Multi-Residue Methods Using LC/TOF-MS for the Analysis of Pesticides in Food

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Introduction

Why do we use Time-of-Flight? Advantages:

- **LC/TOF-MS provides elemental formula composition, specificity and selectivity (high resolving power).**
- **Full-scan data available, even for degradation products!**
- **Retrospective analyses are possible.**
- **Use of Databases (non-targets and lack of standards).**
- **Useful information from isotope pattern (70% pesticides).**
- **Mass defect for A+2 ions highly useful for elemental elucidation.**

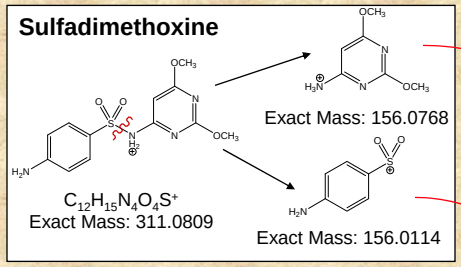
Instrumentation LC/TOF-MS

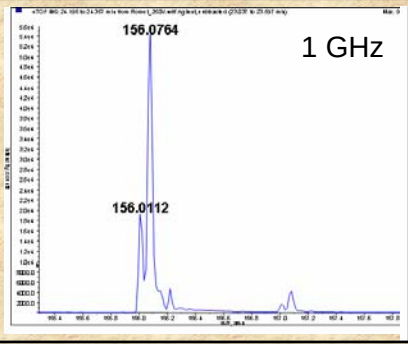
LC/TOF-MS *(TOFFY I)*
Almeria, Spain: Food (2004)

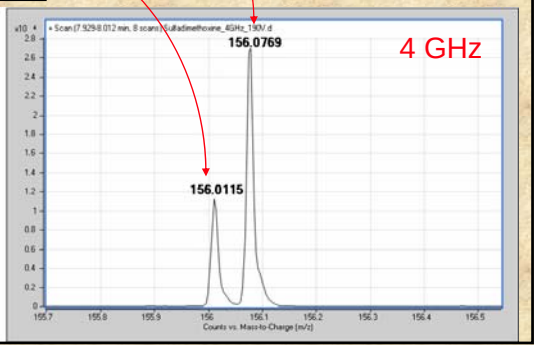
LC/TOF-MS *(TOFFY II)*
Boulder, CO, USA: Water (2008)

- 1 GHz to 4 GHz Detector (increased resolving power)
- Analyst to Mass Hunter Software (Automated Databases, MFE)
- 1100 to 1200 HPLC (Rapid Resolution)
- Improvements in accurate mass (from 3 to sub-1 ppm)

Improvement: 4 GHz Time-of-Flight

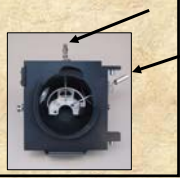






Analytical Methodology

- Extraction procedure:
  - Food samples: Ethyl acetate method or QuEChERS method.
  - Water Samples: Solid-phase extraction with C-18 cartridges.
- LC Conditions:
  - Column
    - Zorbax Eclipse XDB C_8, 4.6 x 150 mm, 5 µm particles
    - Zorbax SB-C_{18}, 4.6 x 50 mm, **1.8 µm** particles
  - Mobile phase
    - Acetonitrile and water with 0.1 % formic acid
    - Gradient: 10 % organic isocratic to 100 % in 30 min.
    - Flow = 0.6 mL/min or 1 mL/min
- MS Conditions:
  - Dual sprayer providing constant low flow of reference solution for continuous auto-calibration.
  - Positive ESI+, Capillary 4000V, Fragmentor 190V.



