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Multiresidue Pesticide Analysis in Food Matrices with an Ultra Inert Splitless Glass Frit Liner by GC/MS/MS

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Abstract

Pesticides in food matrices can be sensitive to inlet conditions, including the type of barrier in the liner, whether this is glass wool, dimples, or sintered frit. A multiresidue pesticide analysis method using gas chromatography/tandem quadrupole mass spectrometry (GC/MS/MS) was used to evaluate the efficacy of the Agilent Ultra Inert splitless sintered glass frit liner (p/n 5190-5112). Calibration linearity was demonstrated for 22 pesticides from 1 to 500 ng/mL using the fritted liners. The fritted liners also had comparable response and peak shape to commonly used wool liners for the first set of matrix injections, but retained greater peak response through 70 matrix injections.

Introduction

Pesticides are sensitive to active sites in gas chromatography-tandem mass spectrometry (GC/MS/MS) systems, which makes an inert flowpath critical; however, some food matrices can alter the inertness of the flowpath. Previous studies have shown that matrix-matched calibration curves and liners with barriers, such as glass wool, prolong column and analysis lifetime.[1,2] Inlet liners packed with glass wool are often used to provide a site for volatilization, and act as a barrier for collecting nonvolatile matrix compounds. Glass wool, however, can also introduce active sites, since an irregular surface may not be fully deactivated or new active sites can open at breakage points of the glass wool.

An alternative to glass wool is a sintered glass frit liner, which also provides a barrier and volatilization site without the risk of wool breakage or movement in the liner. The splitless liner has the frit at the bottom of the single taper liner, similar to the wool location in splitless wool liners. The sintered frit liner was tested with matrix-matched calibration curves in the range of 1 to 500 ng/mL for a set of 22 pesticides. For repeatability of injections per liner and reproducibility across a set of liners, the splitless fritted liner and two splitless wool liners were tested with low level (10 ng/mL) pesticides in food matrices by GC/MS/MS. The sintered frit liners were compared to wool liners to determine efficacy in pesticide analysis and similarities or differences in liner performance, as glass wool liners are commonly used for pesticide analysis.

Experimental

Seven food matrices were chosen for the analysis: strawberry, plum, onion, bell pepper, orange, avocado, and spinach. Each matrix was extracted following the QuEChERS method and the matrix appropriate dispersive SPE technique for matrix cleanup. All seven matrices followed the same QuEChERS extraction protocol:

1. Fifteen grams of homogenized food stuff was added to a 50 mL centrifuge tube.

2. Two ceramic homogenizers and 15 mL of water were added, and the sample was vorexed.

3. The samples were then extracted with 15 mL of acidified acetonitrile (ACN with 1% acetic acid) and vortexed for two to five minutes.

4. QuEChERS Extraction salts (part number 5982-7555) were added to each sample, and samples were mechanically shaken for five minutes, then centrifuged for five minutes at 5,000 rpm.

5. Each sample of ACN extract (top layer) was then transferred to the proper dSPE cleanup.

For avocado

Approximately 10 mL of avocado extract was pipetted from the centrifuge tube to a new tube, and 2 mL of water was added. The mixture was shaken well to mix. Using two Captiva EMR—Lipid 6 mL cartridges (part number 5190-1004), the extract/water mix was pipetted into each cartridge and allowed to drip through the cartridges for ~20 minutes into collection tubes. After most of the extract was through the cartridge, a vacuum manifold was used to pull the small amount of remaining liquid through the cartridge into the collection tubes. The liquid from the collection tubes was decanted into a 15 mL centrifuge vial, the Bond Elut EMR—Lipid Polish Pouch (part number 5982-0102) was added, and the contents were centrifuged for five minutes at 5,000 rpm. The final avocado extract was then transferred into a storage vial.

For all other matrices

Eight milliliters of the acetonitrile/food extract was added to a QuEChERS dSPE for general fruit and vegetables (part number 5982-5058) tube, vortexed for two minutes, and centrifuged for five minutes at 5,000 rpm. Each respective final food extract was transferred into individual storage vials.

Pesticide standards

A custom mixture of 22 pesticides at 10 ppm concentration was procured. A set of nine calibration levels were made by diluting the 10 ppm standard with acetone to 1, 2, 5, 10, 20, 50, 100, 200, and 500 ng/mL. A mixture of six deuterated PAHs was added to each calibration mixture at a concentration of 40 ng/mL each as internal standards (ISTDs). Triphenyl phosphate (TPP) was added as a surrogate to each mixture at a concentration of 200 ng/mL. Table 1 contains the pesticide compounds in elution order with internal standards and surrogate listed at the bottom of the table.

Table 1. Pesticide compound names listed in retention time order. Internal standards and surrogate compounds are listed at the end, not in retention time order.

	Compound name
1	Methacrifos
2	Omethoate
3	Ethalfluralin
4	Sulfotep
5	Demeton-S
6	Simazine
7	γ-lindane (γ-BHC)
8	Chlorpyrifos-methyl
9	Fenitrothion
10	Aldrin
11	Pendimethalin
12	Folpet
13	Bupirimate
14	Dieldrin
15	Triazophos
16	Iprodione
17	EPN
18	Phosalone
19	Mirex
20	Coumaphos
21	Pyraclostrobin
22	Deltamethrin
ISTD	1,4-dichlorobenzene-d_4
ISTD	Naphthalene-d_8
ISTD	Acenaphthene-d_{10}
ISTD	Phenanthrene-d_{10}
ISTD	Chrysene-d_{12}
ISTD	Perylene-d_{12}
Surrogate	Triphenyl phosphate

Method parameters

The Agilent 7890B GC was configured with a midcolumn backflush setup, where the two 15-meter DB-5ms UI columns were connected with a purged Ultimate Union (PUU). The column 2 flow was set to a value 0.2 mL/min greater than column 1 along with a bleed line between the electronic pressure control (EPC) module and PUU to prevent reverse flow to the EPC module. Table 2 summarizes the GC/MS/MS instrumentation and consumables used in this study. The GC/ MS/MS method parameters (Table 3) were optimized to provide a 25-minute method. The Agilent 7000D MS/MS was operated in dynamic MRM (dMRM) mode. Acquisition methods were tailored for each matrix with matrix-matched transitions for each pesticide. Sandwich injections, using the Agilent 7693B automatic liquid sampler, were used to shorten sample preparation time.

Instrumentation

Table 2. GC and MSD instrumentation and consumables.

Parameter	Value
GC	Agilent 7890 GC
MS/MS	Agilent 7000D MS/MS with InertPlus EI source
Autosampler	Agilent 7693B automatic liquid sampler with tray
Syringe	Agilent Blue Line 10 µL PTFE-tip plunger tapered syringe (G4513-80203)
Columns	Two Agilent DB-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm (p/n 122-5512UI)
Inlet Septum	Agilent Advanced Green, nonstick 11 mm septum (p/n 5183-4759 for 50 pack)
Vials	Agilent A-Line certified amber (screw top) vials; 100/pk (p/n 5190-9590)
Vial Inserts	Agilent deactivated vial inserts; 100/pk (p/n 5181-8872)
Vial Screw Caps	Agilent screw caps, PTFE/silicone/PTFE septa, cap size: 12 mm; 500/pk (p/n 5185-5862)

Instrument conditions

Table 3. GC and MSD instrument conditions.

