Improving selectivity of triazole derivative metabolites

Using SelexION® Differential Mobility Separation Technology

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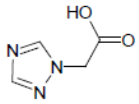
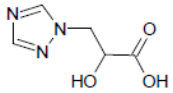
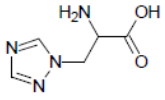
1,2,4-triazole (TRZ), triazole alanine (TAL), triazole acetic acid (TAA) and triazole lactic acid (TLA) are metabolites that commonly occur as plant or soil metabolites of triazole fungicides. They are collectively known as the "triazole derivative metabolites" (Table 1). Therefore, the determination of levels of triazole derivative metabolites in soils and plant materials is the key to assessing the fate of triazole fungicides.

Analysis of these metabolites by LC-MS/MS is challenging because of their polar nature and their poor fragmentation efficiency (fragmentation into a single fragment only). In addition, when dealing with soil and plant extracts, LC-MS/MS analysis typically suffers from high chemical noise and many interferences.

The SelexION® Technology is a planar differential mobility separation device (DMS) that attaches between the curtain plate and orifice plate of the QTRAP® 5500 System. Here, the use of DMS in the form of SelexION Technology[1] coupled to a QTRAP 5500 LC-MS/MS System was used to improve the selectivity of LC-MS/MS detection of triazole derivative metabolites.

Table 1. Structure of studied triazole derivative metabolites.

Compound	Structure
1,2,4-triazole (TRZ)	
Triazole acetic acid (TAA)	
Triazole lactic acid (TLA)	
Triazole alanine (TAL)	

Key features of SelexION Technology for triazole derivative metabolite quantification

- SCIEX SelexION Technology with the QTRAP Systems is a powerful tool to help solve tough selectivity challenges such as resolving chromatographic interferences, eliminating a high baseline, or separating isomers
- Optimization of assays using DMS is straight forward
- Use of SelexION Technology provided similar accuracy in quantification for the triazole derivative metabolites compared to data collected without DMS highlighting the quantitative reproducibility of the technology
- Use of DMS significantly reduced the background observed for the compounds studied in several plant matrices
- Triazole derivative metabolites were quantified at the desired LOQ levels of 0.01 mg/kg with excellent precision and accuracy

Methods

Sample preparation: The following matrices were evaluated in the present study: carrot leaves, carrot roots, 2 different lots of rape green material, rape seeds, lettuce head, grape, and water. Each matrix was extracted using the following procedure:

- Weighed 5 g of material
- Homogenization in methanol/water (4:1)
- Filtration with diatomaceous earth
- Addition of ^{15}N-labeled internal standard
- SPE cleanup using C18 material
- Evaporation of eluate to dryness
- Reconstitution in water

Each sample was prepared at three different concentrations: control (0), recovery LOQ (0.01 mg/kg) and 10x LOQ (0.1 mg/kg).

Chromatography: LC was performed using a Shimadzu UFLCXR system with an Aquasil C18 (3x150 mm; 3 µm) column using a 2 minute gradient of 100% to 90% aqueous. The mobile phase consisted of (A) water + 0.5% acetic acid and (B) methanol + 0.5% acetic acid.

Mass spectrometry: A SCIEX QTRAP® 5500 LC-MS/MS System with Turbo V™ Ion Source and the electrospray ionization (ESI) probe was used. The source was operated at 600°C with Gas 1 and Gas 2 at 40 and 80 psi, respectively. Curtain Gas was set at 20 psi.

For the SelexION Device settings, the separation voltage (SV) was set to 3400 V and compensation voltage (CoV) were tuned for each analyte of interests to obtain highest selectivity (Figure 1). No chemical modifier was introduced. The DMS cell was used in “transparent” mode (SV and CoV turned off) to mimic conventional MS/MS operation. MRM transitions for all compounds, retention time (RT) and CoV values are listed in Table 2.

Table 2. MRM parameters. MRM transitions, optimized CoV values and retention times for each of the triazole derivative metabolites.