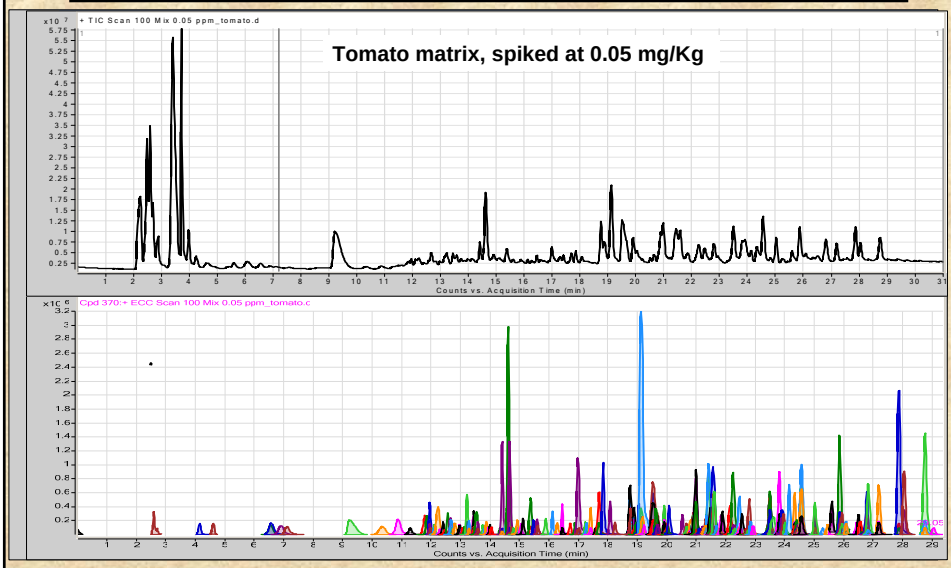
Selection of the 101 Pesticides

- Triazines (13)
- Organophosphorous (11)
- Carbamates (15)
- Conazoles (7)
- Phenylureas (5)
- Chloroacetanilides (4)
- Nereistoxins (4)
- Neonicotinoids (3)
- Benzimidazoles (3)
- Imidazolines (3)
- Other classes (33)

Insecticides (42)
Herbicides (39)
Fungicides (17)

9 Degradation Products Included!

Analysis of 101 pesticides by LC/TOF-MS



I. Ferrer and E.M.Thurman, Journal of Chromatography A, 2007, 1175, 24-37

Accurate Mass Analysis

Table 1
LC/TOF-MS accurate masses for the protonated molecules and the main fragment ions for all the compounds studied (fragmentor voltage 190 V)