Parameter	Value
Injection Type	2-layer sandwich
Injection Volume	1 µL standard and 1 µL matrix
Inlet	Split/splitless 280 °C; Purge 30 mL/min at 0.5 minutes; Switched septum purge 3 mL/min
Carrier Gas and Flow Rates	Helium at 1.20 mL/min (column 1); 1.4 mL/min (column 2), constant flow
Transfer Line Temperature	280 °C
Column Temperature Program	60 °C (1 minute) 40 °C/min to 170 °C 10 °C/min to 310 °C (3 minutes)
Backflush/Post Run Settings	5 minutes at 310 °C and 48 psi (midpoint pressure)
Ion Source Temperature	250 °C
Quadrupole Temperature	150 °C
Acquisition	dMRM
Gain Factor	10
MS Resolution Setting	Wide
Solvent Delay	2 minutes

Sandwich injections

Sandwich injections were used to cut down on sample preparation of combining pesticide standards, internal standards, surrogate (triphenyl phosphate), and matrix.[3] A two-layer injection was selected in the Agilent MassHunter Data Acquisition software (ALS tab). Internal standards and the surrogate were added to each vial of calibration level standard at the concentrations previously stated. For the 70-run matrix study with a two-layer sandwich injection, L1 is the second liquid drawn into the syringe, and first injected into the liner. The liquid was the matrix for the specific injection; for example, the first run used bell pepper. L2 is the first liquid drawn into the syringe, which was the 10 ng/mL pesticide standard (with ISTDs and surrogate). A 0.2 µL air gap was maintained between the two levels after L1 was drawn into the syringe to prevent any liquid from escaping the syringe before injection. The two-layer sandwich injection injects the matrix first and the standards second, permitting the matrix to coat any active sites in the liner.

Liners

Three liner styles were investigated. The Agilent Ultra Inert splitless single-taper liner with frit, a splitless single taper liner with approximately 5 mm of wool at the bottom of the liner (wool A), and a splitless single taper liner with approximately 8 mm of wool at the bottom of the liner (wool B) were tested to compare the efficacy of the fritted liner to the wool liners.

The splitless fritted and splitless wool liners were tested with pesticide standards with and without matrices. The matrix was sandwich injected with a 10 ng/mL pesticide standard to account for matrix enhancement or diminution of compound responses. Ten injections of each matrix and standard were completed in random order for a total of 70 injections per sequence.

Results and discussion

This study demonstrates the linearity and peak consistency of the splitless fritted liner for pesticide analysis. A calibration curve using two-layer sandwich injections of each standard was generated for each matrix. A 100 ng/mL standard was used to check calibration and monitor the peak shape. To monitor the peak shape and recovery over time, a 10 ng/mL standard was sandwich injected with each matrix for 70 total matrix injections (10 per matrix, randomized injections). Following the 70 matrix injections, system maintenance was performed, where the inlet liner and septum were changed. The 100 ng/mL standard was used to determine when to trim the column by tracking peak shape. Typically, the column was trimmed by 0.5 m after

three liners.

Calibration curves

To verify the ability of the fritted liner for pesticide analysis, matrix-matched calibration curves were generated with sandwich injections of the selected matrix and pesticide standards at each concentration level between 1 and 500 ng/mL. The calibration curves were completed individually per matrix, but the concentration levels were injected in random order on the fritted liner. To follow guidance from the European Commission, using SANTE/11813/2017, calibration curves can be fulfilled by an average response factor within ±20% of the concentration level, linear fit or quadratic fit.[4] For the majority of compounds across the matrices, average response factors were used to achieve a calibration curve within the ±20% relative standard deviation (RSD) limits. Table 4 summarizes the number of compounds that pass calibration curves with average response factors and which compounds require linear or quadratic fits per matrix. Even when the compounds pass with average response factors, the linear fits and calibration curve (R^2) coefficients generated by the quantitative analysis software can be used for high level review of results.

Table 4. Summary of the calibration curve results for each matrix, indicating compounds that pass with average response factors, linear fits, or quadratic fits.

	Avocado	Bell Pepper	Onion	Orange	Plum	Spinach	Strawberry
Average Response Factor (RF)	20 compounds	18 compounds	20 compounds	20 compounds	17 compounds	18 compounds	21 compounds
Linear fit	Demeton-S, Deltamethrin	Omethoate, Demeton-S, Folpet, Deltamethrin	Deltamethrin	Deltamethrin, Folpet	Demeton-S, γ-Lindane, Iprodione	Methacrifos, Demeton-S, Foplet, Deltamethrin	
Quadratic fit			Folpet		Folpet, Deltamethrin		Folpet

Table 5 summarizes the coefficient of determination (R^2 values) for a selected set of 12 pesticides across the seven matrices, where calibration coefficients noted with an asterisk (*) represent compounds that achieved calibration with linear fits, and coefficients noted with a cross represent quadratic fits.

The range of R^2 coefficients was 0.9819 to 1.0 across the 22 compounds and seven matrices. Methacrifos had an R^2 value below 0.990 in the avocado matrix, as did pyraclostrobin in plum, but both achieved calibration criteria with average response factors. Deltamethrin required a linear fit in bell pepper matrix with an R^2 value of 0.9819. Even though this calibration coefficient is low, the linear equation had the best fit across the 1 to 500 ng/mL calibration range, compared to other linear equations with fewer calibration levels or a quadratic fit. As highlighted in Table 4, deltamethrin required a linear or quadratic fit in each matrix, except for strawberry. Folpet also required quadratic and linear fits

to pass calibration in all matrices, except for avocado, and demeton-S achieved calibration with linear fits in four matrices. All of these compounds can be challenging and sensitive to matrix effects and active sites in the flowpath. Using quadratic fits for the compounds specified in Table 4 for onion, plum, and strawberry matrices resulted in R^2 values above 0.992. There were several compounds that achieved a high degree of linearity (R^2 = 0.9999 to 1.0); generally, these compounds achieved calibration with average response factors. The bell pepper matrix had the greatest number of compounds with high linearity, which were methacrifos, simazine, γ-lindane, and coumaphos. Other compounds (and matrices) with high linearity included chlorpyrifos-methyl (R^2 = 1.0000) and phosalone (R^2 = 0.9999) in avocado, dieldrin in orange (R^2 = 0.9999), and mirex in onion (R^2 = 1.0000). There were also several compounds in the different matrices with R^2 values greater than 0.9990.

Averaging the R^2 coefficients together indicates how consistently the pesticide compounds interacted to a respective matrix and the inertness of the flowpath. Bell pepper and avocado had the highest average R^2 values of 0.9980 and 0.9975, respectively, which agree with the number of compounds that exhibited high R^2 coefficients. Onion had the lowest average R^2 value of 0.9962, suggesting that onion was a more challenging matrix for the pesticides, even after QuEChERS extraction and dSPE. Average R^2 values for orange (0.9974), plum (0.9964), strawberry (0.9969), and spinach (0.9972) fell inside the range, where the average coefficient for plum was close to onion, suggesting it was somewhat difficult for these pesticides. Overall, these calibration curve coefficients and the low number of compounds that required linear or quadratic fits are excellent with the fritted liner.

