

Pesticide Screening of Food Samples Using the ionKey/MS System

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APPLICATION BENEFITS

- The rugged and easy-to-use ionKey/MS™ System greatly facilitates the utilization of micro-LC in high-throughput food safety laboratories.
- At least a 10x reduction in solvent consumption over existing 2.1 mm I.D. chromatography methods.
- Microliter scale sample and internal standard consumption imparts additional laboratory savings.
- Improvement of signal-to-noise ratio for pesticides, with an average of an 8x improvement in sensitivity over conventional 2.1 mm I.D. chromatography methods.

WATERS SOLUTIONS

ionKey™ Source

[iKey™ Separation Device BEH C18](#)

ACQUITY UPLC® M-Class System

Xevo® TQ-S

MassLynx® Software

TargetLynx™ Application Manager

DisQuE™ QuEChERS Dispersive
Solid Phase Extraction

KEY WORDS

IonKey/MS, pesticide, food safety,
microfluidic

INTRODUCTION

Microfluidic technology offers the capability to integrate several fluidic and instrumental components onto a single device. The process of microfluidic integration has several advantages for the micro-LC user. First, the micro-LC fittings either between the column and ESI source, or between the column and injection valve, must be precisely fitted. Even micrometers of difference between the transfer line and the column can equate to several μL s of void volume, and at $\mu\text{L}/\text{min}$ flow rates can result in large deviations in retention time or peak width due to dispersion. The ability to micromachine and integrate the post-column connections on a planar microfluidic device offers the simplicity of never having to replace or change post-column lines or ESI tips. Secondly, the clamp-on microfluidic fittings make it easy to replace the microfluidic iKey in a matter of seconds. This allows for trouble-free system maintenance, but it can also facilitate the process of method development with different column chemistries. In addition, the integrated heating elements, memory, and ESI tips require minimal programming, providing control of LC-gradients and ESI spray in a customized environment.

Operating at the micro-LC scale provides a number of advantages for minimizing laboratory solvent consumption. For pesticide screening applications, the Waters® ionKey/MS System utilizes a scaled down flow rate of 2.3 $\mu\text{L}/\text{min}$. This substantially lower rate of solvent consumption and consequential reduction of hazardous waste removal can result in significant cost savings to laboratories.

In addition to the reduction in solvent consumption, there are also significant improvements in sensitivity for many analytes. In this application note, a mixture of pesticides was spiked into food matrices of varying levels of complexity. Initial work was undertaken to compare the iKey Separation Device with a conventional 2.1 mm diameter ACQUITY UPLC Column. For the 50 pesticides individually assessed, the sensitivity was improved by an average of 8x over the 2.1 mm format. The ionKey/MS System was further tested for robustness and performance with a range of matrices.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC M-Class
iKey device:	iKey BEH C ₁₈ 1.7 µm, 130Å x 10 cm (p/n 186007258)
iKey temp.:	45 °C
Injection volume:	5 µL
Flow rate:	2.3 µL/min
Mobile phase A:	Water with 10mM ammonium acetate, pH 5.0
Mobile phase B:	Methanol with 10 mM ammonium acetate, pH 5.0
Weak needle wash:	Water
Strong needle wash:	Acetonitrile
Seal wash	90:10 Water:acetonitrile
Gradient:	

Time (min)	Flow (µL/min)	%A	%B	Curve
initial	2.3	98.0	2.0	initial
12.25	2.3	1.0	99.0	6
13.25	2.3	98.0	2.0	6
20.00	2.3	98.0	2.0	6

MS conditions

MS system:	Xevo TQ-S
Acquisition mode:	MRM
Ionization mode:	ESI +
Capillary voltage:	4.0 kV
Source temp.:	120 °C
Cone voltage:	Variable
Dwell time:	0.003 to 0.01 s

Sample preparation

A standard QuEChERS AOAC (2007.01) extraction protocol was performed using the Waters DisQuE Dispersive solid phase extraction (d-SPE) product, first by homogenizing the commodity in a blender, followed by weighing 15 g of the commodity into a vial. Next, 15 mL of extraction buffer consisting of acetonitrile with 1% acetic acid was added to the commodity along with pouch 1 of the DisQuE salt mixture. Vials were shaken for approximately 1 min and centrifuged at 1500 rcf for 1 min. The supernatant was removed and filtered through a 0.2 µm PTFE filter prior to dry down and reconstitution in mobile phase. Standards with 200 pesticides were constituted in extracted food matrix at levels ranging from 1 ppt to 10 ppb with 11 intervals. Matrix blanks from the extracted food matrix were collected along with solvent blanks. These blanks were used to identify the existing presence of pesticides in the commodity.