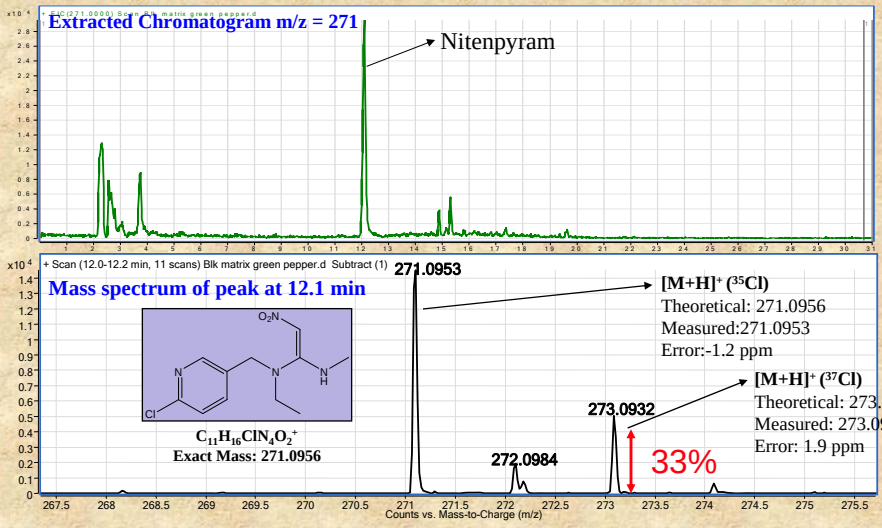
Compound	Retention time (min)	Elemental composition[a]	Accurate mass $[M+H]^+$	Frag ion 1	Frag ion 2	Frag ion 3
Acetamiprid	16.8	$C_{10}H_{11}N_4Cl$	**223.0745**	126.0105		
Acetochlor	26.1	$C_{14}H_{20}NO_2Cl$	270.1255	224.0837	**148.1121**	133.0886
Alachlor	26.1	$C_{14}H_{20}NO_2Cl$	270.1255	238.0993	**162.1277**	
Aldicarb	18.7	$C_7H_{14}N_2O_2S$	213.0668[b]	116.0528	**89.0419**	70.0651
Aldicarb sulfone	11.8	$C_7H_{14}N_2O_4S$	**223.0747**	148.0427	166.0532	86.0600
Aldicarb sulfoxide	6.5	$C_7H_{14}N_2O_3S$	207.0798	132.0478	**89.0419**	
Atrazine	21.4	$C_8H_{14}N_5Cl$	**216.1010**	174.0541	146.0228	
Azoxystrobin	24.3	$C_{22}H_{17}N_3O_5$	**404.1241**	372.0979		
Benalaxyl	26.8	$C_{20}H_{23}NO_3$	**326.1751**	294.1489	208.1332	148.0757
Bendiocarb	20.8	$C_{11}H_{13}NO_4$	224.0917	**167.0703**	109.0284	
Bensultap	21.4	$C_{17}H_{21}NO_4S_4$	**432.0426**	290.0338		
Bromoxynil	21.7	$C_7H_3NOBr_2$	**275.8654**			
Bromuconazole	24.0 + 24.8	$C_{13}H_{12}N_3OCl_2Br$	**375.9614**	158.9763		
Buprofezin	27.4	$C_{16}H_{23}N_3OS$	**306.1635**	201.1056		
Butylate	29.7	$C_{11}H_{23}NOS$	**218.1573**	162.0947		
Captan	24.4	$C_9H_8NO_2SCl_3$	299.9414	**263.9647**	235.9693	
Carbaryl	21.3	$C_{12}H_{11}NO_2$	202.0863	**145.0648**		
Carbendazim	7	$C_9H_9N_3O_2$	**192.0768**	160.0505		
Carbofuran	20.8	$C_{12}H_{15}NO_3$	222.1125	**165.0910**	123.0446	
Cartap	3.1	$C_7H_{15}N_3O_2S_2$		150.0406	**104.9827**	
Chlorfenvinphos	26.5	$C_{12}H_{14}O_4PCl_3$	**358.9768**	204.9373	155.0468	98.9842
Chlorpyrifos methyl	28.2	$C_7H_7NO_3PSCl_3$	**321.9023**	124.9821		
Cyanazine	19.6	$C_9H_{13}N_6Cl$	**241.0963**	214.0854		
Cyproconazole	23.6	$C_{15}H_{18}N_3OCl$	**292.1211**	125.0153	70.0400	
Cyromazine	2.9	$C_6H_{10}N_6$	**167.1040**	108.0556		
DEET	21.3	$C_{12}H_{17}NO$	**192.1383**	119.0491	91.0542	

76 pesticides showed an [M+H]+ peak as a base peak (the rest presented a fragment ion as a base peak).

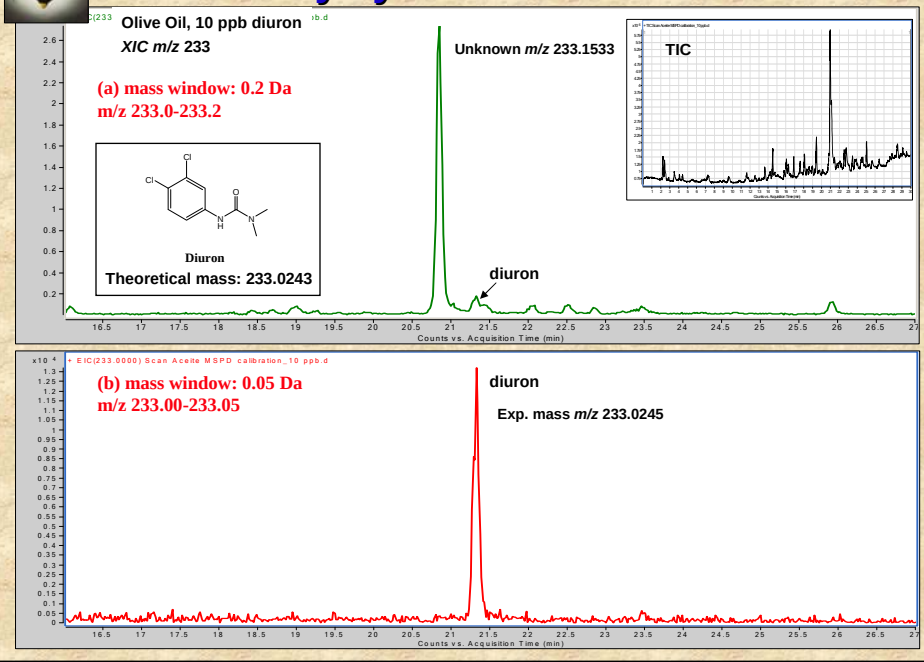
96 pesticides showed at least one fragment ion
49 pesticides showed at least 2 fragment ions
12 pesticides showed as much as 3 fragment ions

