

Improving the LC-MS/MS Selectivity of Triazole Derivative Metabolites with AB SCIEX SelexION™ Technology

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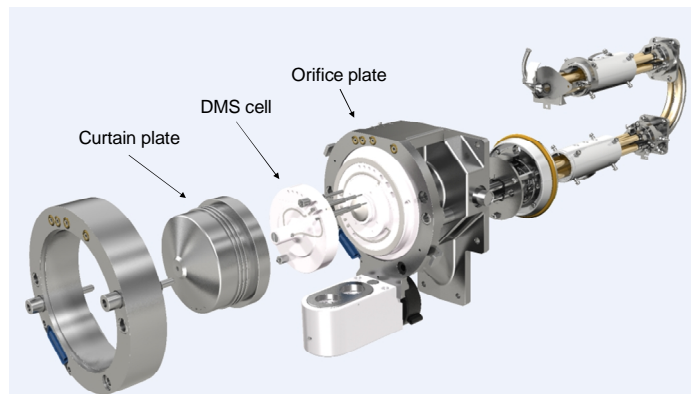
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Introduction

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 and 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 in assessing the fate of triazole fungicides.

However, analysis of these metabolites by Liquid Chromatography coupled to tandem Mass Spectrometry (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. Here we evaluated the use of differential mobility spectrometry (DMS) using the AB SCIEX SelexION™ technology coupled to a QTRAP® 5500 LC/MS/MS system to improve the selectivity of LC-MS/MS detection of triazole derivative metabolites.



SelexION™ Technology

The SelexION™ Technology is a planar differential mobility device (DMS) that attaches between the curtain plate and orifice plate of the 5500 QTRAP® system. Gas draws the ions through the DMS cell towards the orifice while an asymmetric waveform applied to the plates, which alternates between high field and low field. Unlike traditional ion mobility, ions are not separated in time as they traverse the cell. They are separated in trajectory based on difference in their mobility between the high field and low field portions of the applied Separation Voltage (SV). As the ions migrate towards the walls of the DMS cell at different rates, they will be separated. By applying a second voltage offset (the Compensation Voltage, CoV) the trajectory of a desired ions can be corrected along the axis of the DMS cell and transmitted to the mass analyzer (Figure 1).

Chemical modifiers, like isopropanol, methanol, or acetonitrile, can be and introduced into the transport gas via the curtain gas, to alter the separation characteristics of analytes.

The planar design of SelexION™ Technology yields a stable, easy to tune system with high resolving power over a short distance. This gives high speeds and short residence times, resulting in minimal diffusion losses and enabling the use of short MS/MS cycle times. By simply turning off the separation voltage, the cell becomes transparent with ions moving normally

Table 1. Structure of studied triazole derivative metabolites

Compound	Structure
1,2,4-triazole (TRZ)	<chem>C1=NC=CN1</chem>
Triazole acetic acid (TAA)	<chem>CC(=O)OCC1=CN=CN1</chem>
Triazole lactic acid (TLA)	<chem>CC(O)(C(=O)O)CN1=CN=CN1</chem>
Triazole alanine (TAL)	<chem>CC(N)(C(=O)O)CN1=CN=CN1</chem>

along the centre line of the device. Thus it is possible to transmit ions through the mobility cell when not using the DMS mode.

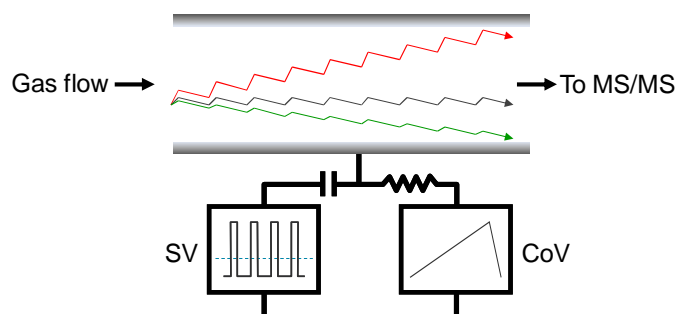


Figure 1. Differential Mobility Separation Process. Innovative planar design of the DMS cell uses an asymmetric RF waveform (SV) to separate ions based on differential mobility between the high and low fields. The compensation voltage (CoV) is used to correct the trajectory of the ion of interest which traverses the cell and into the orifice while interferences are deflected into the cell walls.

Method Details

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:

- Weighing 5g of material
- Homogenization in methanol/water (4/1) with an Ultra Turrax
- Filtration with Celite
- SPE cleanup using C18 material
- Evaporation of eluate to dryness
- Reconstitution in water
- Addition of ¹⁵N-labeled internal standard

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

LC separation

LC was performed using a Shimadzu UFLC_{XR} 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.

