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Ultra-Fast Multiresidues Accurate Mass Screening Strategy

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Introduction

In the area of mass food production and global goods exchange, it is crucial to develop ultra-fast screening to assure raw material food safety. Annually in Europe, for instance, more than 88,000 samples are analyzed for the presence of pesticide residues. Due to that fact, it is crucial to develop rapid methods to facilitate swift safety assessments of raw goods being introduced to the market. Utilizing high resolution accurate mass spectrometry offers a unique opportunity to employ an ultra-fast chromatographic gradient to improve on reliable residue detection in complex matrices.

Here we are presenting a strategy combining a fast LC chromatography gradient using full spectra acquisition with Revident LC/Q-TOF (Figure 1) and its excellent mass accuracy followed by automated data processing and reinjection with different chromatographic conditions and acquisition modes providing some fragmentation information.



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

Experimental

Standards and sample preparation

Vegetable and fruit extracts were prepared following QuEChERS EN standard protocol cartridge (p/n 5982-5650CH) followed by further cleanup using dispersive solid phase extraction (dSPE): broccoli and celery were prepared using the Agilent Bond Elut QuEChERS Pigmented Fruit and Vegetables dSPE kit (part number 5982-5256), and strawberry using the Agilent Bond Elut QuEChERS General Fruits and Vegetables dSPE kit (part number 5982-5056). A pesticide mix comprising over 150 pesticide standards was spiked into the matrices at eight points to make matrix-matched standard curves with concentration ranging from 1 to 100 µg/L.¹

Experimental

LC/MS Analysis

All the samples were analyzed utilizing reverse phase chromatography on a 1290 Infinity II LC and MS only acquisition mode in positive ionization mode for the first-tier pass and All Ions acquisition for the second-tier pass. Two reference ions were used to ensure mass accuracy (Table 1 & 2).

Instrumentation

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

LC Conditions																																										
Analytical Column First Tier	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm 1.8 µm (p/n 959757-302)																																									
Analytical Column Second Tier	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 x 150 mm 1.8 µm (p/n 959759-302)																																									
Injection Volume	4 µL																																									
Sampler Temperature	4 °C																																									
Needle Wash	Standard wash, 10 s, 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	1.0 mL/min	0.45 mL/min																																								
Gradient	<table border="1"> <thead> <tr> <th colspan="2">Fast Screening</th> <th colspan="2">All Ions Chromatography</th> </tr> <tr> <th>Time</th> <th>%B</th> <th>Time</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0.00</td> <td>10</td> <td>0.00</td> <td>2</td> </tr> <tr> <td>0.15</td> <td>10</td> <td>0.50</td> <td>2</td> </tr> <tr> <td>0.85</td> <td>65</td> <td>1.00</td> <td>50</td> </tr> <tr> <td>2.95</td> <td>100</td> <td>4.00</td> <td>65</td> </tr> <tr> <td>3.30</td> <td>100</td> <td>16.00</td> <td>100</td> </tr> <tr> <td>3.35</td> <td>10</td> <td>18.00</td> <td>100</td> </tr> <tr> <td>3.50</td> <td>10</td> <td>18.10</td> <td>2</td> </tr> <tr> <td></td> <td></td> <td>20.00</td> <td>2</td> </tr> </tbody> </table>		Fast Screening		All Ions Chromatography		Time	%B	Time	%B	0.00	10	0.00	2	0.15	10	0.50	2	0.85	65	1.00	50	2.95	100	4.00	65	3.30	100	16.00	100	3.35	10	18.00	100	3.50	10	18.10	2			20.00	2
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		20.00	2																																							
Post-Time	0.5 min and 4 mins, respectively																																									

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

Parameters	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	<i>m/z</i> 121.0509 and 922.0098
First Tier Acquisition mode	MS only
Second Tier Acquisition mode	All Ions with CE20 and CE40V
MS Range	<i>m/z</i> 50–1,000
MS Acquisition Rate	3 Hz
All Ions Acquisition Rate	6 Hz

Mass Accuracy, Isotopic Fidelity, and Extended Dynamic Range for Effective Screening.

150+ pesticides were measured with good mass accuracy in all matrices, all within ± 2 ppm for the MS spectra with the fast LC method (Figure 2). The calibration curves showed good linearity with a $R^2 \geq 0.99$ for all target compounds from 1 ppb to 100 ppb. The %RSD of the abundance were under 20% for all of measurements made at the 5 ppb, 10 ppb and 15 ppb levels for all target compounds. Second tier LC-MC method with All Ions acquisition showed similar results for mass accuracy, calibration curves and %RSD (Figure 3).

