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Routine Pesticide Screening Solution: Data Independent Acquisition with a New LC/Q-TOF and Dedicated Screening Software

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Introduction

Data Independent Acquisition for Routine Pesticide Screening

The requirement for robust and reliable screening methods for pesticides in food matrices continues to emerge as a point of challenge and necessity. With the ever-growing list of compounds of interest and a greater number of matrices being tested every day there is a need for confident screening methods. The utilization of data independent acquisition (DIA) affords scientists the ability to screen for targets while also acquiring non-targeted fragment information, which could be used for retroactive analysis of emerging contaminants and future suspects.

A high-resolution LC/Q-TOF (quadrupole time of flight) facilitates the DIA acquisition while aiding identification with extended dynamic range, stable accurate mass, isotopic fidelity, and acquisition speeds supporting specificity of fragment ions.

Presented here is a workflow for pesticide screening that includes QuEChERs sample preparation, the Agilent 1290 Infinity II LC, the new Revident LC/Q-TOF, and MassHunter Quantitative Analysis 12 Screener Tool for fast and routine pesticide analysis. (Figure 1)

Figure 1. Revident LC/Q-TOF with 1290 Infinity II LC

Key performance elements of the new Revident LC/Q-TOF are a new detector, resulting in better mass accuracies over a broad range of abundances, as well as an increased dynamic range compared to previous instrument generations. In combination with the Thermo-controlled flight tube, which contributes over long-time mass stability, the overall mass accuracy has substantially increased. This makes the Revident LC/Q-TOF extremely suitable for routine screening in food safety as demonstrated in this work.

Experimental

Sample Preparation and All Ions Acquisition for Complex Food Matrices

An organic broccoli matrix was prepared with QuEChERS for pigmented fruits and vegetables, after being homogenized. The supernatant was spiked with over 200 pesticides (PN 5190-0551) at 8 different concentrations from 0.625 ppb to 100 ppb and 4 heavy labeled standards at 50 ppb (n=6).[1] The samples were analyzed utilizing reverse phase chromatography on a 1290 Infinity II LC and All Ions non-targeted acquisition in both positive and negative mode. The collision energies (CE) used were 20 and 40 to fragment molecular ions. Two reference ions were used to ensure mass accuracy.

Table 1. LC method with Agilent 1290 Infinity II LC.

LC Conditions		
Analytical Column	Agilent ZORBAX Eclipse Plus C18, 3.0 x 150 mm 1.8 µm, (p/n 959759-302)	
Guard Column	ZORBAX Eclipse Plus C18, 2.1 mm, 1.8 µm, UHPLC guard column (p/n 821725-901)	
Column temperature	45 °C	
Injection volume	3 µL	
Autosampler temp	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 = ACN + 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1% formic acid	
Flow rate	0.45 mL/min	
Gradient program	Time 0.00 0.50 1.00 4.00 16.00 18.00 18.10 20.00	%B 2 2 50 65 100 100 2 2
Post Time	4 min	

Table 2. Revident LC/Q-TOF acquisition parameters.

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
MS Mode	Positive and Negative
Acquisition	All Ions with 0, 20, and 40 CE experiments
MS Range	*m/z* 50 to 1,000
Reference Mass	*m/z* 121.0509 and 922.0098 (positive) *m/z* 119.0363 and 966.0007 (negative)

The chromatography and analysis allowed for the detection of 214 compounds, including the separation of 5 isomeric pairs in the standard mixture.

The Steppingstones to Confident Screening: Mass Accuracy, Stable Area Counts and Fragments

The 200+ compounds were measured with good mass accuracies, most within ±2 ppm (Figure 2). The %RSD of the abundance were under 20% for > 99% of measurements made at the 5 ppb and 10 ppb levels for all target compounds. As detection limits were pushed the RSDs increased, but the system proved very sensitive even at low limits (Figure 3). The calibration curves showed good linearity with a $R^2 \geq 0.99$ for all target compounds from 0.625 ppb to 100 ppb (Figure 4).

