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Rinse and Shoot: Rapid Pesticide Screening Workflow by GC/MS in Under Five Minutes

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Introduction

Trace-level pesticide and environmental pollutants in the food supply continue to be a worldwide concern and are driving the demand for more rapid and reliable methods of analysis. Part of the challenge is to find technologies that can search for hundreds of pesticides with simple sample preparation and a quick turnaround time.

Simple sample preparation was accomplished with a “rinse and shoot” approach. The solvent rinsate collected from the fruit surface has a favorable pesticide-to-matrix ratio. Because of the limited interferences, the rinsates can be screened with GC coupled with a single quadrupole mass spectrometer (GC/MSD) in full scan mode.

Custom-created and commercial spectral libraries were used for comprehensive screening of the rinsates [1,2]. Confidence in identification was further increased with mass spectral deconvolution and time-filtering.

Quick turnaround time was achieved with a ramp rate of 250 °/C available with the Intuvo 9000 GC.

In this work, the Agilent Intuvo/5977B GC/MSD system was used for a rapid (3.4 min) analysis of fruit rinsates, followed by compound identification based on deconvoluted mass spectral search and time-filtering using linear retention indices (RIs).

Experimental

The system used here was configured to enable the shortest cycle time, avoid carryover and maximize throughput.

The important techniques employed are:

- A 10 m x 0.18 mm x 0.18 µm HP-5MS UI used as column 1 and 1.3 m 0.15 mm deactivated fused silica restrictor as column 2
- Oven ramp rate of 250 °C/min achieved with the Intuvo 9000 GC enabling 3.4 min run time
- Mid-column backflushing to extend the life of the columns and the guard chip. During backflushing, the carrier gas flow through the first column and the guard chip is reversed to carry any high boilers that were in the column and the guard chip at the end of data collection out into the split vent trap
- The Intuvo 9000 GC enables self-configuration when setting up backflush and columns, which are equipped with the column information keys, that significantly simplifies method setup

Experimental, cont.

- The Intuvo PSD Module is a pneumatics module optimized for backflushing. During backflushing, it significantly reduces the flow of helium used compared to previous configurations
- The Intuvo 9000 MMI guard chip prevents high boiling matrix compounds from contaminating the head of the column
- The spectral deconvolution feature in MassHunter Quant 10.1 Unknowns Analysis (MH UA) enables automatic compound identification even in high matrix samples in the presence of coeluting compounds using library match score
- Time filtering using RIs increased compound identification accuracy

Fig. 1. Intuvo 9000/5977B GC/MSD system with a 50-vial capacity 7650A Automatic Liquid Sampler

Sample preparation

The fruits were placed into a glass funnel and rinsed with acetone. The rinsate was collected into a 4 mL amber vial and injected into the GC/MSD system.

This sample prep maximized pesticide-to-matrix ratio.

Fig. 2. Simple sample preparation to accompany fast chromatography for quick screening

Pesticides Found on the Surface of Fruits in 3.4 minutes

The scan files for fruit rinsates were analyzed using MH UA with the deconvoluted components searched against a custom pesticide library that included mass spectra and linear retention index (RI) information for 1,081 entries.

The use of RI makes the screening strategy independent of chromatographic conditions such as the flow path, column flow, and oven ramp rate. When time-filtering is performed with RIs, the library RI values are recalculated to retention times (RTs) using a RI-to-RT calibration. Component RTs are compared with calculated library RTs. RT tolerance range is specified in the method.

RI calibration was performed with a C_8-C_{34} n-alkane ladder.

Below is an example of the pesticide reported in the strawberries, fenhexamid. The deconvoluted mass spectrum (on top) is compared with the library spectrum and the extracted spectrum before deconvolution is shown on the bottom right.

