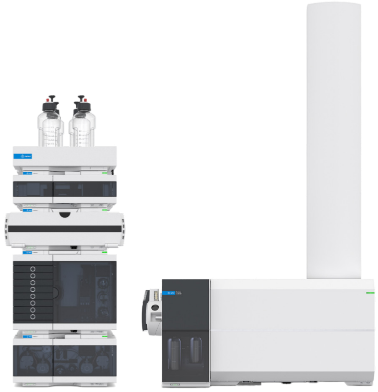
Application Note

Food Safety

Pesticide Screening with Fast Polarity Switching Using Agilent Revident LC/Q-TOF



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Abstract

This application note details the methodology for the screening of pesticides in complex food matrices using the Agilent Revident LC/Q-TOF with Fast Polarity Switching (FPS) and Single Polarity (SP) with Agilent MassHunter acquisition software for LC/MS systems (version 12.1) followed by data analysis with Agilent MassHunter Quantitative Analysis software (version 12.1). This method provides a comparison between FPS and SP in terms of mass accuracy of analyte and overall method performance.

Introduction

Laboratories around the world face the challenge of an ever-increasing sample load, demands for higher productivity, and faster turn-around times. Implementing Fast Polarity Switching (FPS) in MS-only data acquisition mode is one way to increase sample throughput two-fold, but the impact of FPS on mass accuracy, dynamic range, and analytical sensitivity are also important factors to consider when evaluating the merits of FPS versus Single Polarity (SP) mode.

To evaluate the impact of FPS on mass accuracy and dynamic range, a complex mixture of pesticides in black pepper matrix was analyzed in FPS and SP modes on the Agilent Revident LC/Q-TOF, which features both high-resolution and wide in-spectrum dynamic range. In addition, the thermally-inert flight tube on the Revident LC/Q-TOF system provides long-term mass stability for superior performance over time. This application note reports the average and compound-specific mass accuracy differences observed as a function of concentration and overall analytical sensitivity (1 to 100 ppb) between FPS and SP.

Experimental

Chemical and standards

Agilent InfinityLab Methanol for LC/MS (LC/MS-grade, MeOH) (part number 5191-5111), Agilent InfinityLab Acetonitrile for LC/MS (part number 5191-5101), Agilent LC/MS grade formic acid (part number 5191-4549), Agilent ammonium formate (part number G1946-85021), and ultrapure Milli-Q water were used. Acetic acid and ammonium fluoride were purchased from Sigma-Aldrich.

Sample preparation

The ground black pepper sample for the experiment was sourced from a local store. Samples were prepared following the QuEChERS Euronorm (EN) standard protocol. A portion (0.5 g) of ground black pepper was weighed, wetted with water (10 mL) as recommended to improve extraction efficiency of low moisture commodities[1], and extracted with acidified acetonitrile (10 mL with 1% acetic acid) using the Agilent Bond Elut QuEChERS EN extraction kit (part number 5982-5650CH). Further cleanup was carried out using a two-step process: the first step involved using the Agilent Captiva Enhanced Matrix Removal–General Pigmented Dry cartridge (EMR-GPD, part number 5610-2091) and the second step included passing collected eluant through an Agilent Captiva Enhanced Matrix Removal–General Pigmented Fresh cartridge (EMR-GPF, part number 5610-2090).[2] The resulting matrices were stored at –20 °C in scintillation vials.

The Agilent LC/MS Pesticide Comprehensive mix (part number 5190-0551), which contains over 200 compounds, was used as the standard mixture for calibration curve preparation. The standard was spiked into the matrix at eight points between 1 and 100 ppb.

Data acquisition

The samples were analyzed in five replicates using C18 reversed-phase chromatography with a UHPLC guard column (part numbers 959759-302 and 821725-901) on an Agilent 1290 Infinity II LC system with the Revident LC/Q-TOF with MassHunter Acquisition software for LC/MS systems, version 12.1. Data were collected using FPS mode and SP MS-only mode, first in positive ionization mode, followed by negative ionization mode. Two reference ions were used during acquisition to ensure consistent mass accuracy, *m/z* 121.0509 and 922.0098 for positive mode and *m/z* 119.0362 and 966.0007 for negative mode, respectively. Calibration of the instrument was completed before the worklist was started; the instrument ran for over two days without stopping for additional calibration.

Table 1A. LC parameters for the Agilent 1290 Infinity II LC.