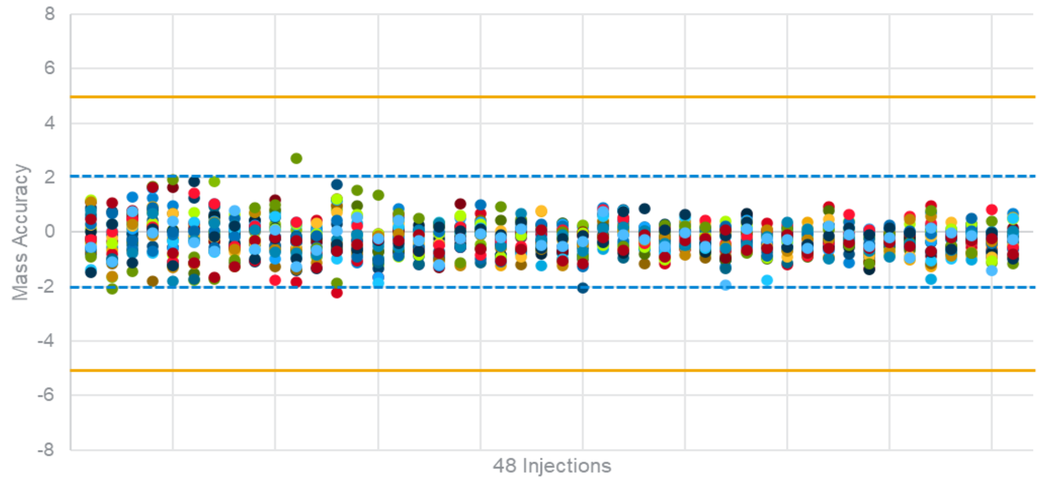


Figure 2. Mass accuracy stability of 25 different analytes (various colors) over the entire calibration range from 48 injections over 18 hours. (blue dotted line ±2 ppm and yellow solid ±5 ppm)

MassHunter Quant 12 offers clear data visualization and calibration curve generation. All Ions data provides fragment ions for confident identification scored for ratio and coelution related to the molecular ion, allowing for processing to distinguish non-targeted noise from specific fragment ions. All the analytes have consistent abundance and mass accuracy over the full calibration range, without stopping the worklist for maintenance or mass calibration over extended periods (Figure 2 and 3).

