

# Improved Quantitation of Fragile Carbamate, Organophosphate and Other Pesticides by Automatic MS/MS Method Generation

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## ABSTRACT

**Purpose:** Many pesticides that are tightly controlled in the food supply are prone to early fragmentation in the high pressure region near the inlet of a mass spectrometer, lowering the sensitivity of targeted quantitation methods. We perform an automatic compound-dependent tuning of the ion optics at the inlet to reduce this effect and gain sensitivity.

**Methods:** Ion funnel RF amplitude was tuned at ion source conditions and flow rates consistent with those used in the intended targeted LC-MS/MS experiment, by sweeping the control voltage and performing kernel intensity function estimation to locate the maximum. The relative advantage of this procedure was evaluated by comparing LC-MS/MS experimental performance across two orders of magnitude of analyte concentration, from 10 to 500 ppb.

**Results:** Automated compound-specific tuning of the ion funnel RF amplitude increased LC-MS/MS method sensitivity for representative organophosphates and carbamates approximately 2.5- to 5-fold and for a modestly fragile chloroanilide herbicide by approximately 50%.

## INTRODUCTION

While still widely used in food production, the use of carbamate and organophosphate pesticides is highly regulated due to acute and long-term cumulative toxicity in humans.<sup>1,2</sup> In some jurisdictions, certain pesticides from these classes have been banned.<sup>3,4</sup> Correspondingly low maximum residue limits (e.g. 20 µg/kg aldicarb in fruit in the EU<sup>5</sup>) require analytical methods to have high sensitivity.

Many carbamate and organophosphate pesticides and certain other pesticides are “fragile” in the sense that they are prone to early fragmentation in the high pressure region containing the entrance optics for the mass spectrometer, thus lowering the sensitivity of quantitation methods. For example, the preliminary analysis of aldicarb + H<sup>+</sup>, optimizing RF amplitude in tandem with ion source conditions at a flow rate of 5 µL/min, found that lowering the ion funnel RF amplitude of a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer from the 60 V amplitude determined during instrument calibration (considering ion mass alone) to the 30 V suggested by an automatic compound optimization procedure obtained 1.4-fold improvement in the SRM signal intensity of continuously infused analyte.

Using an automated method-generation procedure, we optimized the ion funnel RF amplitude of a Thermo Scientific™ TSQ Altis™ mass spectrometer and compared the sensitivity across two orders of magnitude, finding RF amplitude optimization increases sensitivity by compound-dependent factors ranging from 1.5 to 5-fold and additionally decreases the background intensity.

## MATERIALS AND METHODS

### Sample Preparation

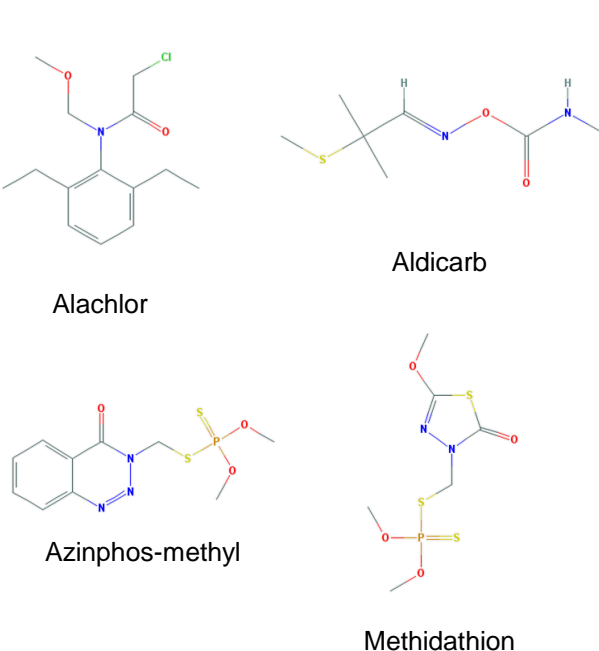
A panel of representative fragile pesticides (Table 1, Figure 1) were purchased from Sigma-Aldrich® and prepared as concentrated stock solutions in water from which mixtures were prepared for analysis. Chromatographic mobile phase was prepared as follows:

- A: 0.1% Formic acid, 2% MeOH and 5 mM ammonium formate in water  
B: 0.1% Formic acid, 2% water and 5 mM ammonium formate in MeOH

**Table 1. Fragile pesticides investigated, specifying the particular adduct identity and precursor mass for which the ion funnel was tuned and the sensitivity was evaluated.**

Compound	Chemical Formula	Monoisotopic Mass	Precursor Mass	Adduct	Classification
Alachlor	C <sub>14</sub> H <sub>20</sub> ClN <sub>2</sub> O <sub>2</sub>	265.118	270.088	H <sup>+</sup>	aromatic amide
Aldicarb	C <sub>7</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S	190.078	208.024	NH <sub>4</sub> <sup>+</sup>	carbamate ester
Azinphos-methyl	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> PS <sub>2</sub>	317.008	317.008	H <sup>+</sup>	organic thiophosphate
Methodathion	C <sub>8</sub> H <sub>11</sub> N <sub>2</sub> O <sub>4</sub> PS <sub>2</sub>	301.982	319.908	NH <sub>4</sub> <sup>+</sup>	organic thiophosphate

**Figure 1. Skeletal formulas.**



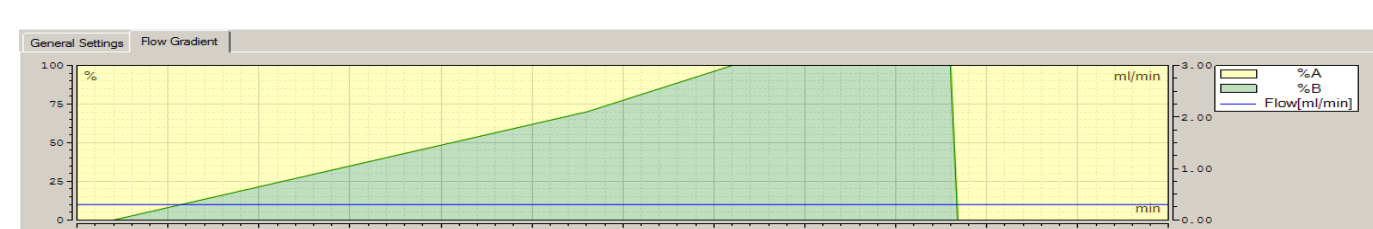
### Method Generation

MS/MS methods were generated using the batch Compound Optimization feature of TSQ Altis 3.0 Tune instrument control software. In brief: 10 µg/mL pesticide standards delivered at 5 µL/min flow rate by a syringe pump were teed in to 50:50 chromatographic mobile phase A and B delivered at flow 100 µL/min with source conditions identical to those used (inf.) in acquisition. Tuning curves were acquired (automatically) by parking the first quadrupole mass filter at the precursor ion *m/z*, rapidly sweeping the ion funnel RF control voltage across its operating range, and applying kernel intensity function estimation to the Q1 SIM signal.<sup>6</sup>

### Identification and Quantitation

Pesticides were separated, identified, and quantitated on a Thermo Scientific™ Vanquish™ HPLC and a TSQ Altis triple quadrupole mass spectrometer using a HESI source and Thermo Scientific™ Accucore™ aQ column (100 x 2.1 mm, 2.6 µm) held at 25 °C. Flow rate, injection volume, and source conditions were as follows: 300 µL/min, 1 µL injection per run, 350 °C vaporizer temperature, 325 °C ion transfer tube temperature, sheath gas 30, aux gas 6, sweep gas 1. Mobile phase gradient was as below (Figure 2). The identification of the pesticide was based on retention time, a minimum of two product ions, and ion-ratio confirmation using selected reaction monitoring (SRM). Product ions monitored were in both the optimized and unoptimized case. Those selected by the batch compound optimization procedure and generated at the collision energy determined during the optimization. Three replicates were run at each concentration level.

**Figure 2. Mobile phase composition gradient.**



## RESULTS

### Optimization Results

Each of the compounds investigated in this study optimized at a much lower ion funnel RF amplitude than that determined during the instrument tuning procedure by analysis of calibration mix (Table 2). The optimal amplitudes do not order by mass; ion funnel tuning in the high pressure entrance region of our mass spectrometer is truly compound-dependent. Note that even within the same family of compound, there does not appear to be an order; the methidathion + NH<sub>4</sub><sup>+</sup> ion has a lower *m/z* than azinphos-methyl + H<sup>+</sup> yet optimizes at a higher voltage.