Recovery

To test recovery, repeatability, and reproducibility across many randomized matrix injections and liners, a sequence of 70 runs was completed. Six fritted liners were tested in this manner, as were three wool A and three wool B liners. The 70-run sequence data were used to calculate recovery of the pesticides to evaluate the different liner styles, since pesticides were sandwich-injected with the matrices. The samples for the 70-run data sets were 10 ng/mL pesticide standards, and recovery was calculated using the calibration curve average response factor, linear fit or quadratic fit per each compound and matrix. Figure 1 illustrates the average recovery of the 10 ng/mL standard throughout 70 injections averaged across six liners for a select set of matrices: avocado (high oil matrix), bell pepper, spinach (high pigment matrix), and strawberry.

Table 5. R^2 values of 12 selected pesticides for all seven matrices.

| Compound	R² Calibration Coefficients						
	Avocado	Bell Pepper	Onion	Orange	Plum	Spinach	Strawberry
Methacrifos	0.9849	0.9999	0.9996	0.9993	0.9992	0.9957*	0.9995
Omethoate	0.9985	0.9949*	0.9982	0.9996	0.9928	0.9911	0.9944
Sulfotep	0.9990	0.9997	0.9994	0.9991	0.9967	0.9967	0.9995
Simazine	0.9984	0.9999	0.9992	0.9989	0.9972	0.9951	0.9973
Fenitrothion	0.9994	0.9980	0.9962	0.9954	0.9987	0.9989	0.9994
Aldrin	0.9958	0.9987	0.9992	0.9994	0.9987	0.9993	0.9998
Dieldrin	0.9991	0.9998	0.9996	0.9999	0.9975	0.9975	0.9995
Iprodione	0.9997	0.9996	0.9959	0.9985	0.9925*	0.9990	0.9994
EPN	0.9998	0.9960	0.9931	0.9934	0.9921	0.9975	0.9949
Mirex	0.9967	0.9998	1.0	0.9994	0.9988	0.9996	0.9938
Pyraclostrobin	0.9984	0.9996	0.9958	0.9981	0.9896	0.9992	0.9991
Deltamethrin	0.9965*	0.9819*	0.9930*	0.9981*	0.9975†	0.9942*	0.9907

* Coefficient of determination for compound requiring linear fit
† Coefficient of determination for compound requiring quadratic fit

Some analytes, such as folpet, show a large degree of variability in recovery across the different matrices, or lower recoveries, as with deltamethrin; however, most pesticides show recoveries between 70 to 130% for a majority of the seven matrices. The average recovery for the target pesticides across the seven matrices was 106%, and the average RSD across these seven matrices was 10.9%. Bell pepper, spinach, and strawberry matrices (Figures 1B, 1C, and 1D, respectively)

had excellent recoveries on the fritted liners, as all compounds fell between 70 to 130% recovery for the spinach matrix, and only one compound was outside the typical recovery range for bell pepper (bupirimate) and strawberry (pyraclostrobin). Avocado proved to be a good matrix for pesticide recovery (Figure 1A), in which only three compounds fell outside the typical bounds, with simazine reporting high at 132% and the challenging compounds of folpet and deltamethrin

reporting low. Plum, like avocado, had two compounds on the lower end of recovery with omethoate and folpet, while coumaphos reported a higher recovery. The orange matrix had four compounds that were above the 130% recovery bound, while 18 compounds and the surrogate reported in the 70 to 130% recovery range. The onion matrix generally reported high recoveries across the board with most compounds in the 70 to 130% range; however, several compounds went above 130% recovery.
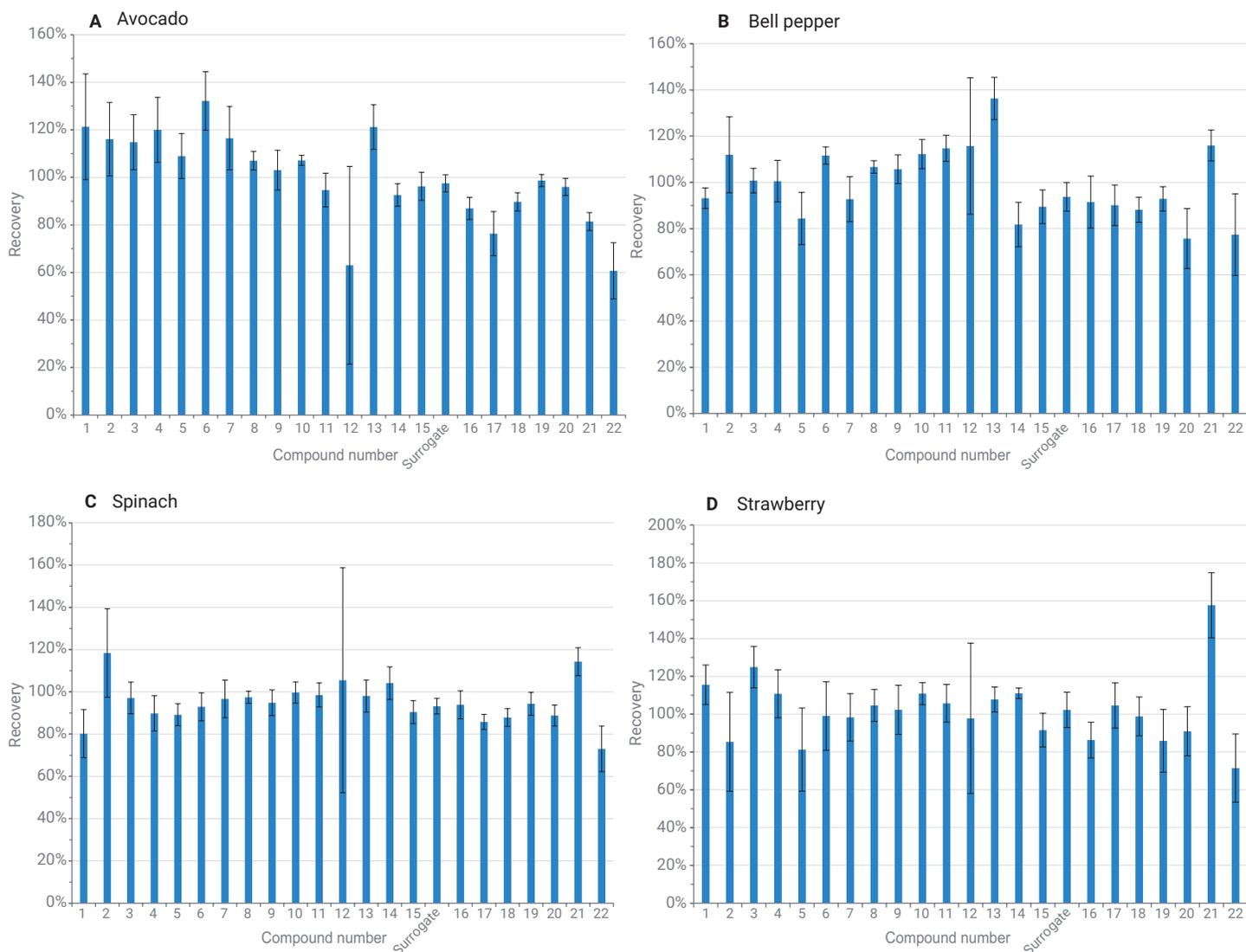
Figure 1. Average recoveries for 22 pesticides and the surrogate compound from six fritted liner sets of 70-run sequences for A) avocado, B) bell pepper, C) spinach, and D) strawberry matrices. Errors bars denote the standard deviation for recoveries of each pesticide in the respective matrix. Table 1 shows compound numbers.