Compound	Q1 (Da)	Q3 (Da)	Compensation voltage (CoV)	Retention Time (min)
TRZ	*70*	*43*	*-17.0*	*1.70*
TAA	*128*	*70*	*-4.0*	*1.91*
TLA	*158*	*70*	*0.5*	*1.96*
TAL	*157*	*70*	*2.0*	*1.30*

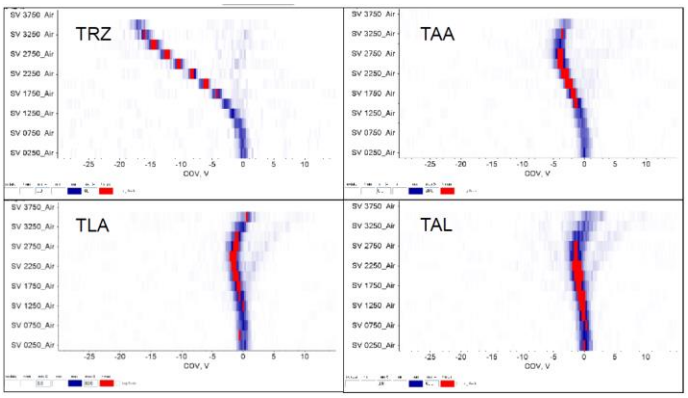


Figure 1. Optimization of the separation and compensation voltages. The SV and the CoV of each triazole derivative metabolite was optimized to obtain highest sensitivity and selectivity.

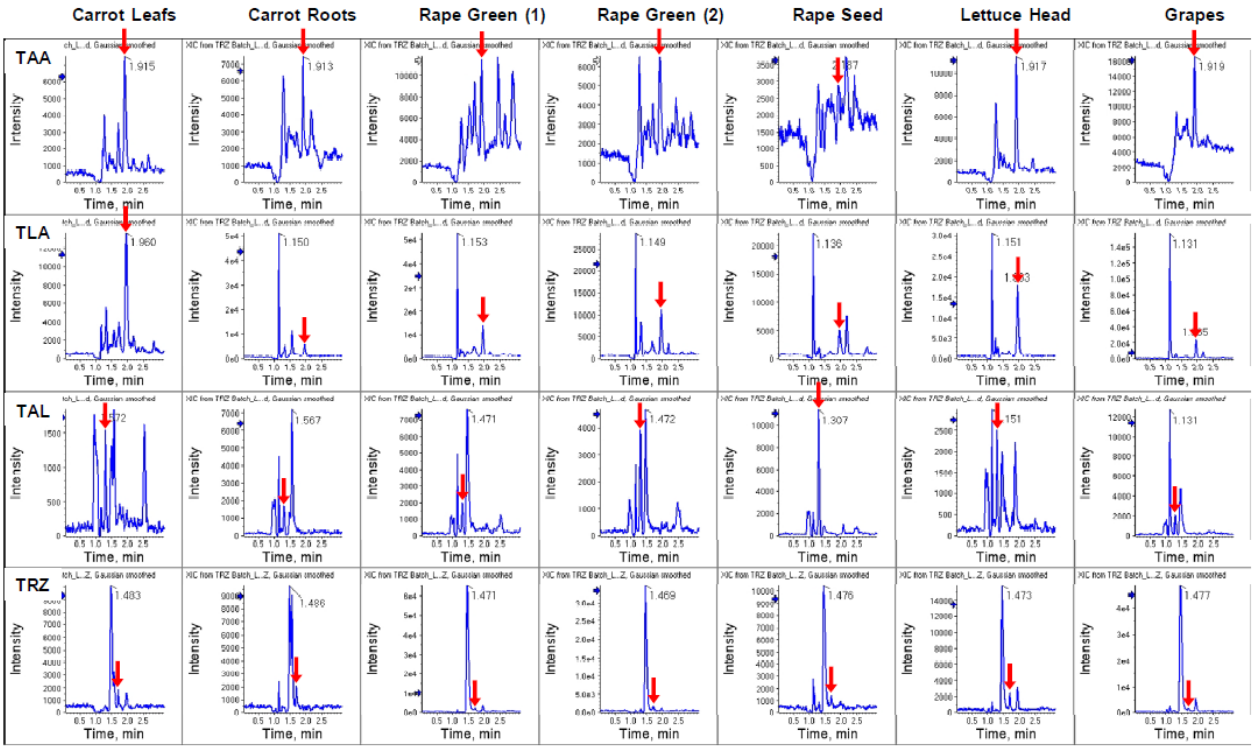
Improved selectivity with optimized DMS parameters

High background and matrix interferences is a pervasive analytical challenge associated with the LC-MS/MS analysis of triazole derivative metabolites in plant matrices (Figure 2, top). Each analyte exhibits variable interferences in the form of high background levels as well as multiple LC peaks, which depend on the matrix being analyzed. Furthermore, minimal chromatographic separation was achieved due to the polar nature of the analytes.