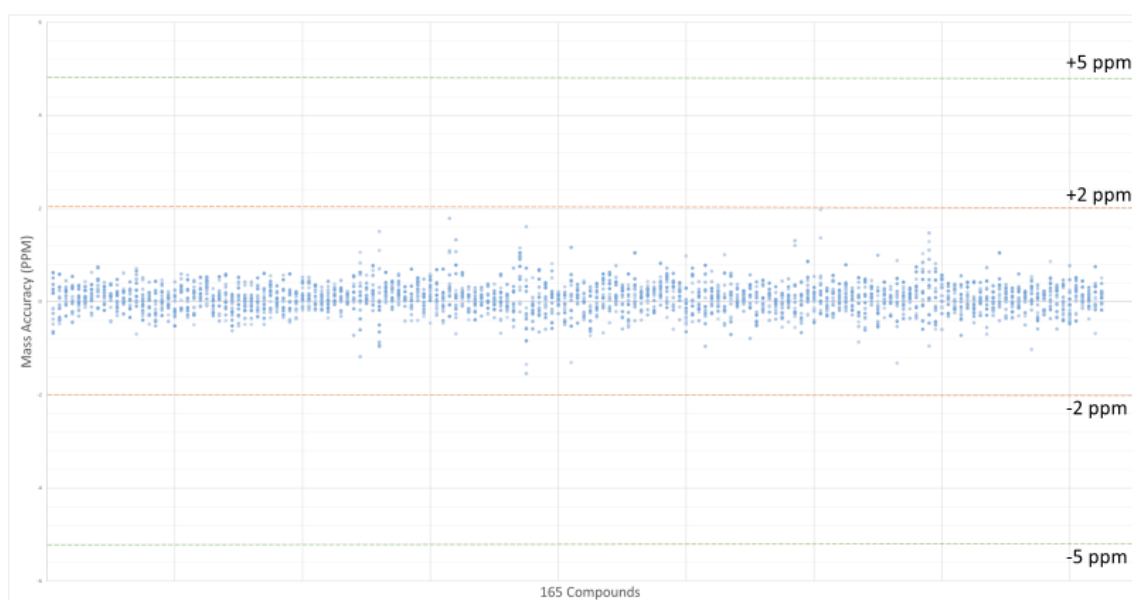


Figure 2. Mass accuracy of 165 pesticides with fast LC method (First Tier) in strawberry matrix over the entire calibration range with triplicate injection.