This high recovery is likely due to matrix enhancement, and standard addition may be necessary for more accurate quantitation.

Response changes

Splitless fritted and the two styles of splitless wool liners, wool A and wool B, were tested with pesticide standards with and without matrices. The matrices were sandwich injected with a 10 ng/mL pesticide standard to account for matrix enhancement or diminution of compound responses, especially with possible matrix build-up in the liners. It was expected to see signal changes in the form of decreased area and loss of ideal peak shape throughout the 70-injection sequence, especially for sensitive compounds such as omethoate, folpet, and pyraclostrobin. The surrogate compound and ISTD areas were also tracked to check if the entire sample was affected similarly across the various pesticides. Examples of select peaks and how the peak areas and shapes change from the first to last run of a respective matrix were collected in Figures 2 and 3 for avocado and onion matrices, respectively; other select peaks for the two additional matrices are found in the Appendix (Figures A2 and A3). Each figure contains the overlays for tested liner styles and are listed as follows: A) frit liner, B) wool A liner, C) wool B liner, D) frit liner, E) wool A liner, and F) wool B liner.
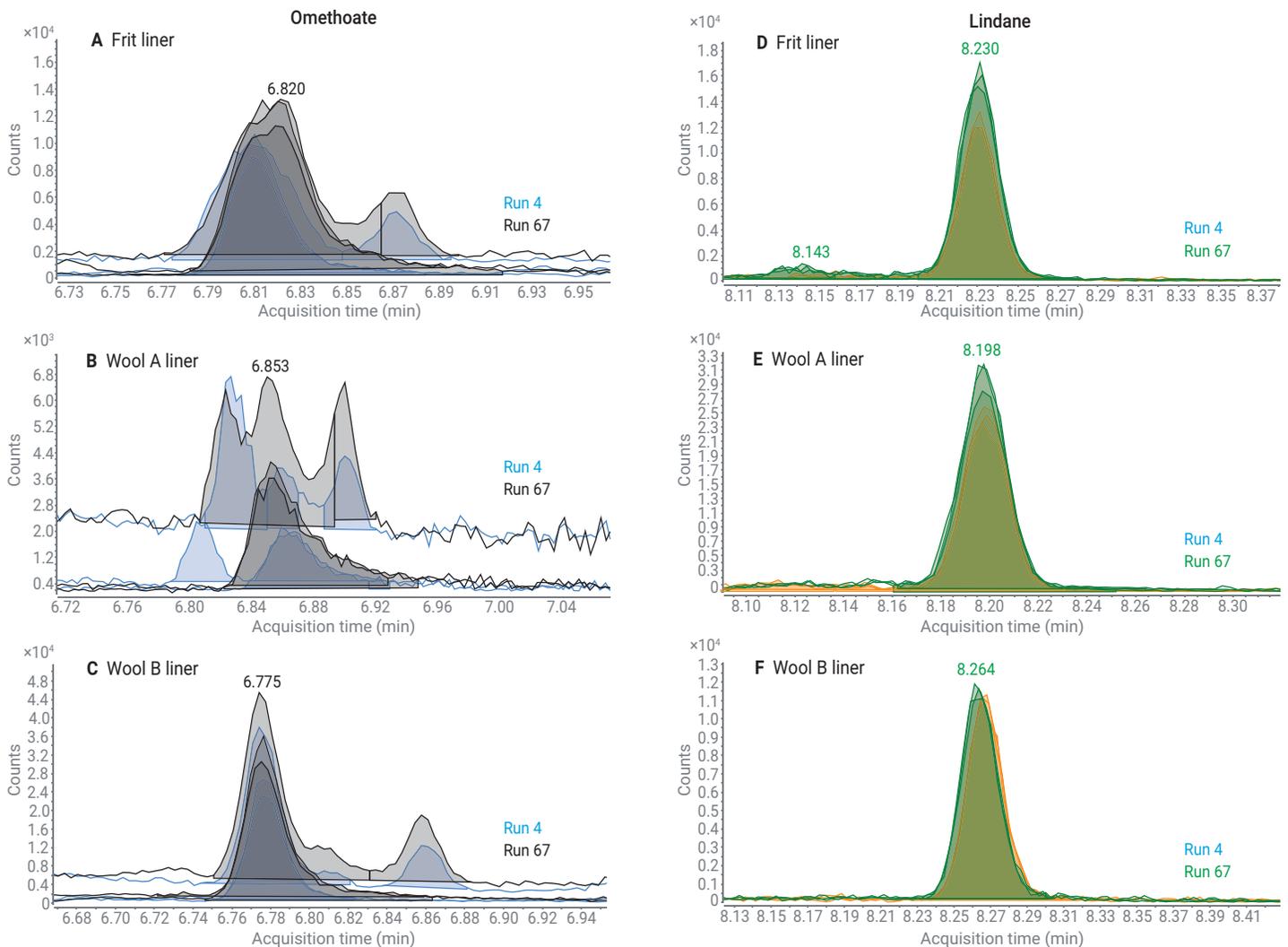
Figure 2. Extracted ion chromatogram overlays of the MRM transitions for the first (run 4) and last (run 67) avocado matrix runs of selected compounds on the fritted liner (A, D), wool A liner (B, E), and wool B liner (C, F). Pesticides at 10 ng/mL were sandwich injected with the avocado matrix.
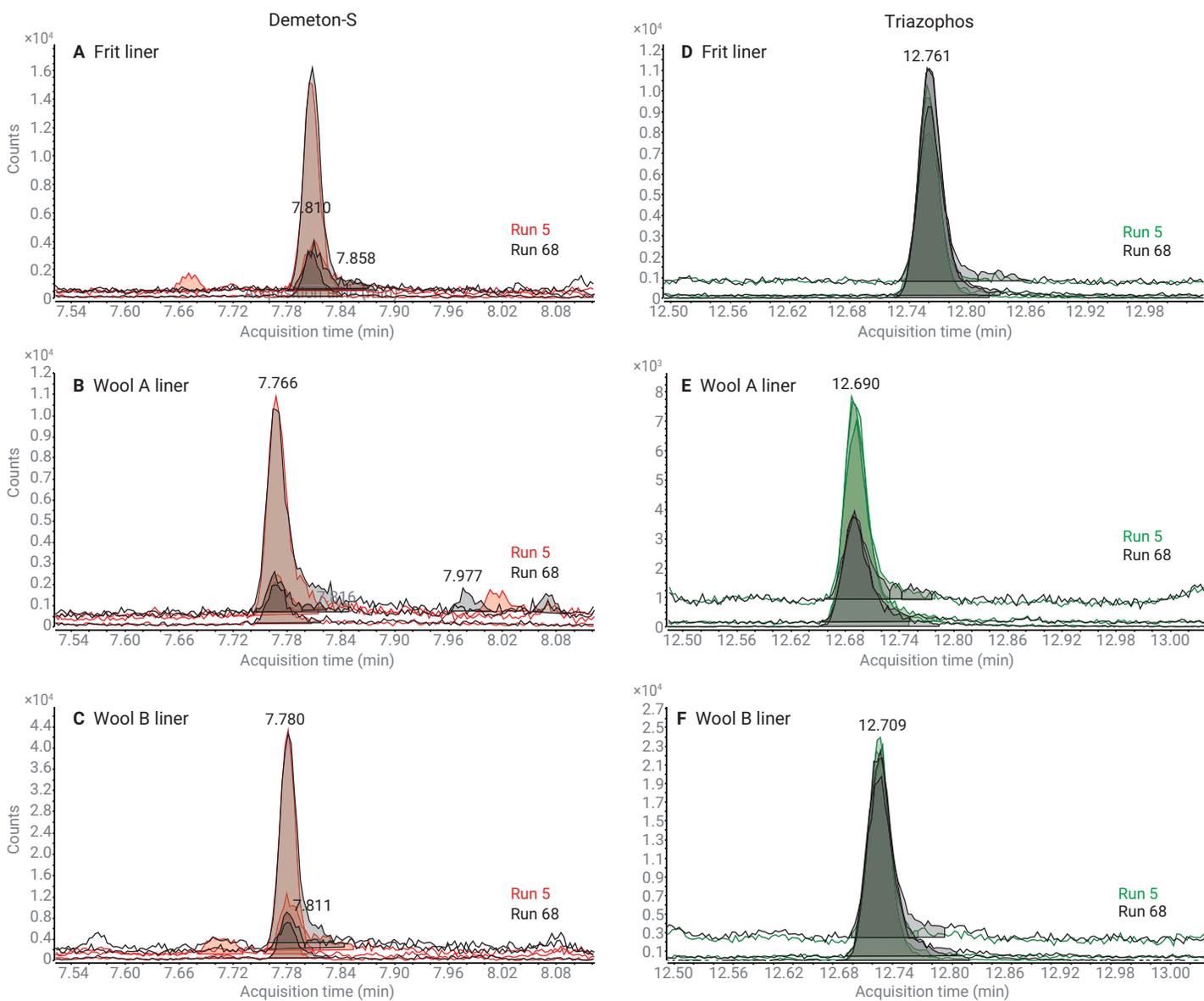
Figure 3. Extracted ion chromatogram overlays of the MRM transitions for the first (run 5) and last (run 68) onion matrix runs of selected compounds run on the fritted liner (A, D), wool A liner (B, E), and wool B liner (C, F). Pesticides at 10 ng/mL were sandwich injected with the onion matrix.