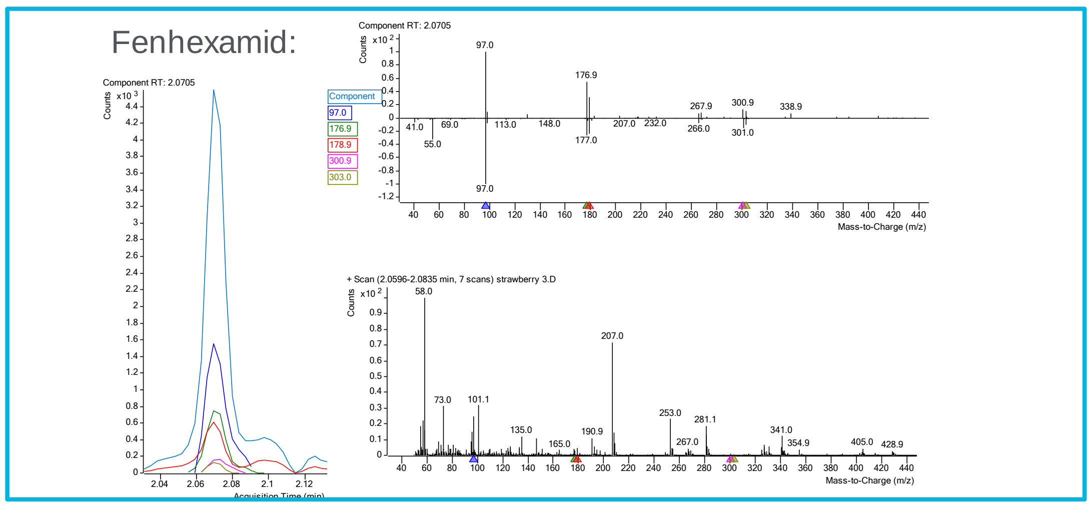
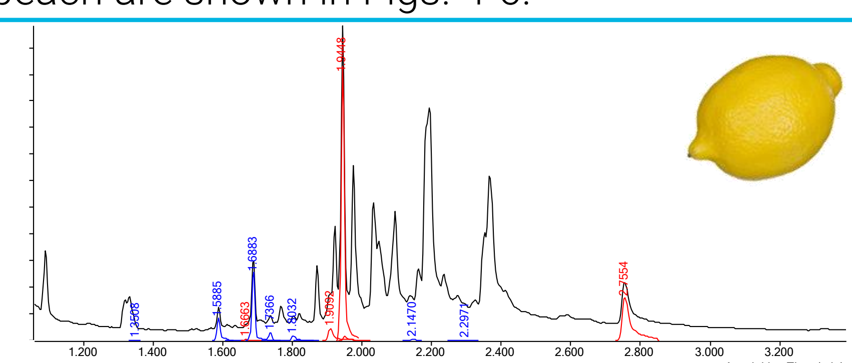


Fig. 3. Identification of fenhexamid in the strawberry rinsate with MassHunter Unknowns Analysis.

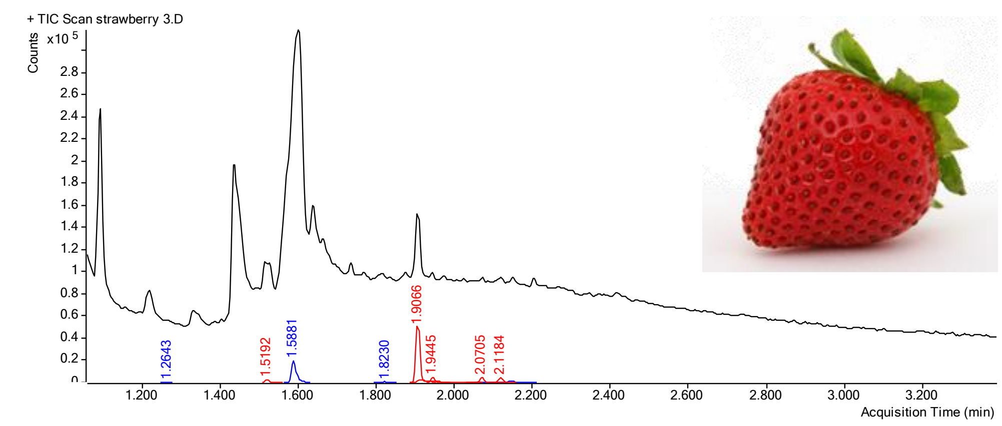
Pesticides identified in the fruit rinsates are highlighted in red in the chromatograms and in blue in the tables. Screening results for lemon, strawberry, banana, cherry, and peach are shown in Figs. 4-6.