MS/MS Detection

An AB SCIEX QTRAP[®] 5500 system with Turbo V[™] 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.

SelexION[™] Settings

SV was set to 3400 V and CoV were tuned for each analyte of interests to obtain highest selectivity (Figure 2). 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 transitions, optimized CoV, and retention time (RT) of each triazole derivative metabolite

Compound	MRM	CoV	RT (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

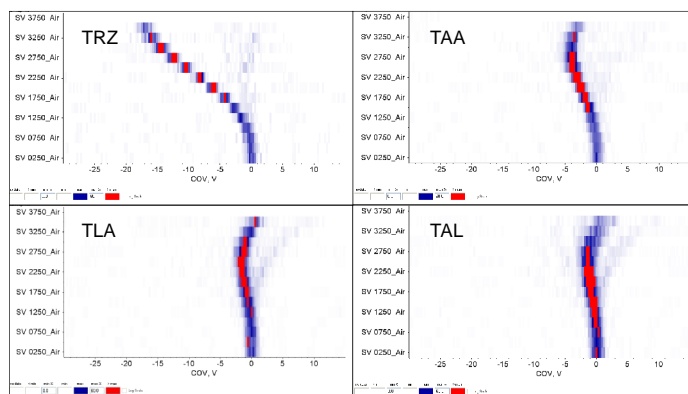


Figure 2. Optimization of CoV of each triazole derivative metabolite to obtain highest selectivity

Results

High background and matrix interferences are the analytical challenges associated with the LC-MS/MS analysis of triazole

derivative metabolites (Figure 3). As can be seen, each analytes exhibits variable interferences (high background levels as well as multiple LC peaks) that depends on the matrix analyzed.

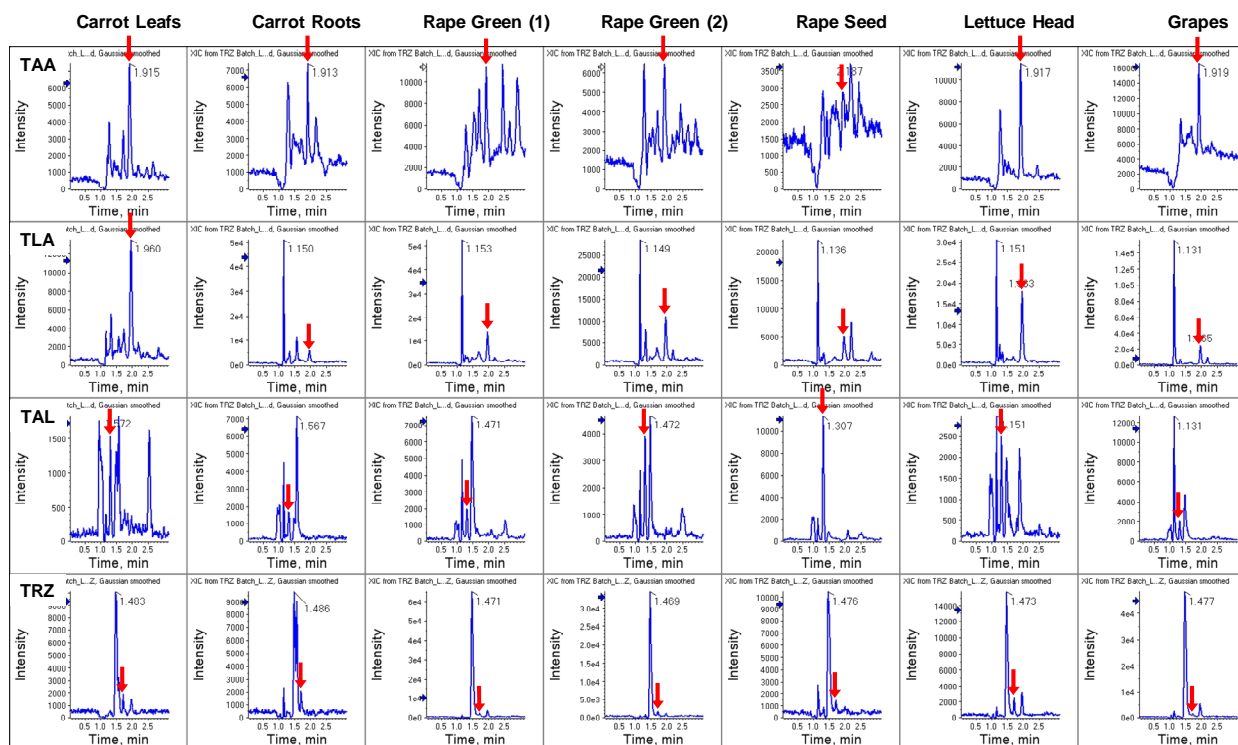


Figure 3. MRM traces for recovery LOQ (0.01 mg/kg) in various matrices when acquisition was performed with DMS cell in transparent mode