RESULTS AND DISCUSSION

In order to determine the feasibility of the ionKey/MS System for pesticide residue analysis, a method that incorporated 360 MRM transitions was employed, even though not all of the pesticide standards for the method were spiked. This enabled assessment of the data quality that would be obtained with a typical multi-residue pesticide method. Figure 1 illustrates a total ion chromatograms (TIC) containing 99 pesticides in onion matrix separated and detected using the ionKey/MS System. Several matrices were analyzed with this system including infant formula, summer squash, onion, and tomato.

Peak widths at half height were similar to the 2.1 mm separations ranging from 3 to 6 s. However, the sensitivity of the ionKey/MS System was on average 8x greater compared to regular analytical columns. The improvement in sensitivity is due to the improved electrospray at low flow and the reduced dilution that occurs within the iKey Separation Device. These two factors help increase the number of ions entering the Xevo TQS, resulting in improved sensitivity. The sensitivity enhancement observed in the ionKey/MS is illustrated in Figures 2 and 3. Figure 2 shows a series of six pesticides in infant formula displaying different improvements in sensitivity over 2.1 mm I.D. chromatography. The differences in ionization efficiency are based on molecular structure, hydrophobicity, and acid-base functionality. Pesticides that have non-polar functionalities are driven towards the surface of the droplet. If pesticides reside at the surface of a droplet for longer periods of time they are more likely to enter the gas phase as an ion. The greatest improvement was observed with fenpropimorph with a 25x improvement seen in infant formula. In Figure 3, MRM chromatograms of four different pesticides are displayed with the iKey peaks overlaid with the 2.1 mm I.D. peaks. As can be seen from these chromatograms, the ionKey/MS offers improved signal-to-noise ratio over the 2.1 mm I.D. chromatography.

The dynamic range of ionKey/MS was also shown to be >3 orders of magnitude for the majority of compounds investigated here. Figures 4A and 4B show the linearity of the calibration curves for dicrotophos and flutolanil, which demonstrated R^2 values >0.99. The MRM chromatograms of both the primary and secondary ions for both dicrotophos and flutolanil at their LOQs (2.5 and 12.5 ng/L, respectively) are also shown. The calculated peak-to-peak signal-to-noise (S/N) for the dicrotophos primary ion was 12 and 35 for flutolanil.

Peak repeatability and robustness

Microfabrication of fluidic connections allows for precise connections with limited to no dead volume, and limited variation from chip to chip. Using TrendPlot™ we plotted peak area and retention time reproducibility, shown in Figures 5 and 6. We found peak area and retention time reproducibility to be below 13% RSD and 1% RSD, respectively, for boscalid, flutolanil, chlortoluron, and dicrotophos at 1 µg/L in tomato matrix. In addition to investigating peak area and retention time reproducibility, we also investigated ruggedness of use. After 1000 injections of infant formula extract we found negligible increases in pressure and reproducible peak retention times. Figure 7 illustrates peak retention reproducibility of buprofezin and monocrotophos at injection 1, 500, and 1000.