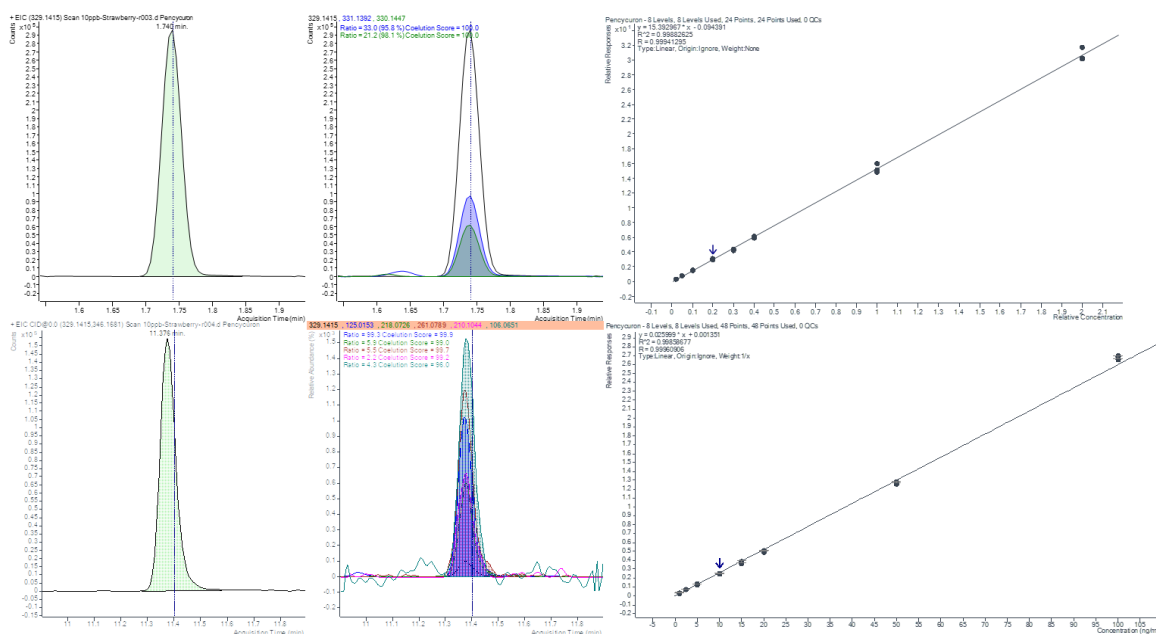


Figure 3. Pencycuron analyte with molecular ions (left), isotopic pattern for MS only (top center), fragment ions with ratio and coelution score (bottom center) for All Ions at 10 ppb in matrix, and corresponding full calibration curves (right).

Intelligent Reflex Fast Screening for Efficient Assessment of Food Matrices.

Automated worklist intervention was utilized to screen food matrices for pesticides first with an ultra-fast chromatographic method using MS Only acquisition. Dependent on detection of pesticide residues, as analyzed automatically by MassHunter Quantitative Analysis 12.1, samples with target hits are reinjected using a longer chromatographic method and All Ions acquisition. The All Ions acquisition includes multiple collision energies affording the analyst fragmentation information pertinent to the confident identification of the hit. This workflow is displayed in Figure 4.