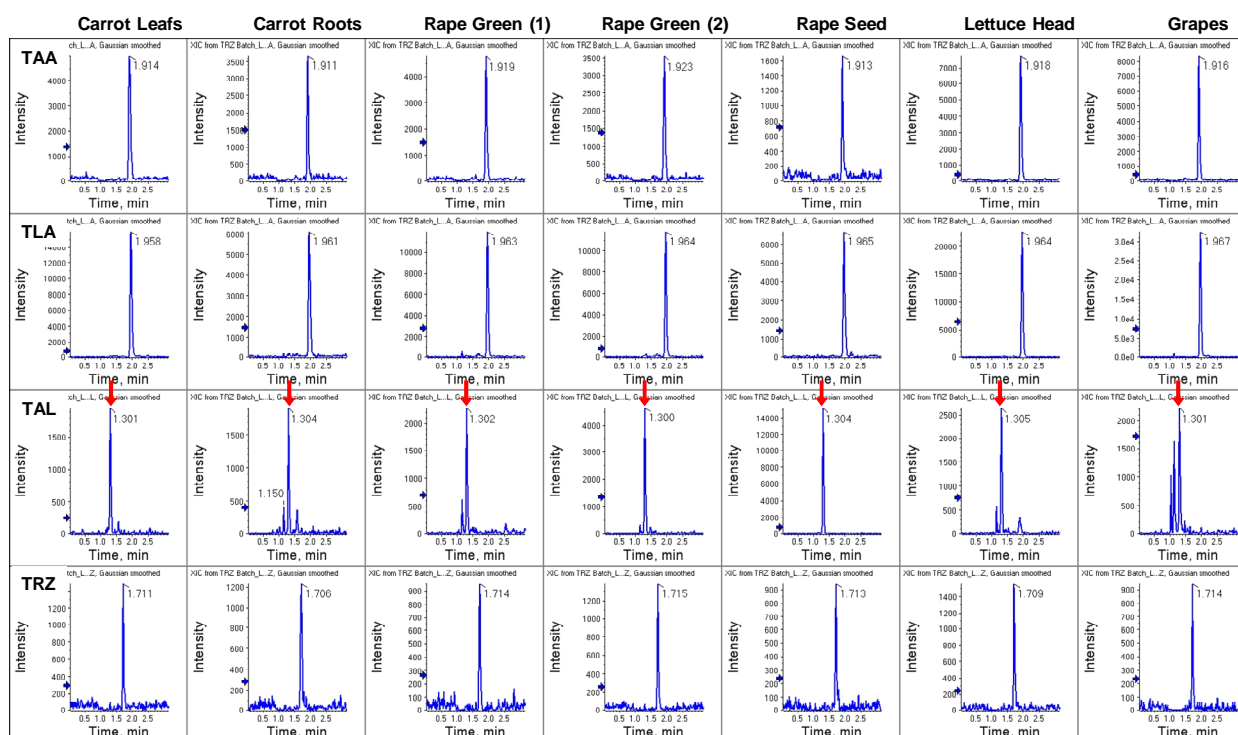


Figure 4. MRM traces for recovery LOQ (0.01 mg/kg) in various matrices when acquisition was performed with DMS cell optimized for each analyte

Furthermore, minimal chromatographic separation was achieved due to the polar nature of the analytes, but still required to minimize isotope contribution in MRM channels.

Figure 4 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.

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 5 shows the results obtained for all spiked samples when DMS was operated in transparent mode (A) and optimized for each analyte (B).

Figure 5 A shows that the noise around the LC peaks is elevated since the ratio is still <0.7 in many cases even when the analytes are spiked at 10x LOQ. In contrast, Figure 5 B 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, the SelexION™ Technology provided additional selectivity that increases confidence in the detection of triazole derivative metabolites, reduced the LC requirements, and simplified the data review and peak integration process.

Figure 6 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 6 clearly shows that the analytes of interest are clearly separated from the chemical interferences in terms of CoV values, in addition to LC time.