Accurate Mass Information of Isotopes

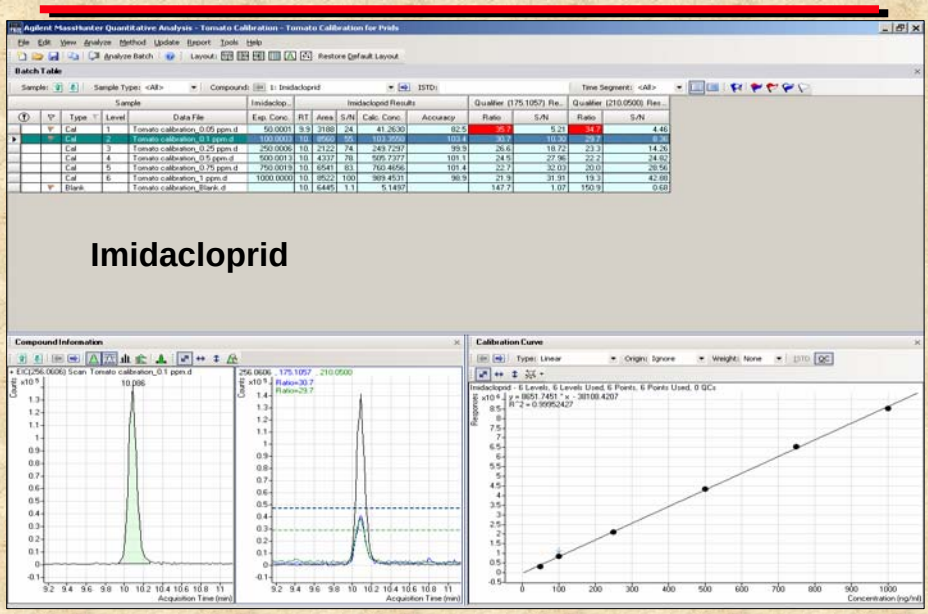
For 70% of the pesticides, the accurate mass value of the A+2 ion (Cl, Br or S) is highly useful for correct identification.