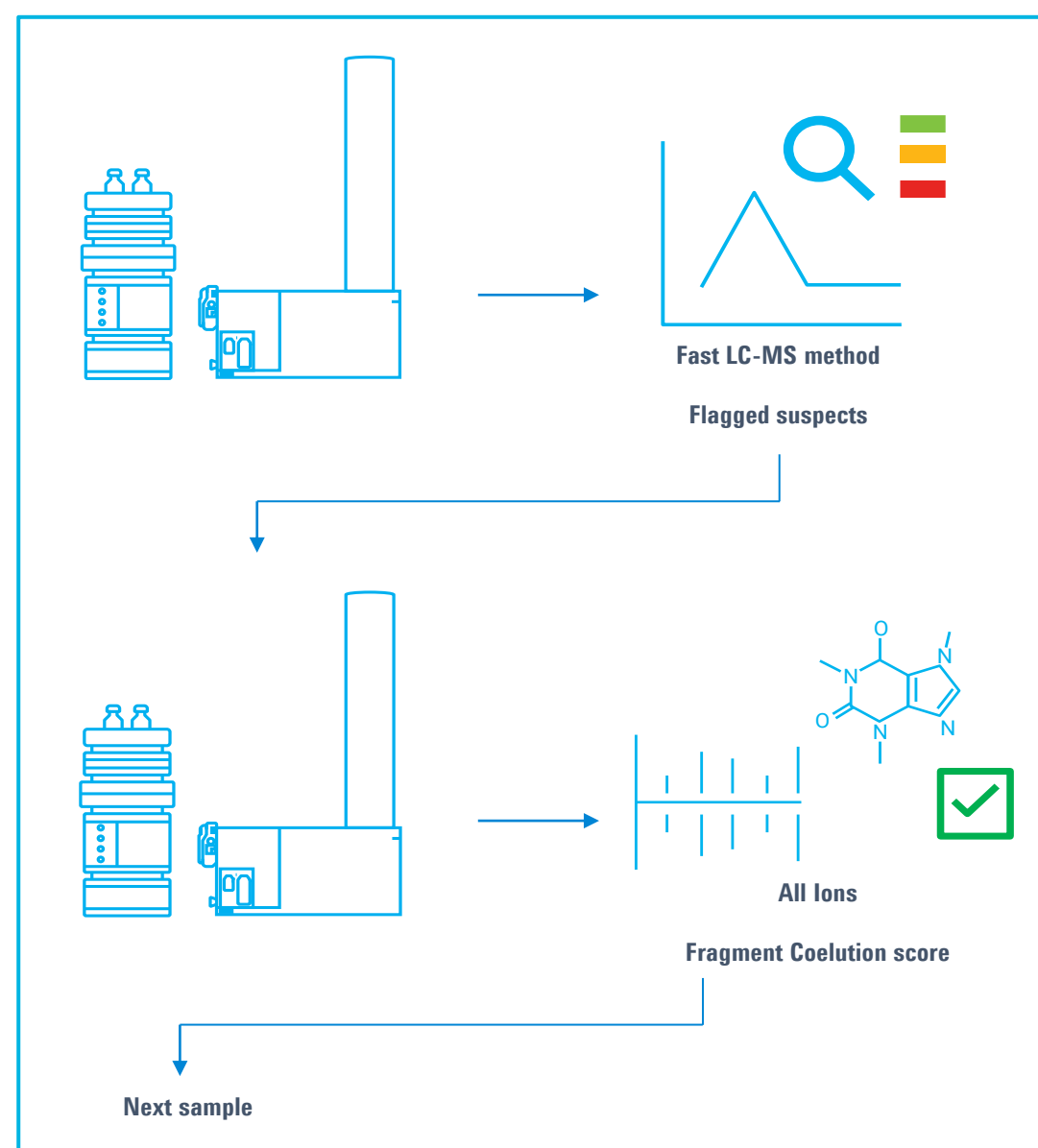


Figure 4. Intelligent reflex reinjection workflow with initial ultra-fast chromatography followed by All Ions data independent acquisition.

ChemVista Library Management Software for Library Curation.

In order to facilitate creation of a dedicated library, ChemVista library management software allows users to curate libraries with spectra collected in their own labs, acquired from Agilent libraries, and from 3rd party open sources, storing method specific retention times and engaging multivariable searches for streamlined list creation.

Results and Discussion

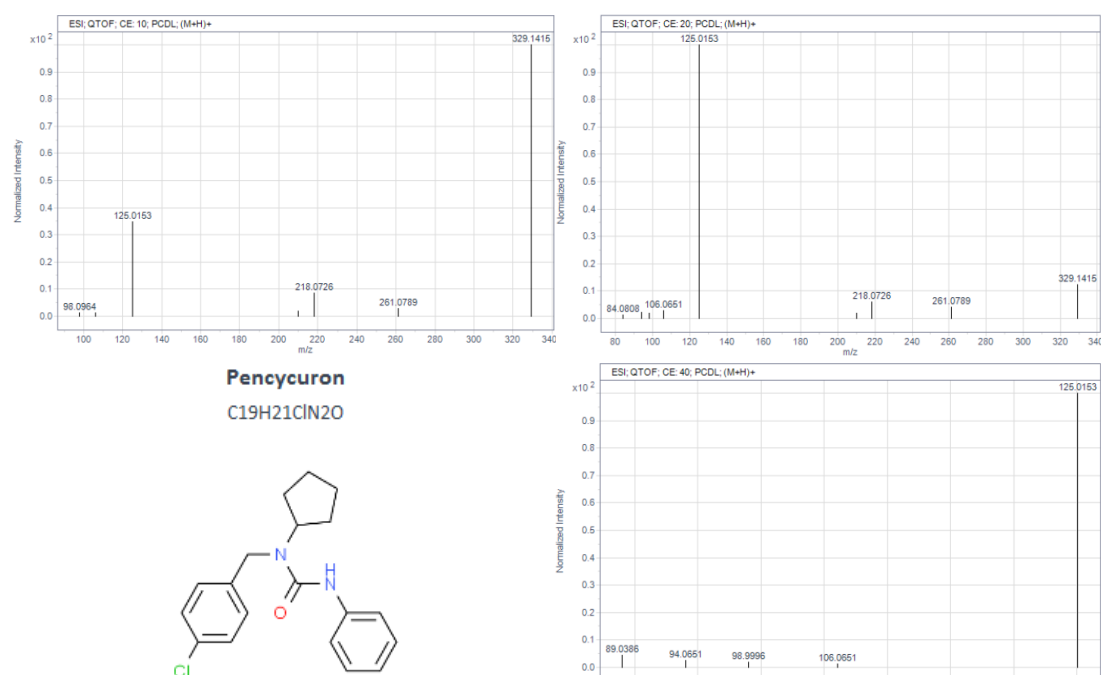


Figure 5. Spectra used for transfer of fragment ion information to MassHunter Quantitative Analysis and targeted screening for pencycuron experimental data shown in Figure 7 (bottom).

Screening Metrics for MS Only and All Ions Acquisition.

Intelligent reflex workflow will automatically carry out data analysis and if any analytes are detected, worklist will be appended with the second tier LC-MS method and data analysis will be automatically performed and reviewed with LC Screener Tool (Figure 6). Appended data files are renamed, clearly designating the reinjection and within the worklist appended injections are highlighted to be easily visible to the analyst.