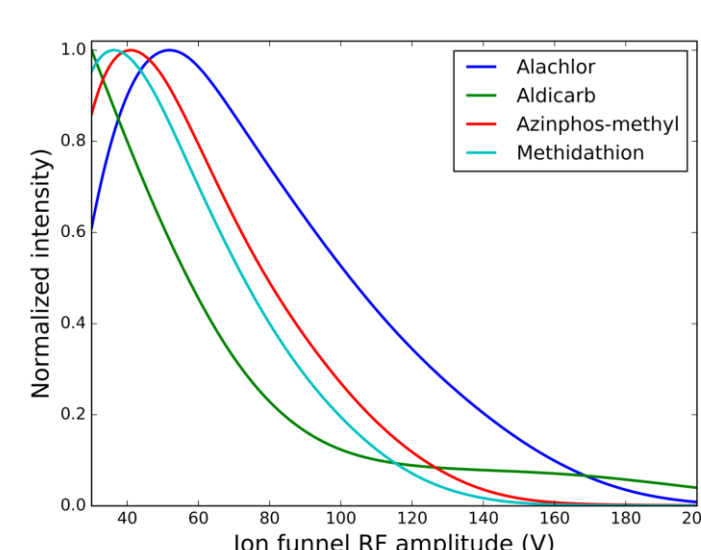
In Figure 3, the tuning curve for aldicarb has no local optimum, so the global optimum is selected instead. The lower operating limit of the RF amplitude of a TSQ Altis mass spectrometer is 30 V. We expect that, were it possible to lower the amplitude further, we could increase intensity by setting the RF amplitude lower. Aldicarb is the most fragile of the compounds tested and, accordingly, also benefits the most from compound optimization (Table 3, Figures 4 and 6.) Likewise the difference between the optimal voltage and the system tuning is the least for alachlor and it also benefits (per Table 3) considerably less than the others from compound optimization.

High *m/z* ions (approximately 1000 Th and greater), not shown, tune in a qualitatively different manner, with a plateau at high RF amplitude. The tunings shown in Figure 3 are representative of the behavior of low or mid-range *m/z* ions.

**Table 2. Optimal ion funnel RF amplitudes found by the batch compound optimization feature, compared with the instrument tuning at the precursor *m/z*. Instrument tuning values are under normal operation determined by linear interpolation between set points at the instrument calibrant masses, and were retrieved from the TSQ Altis instrument by the function calls that are used while setting up acquisition.**

Compound	Ion Funnel RF Amplitude Instrument Tuning	Optimized
Alachlor	74.3	51.9
Aldicarb	65.7	30.0
Azinphos-methyl	80.5	41.1
Methodathion	80.7	36.9

**Figure 3. Tuning curves collected by the batch compound optimization routine, used to infer the optimal ion funnel RF amplitude. 30 V is the lower operating limit of the ion funnel electronics.**