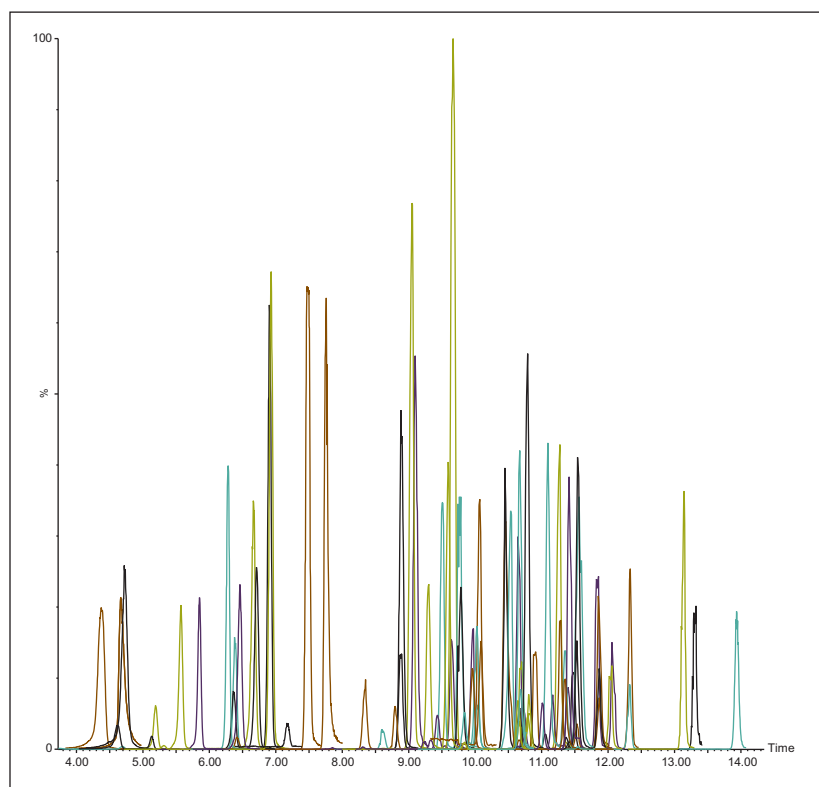


Figure 1. Overlay showing TIC chromatograms of 99 pesticides spiked into an onion extract; concentration of pesticides was 1 µg/L.

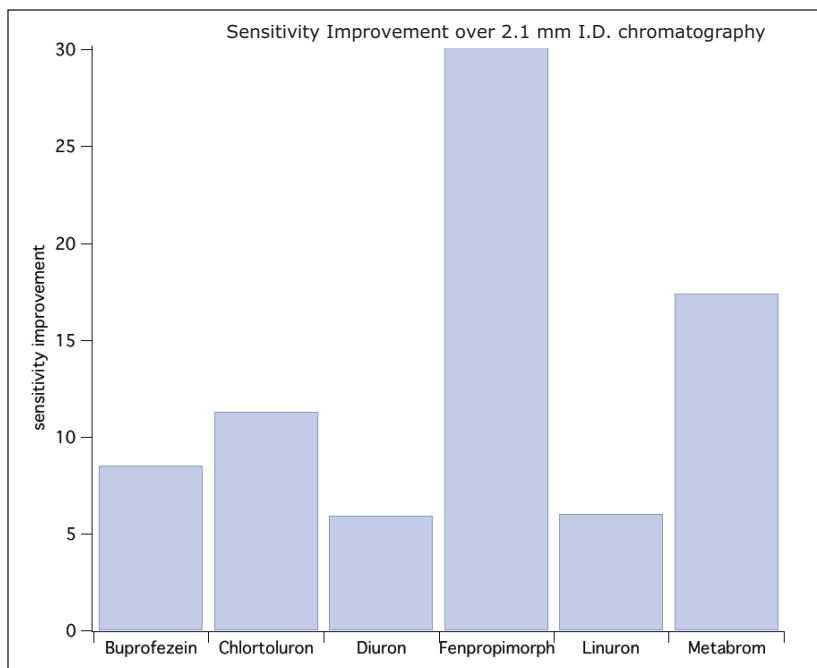


Figure 2. Comparison of signal-to-noise ratio observed between series of six pesticides in baby formula measured using ionKey/MS and 2.1 mm I.D. chromatography. The concentration of pesticides was 1 µg/L.