Components

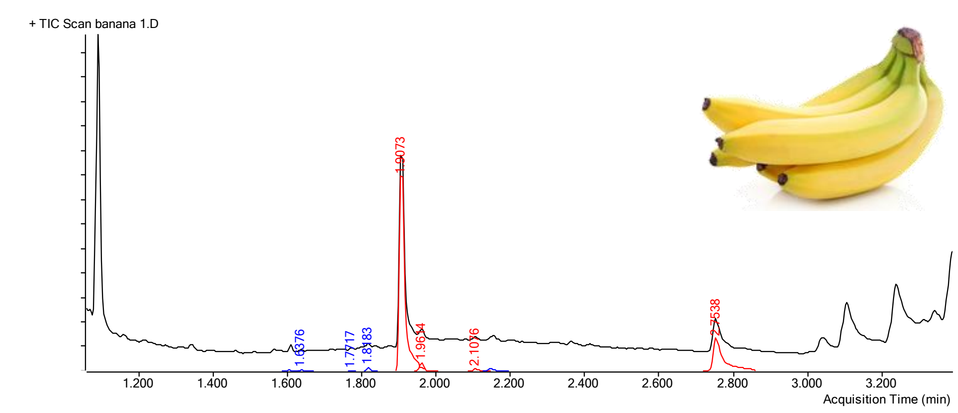
Component RT	Library RT	Delta RT	Compound Name	Match Factor	Best Hit	Formula	Component Area
1.3508	1.4072	0.0564	4-Isopropylaniline	58.5	✓	C9H13N	304.7
1.5885	1.5756	-0.0128	Cashmeran	61.6	✓	C14H22O	66621.3
1.6373	1.8044	0.1670	Di-n-butylphthalate	66.3	✓	C16H22O4	551.8
1.6663	1.6501	-0.0161	Chlorpropham	57.2	✓	C10H12Cl...	2240.5
1.6883	1.6877	-0.0006	Empenthrin IV {CAS ...	56.4	✓	C18H26O2	186521.1
1.7366	1.7399	0.0032	Exaltolide [15-Penta...	62.5	✓	C15H28O2	22674.1
1.8032	1.7902	-0.0131	Musk Ambrette	71.1	✓	C16H28O2	13633.6
1.8188	1.8044	-0.0144	Di-n-butylphthalate	66.8	✓	C16H22O4	3270.9
1.9092	1.8537	-0.0555	Thiabendazole	88.9	✓	C10H7N3S	55167.9
1.9448	1.9161	-0.0286	Fludioxonil	96.4	✓	C12H6F2...	782955.8
1.9502	1.9092	-0.0409	Imazalil	65.6	✓	C14H14Cl...	17839.8
2.1470	2.1097	-0.0373	Dicyclohexyl phthalate	59.6	✓	C20H26O4	4686.6
2.2971	2.4582	0.1611	Di-n-nonyl phthalate	65.0	✓	C26H42O4	1401.2
2.7554	2.6818	-0.0736	Azoxystrobin	89.6	✓	C22H17N...	295826.5

Fig. 4. Screening results for a lemon rinsate.