To address this issue, DMS was used in the development of a quantitative assay for these analytes. The goal is to determine combination of the separation voltage (SV) and the compensation voltage (CoV) that best improve selectivity for each analyte by removing interferences. This can be done by infusion of the analyte and generating a response map (Figure 1). It is imperative that the optimized compensation voltages be tested in matrix to ensure good separation from matrix interferences.

Figure 2 (bottom) shows the same matrix spiked samples analyzed with DMS, optimized for each triazole derivative metabolite. Due to the increased selectivity, single LC peaks were observed for each analyte, with the exception of TAL in some matrices. Even in cases where LC interferences were observed, the dominant LC peaks were associated with TAL. In addition, the noise level was significantly reduced.

DMS off



DMS on

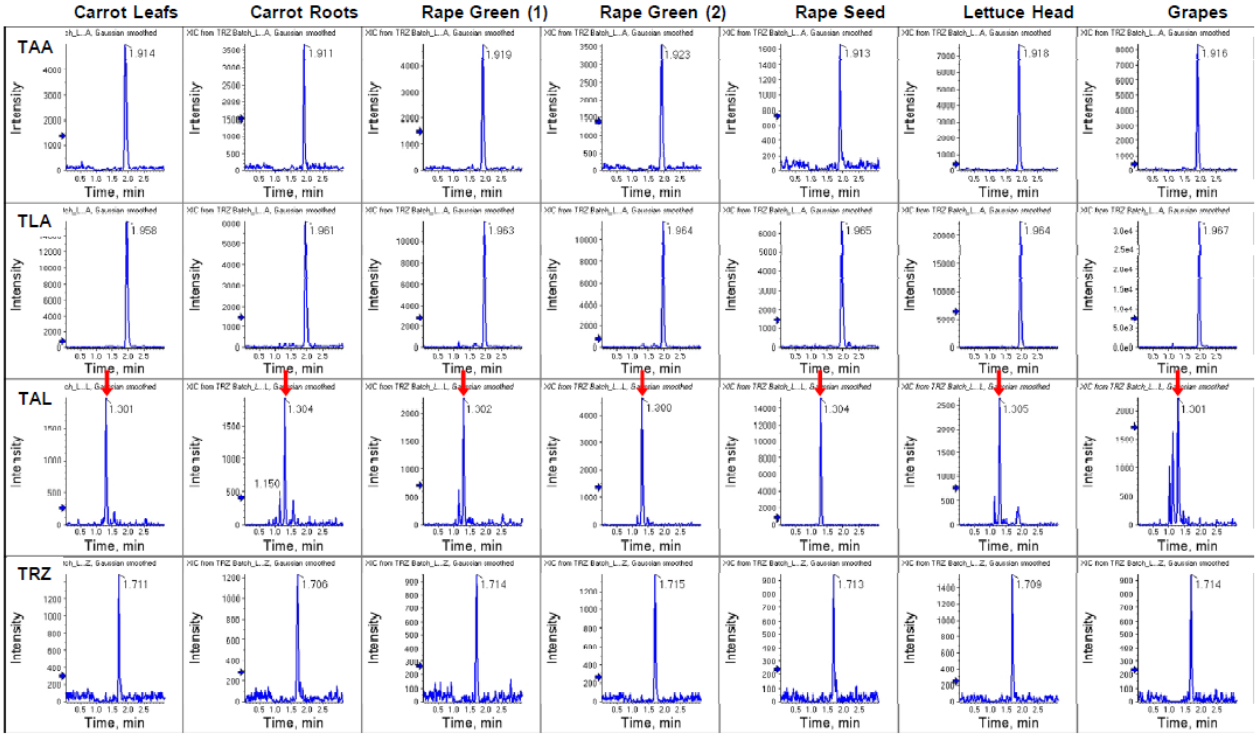


Figure 2. Signal for triazole derivative metabolites in various plant matrices. MRM traces for each metabolite is shown for recovery LOQ (0.01 mg/kg) in various matrices. (Top) Acquisition was performed with the DMS cell in transparent mode (DMS off) to show the high background possible in various plant matrices. (Bottom) Acquisition was repeated with the DMS cell operating with optimized CoV for each analyte. Significant improvements in selectivity were observed for each analyte in all matrices.