As expected, the more sensitive compounds, such as omethoate or pyraclostrobin, tend to have a loss of ideal peak shape with increased matrix injections. For example, omethoate tailing tends to increase with matrix injections, at least for the fritted and wool A liner (Figures 2A and 2B), while wool B liner maintained more of a Gaussian shape (Figure 2C). For all three liner styles, the pyraclostrobin peak tails more with increased matrix injections (Figure A2 D-F). Comparatively, many other peaks maintain Gaussian peak shapes through the full set of injections, such as lindane (Figure 2D-F) in avocado, demeton-S in onion (Figure 3A-C), and mirex in strawberry (Figure A3).

Reviewing the data for all three liner styles, many compounds across the various matrices increased in area from the first run to final run per matrix, which countered the expectation that areas would decrease with more matrix injections. The specific compounds that increase in area are different per matrix and liner style, but trends can be identified with respect to the liner styles. In five of the matrices for all three liner styles, more than half of the compounds, including the internal standards, increase or remain at the same area from the first to the final run of each matrix.

In orange and strawberry matrices, fritted and wool B liners have more than half of the compounds increasing in area with more matrix injections, while wool A results have more than half of the compounds decreasing in area. Examples of these differences in pesticide responses between the liners are shown in Figures 3D-3F, A2, and A3. For triazophos in onion, the fritted liner (Figure 3D) and wool B liner (Figure 3F) show a similar peak area, height, and shape throughout the 70-injection sequence, while triazophos has a significant decrease in area from the first onion run to the last onion run with wool A (Figure 3E). Coumaphos, which can be sensitive to active sites, also highlights peak response differences in the strawberry matrix. In Figure A3-D, coumaphos maintains a similar peak area for the fritted liner from run three to run 66, even though the peak became slightly broader with a rounded apex. Comparatively, wool A and wool B liners show significant decreases in area for coumpahos (Figures A3-E and A3-F) along with broader peaks. In the bell pepper matrix, wool A and wool B also illustrate similarities in liner styles and peak changes, in which bupirimate shows maintenance of peak area and shape throughout the 70 runs (Figures A2-B and A2-C), while the fritted liner shows an increase in peak area (Figure A2-A), which matches the internal standard area changes and maintains similar response factors throughout the sequence.

Across all matrices, wool A has the most compounds that decrease in area (during the 70-injection sequence), while the fritted liner has the lowest number that decrease. The fritted liner only had one to three compounds decrease in any of the matrices, while the other 19 to 21 compounds either maintained the same area or increased throughout the 70-injection sequence. Wool B liner tended to sit in between these two liner

styles, except for a couple matrices where wool B results would match the fritted or wool A results. For the avocado matrix, both wool B and fritted liners only have one compound each that decreases in area, while in the bell pepper data, wool A and wool B liners had three compounds decrease in area. The general trend of decreasing area may be a result of the interplay between barrier type, size, and deactivation of the (barrier) surface. Wool A had a smaller plug of wool (approximately 5 mm long), while the wool B plug was larger (approximately 8 mm); this size difference may have provided more surface area to spread the matrix. The fritted liner had the lowest number of decreasing compounds, which may indicate a more complete deactivation of the glass frit compared to the irregular glass wool. Better surface deactivation would result in fewer active sites and better recoveries and peak responses.

Internal liner repeatability

The 70-injection data were used to review the liner repeatability for six fritted liners, three wool A liners and three wool B liners, where repeatability shows the internal consistency of each liner in a set of injections. The 10 ng/mL concentration was selected as a more challenging and sensitive probe for active sites in the liners, as higher concentrations can mask issues. The sintered frit liners were compared

to wool liners to determine efficacy in pesticide analysis and similarities or differences of liner performance, as glass wool liners are commonly used for pesticide analysis. Internal liner repeatability across the 10 injections per matrix were calculated with respect to response factors and % RSD values. A selection of fritted and wool liner repeatability data are shown in Table 6, and all data are shown in Table A2 (fritted liners) and Table A3 (wool A and wool B liners).

Internal liner repeatability for each matrix was under 20% RSD for all six fritted liners, three wool B liners, and for most matrices with wool A liners. Wool A liners exhibited % RSDs below 25% for the onion matrices, plum data sets, and two strawberry data sets. Only one wool A with strawberry matrix data set had a result above 30% RSD, indicating that there may have been issues with the injections, since all other matrices tested on that liner had % RSD values below 25%. All six fritted liners were repeatable within 16% RSD for each matrix; furthermore, the % RSDs range was between 8 to 16% across the various matrices and liners, indicating consistent deactivation, pore size, and pesticide interactions with the fritted liner style. Overall, fritted and most wool liners have excellent repeatability, with a majority of % RSD values below 15%, indicating good internal consistency even as more matrix was being deposited into the liner.

