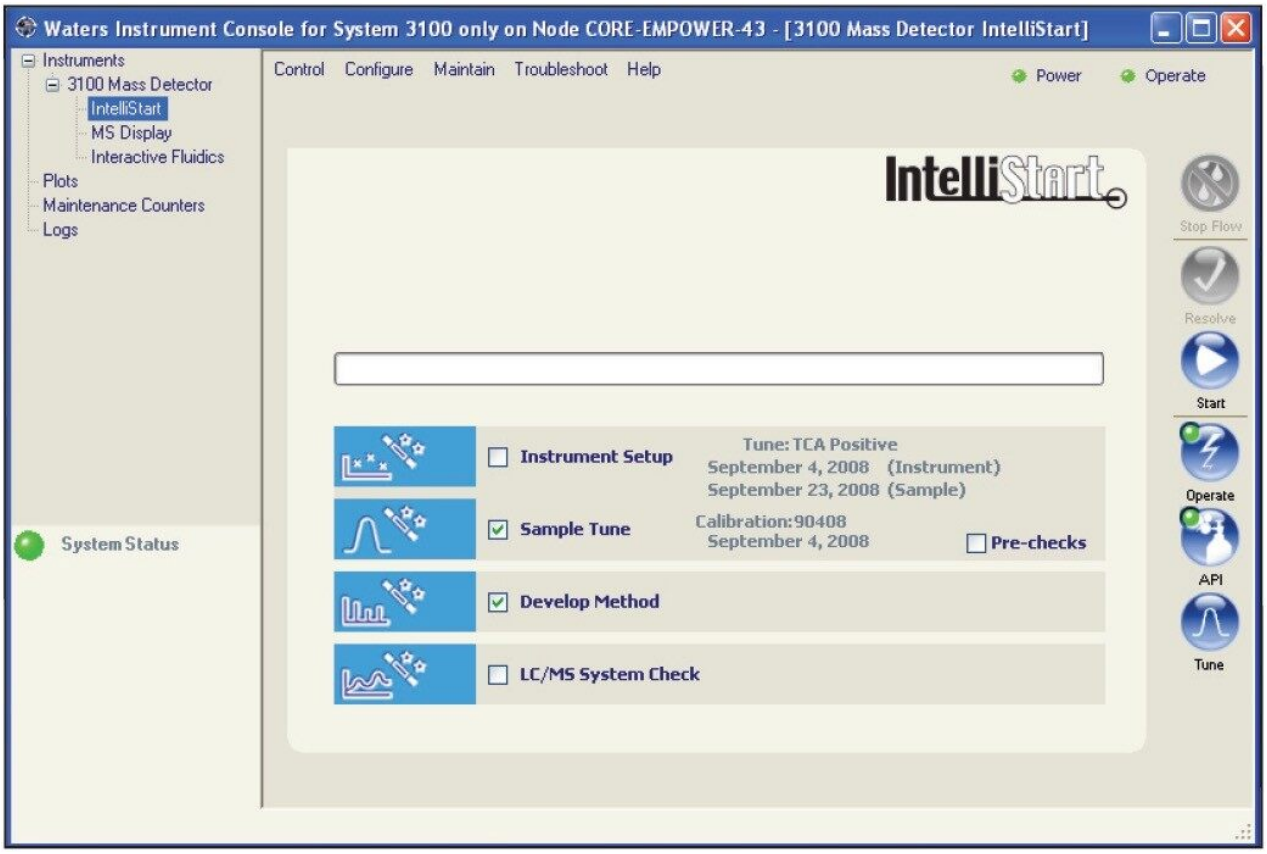


Figure 2. IntelliStart Interface.

For the series of tricyclic antidepressants, the screened parameters included the column (SunFire C_{18}, Atlantis T3 C_{18}, and XBridge C_{18}), pH (ammonium formate at pH 3 and ammonium bicarbonate at pH 10), and organic modifier (methanol and acetonitrile). The resulting matrix of chromatographic runs can be found in Figure 3. The data was collected on both the 2998 PDA and 3100 MS detectors for comparison of peak tracking capabilities.