Quantifying noise reduction

In order to quantify the reduction of the noise level, all spiked samples (at 0.01 and 0.1 mg/kg) were integrated by summing all intensities within a 15 sec window around the retention time of the analyte (LC peak width at peak base). This value was divided by the sum of all intensities within a 60 sec window (4x LC peak width). If the noise levels (either chromatographically resolved or unresolved) around the peak of interest is low, than this ratio approaches a value of 1. A value significantly below 1 indicates strong matrix interferences. Figure 3 shows the results obtained for all spiked samples when DMS was operated in transparent mode (A) and optimized for each analyte (B).

Figure 3A shows that the noise around the LC peaks is elevated, with a ratio below 0.7 in many cases, even when the analytes are spiked at 10x LOQ. In contrast, Figure 3B shows that the ratio is greater than 0.8 in all but 3 cases (TAL in 3 matrices), at both the LOQ and 10x LOQ level when DMS is used. Thus, DMS provided additional selectivity that increases confidence in the detection of triazole derivative metabolites, reduced the LC separation requirements, and simplified the data review and peak integration process.

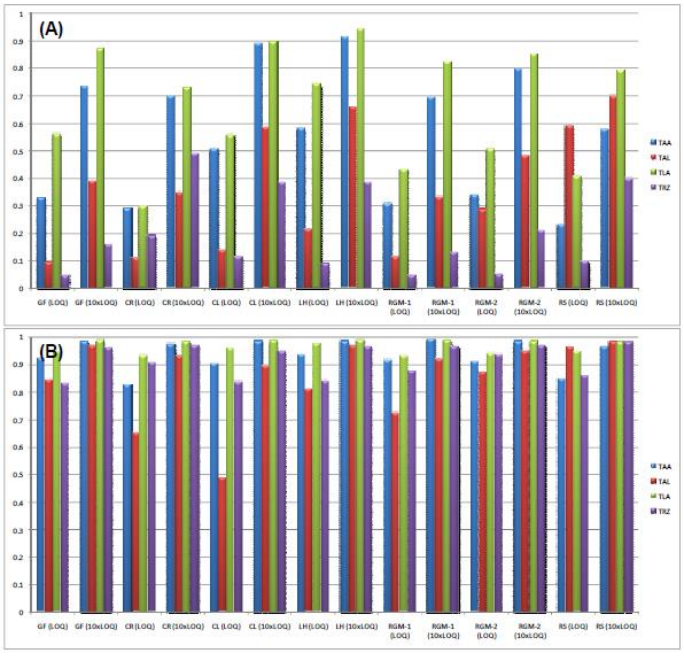


Figure 3. Complexity of noise around LC peak of interest across for all matrices. The amount of signal in a 15sec window around the peak is divided by a 60sec window to represent the amount of noise present. This is done at both the LOQ (right) and 10x LOQ (left). (A) Data collected with DMS in transparent mode shows values below 1 indicating significant competing signal. (B) Data collected with DMS on and optimized for each analytes shows values near 1 indicating that most signal around the elution time is the analyte of interest.

Figure 4 shows the MRM signal across multiple CoV values over the entire LC analysis. This is performed by monitoring the MRM transition while ramping CoV throughout the chromatographic run. This provides a “map” in CoV space of the analyte versus interferences of the same MRM. Rape green spiked at 10x LOQ was used to generate the CoV map of TRZ and TAA. Figure 4 shows that the analytes of interest are clearly separated from the chemical interferences in terms of CoV values, in addition to LC time.

Quantitative performance

Finally, quantitative performance under three different LC-MS/MS configurations was compared: DMS on, DMS off (cell mounted and operated in transparent mode) and DMS removed (cell physically removed). Linearity (linear regression with 1/x weighting), precision and accuracy were found to be similar using all three configurations (Table 3), confirming that the SelexION Device can be used with the same quantitative abilities of traditional LC-MS/MS analysis.

Conclusions

The combination of DMS with LC-MS/MS provides a high degree of selectivity for the analysis to triazole derivative metabolites across several extracted plant matrices. Significant reduction in noise levels was obtained when using the SelexION Device. Single LC peaks were obtained for TRZ, TAA, and TLA in all matrices and for TAL in most matrices.