Selectivity by LC/TOF-MS



Automated Quantitation



Imidacloprid

Analytical Performance

Compound	Calibration curve	R^2	LODs (µg/kg)
Diazinon	$y = 6.95 \times 10^3 C - 9.03 \times 10^5$	0.996	0.05
Dichlorvos	$y = 6.67 \times 10^3 C - 1.33 \times 10^3$	0.994	0.5
Difeconazole	$y = 2.68 \times 10^4 C - 6.21 \times 10^4$	0.995	0.5
Difenoxuron	$y = 1.46 \times 10^5 C + 3.51 \times 10^5$	0.998	0.4
Diflubenzuron	$y = 1.03 \times 10^3 C - 586$	0.996	12
Dimethenamide	$y = 2.84 \times 10^4 C + 1.62 \times 10^4$	0.998	1
Dimethoate	$y = 1.03 \times 10^4 C - 914$	0.999	1.5
Dimethomorph	$y = 5.04 \times 10^4 C + 5.43 \times 10^5$	0.998	4
Diuron	$y = 1.6 \times 10^4 C + 2.65 \times 10^3$	0.998	0.6
Ethiofencarb	$y = 7.61 \times 10^4 C + 1.15 \times 10^4$	0.999	4
Fenamiphos	$y = 1.53 \times 10^5 C - 7.84 \times 10^4$	0.997	0.1
Fenuron	$y = 2.9 \times 10^4 C + 7.52 \times 10^4$	0.997	10
Flufenacet	$y = 9.97 \times 10^3 C + 5.79 \times 10^3$	0.998	3
Flufenoxuron	$y = 1.17 \times 10^3 C - 3.08 \times 10^3$	0.999	6
Fluoroacetamide	$y = 1.85 \times 10^3 C - 5.91 \times 10^4$	0.986	80
Fluoroxypyr	$y = 3.93 \times 10^2 C - 1.04 \times 10^3$	0.988	45
Hexaflumuron	$y = 7.69 \times 10^2 C - 1.38 \times 10^3$	0.993	8
Hydroxyatrazine	$y = 9.74 \times 10^4 C - 6.39 \times 10^4$	0.992	0.4
Imazalil	$y = 9.66 \times 10^4 C - 1.38 \times 10^5$	0.999	0.3
Imazapyr	$y = 7.44 \times 10^4 C - 2.49 \times 10^4$	0.998	5
Imazaquin	$y = 1.36 \times 10^5 C - 9.31 \times 10^4$	0.997	0.7
Imidacloprid	$y = 5.84 \times 10^3 C - 374$	0.998	2
Ioxynil	$y = 1.05 \times 10^3 C + 454$	0.992	15
Iprodione	$y = 1.55 \times 10^3 C - 261$	0.987	4
Irgarol 1051	$y = 2.89 \times 10^5 C - 4.59 \times 10^5$	0.998	0.1
Irgarol metabolite	$y = 9.48 \times 10^4 C - 1.01 \times 10^5$	0.997	0.5
Isoproturon	$y = 1.63 \times 10^5 C - 1.88 \times 10^4$	0.999	0.7

- Calibration Curves >0.99
- LOD's ranged from 0.04 µg/Kg (Benalaxyl) to 120 µg/Kg (Spiromesifen).
- Average LOD's were around 3 µg/Kg.
- Less sensitive compounds: bromoxynil, captan, chlorpyrifos-methyl, fluoroacetamide, fluoroxypyr, spiromesifen, teflubenzuron and trifluralin.

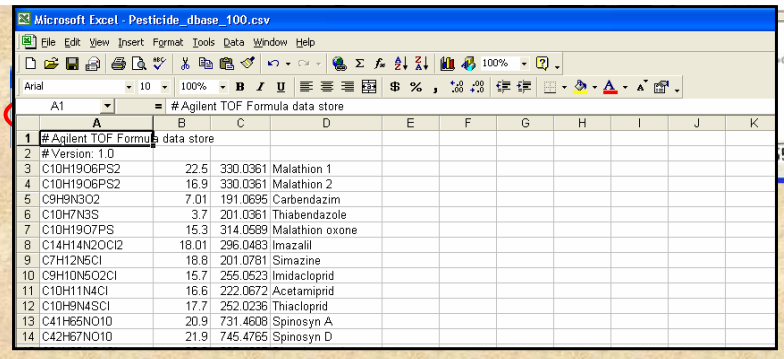
Importance of Databases

- First attempt of database creation (using Access Microsoft Office for 350 pesticides)

  Thurman et al. Food Additives and Contaminants, 2006, 23, 1169-1178.