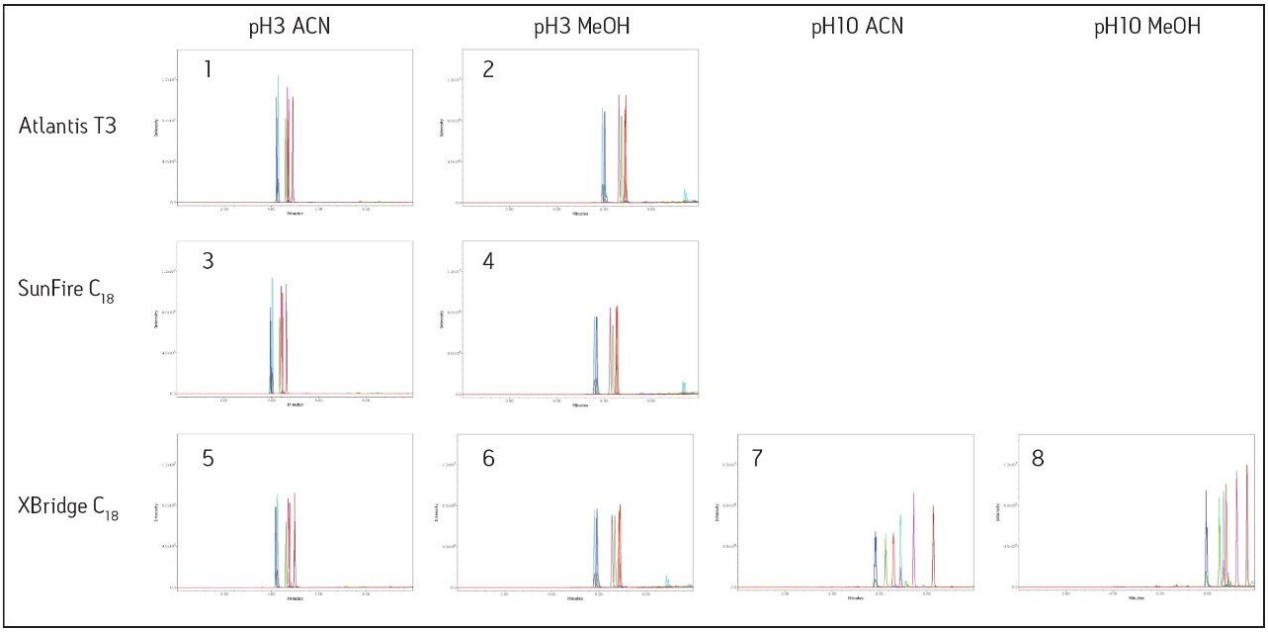


Figure 3. Resulting chromatograms of the automated screening process on the Alliance XC System with the 3100 MS Detector. Traces are the overlay of the MS SIR channels.
Note: Only high pH stable columns were screened at pH 10.

The resulting chromatograms contain numerous co-elutions of peaks. As shown in Figure 3, by monitoring the masses of each of the six components peak tracking is easily achieved. Figure 4 shows the expanded view of two of these traces (runs 3 and 7), comparing the MS SIR channels and the extracted UV channel at 250 nm. From the analysis of the data collected on the 2998 PDA Detector, it is evident that peak tracking is not as definitive as was observed by the 3100 MS Detector. To demonstrate the success and failure of the 2998 PDA Detector for peak tracking during method development, a library of the spectra of the six compound standards was created using the conditions from run 7 (XBridge C_{18} Column, pH 10, acetonitrile). This library provided excellent matching results when compared across multiple subsequent injections at the same chromatographic conditions. Figure 5 shows the spectrum index plot from this run. The spectra from nordoxepin (peak 1) and doxepin (peak 4) are very spectrally similar, as these compounds only differ by a single methyl group. Under these conditions, spectral contrast theory is able to differentiate between the two compounds. This example shows that if the UV spectra do not suffer from co-elutions or spectral shifts from the mobile phase, then definitive peak tracking of very spectrally similar compounds is possible. Figure 6 shows a similar spectrum index plot for the conditions in run 3 (SunFire C_{18} Column, pH 3, acetonitrile). Again, the spectra for nordoxepin and doxepin are very similar; however the spectral overlay shows that these are both different from the spectra collected using the conditions for run 7 (which are the spectra stored in the library used for identification). Most of these spectral differences, are due to the change in buffer. Due to these