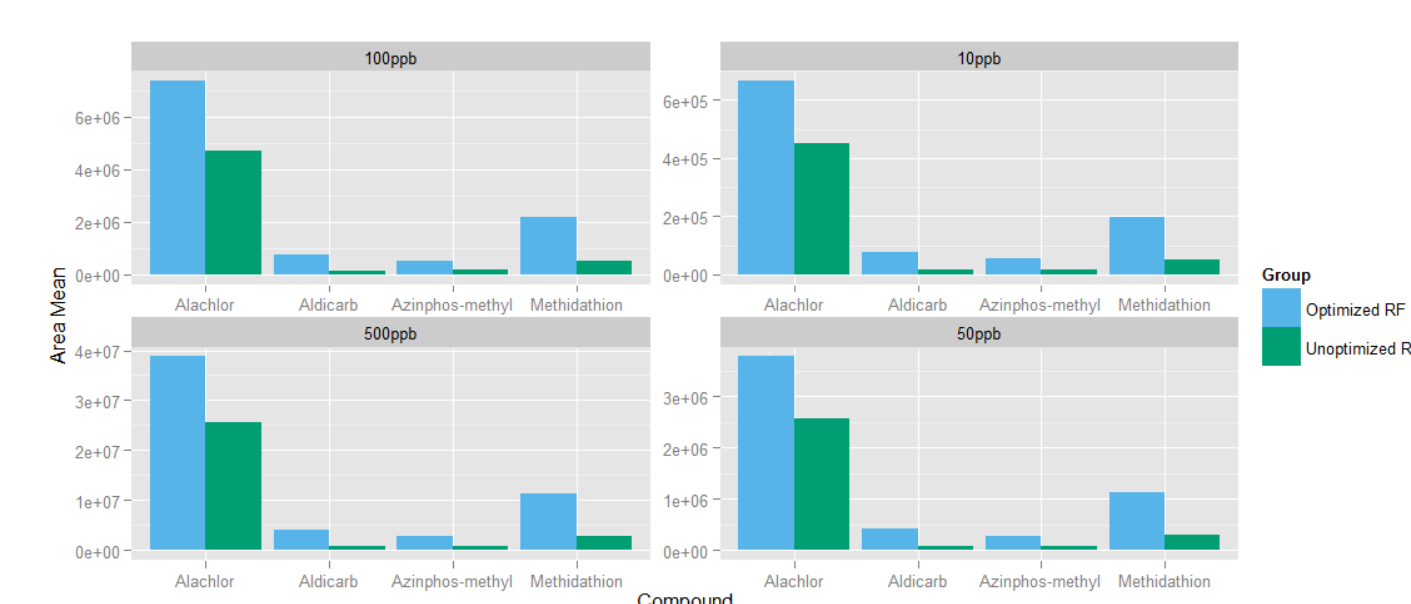


### Sensitivity Gain from Compound Optimization

Using the ion funnel RF amplitudes determined by the automated method generation procedure caused marked increase in the chromatographic peak area for all compounds under study (Figure 4). The magnitude of the improvement ranged from 1.5 x for alachlor to 5.3 x for aldicarb (Table 3). The two organophosphate compounds tested exhibited similar factors of improvement.

Detector response was linear across the range of concentrations measured. (Figure 6, right-most column.) Accordingly, the ratio of chromatographic peak areas for optimized and unoptimized acquisitions are identical across the range of concentrations investigated (Figure 5.)

**Figure 4. Peak area comparison.**



**Table 3. Ratio of peak area with optimized RF amplitude to that with unoptimized RF amplitude. Computed from 500 ppm data.**

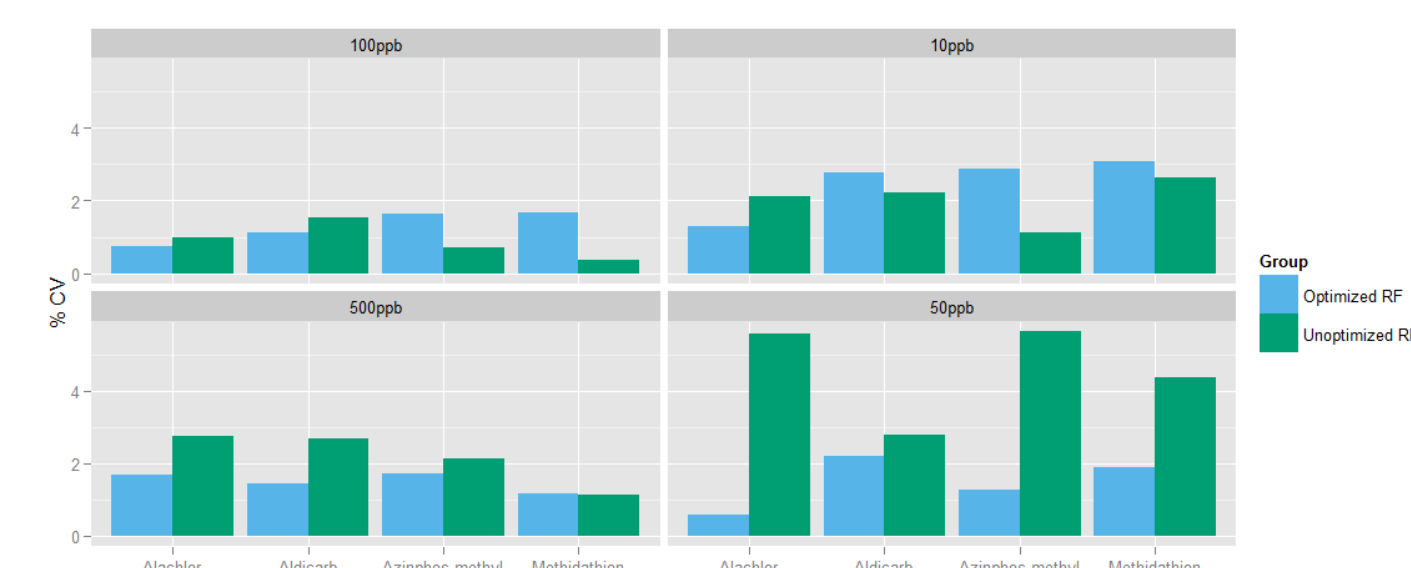
Compound	Improvement Factor
Alachlor	1.5
Aldicarb	5.3
Azinphos-methyl	3.2
Methodathion	3.9