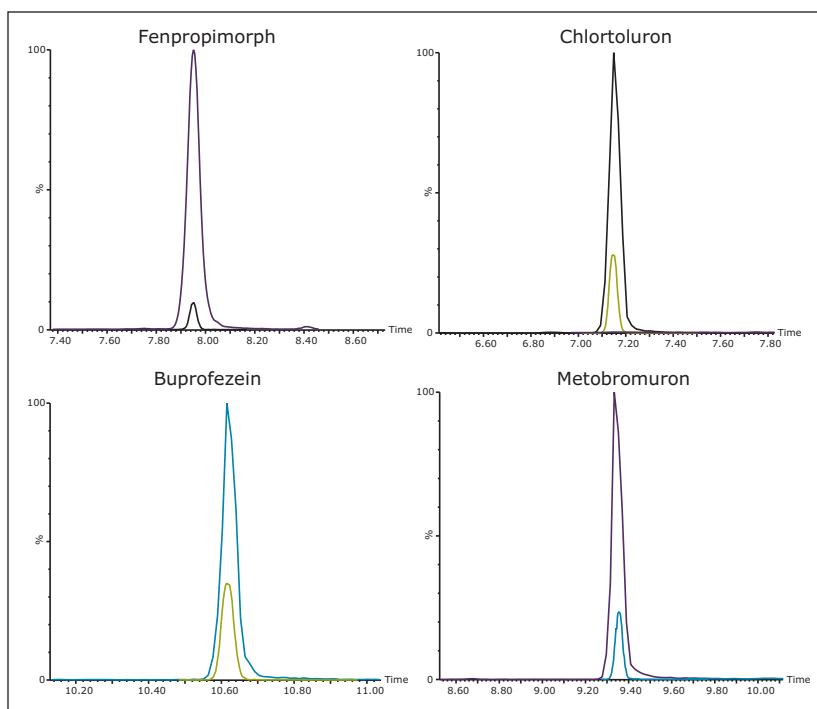


Figure 3. Superimposed chromatograms of four pesticides showing comparison between ionKey/MS and 2.1 mm I.D. chromatography format. The concentration of pesticides was 1 µg/L.