significant differences, spectral contrast theory was not able to correctly identify these peaks. In fact, not only did library matching fail, but in some cases, peak purity did not return a reasonable value. Despite the fact that doxepin and nordoxepin co-eluted, peak purity evaluation indicated no co-elution was present due to the spectral similarity of these two components.

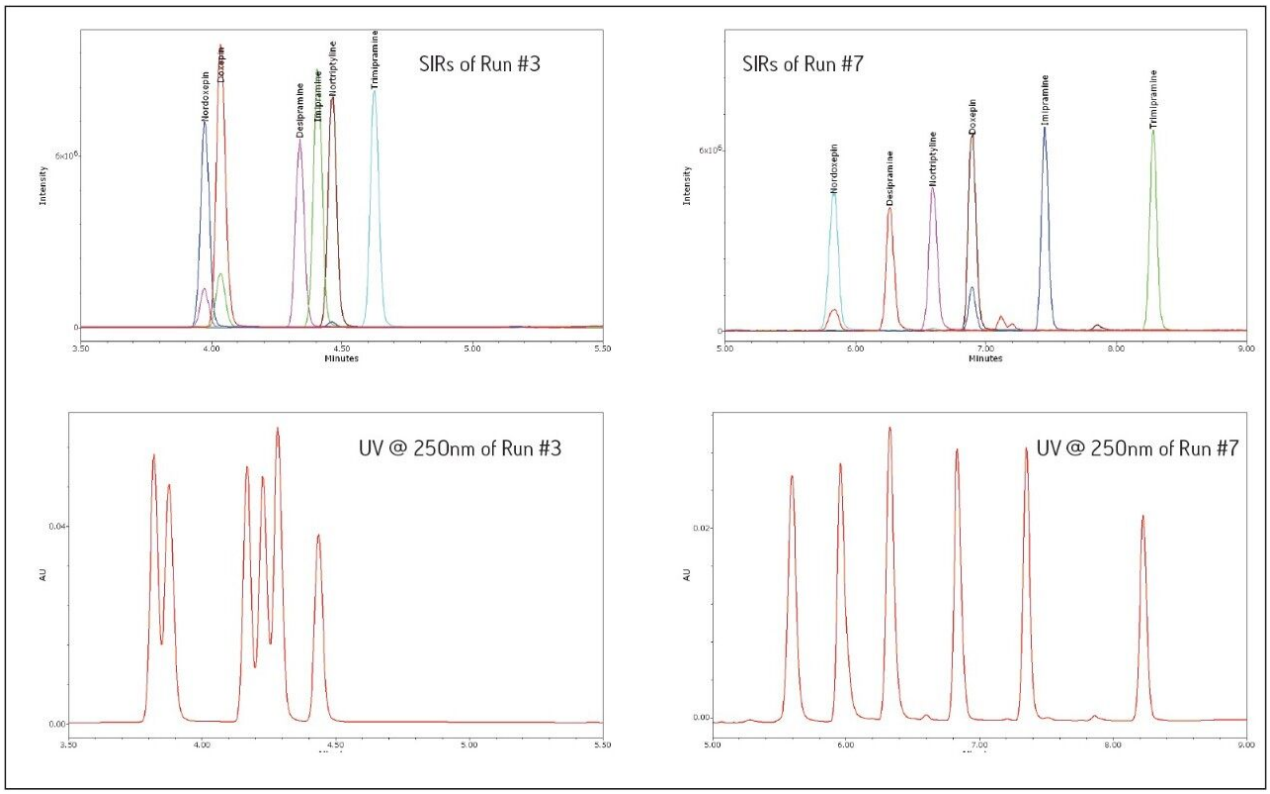
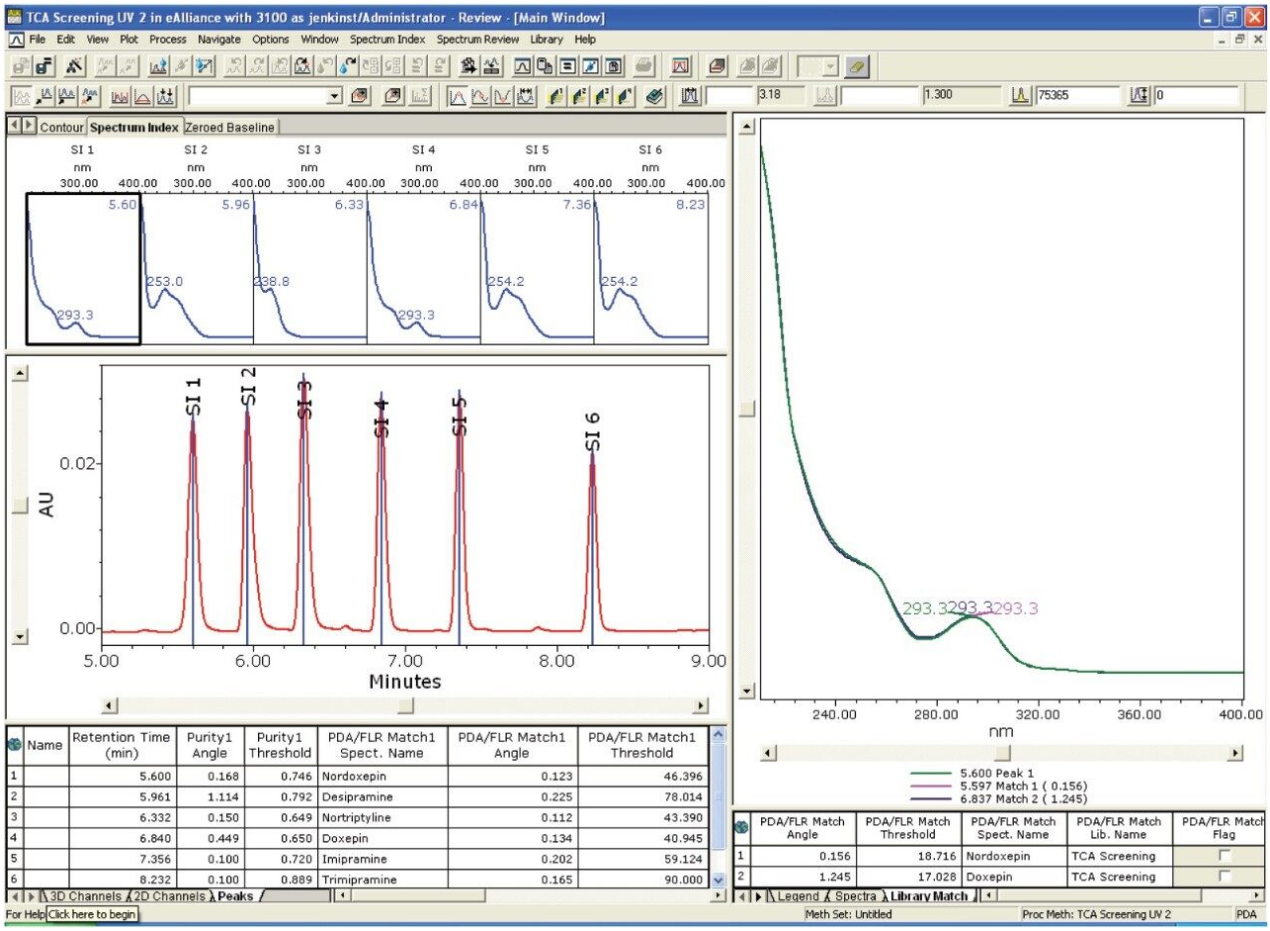


Figure 4. Chromatograms resulting from overlaid MS SIR channels and UV channel. (A) MS SIR on SunFire C $_{18}$ Column at pH 3 with acetonitrile; (B) UV on SunFire C_{18} Column at pH3 with acetonitrile; (C) MS SIR on XBridge C_{18} Column at pH 10 with acetonitrile; (D) UV on XBridge C_{18} Column at pH 10 with acetonitrile.