Table 6. Repeatability (% RSD of average response factors) across 10 injections of 10 ng/mL pesticides with matrix, randomized in a 70-run matrix-matched sequence for selected set of fritted, wool A, and wool B liners.

	% RSDs for Average Response Factors (10 Replicates at 10 ng/mL)				
Matrix/Liner	Frit 2	Frit 4	Frit 6	Wool A2	Wool B2
Avocado	7.23%	15.93%	8.57%	10.84%	5.95%
Bell Pepper	9.47%	13.73%	11.70%	11.34%	10.36%
Onion	9.11%	11.09%	10.02%	17.38%	11.22%
Orange	8.94%	12.88%	11.35%	13.77%	8.24%
Plum	9.50%	12.10%	12.08%	23.69%	10.03%
Spinach	8.47%	10.65%	9.68%	12.34%	6.12%
Strawberry	9.12%	11.80%	11.15%	21.70%	9.99%

Liner reproducibility

Liner reproducibility was evaluated using % RSD of all response factors for each matrix and each liner, which tests the consistency across multiple liners of the same type. To calculate average response factors and % RSDs when determining the similarity of results across the individual liners, 60 data points for the fritted liner style (and 30 data points per wool liner style) were averaged together. Low % RSD values indicate consistent deactivation and pesticide interaction with the liner. High % RSD values suggest inconsistent deactivation or different pesticide interactions over the many matrix injections. Overall, all % RSD values were below 20%, indicating excellent reproducibility across different liners. The % RSD values were under 15% for all matrices with the fritted and wool B liners, and most matrices with the wool A liner. The onion, plum, and strawberry matrices with the wool A liner had % RSDs above 15%, but below 20%. It is difficult to identify one liner style as the best with respect to reproducibility. The only exceptions occur with wool A liner and the onion, plum, and strawberry matrices, since the % RSD values were higher than those for the fritted or wool B liners. Most of the % RSD values indicate consistent deactivation and pesticide interaction across the various matrices and three liner styles.

Table 7. Reproducibility results in the form of % RSD for the response factors (RFs) across six frit liners, three wool A liners, and three wool B liners.

Matrix	Frit Liners: % RSD of Response Factors (RFs)	Wool A Liners: % RSD of RFs	Wool B Liners: % RSD of RFs
Avocado	7.00%	6.94%	9.35%
Bell Pepper	8.35%	8.86%	8.25%
Onion	13.62%	15.98%	11.38%
Orange	8.59%	10.18%	7.97%
Plum	9.34%	16.12%	9.68%
Spinach	7.40%	8.54%	12.44%
Strawberry	12.69%	17.24%	8.85%

Conclusion

The Agilent Ultra Inert splitless fritted liner (p/n 5190-5112) is a suitable choice for testing pesticides in food by GC/MS/MS. The fritted liners successfully achieved calibration from 1 to 500 ng/mL with average calibration coefficients (R^2) of 0.996 across 22 targeted pesticides. Fritted liners provided similar responses to the splitless wool liners, but tended to have better retention of peak area with increased matrix injections across 70 matrix-matched injections than the wool liners. There was excellent liner repeatability and reproducibility with % RSD values below 16% for all fritted liners, and below 20% for wool B liners, indicating consistent deactivation and interaction of pesticides with the liners.

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Appendix

Table A1. Calibration curve (1 to 500 ng/mL) linearity coefficients (R^2 values) for all 22 pesticides across the seven matrices.