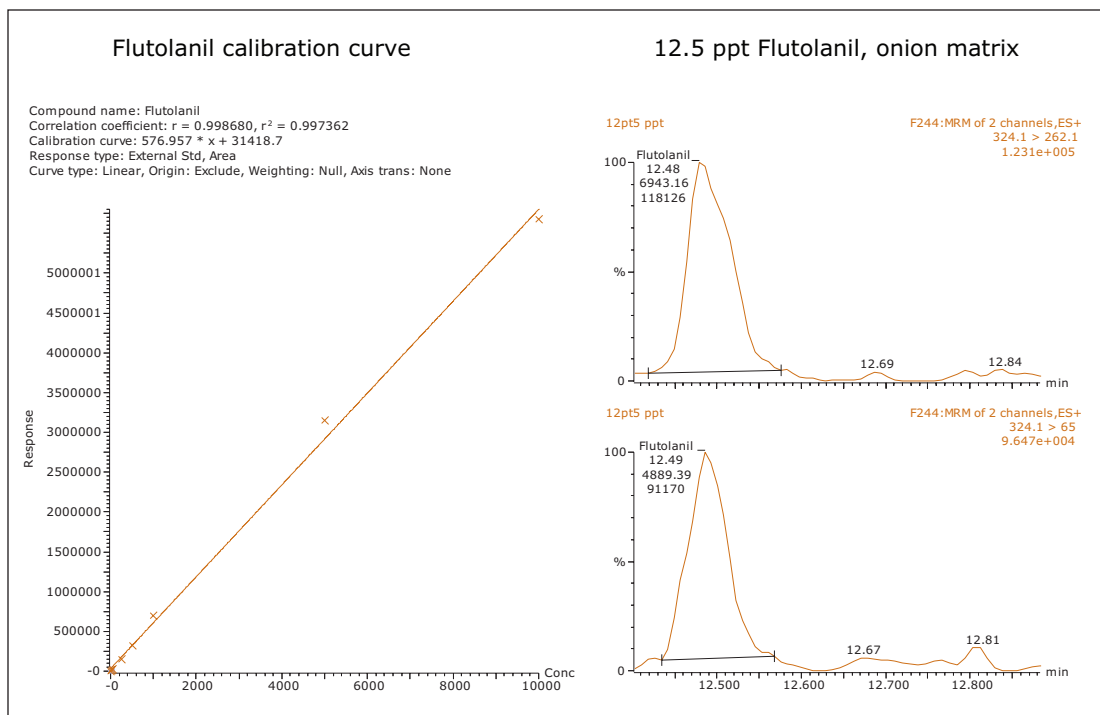


Figure 4A. (Left) Linearity plot of dicrotophos in onion matrix. (Right) MRM transition for the primary ion (peak-to-peak S/N of 12) and the secondary ion at 2.5 ng/L.

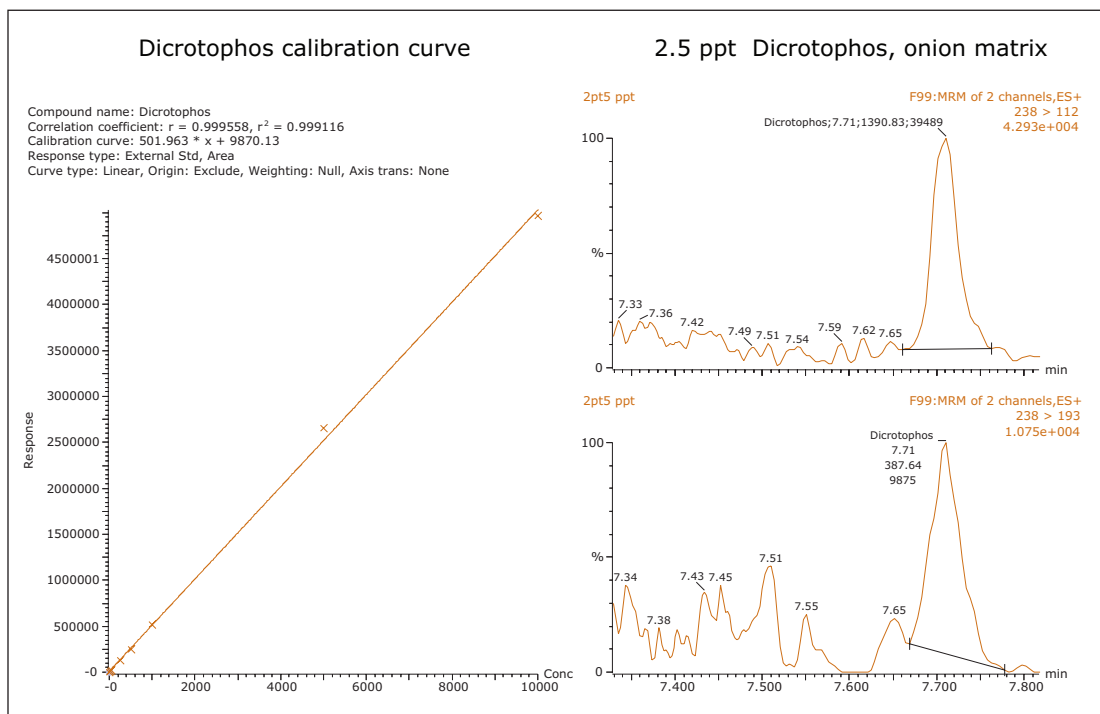


Figure 4B. (Left) Linearity plot of flutolanil in onion matrix. (Right) MRM transition for the primary ion (peak-to-peak S/N of 35) and secondary ion at 12.5 ng/L.