	Name	Retention Time (min)	Purity1 Angle	Purity1 Threshold	PDA/FLR Match1 Spect. Name	PDA/FLR Match1 Angle	PDA/FLR Match1 Threshold
1		5.600	0.168	0.746	Nordoxepin	0.123	46.396
2		5.961	1.114	0.792	Desipramine	0.225	78.014
3		6.332	0.150	0.649	Nortriptyline	0.112	43.390
4		6.840	0.449	0.650	Doxepin	0.134	40.945
5		7.356	0.100	0.720	Imipramine	0.202	59.124
6		8.232	0.100	0.889	Trimipramine	0.165	90.000

	PDA/FLR Match Angle	PDA/FLR Match Threshold	PDA/FLR Match Spect. Name	PDA/FLR Match Lib. Name	PDA/FLR Match Flag
1	0.156	18.716	Nordoxepin	TCA Screening	
2	1.245	17.028	Doxepin	TCA Screening	

Figure 5. UV spectrum index plot and library matching results of the TCA separation on XBridge C_{18} Column at pH 10 with acetonitrile. Although compounds are spectrally similar, spectral contrast theory is able to identify the peaks when the same chromatographic conditions are used for the standards and unknowns. The 2998 PDA Detector match name assigned for all of the unknowns corresponded with the results by MS.