Status	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Inj Vol (µL)	Intelligent Reflex Type	Intelligent Refl	First Pico Quant	Second Pico Quant Meth	Screening Status
Completed	1st reinjection	No Injection	MSOnly-PeactC-4min-05-Apr-24.n	No Injection-051.d	Blank	4	4	As Method				
Completed	ACN Blank	P1-E1	MSOnly-PeactC-4min-05-Apr-24.n	ACN Blank-051.d	Blank	4	4	No Intelligent Reflex Workflow	PeactC-Lung	D:\Project\SPC_Novem		
Completed	ACN Blank	P1-E1	MSOnly-PeactC-4min-05-Apr-24.n	ACN Blank-051.d	Blank	4	4	No Intelligent Reflex Workflow	PeactC-Lung	D:\Project\SPC_Novem		
Completed	ACN Blank	P1-E1	MSOnly-PeactC-4min-05-Apr-24.n	ACN Blank-051.d	Blank	4	4	No Intelligent Reflex Workflow	PeactC-Lung	D:\Project\SPC_Novem		
Completed	ACN Blank	P1-E1	MSOnly-PeactC-4min-05-Apr-24.n	ACN Blank-051.d	Blank	4	4	No Intelligent Reflex Workflow	PeactC-Lung	D:\Project\SPC_Novem		
Completed	ACN Blank	P1-E1	MSOnly-PeactC-4min-05-Apr-24.n	ACN Blank-051.d	Blank	4	4	No Intelligent Reflex Workflow	PeactC-Lung	D:\Project\SPC_Novem		
Completed	Strawberry Blank	P1-D1	MSOnly-PeactC-4min-05-Apr-24.n	Strawberry Blank-0.d	Blank	4	4	No Intelligent Reflex Workflow	PeactC-Lung	D:\Project\SPC_Novem		
Completed	Strawberry QC 20ppb	P1-D7	MSOnly-PeactC-4min-05-Apr-24.n	Strawberry QC 20ppb.d	QC	4	4					
Completed	Strawberry sample 1	P1-F5	MSOnly-PeactC-4min-05-Apr-24.n	Strawberry sample 1.d	Sample	4	4	TS Confirmation	PeactC-Lung	D:\Project\SPC_Novem		Green
Completed	Strawberry sample 2	P1-F6	MSOnly-PeactC-4min-05-Apr-24.n	Strawberry sample 2.d	Sample	4	4	TS Confirmation	PeactC-Lung	D:\Project\SPC_Novem		Green
Completed	ACN Blank	P1-E1	MSOnly-PeactC-4min-05-Apr-24.n	ACN Blank-051.d	Blank	4	4					
Completed	ACN Blank	P1-E1	MSOnly-PeactC-4min-05-Apr-24.n	ACN Blank-051.d	Blank	4	4					
Completed	Strawberry sample 2-Intellig	P1-D1	Pesticide-Lung-gradient-1700mg-6hr.n	Strawberry sample 2-Intellig-Reflex.d	Blank	4	4	No Intelligent Reflex Workflow				
Completed	Strawberry sample 2-Intellig	P1-F6	Pesticide-Lung-gradient-1700mg-6hr.n	Strawberry sample 2-Intellig-Reflex.d	Sample	4	4	No Intelligent Reflex Workflow				

Figure 6. Appended worklist with intelligent reflex reinjection for sample 2 spiked with a subset of pesticides and with sample 1 blank matrix..

The LC Screener Tool, embedded into MassHunter Quantitative Analysis 12.1, equips the user with an interface to quickly survey data files against the imported library targets. For first tier acquisition, isotopic fidelity can be easily evaluated using theoretical isotope pattern boxes, mass accuracy, and retention time and second tier uses those same metrics as first tier and verified ions and coelution scores. These are summarized in table format and visible in clearly displayed spectra for all compounds and data files (Figure 7).



Figure 7. LC Screener Tool results for first tier (top) and second tier acquisition (bottom).

Conclusions

Fast Pesticide Screening followed by Automated Reinjection with All Ions Acquisition and extended gradient

Over 150 pesticides screened for in 3 different matrices from 1 ppb to 100 ppb in 4 min per injection.

- Excellent Mass Accuracy from Revident LC/Q-TOF
- Intelligent reflex workflow combining short LC runtime with MS only followed by longer LC runtime with All Ions acquisition method with fragment information
- Sample preparation and detection in complex matrices
- Data analysis with MassHunter Quant 12.1 and LC Screener demonstrate ease of use and flexibility.

References

1 Simmermaker, C.; et al Intelligent Reflex Targeted Reinjection Workflow for Pesticide Screening in Food Matrices, *Agilent Technologies Application Note*, [5994-7312EN](https://www.agilent.com/en/promotions/asms), 2024.

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

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