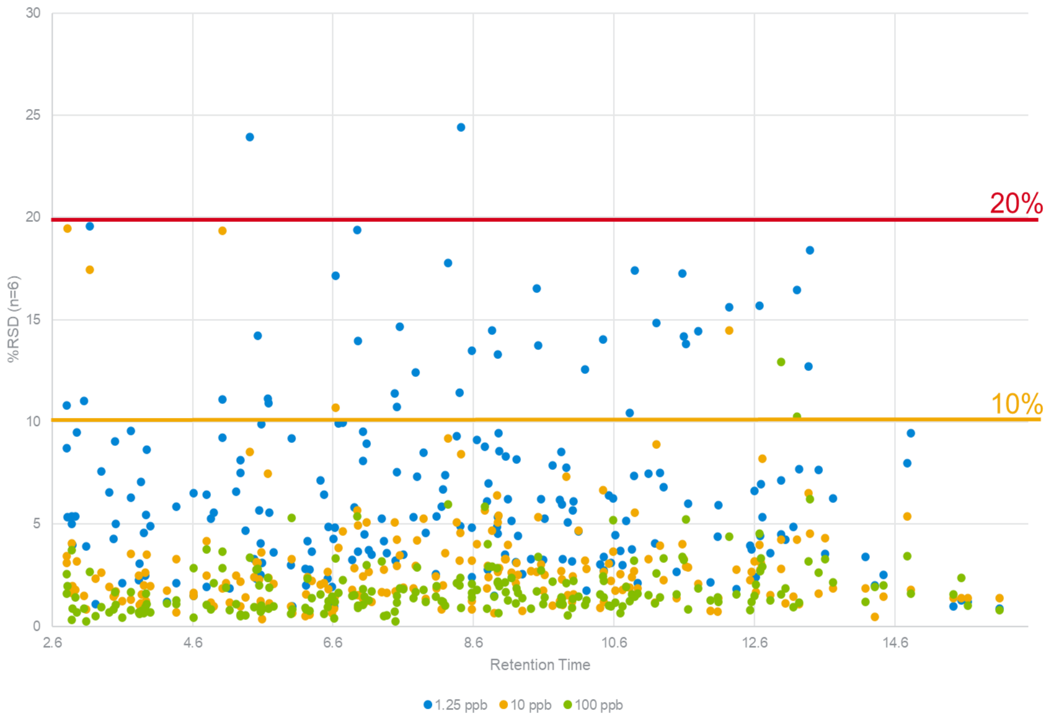


Figure 3. %RSDs (n = 6) at 1.25, 10, and 100 ppb for compounds screened in complex matrix as identified, plotted against RTs.

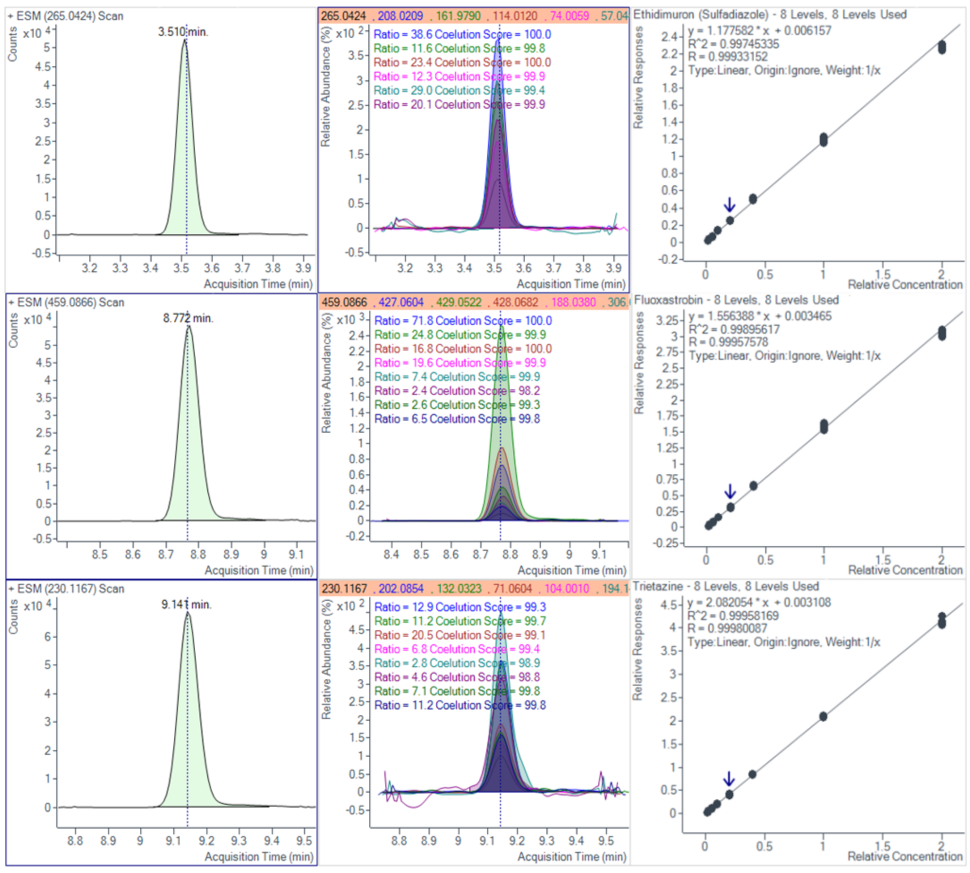
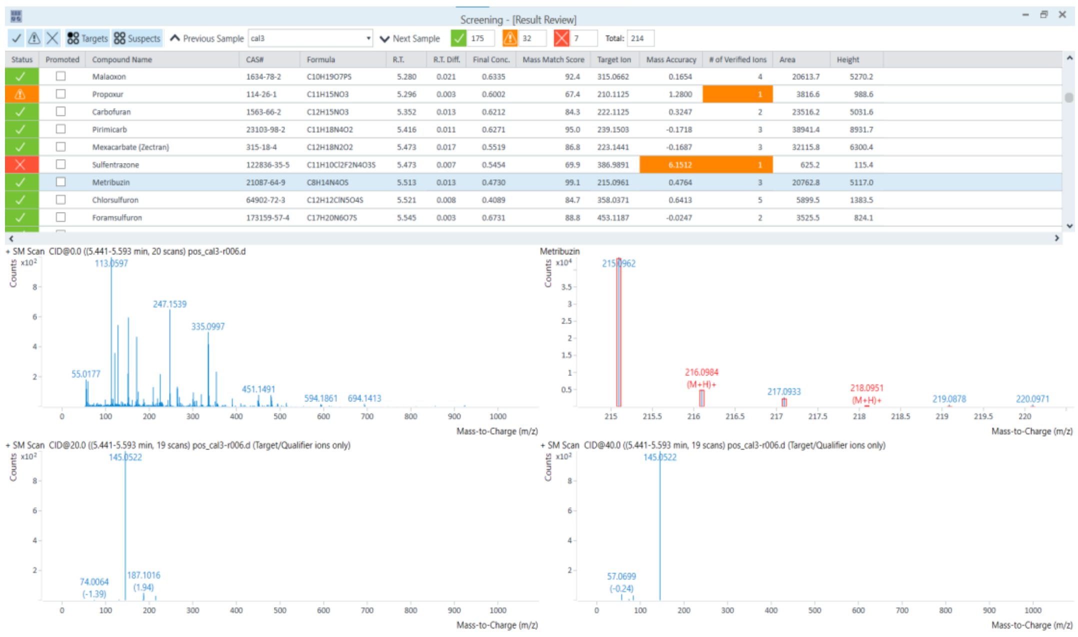