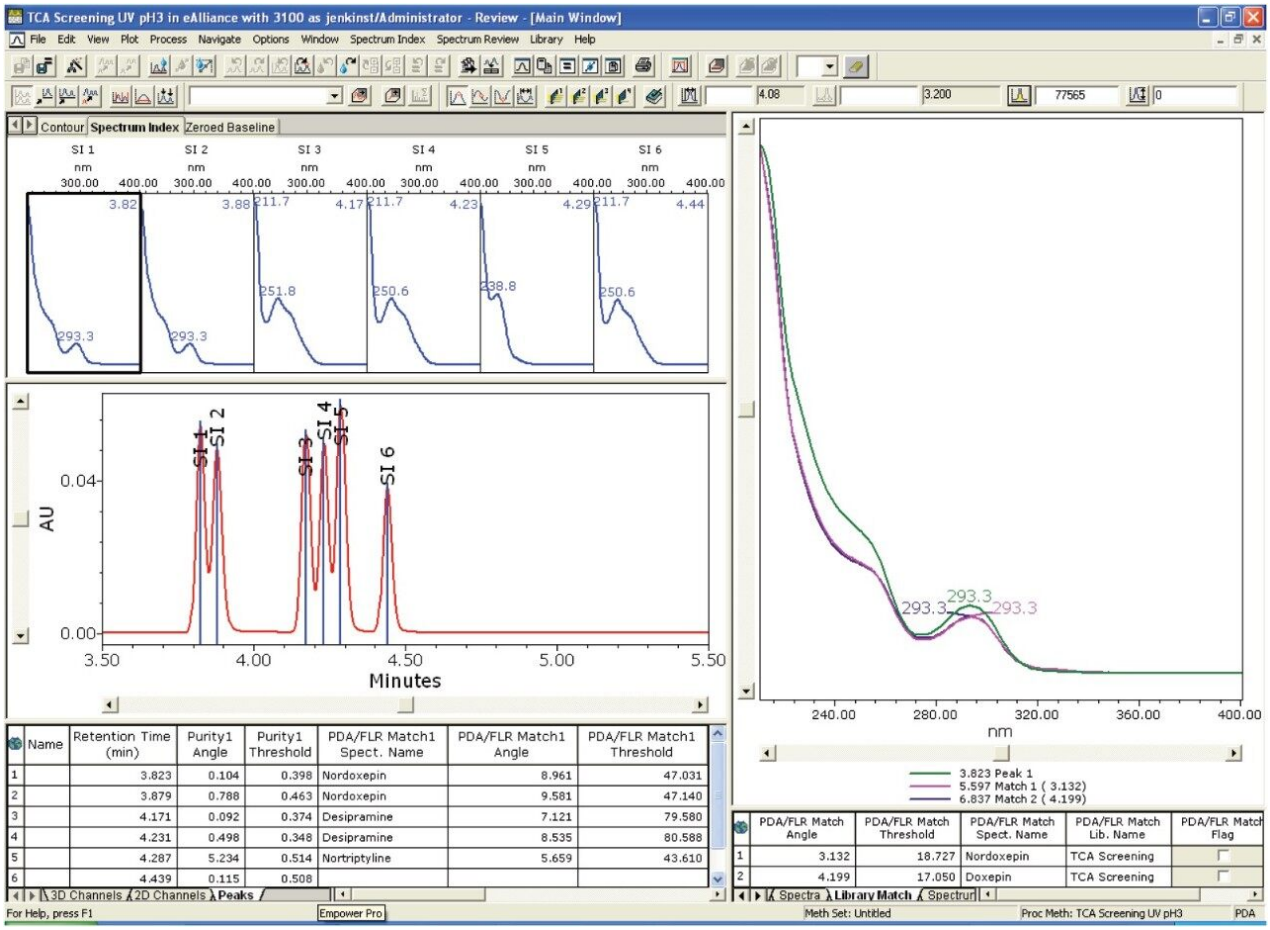


Figure 6. UV spectrum index plot and library matching results of the TCA separation on SunFire C_{18} Column at pH 3 with acetonitrile. The spectra of the unknowns from this separation were compared against the same library as the unknowns in Figure 5. However, the spectra differ significantly from the library spectra and peak tracking becomes impossible. The same PDA match name was assigned to both doxepin and nordoxepin, incorrectly identifying these peaks.

Because of the failure of spectral contrast theory to track the peaks of interest during the method development of these TCAs, it would be necessary to inject individual standards of each of the method screening conditions. If the screening process relied on single injections of each sample and individual standards, then using PDA as the source of peak tracking would have required 56 injections (1 sample + 6 standards x 8 conditions) for a total analysis time of 16 hours (which includes column equilibration times of 15 minutes at column switch and 5 minutes post injection). If the same method development screening process is run using the 3100 MS Detector for peak tracking, it would require only eight injections for a total analysis time of 3 hours, 20 minutes (including column equilibration time of 15 minutes at column switch), a time-savings of 80%.

One additional point of interest is that some of the SIRs for the TCAs contained multiple peaks. A scan was performed to look at the MS spectra of each compound (Figure 7). The TCAs are very close in molecular weight. Therefore, in the SIR at 267, the C12 M+1 peak of desipramine and the C13 M+1 peak of nordoxepin are observed. Similarly in the SIR at 281, the C12 M+1 peak of imipramine and the C13 M+1 peak of doxepin are observed.