Parameter	Value
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 × 150 mm 1.8 µm (p/n 959759-302)
Guard Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, UHPLC guard column (p/n 821725-901)
Column Temperature	45 °C
Injection Volume	4 µL
Sampler Temperature	4 °C
Needle Wash	Standard wash, 10 sec, MeOH:IPA (50:50)
Mobile Phase	A: Water + 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1% formic acid B: MeOH + 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1% formic acid
Flow Rate	0.45 mL/min
Gradient Program	Time (min) %B 0.00 2 0.50 2 1.00 50 4.00 65 16.00 100 18.00 100 18.10 2 20.00 2
Posttime	4 min
Total Run Time	24 min

Table 1B. MS parameters for the Revident LC/Q-TOF.

Source Parameter	Value
Sheath Gas Temperature	375 °C
Sheath Gas Flow	12 L/min
Gas Temperature	325 °C
Gas Flow	10 L/min
Nebulizer	35 psi
Capillary Voltage	2500 V
Nozzle Voltage	200 V
Reference Mass	*m/z* 121.0509 and 922.0098 (positive) *m/z* 119.0362 and 966.0007 (negative)
Acquisition Rate	FPS: 2 spectra/sec SP: 2 spectra/sec
MS Range	*m/z* 50 – 1,000

Screening parameters

Suspect screening data analysis was performed with Agilent MassHunter Quantitative Analysis software, version 12.1, applying the embedded LC Screener tool. The LC Screener tool summarizes subsets of information based on the presence, absence, or possible detection as decided by modifiable screening settings based on attributes of data such as mass accuracy, signal-to-noise ratio (S/N), mass match score, and retention time (Table 2).

Table 2. LC Screener tool outlier settings.

LC Screener Outlier Parameter	Value
Retention Time Window	10%
Minimum Signal to Noise	3
Coelution Score Limit	65
Mass Accuracy Limit	5 ppm
Mass Match Score Minimum	50
# of Verified Ions Minimum	1

Results and discussion

During mass spectrometric analysis, many compounds respond better to a particular ionization mode to produce mostly positive or mostly negative ions, creating an analytical challenge when a sample mix contains both analytes that favor positive ionization and analytes that favor negative ionization. To meet this challenge for high-resolution analysis, FPS data acquisition can be used to detect analytes in a mixture regardless of their preferred polarity. The faster a mass spectrometer can switch polarities, the more scans it can acquire from a particular chromatographic peak.

Standards for 200 pesticides in black pepper matrix were analyzed using the Revident LC/Q-TOF in both FPS and SP modes for MS-only data acquisition. Both data acquisition methods gave strong results with most mass accuracies within ±1 ppm (Figure 1). In addition, the %RSDs of the abundances were under 20% for > 98% of measurements made at the 5 and 10 ppb levels for all measured pesticides in both polarities using both FPS and SP modes.