### Data Quality Improvement

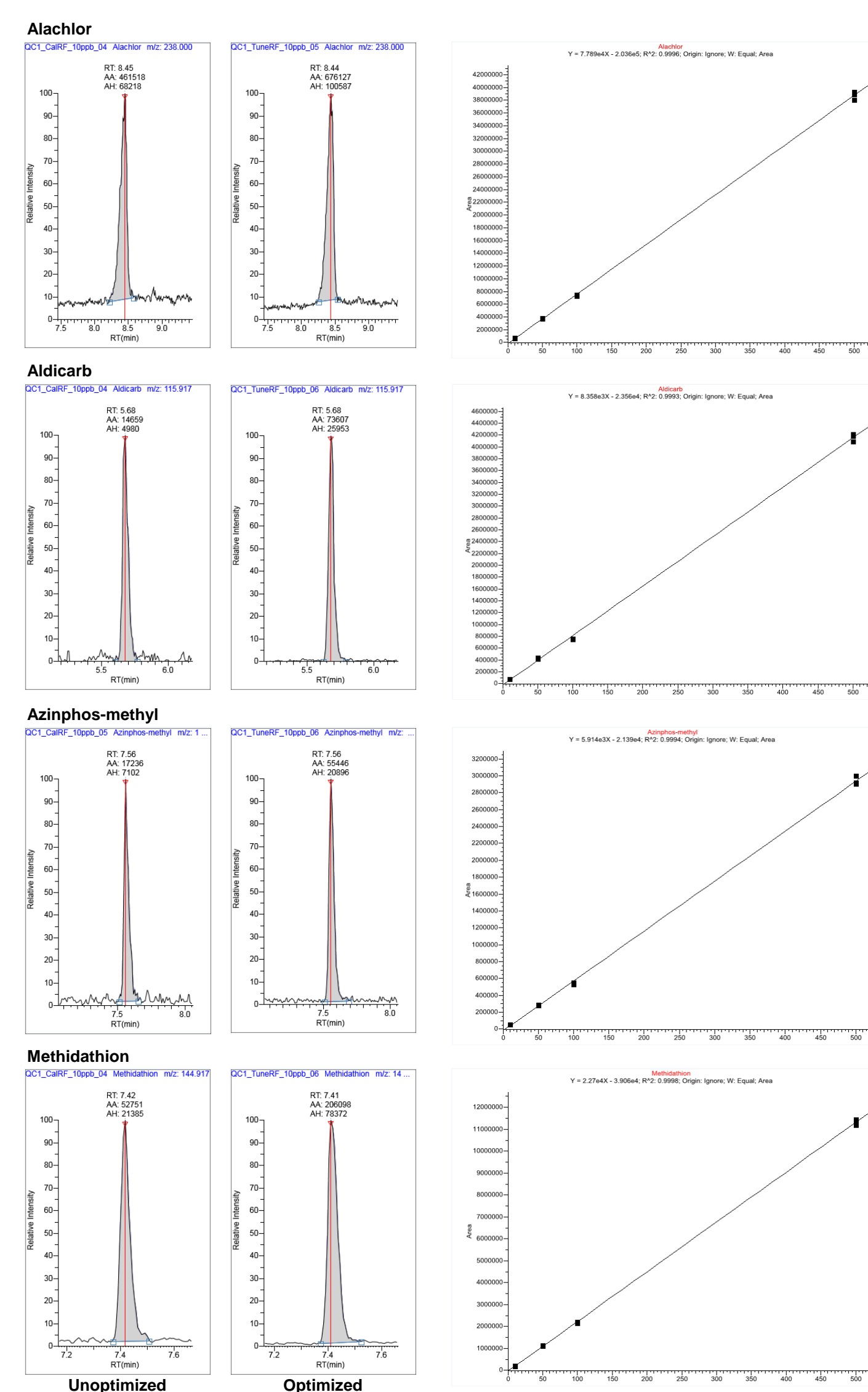
Coefficient of variation (Figure 5) tends to be better for the optimized method but there is some inconsistency, especially at the lowest concentration investigated. More than three replicates would have to be collected to estimate CV with certainty.