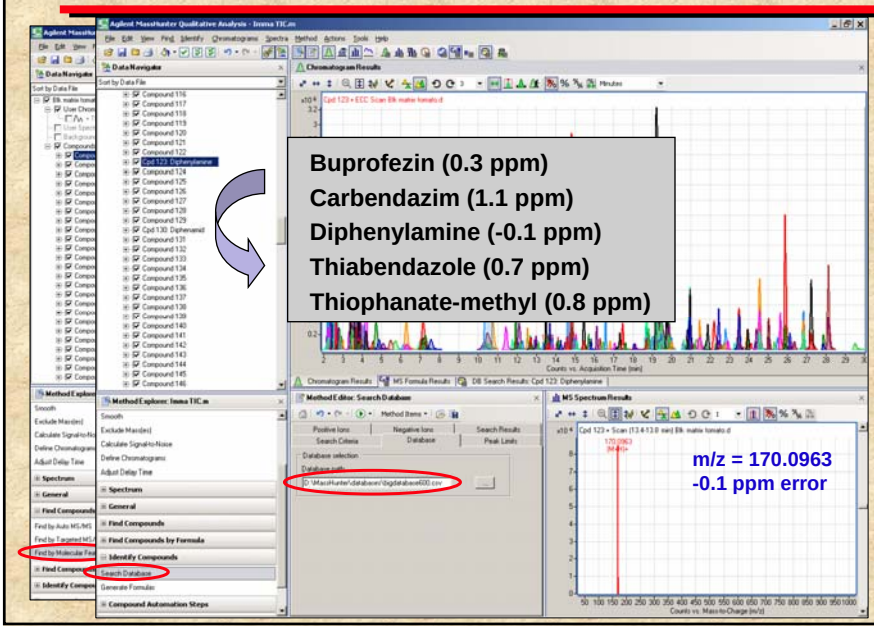
- Second attempt of database creation (using a Molecular Feature Database, csv files and Agilent software)

  Ferrer et al. Rapid Commun. Mass Spectrom, 2006, 20, 3659-3668.

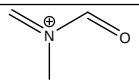
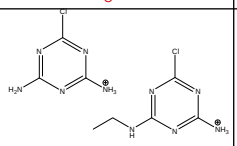


	A	B	C	D
1	# Agilent TOF Formula data store			
2	# Version: 1.0			
3	C10H19O6PS2	22.5	330.0361	Malathion 1
4	C10H19O6PS2	16.9	330.0361	Malathion 2
5	C9H9N3O2	7.01	191.0695	Carbendazim
6	C10H7N3S	3.7	201.0361	Thiabendazole
7	C10H19O7PS	15.3	314.0589	Malathion oxone
8	C14H14N2OCl2	18.01	296.0483	Imazalil
9	C7H12N5Cl	18.8	201.0781	Simazine
10	C9H10N5O2Cl	15.7	255.0523	Imidacloprid
11	C10H11N4Cl	16.6	222.0672	Acetamiprid
12	C10H9N4SCl	17.7	252.0236	Thiacloprid
13	C41H65NO10	20.9	731.4608	Spinosyn A
14	C42H67NO10	21.9	745.4765	Spinosyn D