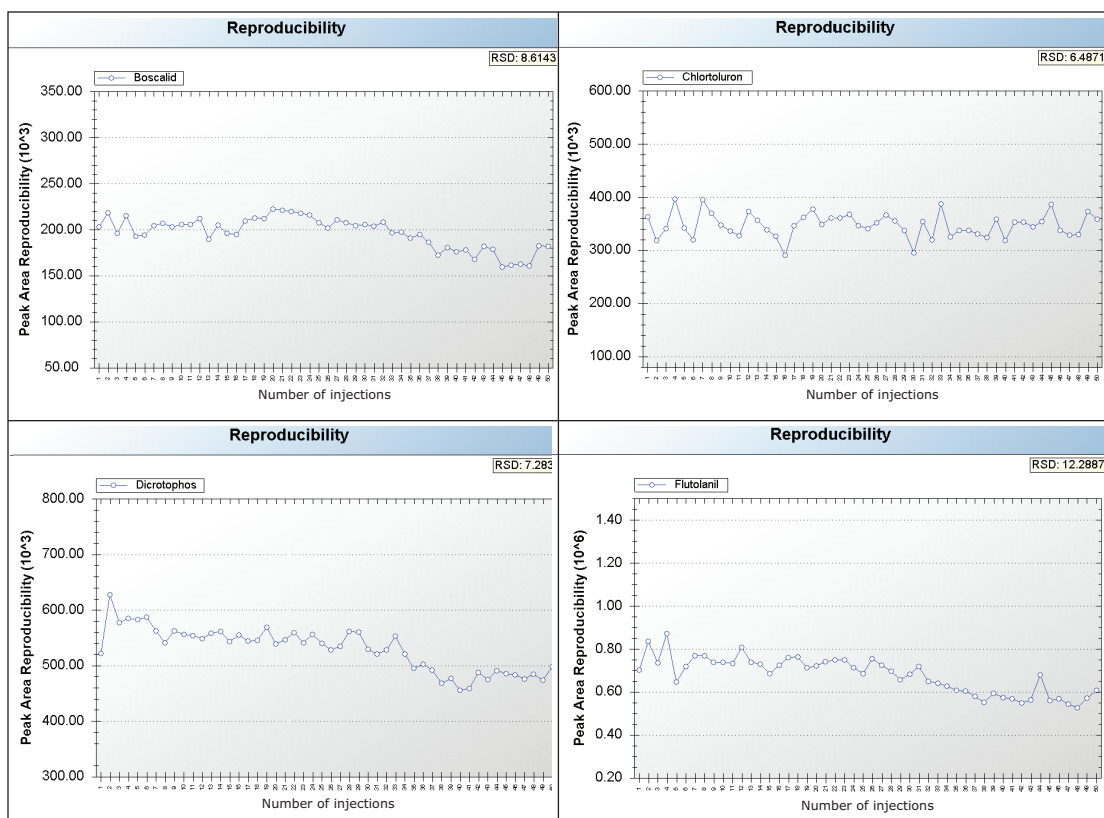


Figure 5. Peak area reproducibility for pesticide tomato matrix standards at 1 µg/L.