Figure 4. Analyte examples, molecular ions (left), fragment ions with ratio and coelution score at 10 ppb in matrix (center), and full calibration curve (right).

All the data was analyzed using SureMass, a novel chemical feature detection algorithm which speeds up processing and enhances extended quantitative accuracy while maintaining trace analysis.

MassHunter Quant and LC Screener Workflow

The simple and customizable workflow available in MassHunter Quant 12 affords the analysis of various adducts from the same origin PCDL, confident optimization of method parameters to suit the performance of your analytes, and a simple user interface for quick data analysis of dense Q-TOF data (Figure 5).



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
✓	☐	Malaoxon	1634-78-2	C10H19O7PS	5.280	0.021	0.6335	92.4	315.0662	0.1654	4	20613.7	5270.2
⚠	☐	Propoxur	114-26-1	C11H15NO3	5.296	0.003	0.6002	67.4	210.1125	1.2800	1	3816.6	988.6
✓	☐	Carbofuran	1563-66-2	C12H15NO3	5.352	0.013	0.6212	84.3	222.1125	0.3247	2	23516.2	5031.6
✓	☐	Pirimicarb	23103-98-2	C11H18N4O2	5.416	0.011	0.6271	95.0	239.1503	-0.1718	3	38941.4	8931.7
✓	☐	Mexacarbate (Zectran)	315-18-4	C12H18N2O2	5.473	0.017	0.5519	86.8	223.1441	-0.1687	3	32115.8	6300.4
✕	☐	Sulfentrazone	122836-35-5	C11H10Cl2F2N4O3S	5.473	0.007	0.5454	69.9	386.9891	6.1512	1	625.2	115.4
✓	☐	Metribuzin	21087-64-9	C8H14N4OS	5.513	0.013	0.4730	99.1	215.0961	0.4764	3	20762.8	5117.0
✓	☐	Chlorsulfuron	64902-72-3	C12H12ClN5O4S	5.521	0.008	0.4089	84.7	358.0371	0.6413	5	5899.5	1383.5
✓	☐	Foramsulfuron	173159-57-4	C17H20N6O7S	5.545	0.003	0.6731	88.8	453.1187	-0.0247	2	3525.5	824.1

Figure 5. LC Screener user interface with easy to analyze colorful designations allows for navigation from sample summary to compound data for in depth review.

Screening outliers were set in consideration of SANTE guidelines[2], including the requirement of mass accuracy ≤ 5 ppm, fragment coelution score above 80%, the signal-to-noise ≥ 3, and the 2 ions verified with mass accuracy.