Compound	RT (min)	R^2 values for Calibration Curves 1 to 500 ng/mL						
		Avocado	Bell Pepper	Onion	Orange	Plum	Spinach	Strawberry
Methacrifos	6.029	0.9849	0.9999	0.9996	0.9993	0.9992	0.9957	0.9995
Omethoate	6.838	0.9985	0.9949	0.9982	0.9996	0.9928	0.9911	0.9944
Ethalfluralin	7.047	0.9993	0.9994	0.9970	0.9983	0.9998	0.9968	0.9997
Sulfotep	7.288	0.9990	0.9997	0.9994	0.9991	0.9967	0.9967	0.9995
Demeton-S	7.772	0.9946	0.9958	0.9975	0.9971	0.9990	0.9921	0.9966
Simazine	7.891	0.9984	0.9999	0.9992	0.9989	0.9972	0.9951	0.9973
γ-Lindane (γ-BHC)	8.177	0.9954	0.9999	0.9963	0.9939	0.9991	0.9958	0.9987
Chlorpyrifos-methyl	9.072	1.0	0.9998	0.9934	0.9995	0.9997	0.9994	0.9995
Fenitrothion	9.602	0.9994	0.9980	0.9962	0.9954	0.9987	0.9989	0.9994
Aldrin	10.026	0.9958	0.9987	0.9992	0.9994	0.9987	0.9993	0.9998
Pendimethalin	10.451	0.9986	0.9981	0.9950	0.9929	0.9931	0.9988	0.9977
Folpet	10.966	0.9969	0.9966	0.9965	0.9911	0.9997	0.9933	0.9924
Dieldrin	11.847	0.9991	0.9998	0.9996	0.9999	0.9975	0.9975	0.9995
Bupirimate	11.759	0.9965	0.9993	0.9984	0.9994	0.9985	0.9972	0.9992
Triazophos	12.711	0.9996	0.9991	0.9913	0.9979	0.9956	0.9953	0.9973
Iprodione	13.826	0.9997	0.9996	0.9959	0.9985	0.9925	0.9990	0.9994
EPN	14.035	0.9998	0.9960	0.9931	0.9934	0.9921	0.9975	0.9949
Phosalone	14.65	0.9999	0.9994	0.9904	0.9978	0.9932	0.9997	0.9972
Mirex	15.147	0.9967	0.9998	1.0	0.9994	0.9988	0.9996	0.9938
Coumaphos	15.904	0.9982	0.9999	0.9908	0.9956	0.9921	0.9998	0.9932
Pyraclostrobin	17.63	0.9984	0.9996	0.9958	0.9981	0.9896	0.9992	0.9991
Deltamethrin	18.265	0.9965	0.9819	0.9930	0.9981	0.9975	0.9942	0.9907
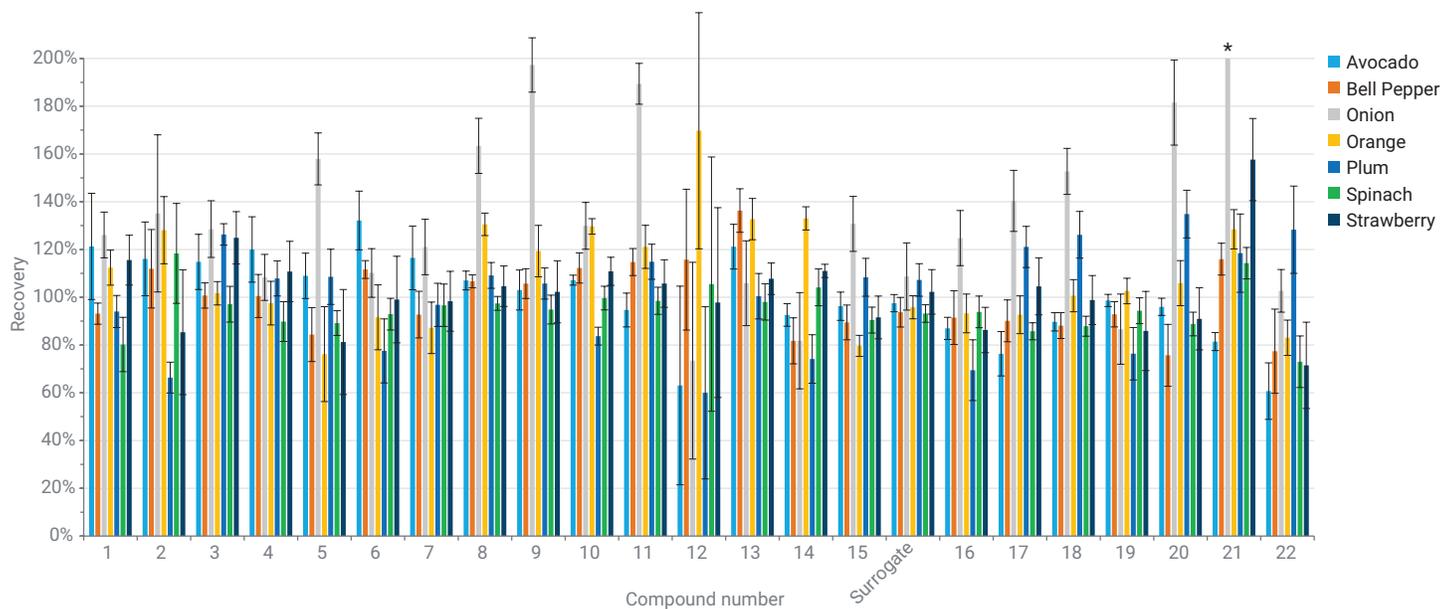
Figure A1. Average recoveries of seven matrices show good recoveries across most matrices. Seventy injections were completed with 10 randomized injections per matrix, and results were averaged across six liners. Compound numbers are from Table 1; the asterisk above pyraclostrobin in onion matrix (compound 21, gray bar) denotes off-scale recovery (>250%).
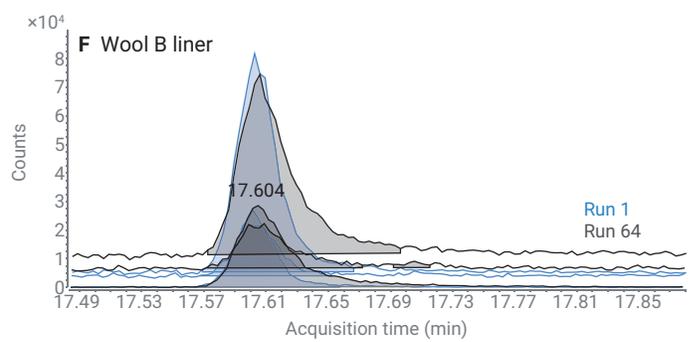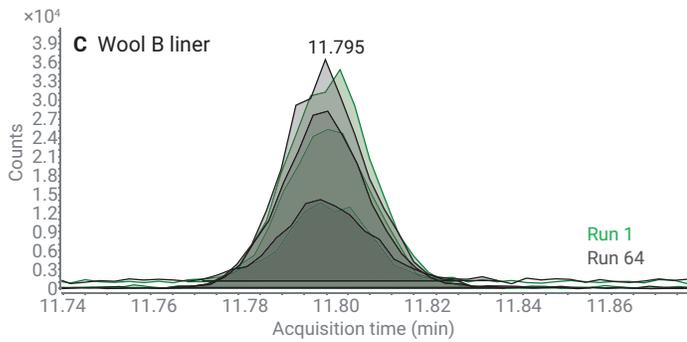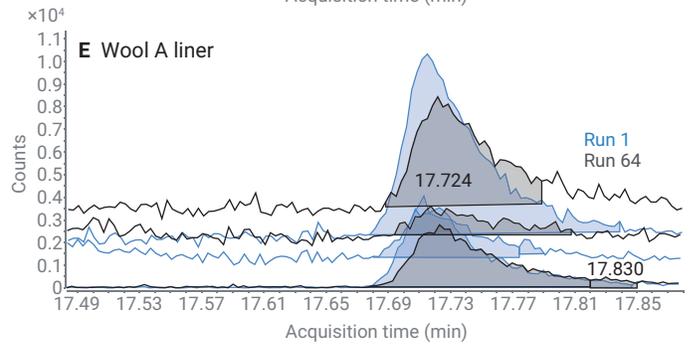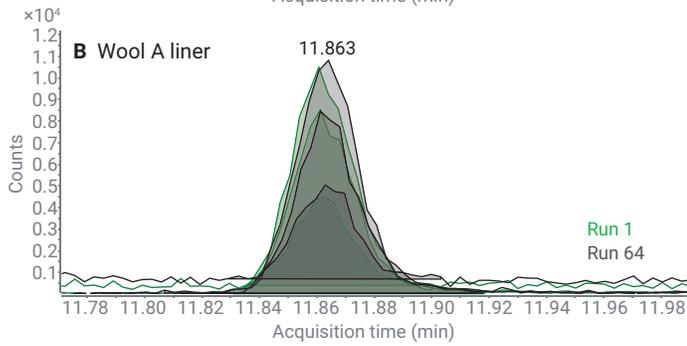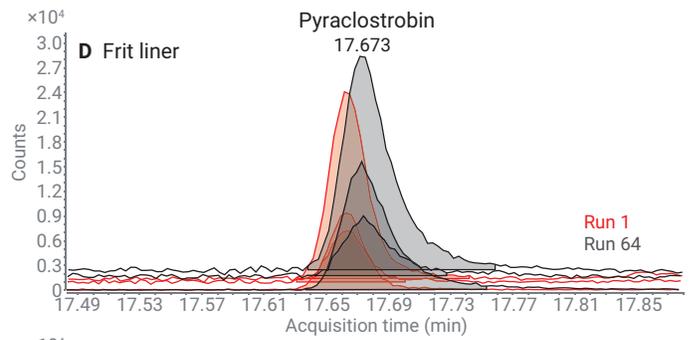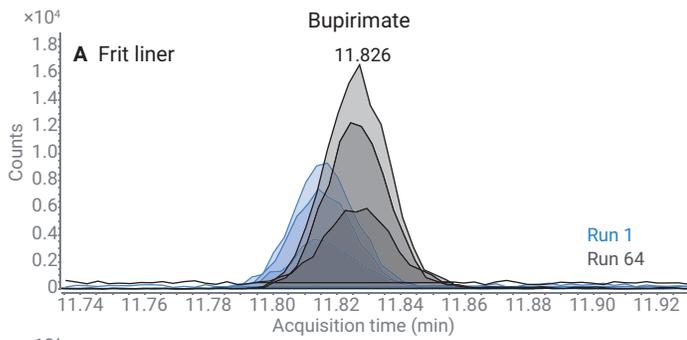
Figure A2. Extracted ion chromatogram overlays of the MRM transitions for the first (run 1) and last (run 64) bell pepper matrix runs of selected compounds on the fritted liner (A, D), wool A liner (B, E), and wool B liner (C, F). Pesticides at 10 ng/mL were sandwich injected with the bell pepper matrix.
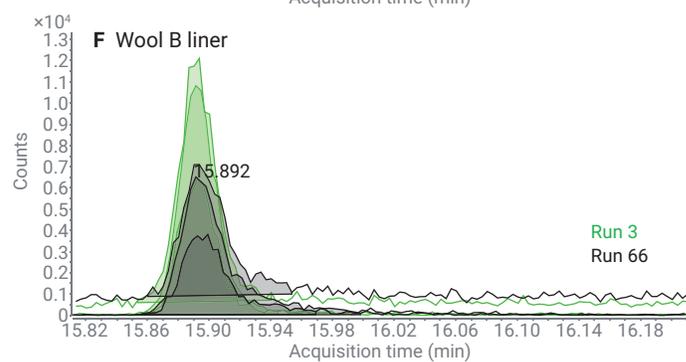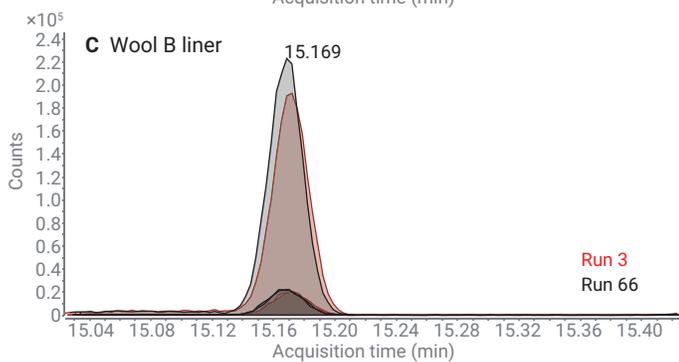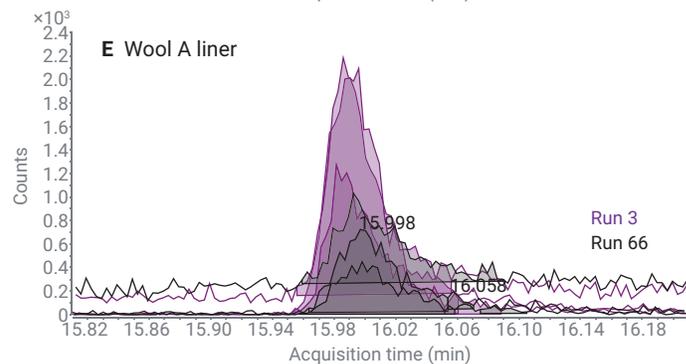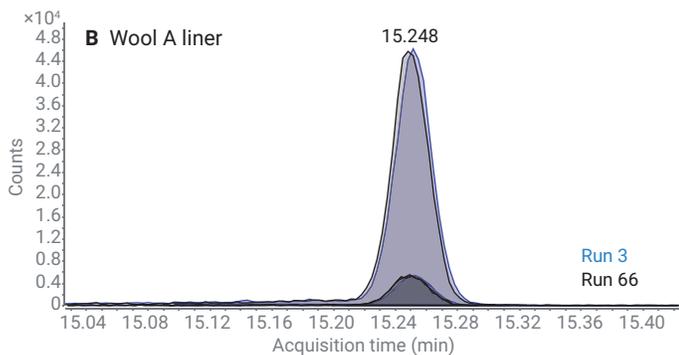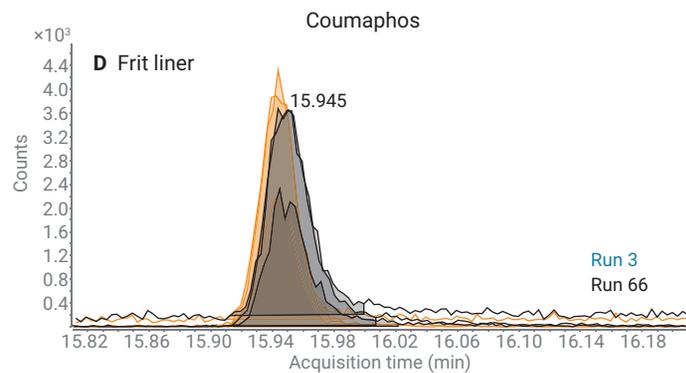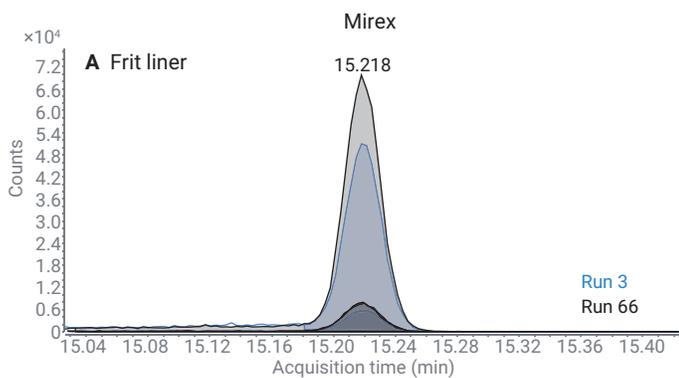
Figure A3. Extracted ion chromatogram overlays of the MRM transitions for the first (run 3) and last (run 66) strawberry matrix runs of selected compounds on the fritted liner (A, D), wool A liner (B, E), and wool B liner (C, F). Pesticides at 10 ng/mL were sandwich injected with the strawberry matrix.