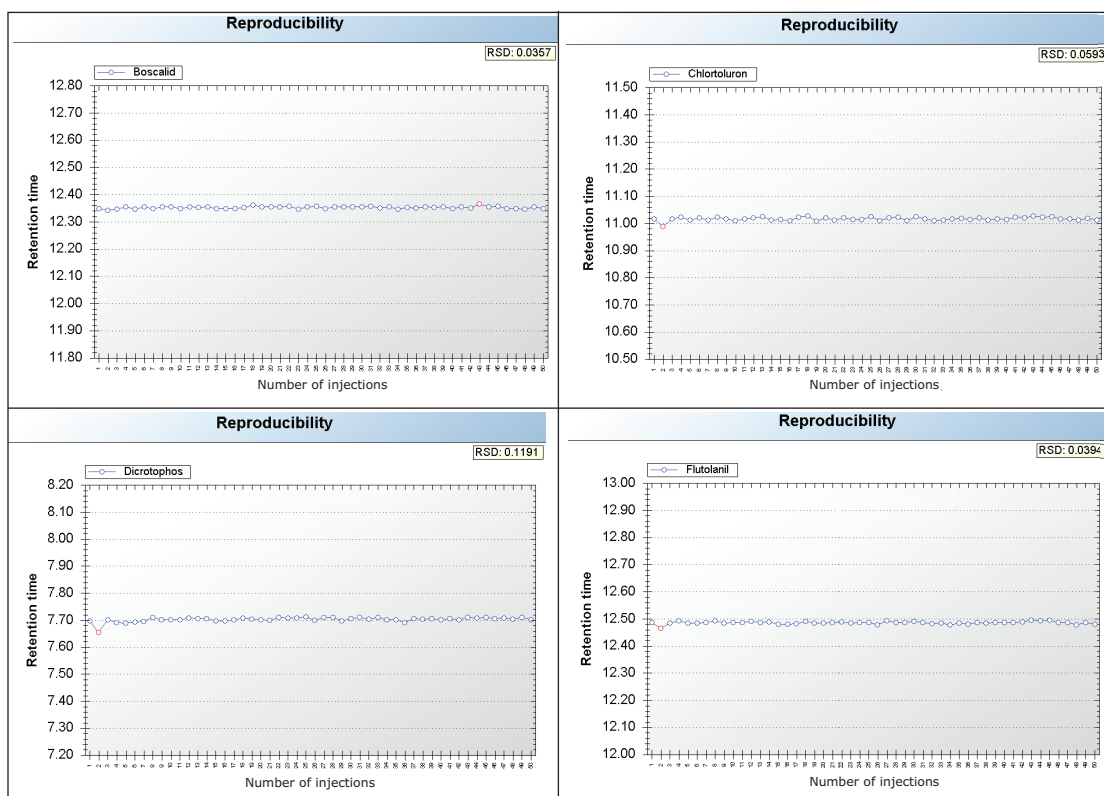


Figure 6. Retention time reproducibility for pesticide tomato matrix standards at 1 µg/L.

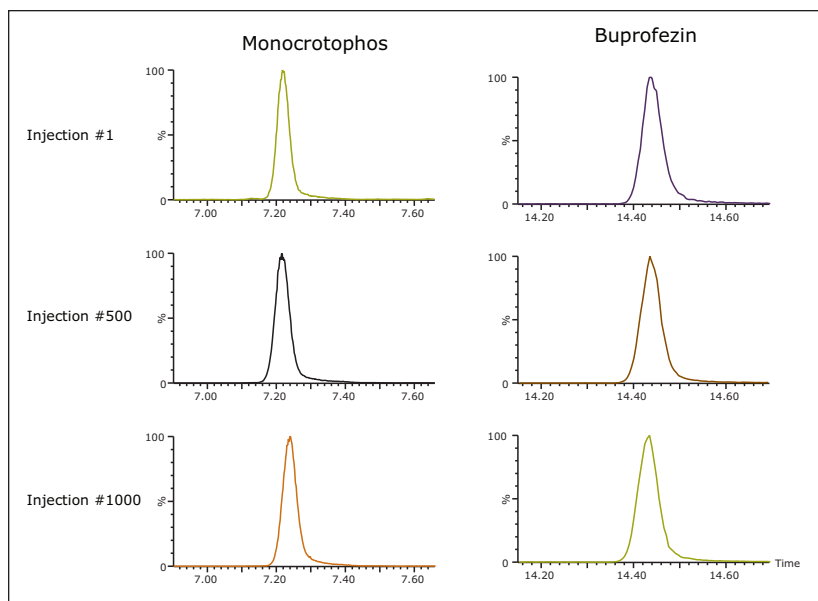


Figure 7. MRM chromatograms of two pesticides, monocrotophos and buprofezin at 1 µg/L. Injections injection 1, 500, and 1000 are shown (top to bottom).

CONCLUSIONS

The ionKey/MS System is a novel microfluidics-MS platform for rugged and easy-to-use micro-LC analysis of pesticides in food samples. The iKey enables highly reproducible LC separation with comparable resolution to analytical scale LC-MS analysis. This was demonstrated in both peak area reproducibility and retention times for pesticides in a variety of matrices.

An average of 8x improvement in sensitivity over 2.1 mm high-flow chromatography can help food and beverage laboratories meet the increasing demands of international regulatory agencies.

Low flow rates of 2.3 µL/min allow for 10x savings in solvent consumption and costly hazardous waste disposal charges to improve a laboratory's bottom line.

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