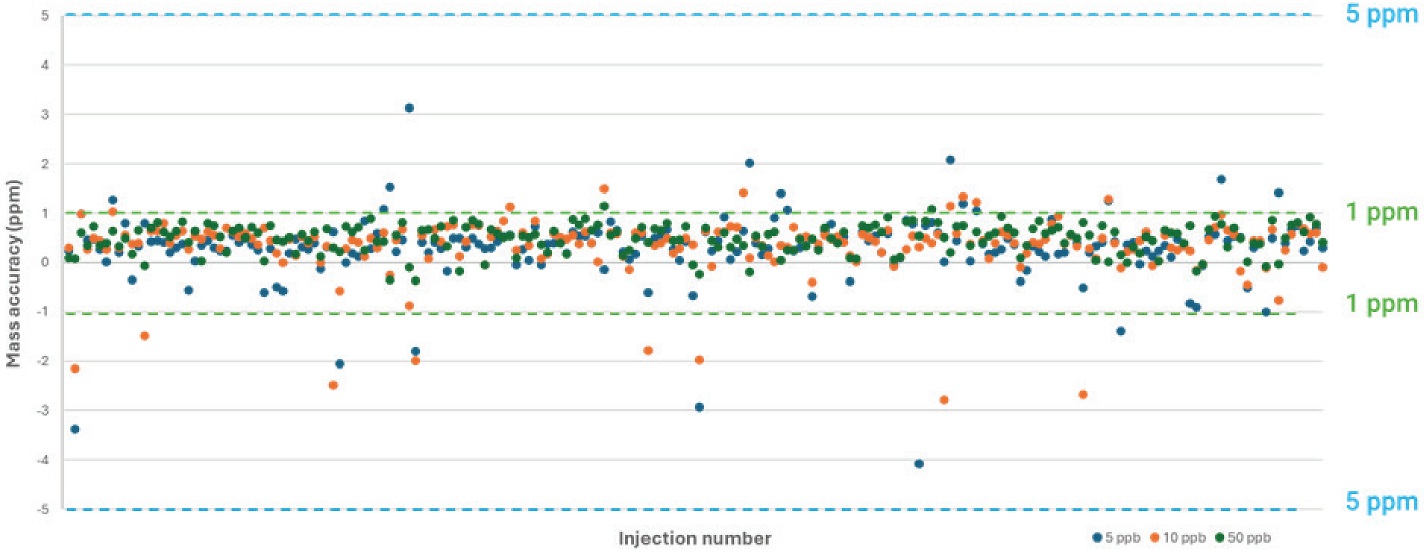


Figure 1. Mass accuracy stability for 200 pesticides across 18 hours of injections (corresponding to 45 consecutive injections) in Fast Polarity Switching (FPS) mode at 5, 10, and 50 ppb levels. > 93% of measurements fell within ±1 ppm (green lines) and 100% within ±5 ppm (blue lines).

Both FPS and SP data acquisition yielded calibration curves with $R^2 > 0.99$ for all identified compounds, with similar performance for all acquisition modes across both positive and negative polarity data at concentrations ranging from 1 to 100 ppb (Figures 2 and 3).