Results and Discussion

Enhanced Screening for Suspects: How MassHunter Quant 12 Addresses Various Levels of PCDL Info

Robust PCDL information can bolster the efficiency of DIA screening methods. MS/MS spectra and RTs built from standards are not always available. Independent of the information available in the PCDL the screening method needs to be able to offer information on the presence of the analyte. The ability of the instrument and software to enable this identification workflow, without standards, is essential to supporting future purchase of necessary analytes and library building.

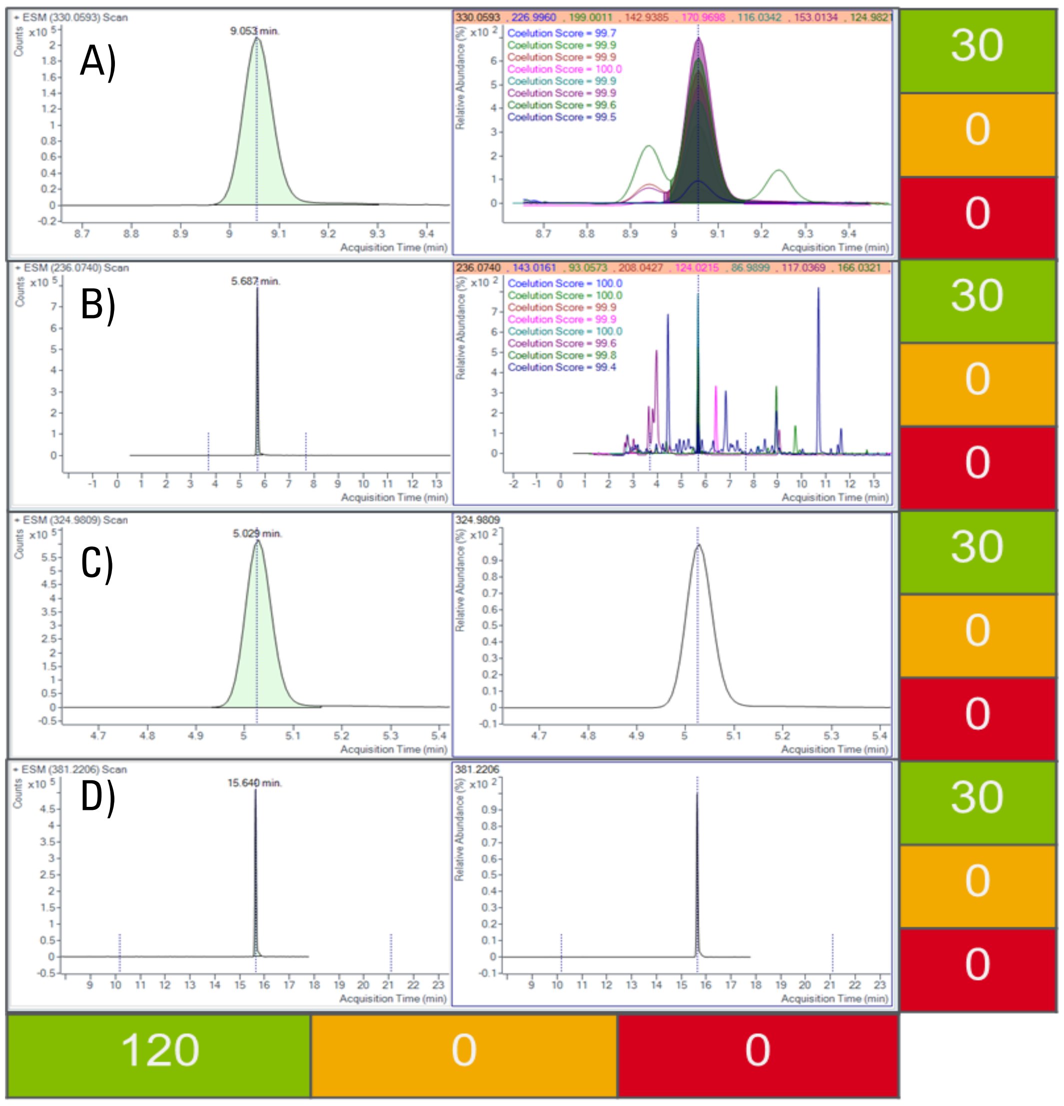


Figure 6. MassHunter Quant 12 evaluation of DIA screening when various levels of information is provided in the PCDL. (A) RT and spectra, (B) no RT, (C) no spectra, and (D) no RT or spectra. The right shows all true positive hits for each scenario with LC Screener at 5 ppb level.