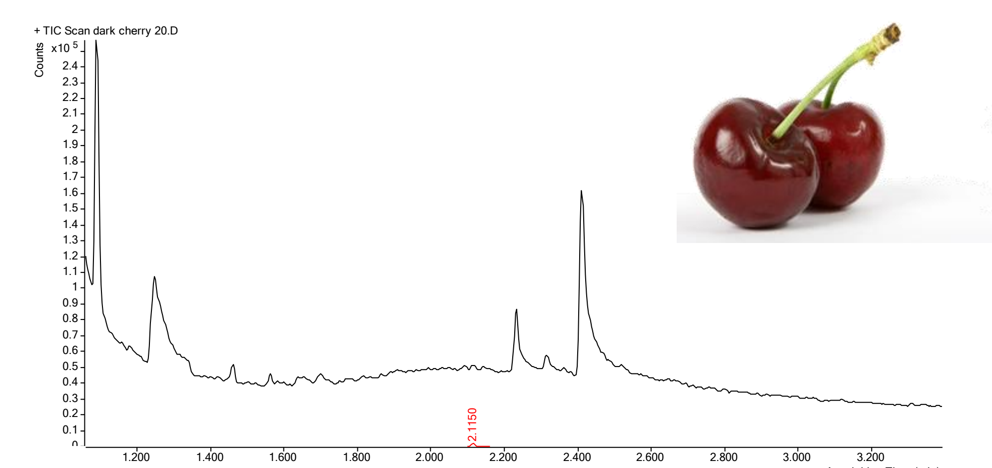
Components

Component RT	Library RT	Delta RT	Compound Name	Match Factor	Best Hit	Formula	Component Area
1.2643	1.2957	0.0314	4-Methylphenol	56.8	✓	C7H8O	125.3
1.5192	1.5094	-0.0098	Novaluron	60.8	✓	C17H9ClF...	1755.9
1.5881	1.5607	-0.0274	cis-1,2,3,6-Tetrahydr...	77.3	✓	C8H9NO2	18090.7
1.8230	1.7755	-0.0476	Tolclofos-methyl	56.8	✓	C9H11Cl2...	539.5
1.9066	1.8585	-0.0482	Captan	88.3	✓	C9H8Cl3N...	37205.7
1.9154	1.8537	-0.0617	Thiabendazole	63.7	✓	C10H7N3S	6137.1
1.9445	1.9161	-0.0284	Fludioxonil	64.2	✓	C12H6F2...	3764.8
2.0705	2.0062	-0.0644	Fenhexamid	63.6	✓	C14H17Cl...	4593.0
2.1184	2.0849	-0.0335	Fluxapyroxad	63.2	✓	C18H12F5...	3811.7
2.1462	2.1097	-0.0365	Dicyclohexyl phthalate	56.8	✓	C20H26O4	2573.3



Components

Component RT	Library RT	Delta RT	Compound Name	Match Factor	Best Hit	Formula	Component Area
1.6035	1.5805	-0.0230	o-Phenylphenol	61.8	✓	C12H10O	1334.6
1.6376	1.8044	0.1667	Di-n-butylphthalate	66.0	✓	C16H22O4	1350.7
1.7717	1.9034	0.1316	Diamyl phthalate	62.6	✓	C18H26O4	698.8
1.8183	1.8044	-0.0139	Di-n-butylphthalate	78.8	✓	C16H22O4	5418.2
1.9073	1.8537	-0.0536	Thiabendazole	96.7	✓	C10H7N3S	405205.6
1.9614	1.9284	-0.0330	Buprofezin	75.0	✓	C16H23N...	12812.3
2.1076	2.0862	-0.0215	Bifenthrin	74.2	✓	C23H22Cl...	3833.6
2.1461	2.1285	-0.0176	Bis(2-ethylhexyl)phth...	63.1	✓	C24H38O4	4977.6
2.7538	2.6818	-0.0720	Azoxystrobin	89.3	✓	C22H17N...	130424.7



Components

Component RT	Library RT	Delta RT	Compound Name	Match Factor	Best Hit	Formula	Component Area
2.1150	2.0849	-0.0301	Fluxapyroxad	69.0	✓	C18H12F5...	1272.8