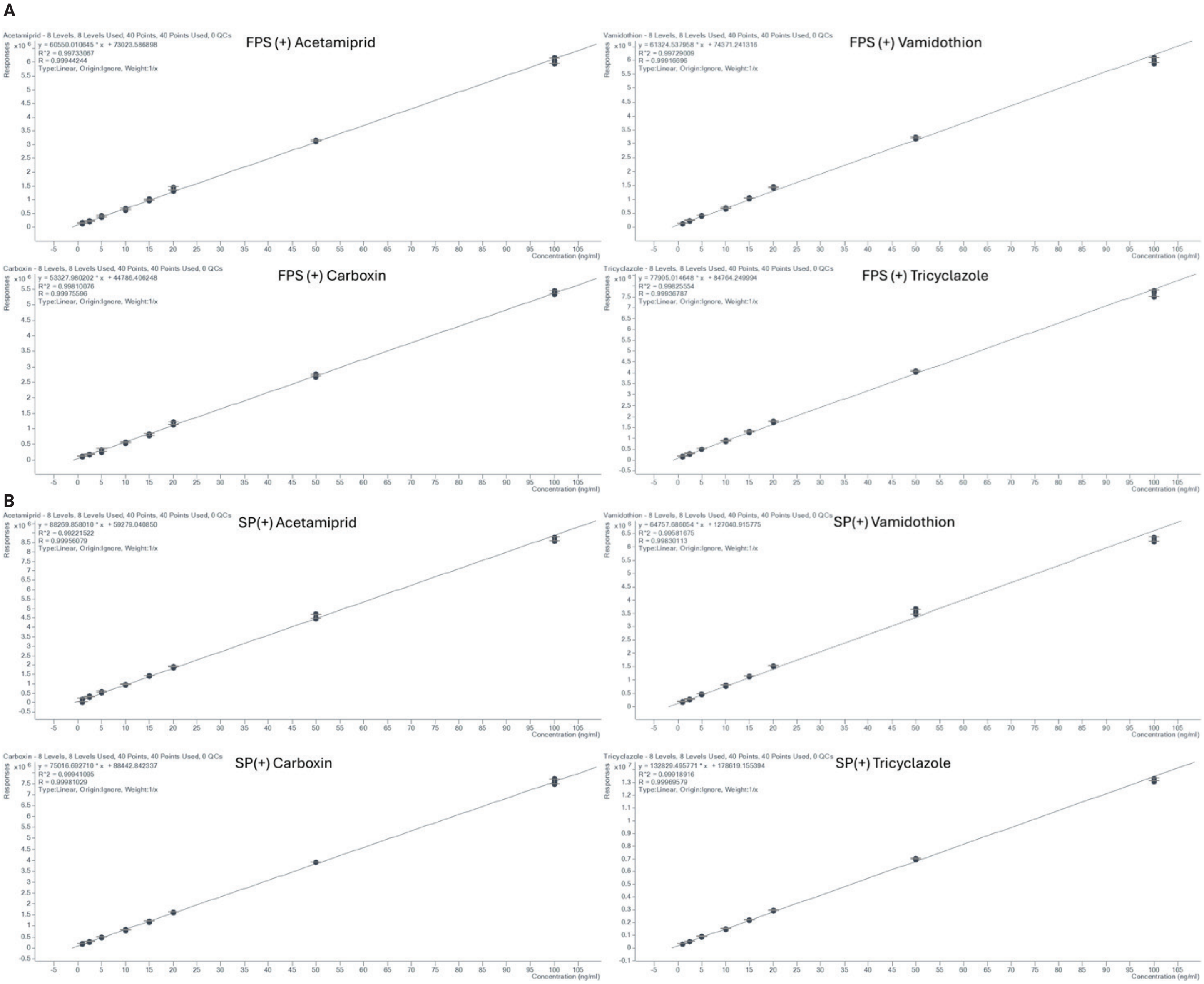


Figure 2. Calibration curves for selected positive ion compounds with FPS (A) and SP (B) demonstrating good linearity from 1 to 100 ppb.