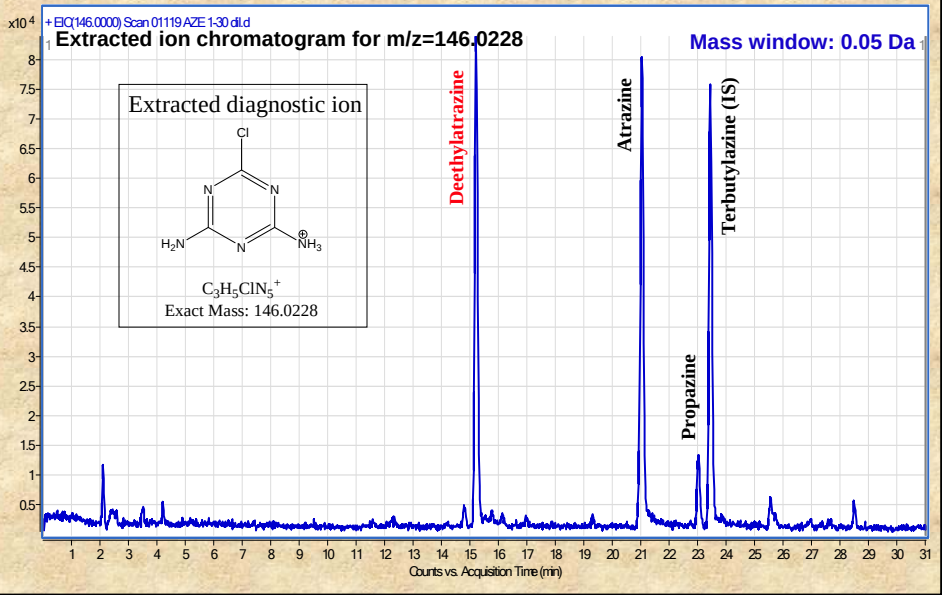
Use of Molecular Feature and Databases



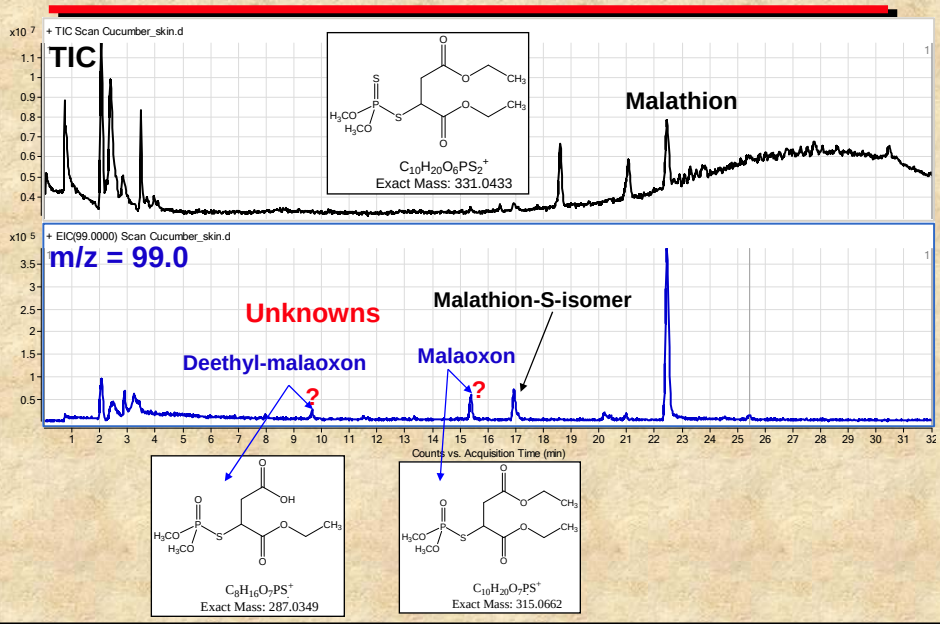
Accurate Mass of Diagnostic Frag. Ions

Pesticide Family	Compound	Diagnostic Ion	Accurate Mass of Diagnostic Ion
Phenylureas	Diuron Fenuron Isoproturon Monuron		72.0444
Organophosphates	Chlorpyrifos methyl Dimethoate Malathion		124.9821
Triazines	Deethylatrazine Deethylterbuthylazine Deisopropylatrazine Atrazine Terbuthylazine		146.0228 146.0228 + 174.0541
Fluorobenzylureas	Diflubenzuron Lufenuron Flufenoxuron Teflubenzuron Hexaflumuron		158.0412
Neonicotinoids	Acetamiprid Thiacloprid		126.0105
Conazole Fungicides	Bromuconazole Propiconazole Imazalil		158.9763