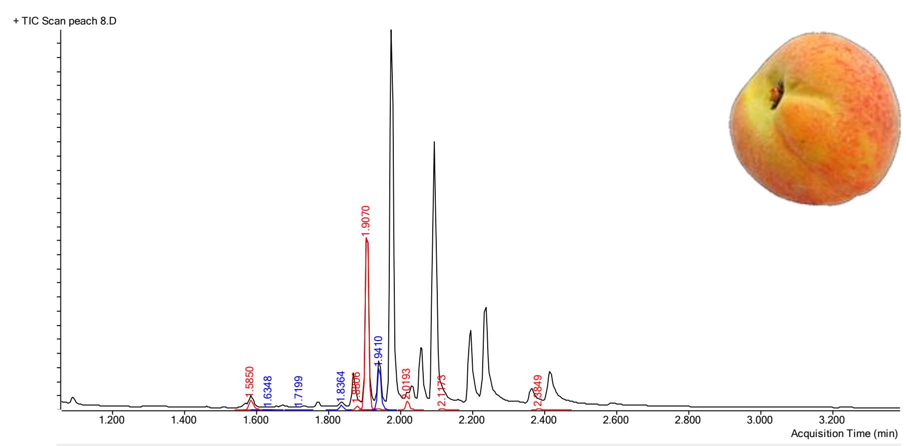
Fig. 5. Screening results for banana and cherry rinsates identified against the pesticide library.

MH UA can also be used to search the deconvoluted components against the NIST 17 library, which contains over 260,000 spectra. NIST 17 contains RIs experimentally determined on "Semi-standard non-polar" columns of the type used here for many of the entries.

Identity Confirmation with Increased Chromatographic Resolution

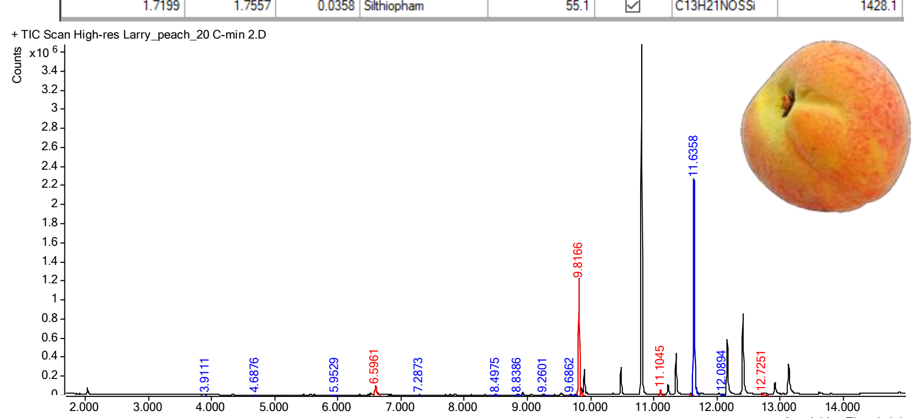
With the hardware employed, the oven ramp rate can be lowered to yield a significant increase in a chromatographic resolution. For example, to more closely evaluate a screening result and increase confidence in compound identification, chromatographic and spectral interference can be reduced by using the slower oven ramp.

If the rapid screening analysis finds compounds of high concern, the confirmation analysis can be used to confirm the results. Fig. 6 shows the utility of this optional capability.