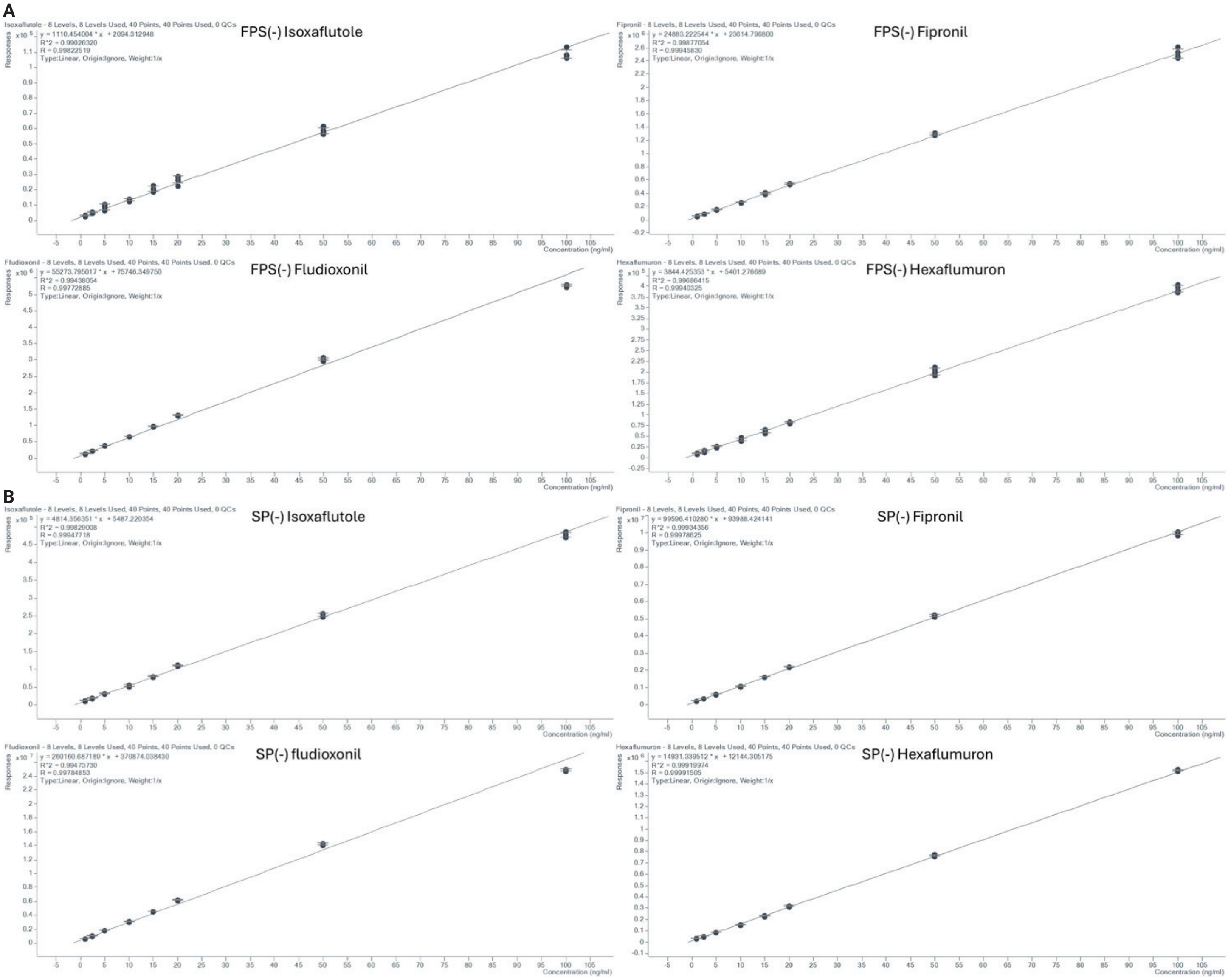


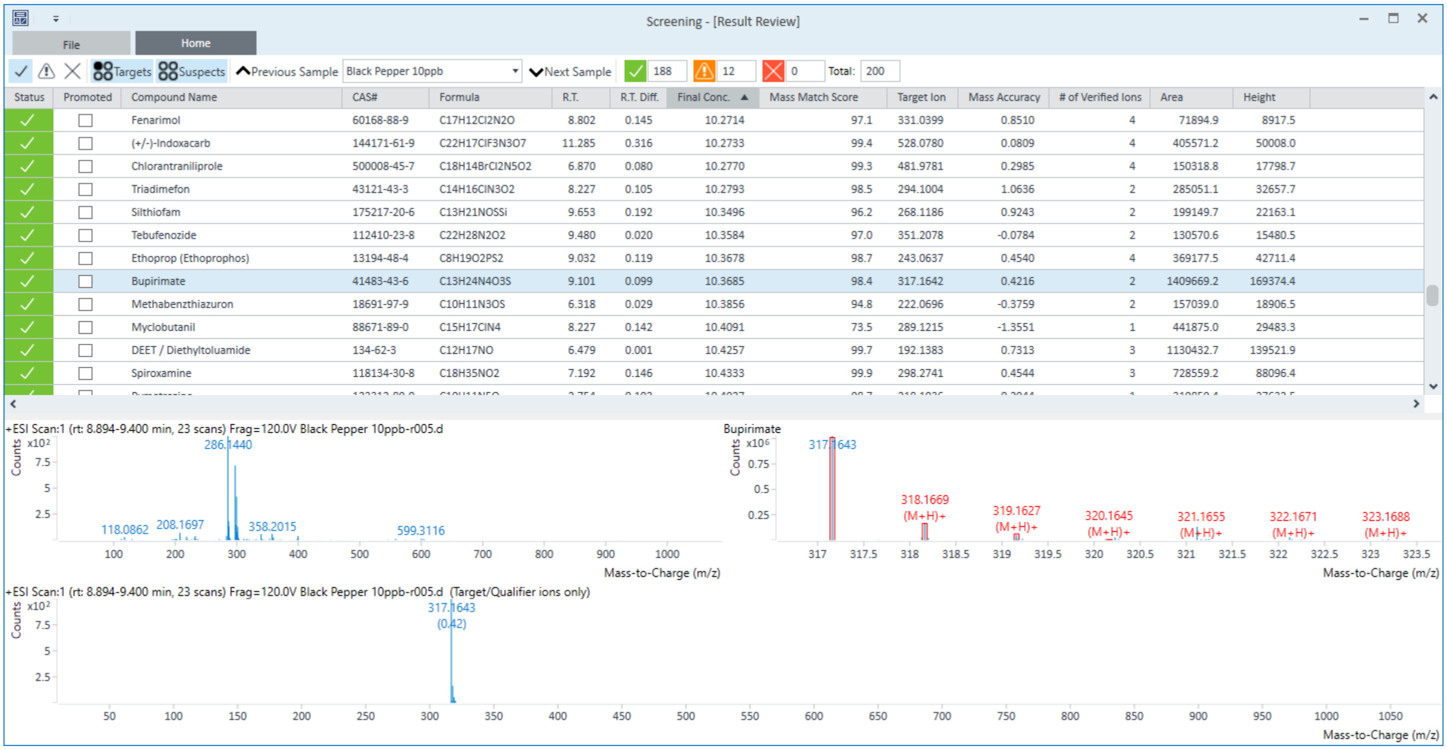
Figure 3. Calibration curves for selected negative ion compounds with FPS (A) and SP (B) demonstrating good linearity from 1 to 100 ppb.