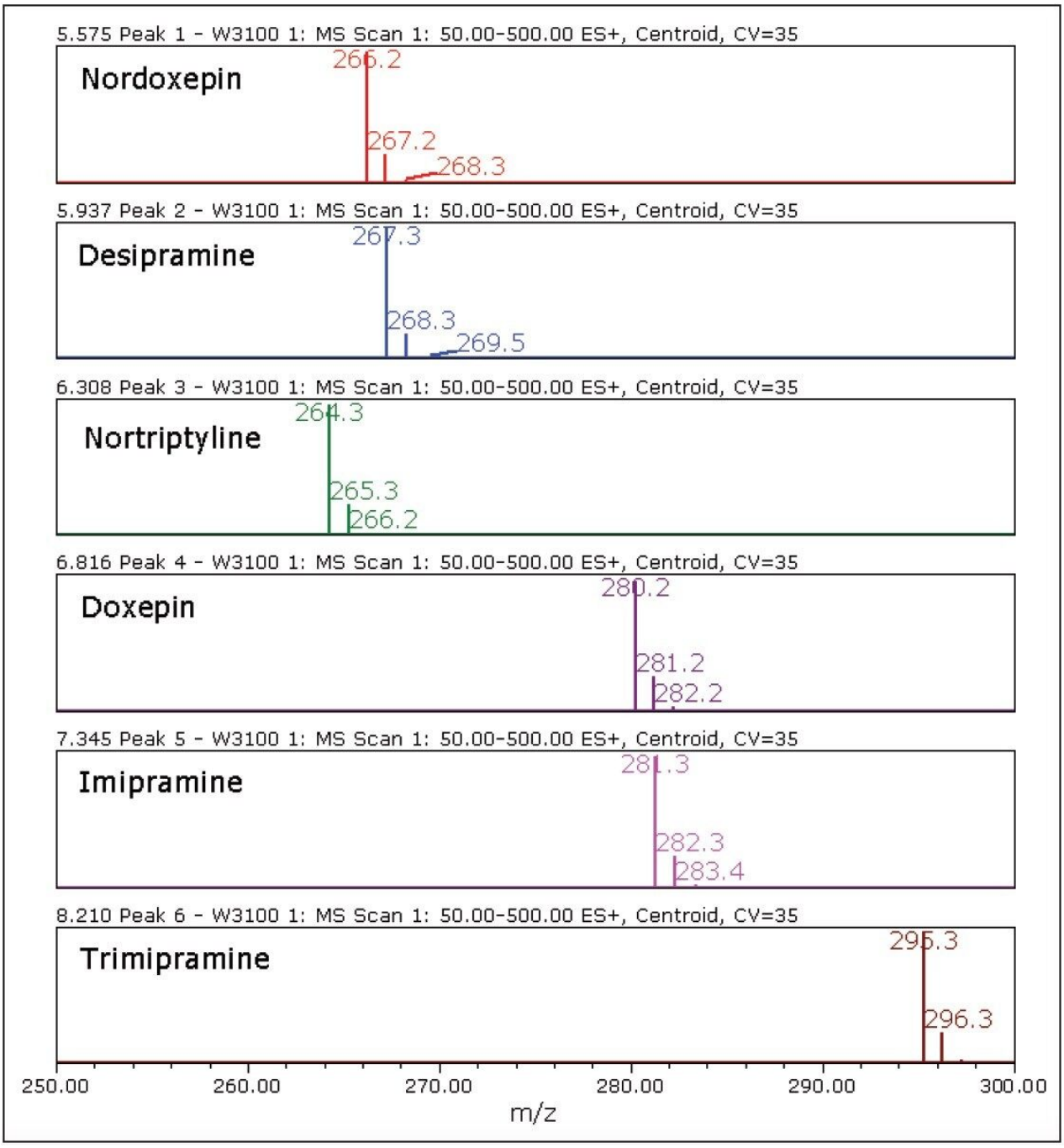


Figure 7. MS Spectra of the TCA demonstrating the C12 and C13 peaks of some the TCAs are at the same nominal mass and therefore result in multiple peaks in the SIRs.

Conclusion

- The method development capabilities of the Alliance HPLC System with column selection capabilities and the combined detection capabilities of the Waters 2998 PDA and 3100 MS detectors yield a very powerful method development tool.
- Utilizing a systematic screening process for method development can dramatically reduce method development timelines.
- The 3100 Mass Detector yields invaluable information for method development, especially when traditional peak tracking methods, such as UV spectra, do not provide adequate tracking capabilities.

References

1. Jenkins, K., ***et. al***. Streamlining HPLC Method Development, Waters Poster, Literature Number WA43196.

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Alliance HPLC System <https://www.waters.com/534293>

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

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