As a proof of concept, a subset of analytes were processed with different PCDL information settings. For each state 30 random compounds, distributed evenly among RTs, were included. (A) with a recorded RT and spectra, (B) with no RT but with spectra, (C) with no spectra but with RT, and (D) with no RT or spectra. The LC Screener evaluations were modified to allow for the new information levels, criteria was changed to “close rt” for (C) and “greatest response” for (B) and (D) (Figure 6). Required verified ions were modified to 1 for all states without spectra. Of the 120 analytes, 30 in each state, all were screened as identified for the top 6 concentrations, and no analytes were tagged as unidentified at any targeted concentration.

Accurate and confident screening of suspect compounds, or those without a calibration curve is important to the detection of emerging contaminants. As compounds of interest are added to lists the DIA acquisition mode allows for the retroactive analysis of past data, easy expansion of the analysis method, and evidence to determine whether this compound needs to become a target, requiring the addition of a standard and MS/MS data loaded into the accompanying PCDL.

Tools in MassHunter Quant 12 to Simplify and Support Confident Screening

The Update RT Tool and LC Screener Setup were utilized in the processing of the complex LC/Q-TOF data to generate quick answers with understandable analysis settings (Figure 7).

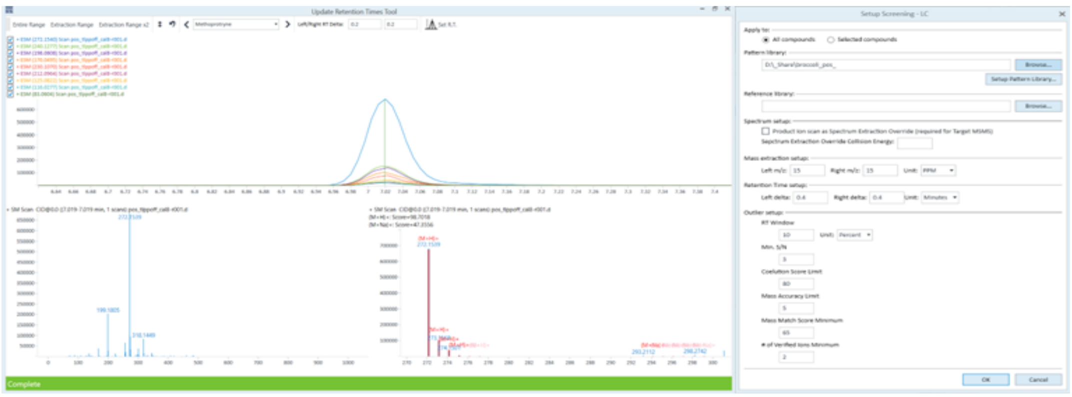


Figure 7. Update RT Tool (left) allows for individual optimization of retention times and LC Screener Setup (right) allows customization of screening on a per analyte or group basis.

Conclusions

Confident Pesticide Screening with All Ions Acquisition and Updated LC/Q-TOF Hardware and Software

Over 200 pesticides screened for, using a DIA mode of acquisition from 0.625 ppb to 100 ppb

- New Agilent Revident LC/Q-TOF
- Good Mass Accuracy, Low RSD’s, Scored Coelution of Fragment Ions
- Sample Preparation and Detection in Complex Matrix
- Analysis with MassHunter Quant 12 Showing Ease of Screening regardless of PCDL Input Information

References

[1] Yannell, K.; *et al.* Enhanced Food Safety Testing: A Pesticide Screening Methodology Using the Agilent 6546 LC/Q-TOF and MassHunter Quantitative Analysis Software 10.0 LC/Q-TOF Screener Tool, *Agilent Technologies Application Note,* 5994-0738EN, 2019.

[2] Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed SANTE 11312/2021.

https://www.agilent.com/en/promotions/asms

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