Table A2. Repeatability (% RSD of average response factors) across 10 injections of 10 ng/mL pesticides with matrix, randomized in a 70-run matrix-matched sequence for six fritted liners.

Matrix/Liner	% RSDs for Average Response Factors (10 Replicates at 10 ng/mL)					
	Frit 1	Frit 2	Frit 3	Frit 4	Frit 5	Frit 6
Avocado	10.40%	7.23%	10.72%	15.93%	12.65%	8.57%
Bell Pepper	11.45%	9.47%	9.60%	13.73%	11.90%	11.70%
Onion	10.14%	9.11%	13.21%	11.09%	12.16%	10.02%
Orange	11.14%	8.94%	9.91%	12.88%	14.10%	11.35%
Plum	10.35%	9.50%	12.63%	12.10%	13.82%	12.08%
Spinach	11.72%	8.47%	9.18%	10.65%	14.33%	9.68%
Strawberry	12.57%	9.12%	11.14%	11.80%	17.12%	11.15%

Table A3. Repeatability (% RSD of average response factors) across 10 injections of 10 ng/mL pesticides with matrix, randomized in a 70 run matrix-matched sequence for three wool A and three wool B liners.

Matrix/Liner	% RSDs for Average Response Factors (10 Replicates at 10 ng/mL)					
	Wool A Liner 1	Wool A Liner 2	Wool A Liner 3	Wool B Liner 1	Wool B Liner 2	Wool B Liner 3
Avocado	14.45%	10.84%	8.08%	8.96%	5.95%	12.55%
Bell Pepper	14.30%	11.34%	8.91%	10.51%	10.36%	9.86%
Onion	22.41%	17.38%	14.24%	11.15%	11.22%	18.55%
Orange	16.13%	10.11%	11.00%	12.76%	8.24%	10.45%
Plum	24.51%	23.69%	12.50%	12.61%	10.03%	13.64%
Spinach	12.56%	12.34%	7.99%	8.98%	6.12%	13.21%
Strawberry	32.19%	21.70%	11.61%	10.14%	9.99%	16.21%

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