Overall, combining the DMS with the SCIEX QTRAP® 5500 System enabled the detection of triazole derivative metabolites with high confidence (at desired LOQ levels of 0.01 mg/kg) and good accuracy. This technique proved to be extremely useful in the detection and monitoring of these species.

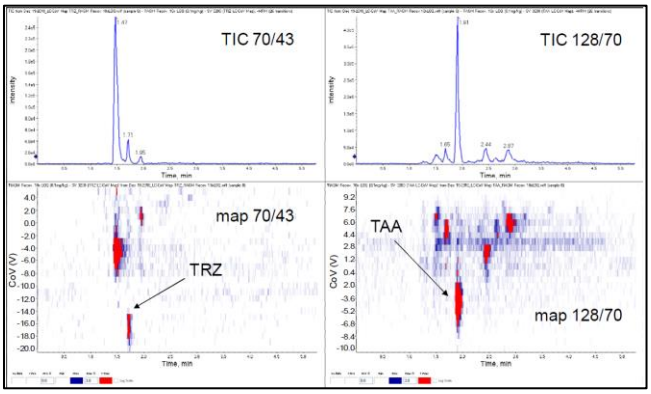


Figure 4. Compensation voltage maps. Separation of interferences of TRZ (left) and TAA (right) at 10x LOQ in rape green in the CoV space and on the LC time scale.

Table 3. Accuracy obtained for single injections of solvent standards using three instrument configurations (DMS on, DMS off in transparent mode, and DMS removed).

Compound		TAA		TLA		TAL		TRZ	
	Actual conc. (ng/mL)	Calibrated conc. (ng/mL)	Accuracy	Calibrated conc. (ng/mL)	Accuracy	Calibrated conc. (ng/mL)	Accuracy	Calibrated conc. (ng/mL)	Accuracy
DMS on	0.5	0.57	*114.2*	*0.53*	*106.0*	*0.58*	*115.7*	*0.50*	*100.1*
	1.0	*1.07*	*107.1*	*0.94*	*94.4*	*0.89*	*89.0*	*1.03*	*102.5*
	2.5	*2.42*	*96.7*	*2.52*	*100.6*	*2.43*	*97.4*	*2.59*	*103.4*
	5.0	*4.66*	*93.1*	*4.88*	*97.6*	*4.90*	*98.0*	*4.84*	*96.8*
	10	*8.54*	*85.4*	*10.2*	*101.5*	*9.95*	*99.5*	*9.65*	*96.5*
	50	*51.8*	*103.5*	*50.0*	*100.0*	*50.3*	*100.5*	*50.4*	*100.8*
DMS off	0.5	*0.55*	*110.6*	*0.55*	*109.0*	*89.6*	*89.6*	*0.42*	*84.5*
	1.0	*1.02*	*101.8*	*0.97*	*96.6*	*105.8*	*105.8*	*1.16*	*115.8*
	2.5	*2.43*	*97.4*	*2.43*	*97.3*	*94.8*	*94.8*	*2.67*	*106.8*
	5.0	*4.63*	*92.6*	*4.96*	*99.3*	*102.2*	*102.2*	*4.73*	*94.6*
	10	*9.62*	*96.2*	*9.72*	*97.2*	*109.5*	*109.5*	*9.79*	*97.9*
	50	*50.8*	*101.5*	*50.4*	*100.8*	*98.1*	*98.1*	*50.2*	*100.5*
DMS removed	0.5	*0.386*	*77.3*	*0.30*	*59.1*	*59.1*	*59.1*	*0.48*	*96.3*
	1.0	*1.05*	*105.0*	*0.98*	*97.5*	*97.5*	*97.5*	*1.03*	*102.5*
	2.5	*2.60*	*104.1*	*2.77*	*110.8*	*110.8*	*110.8*	*2.29*	*91.5*
	5.0	*5.30*	*106.0*	*5.90*	*120.5*	*118.0*	*118.0*	*5.28*	*105.6*
	10	*11.0*	*110.4*	*12.1*	*120.5*	*120.5*	*120.5*	*10.5*	*105.2*
	50	*48.6*	*97.3*	*47.0*	*94.0*	*94.0*	*94.0*	*49.4*	*98.8*

References

1. SelexION® Technology: The solution to selectivity challenges in quantitative analysis. SCIEX technical note RUO-MKT-02-3251-A.

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