Components

Component RT	Library RT	Delta RT	Compound Name	Match Factor	Best Hit	Formula	Component Area
1.9070	1.8585	-0.0485	Captan	93.2	☑	C9H8Cl3NO2S	1679701.3
1.5850	1.5607	-0.0243	cis-1,2,3,6-Tetrahydr...	92.1	☑	C8H9NO2	126991.5
2.0193	2.0155	-0.0038	Trifloxystrobin	90.3	☑	C20H19F3N2O4	95054.7
1.8806	1.8633	-0.0173	Fluopyram	76.7	☑	C16H11ClF6N2O	43718.3
2.1173	2.0849	-0.0324	Fluxapyroxad	76.1	☑	C18H12F5N3O	13847.8
1.8364	1.7399	-0.0965	Exaltolide [15-Penta...	74.7	☑	C15H28O2	51549.8
1.9095	1.8795	-0.0300	Pyrene	63.0	☑	C16H10	1852.8
1.6348	1.6205	-0.0144	Diethyl phthalate	60.6	☑	C12H14O4	1380.7
1.5562	1.5929	0.0367	Isoprocarb	60.4	☑	C11H15NO2	1205.7
1.6091	1.6985	0.0894	4-Aminodiphenyl	57.5	☑	C12H11N	477.2
2.3849	2.3091	-0.0758	Fenbuconazole	56.4	☑	C19H17ClN4	22035.1
1.9410	1.7902	-0.1508	Musk Ambrette	55.7	☑	C16H28O2	421548.5
1.7199	1.7557	0.0358	Silthiopham	55.1	☑	C13H21NOSSi	1428.1



Components

Component RT	Library RT	Delta RT	Compound Name	Match Factor	Best Hit	Formula	Component Area
6.5961	6.6196	0.0235	Tetrahydrophthalimide, cis-1,2,3,6-	94.9	☑	C8H9NO2	173839.5
9.8166	9.7883	-0.0283	Captan	94.4	☑	C9H8Cl3N...	1567145.9
9.8531	9.8361	-0.0171	Fluopyram	88.5	☑	C16H11Cl...	64256.2
11.1045	11.0972	-0.0073	Trifloxystrobin	86.8	☑	C20H19F3...	74935.7
11.5842	11.5677	-0.0165	Fluxapyroxad	73.4	☑	C18H12F5...	11459.8
12.7251	12.7289	0.0038	Fenbuconazole	66.3	☑	C19H17Cl...	37054.1
12.0894	12.0764	-0.0130	Cyhalothrin I (lambda)	64.6	☑	C23H19Cl...	13391.1
12.0894	12.0802	-0.0092	Cyhalothrin (Gamma)	64.0	☑	C23H19Cl...	12061.4

Fig. 6. Screening results for a peach rinsate with a rapid 3.4 min analysis (top) and a 15-min confirmation run (bottom).

As expected, LMS for some compounds like fluopyram and fenbuconazole were improved from 76.7 to 88.5 and from 56.4 to 66.3, respectively, with a slower oven ramp rate due to the decreased interferences.

System Robustness with 210 Injections of Peach Rinsate

To demonstrate the robustness of the system, 210 injections of a peach rinsate were performed.

These injections of sample led to a response loss, especially for high-boiling compounds, and a small RT shift towards earlier times.

System maintenance was performed, including liner, septum, and guard chip replacement. Next, the electron multiplier gain was updated and an alkane ladder (C_9-C_{34}) was analyzed to update the RI calibration.

This restored the system response and corrected for the small RT shift.

Conclusions

The Agilent Intuvo 9000/5977B GC/MSD system enables rapid screening for pesticides found on the surface of fruits and berries in 3.4 minutes.

The Intuvo 9000 GC provides oven ramping at a rate of 250 °C/min without requiring special electrical service (V or A) at the bench.

Rapid and reliable identification of pesticides is achieved by library searching of deconvoluted spectra coupled with time-filtering using RIs.

The Intuvo 9000 guard chip extends column lifetime and its replacement does not alter RI calibration.

Backflushing allows for extending maintenance-free uptime and ensures no carryover is observed, eliminating the need for extended column bakeout.

The screening workflow described herein provides the means for identifying those pesticides that should be included in subsequent quantitative targeted analysis.

References

[1]Andrianova, A.; et al. Agilent Technologies Application Note, publication number 5994-0915EN, 2019

[2]Churley, M.; et al. Agilent Technologies Application Note, publication number 5994-1505EN, 2019.

Acknowledgements

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