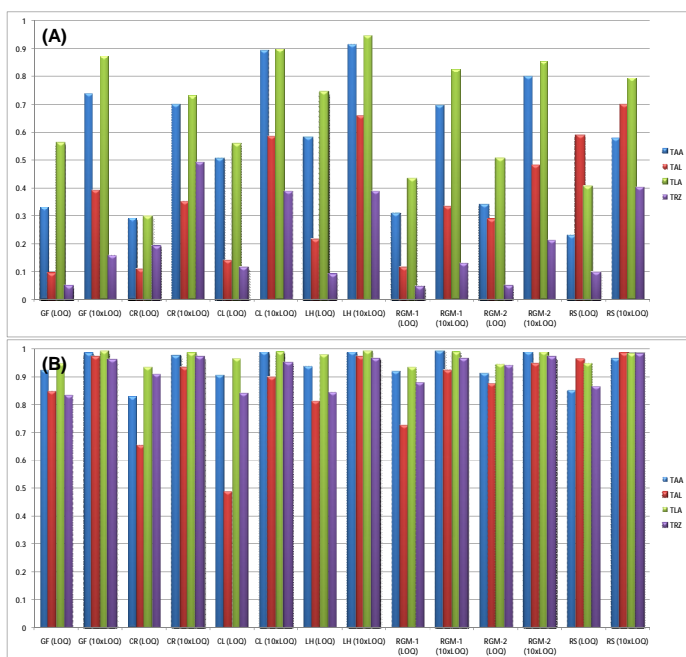


Figure 5. Complexity of noise around LC peak of interest across for all matrices at the LOQ and 10x LOQ with DMS operated in (A) transparent mode and (B) optimized for each analytes

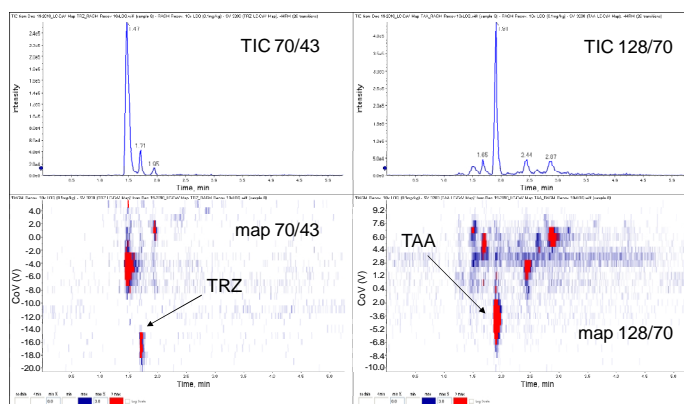


Figure 6. Separation of interferences of TRZ (left) and TAA (right) at 10x LOQ in rape green in the CoV space and on the LC time scale

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).

Table 3. Precision and accuracy obtained for single injections of solvent standards using three instrument configurations (DMS on, DMS off, and DMS removed)

Compound	TAA			TLA		TAL		TRZ	
	Actual conc. (ng/mL)	Calculated conc. (ng/mL)	Accuracy	Calculated conc. (ng/mL)	Accuracy	Calculated conc. (ng/mL)	Accuracy	Calculated conc. (ng/mL)	Accuracy
<i>DMS on</i>	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
<i>DMS off</i>	0.5	0.55	110.6	0.55	109.0	0.45	89.6	0.42	84.5
	1.0	1.02	101.8	0.97	96.6	1.06	105.8	1.16	115.8
	2.5	2.43	97.4	2.43	97.3	2.37	94.8	2.67	106.8
	5.0	4.63	92.6	4.96	99.3	5.11	102.2	4.73	94.6
	10	9.62	96.2	9.72	97.2	10.1	109.5	9.79	97.9
	50	50.8	101.5	50.4	100.8	49.1	98.1	50.2	100.5
<i>DMS removed</i>	0.5	0.386	77.3	0.30	59.1	0.30	59.1	0.48	96.3
	1.0	1.05	105.0	0.98	97.5	0.98	97.5	1.03	102.5
	2.5	2.60	104.1	2.77	110.8	2.77	110.8	2.29	91.5
	5.0	5.30	106.0	5.90	120.5	5.90	118.0	5.28	105.6
	10	11.0	110.4	12.1	120.5	12.1	120.5	10.5	105.2
	50	48.6	97.3	47.0	94.0	47.0	94.0	49.4	98.8

Summary

The combination of LC-DMS-MS/MS provides a high degree of selectivity for the analysis to triazole derivative metabolites across several matrices extracted. Significant reduction in noise levels was obtained when using the AB SCIEX SelexION™ Technology. 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 AB SCIEX QTRAP® 5500 system enabled the detection of triazole derivative metabolites

with high confidence at desired LOQ levels of 0.01 mg/kg and excellent precision. This technique proved to be extremely useful in the detection and monitoring of these species.

References

- ¹ B.B. Schneider, T. R. Covey, S.L. Coy, E.V. Krylov, E.G. Nazarov: *Int. J. Mass Spectrom.* 298 (2010) 45-54
- ² B.B. Schneider, T. R. Covey, S.L. Coy, E.V. Krylov, E.G. Nazarov: *Anal.Chem.* 82 (2010) 1867-1880

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