The LC Screener tool in MassHunter Quantitative Analysis 12.1 was used to summarize data based on the presence (green), absence (red), or possible detection (orange) as decided by modifiable screening settings based on attributes of data including mass accuracy, signal-to-noise ratio, mass match score, and retention time (Figure 4). Screening parameters for pesticide data analysis are described in Table 2.

The number of pesticides identified with FPS data acquisition was nearly identical to the number of pesticides identified through a combination of both positive and negative SP data acquisition across multiple concentration levels, as shown in Table 3. These results demonstrate that the higher throughput achieved with FPS data acquisition does not limit compound identification, even in complex matrix with a combination of analytes that favor positive ionization and analytes that favor negative ionization.

Table 3. Number of pesticides identified at 5, 10, and 50 ppb with FPS and SP data acquisition modes.

Number of Pesticides Identified			
Level	FPS	Positive Only	Negative Only
5 ppb	189	160	38
10 ppb	194	160	39
50 ppb	198	160	40



Status	Promoted	Compound Name	CAS#	Formula	R.T.	R.T. Diff.	Final Conc.	Mass Match Score	Target Ion	Mass Accuracy	# of Verified Ions	Area	Height
✓	☐	Fenarimol	60168-88-9	C17H12Cl2N2O	8.802	0.145	10.2714	97.1	331.0399	0.8510	4	71894.9	8917.5
✓	☐	(+/-)-Indoxacarb	144171-61-9	C22H17ClF3N3O7	11.285	0.316	10.2733	99.4	528.0780	0.0809	4	405571.2	50008.0
✓	☐	Chlorantraniliprole	500008-45-7	C18H14BrCl2N5O2	6.870	0.080	10.2770	99.3	481.9781	0.2985	4	150318.8	17798.7
✓	☐	Triadimefon	43121-43-3	C14H16ClN3O2	8.227	0.105	10.2793	98.5	294.1004	1.0636	2	285051.1	32657.7
✓	☐	Silthiofam	175217-20-6	C13H21NOSSi	9.653	0.192	10.3496	96.2	268.1186	0.9243	2	199149.7	22163.1
✓	☐	Tebufenozide	112410-23-8	C22H28N2O2	9.480	0.020	10.3584	97.0	351.2078	-0.0784	2	130570.6	15480.5
✓	☐	Ethoprop (Ethoprophos)	13194-48-4	C8H19O2PS2	9.032	0.119	10.3678	98.7	243.0637	0.4540	4	369177.5	42711.4
✓	☐	Bupirimate	41483-43-6	C13H24N4O3S	9.101	0.099	10.3685	98.4	317.1642	0.4216	2	1409669.2	169374.4
✓	☐	Methabenzthiazuron	18691-97-9	C10H11N3OS	6.318	0.029	10.3856	94.8	222.0696	-0.3759	2	157039.0	18906.5
✓	☐	Myclobutanil	88671-89-0	C15H17ClN4	8.227	0.142	10.4091	73.5	289.1215	-1.3551	1	441875.0	29483.3
✓	☐	DEET / Diethyltoluamide	134-62-3	C12H17NO	6.479	0.001	10.4257	99.7	192.1383	0.7313	3	1130432.7	139521.9
✓	☐	Spiroxamine	118134-30-8	C18H35NO2	7.192	0.146	10.4333	99.9	298.2741	0.4544	3	728559.2	88096.4

Figure 4. The Agilent LC Screener tool provides an easy-to-navigate table with a clear, color-coordinated display that connects seamlessly with the corresponding spectral displays.

Conclusion

Streamlined screening of 200 pesticides with both FPS and SP data across concentration levels was facilitated by a robust library of pesticides complete with accurate mass and retention time information, LC Screener tool settings of mass accuracy ≤ 5 ppm, coelution score above 65%, and signal-to-noise ≥ 3. Good mass accuracy is achieved with both fast polarity switching and single polarity MS acquisition. These results demonstrate the time-saving ability of FPS data acquisition without loss of data quality with the Agilent Revident LC/Q-TOF, which makes this instrument ideal for high-level suspect screening.

References

1. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed, SANTE/11312/2021v2, https://food.ec.europa.eu/system/files/2023-11/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf
2. Analysis of 510 Pesticides in Black Pepper Using Captiva EMR Sequential Passthrough Cleanup and LC/MS/MS. *Agilent Technologies application note*, publication number 5994-4768EN, **2023**.

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