Careful inspection of the baselines of the chromatograms in Figure 6 (all acquired at 10 ppb concentration) shows that optimization of the ion funnel RF amplitude not only raises the intensity of the ion of interest, but it also reduces, at least relatively, the background intensity. This is due to the compound-specific nature of the tuning and provides a necessary control. Were the compound optimization procedure merely correcting a mistuned instrument, the background and peak would increase by approximately the same factor. As would be expected, the reduction of background is most dramatic for aldicarb and only barely perceptible for alachlor.

**Figure 5. Data quality as quantified by coefficient of variation (% RSD) shows improvement from optimization of the ion funnel RF amplitude in most cases. More replicates trends toward improvement by optimization. More replicates should be run to provide a definitive answer.**



**Figure 6. Chromatograms and calibration curves, acquired at 10 ppb concentration, for each of the four compounds tested in this investigation. Note the difference in the baseline background ion signal between the optimized and unoptimized methods.**



## CONCLUSIONS

Compound-specific optimization of the ion funnel RF amplitude in the entrance region of the TSQ Altis mass spectrometer, using the optimization tool built in to its instrument control software, gave a 3- to 4-fold improvement in peak area for two organophosphates and a greater than 5-fold improvement in peak area for a representative carbamate pesticide, together with a reduction in the relative intensity of the background signal. We strongly recommend that tuning the ion funnel or stacked ring ion guide (SRIG or s-lens) in the entrance region of Thermo Scientific™ mass spectrometers—and similar RF devices in the entrance region of other mass spectrometers—be a routine step in development of analytical methods for compounds in these classes and others that are prone to CID (collision-induced dissociation) at low energy in high pressure regions of a mass spectrometer's ion optical beam path.

Results (not shown) of iterative optimization of HESI spray voltage, gas flows, and the ion funnel RF amplitude for these compounds show a positive dependence of the optimal ion funnel RF amplitude on the spray voltage, suggesting that kinetic energy, and not just mass to charge ratio and collisional ability, must be accounted for in tuning the RF amplitude. SRIG or ion funnel RF amplitude tuning in the entrance region of a mass spectrometer trades off between a number of effects and should, in general, be done not only per compound but using or approximating the source conditions to be used in the intended acquisition experiment.

The optimization algorithm and batch optimization tool used in this analysis are available in version 2.1 or greater of the instrument control software for Thermo Scientific™ TSQ Endura™, TSQ Quantiva™, TSQ Altis™, and TSQ Quantis™ triple quadrupole mass spectrometers.

## REFERENCES

- Gupta, R. C., ed. Toxicology of Organophosphate and Carbamate Compounds. Elsevier Academic Press, Burlington, Mass. (2006).
- Rotterdam Convention, Annex III.
- Scott, A. "Europe rejects appeal for use of azinphos-methyl pesticide." Chem. Week. 4 August 2008.
- Cone, M. "Toxic pesticide banned after decades of use." Sci. Am. 18 August 2010.
- Commission Regulation (EU) No 310/2011.
- Kalafut, B. "Automatic optimization of MS and MS/MS assays for dilute samples or weak transitions." Proceedings of the 63rd ASMS Conference on Mass Spectrometry and Allied Topics. St. Louis, Mo. 2015.

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## TRADEMARKS/LICENSES

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