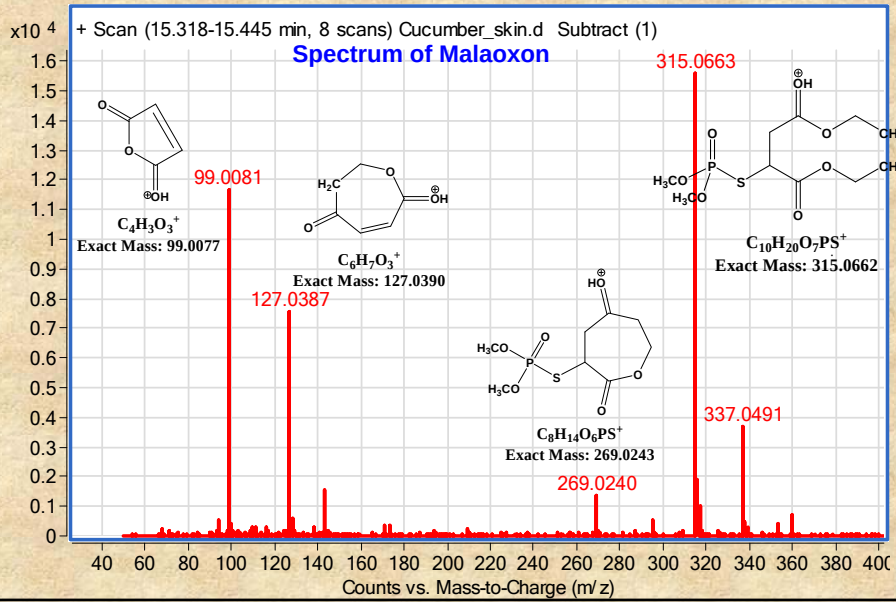
Using Accurate Mass Diagnostic Ions



Accurate Mass of Degradation Products

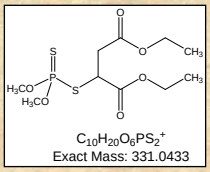


Accurate Mass Fragments of Degradation Products



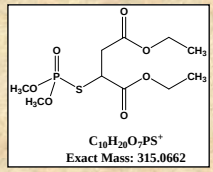
Isotope Signature and Accurate Mass

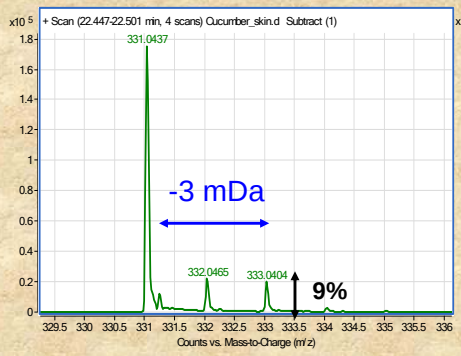
Malathion

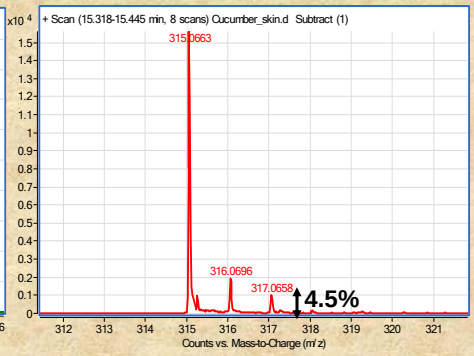


$^{32}S = 31.97207069$
$^{34}S = 33.96786683$

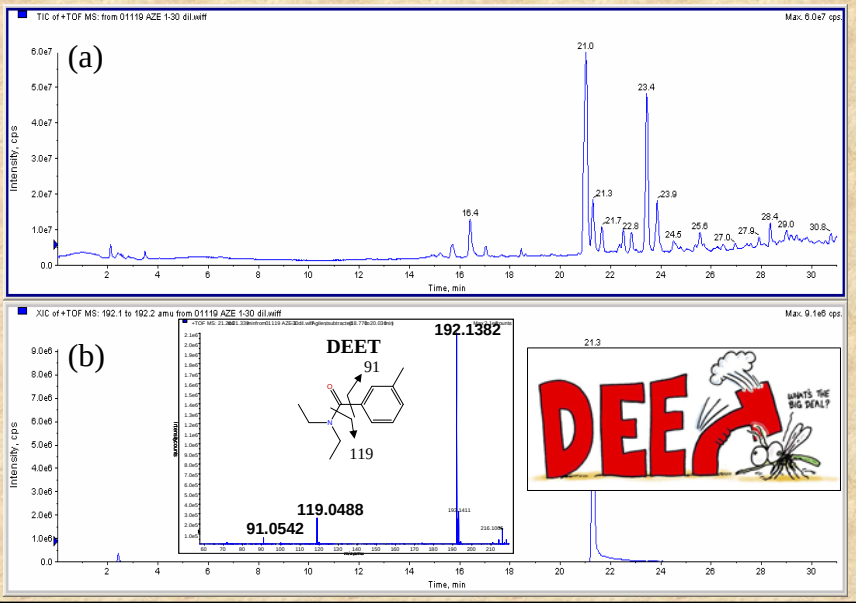
Malaoxon





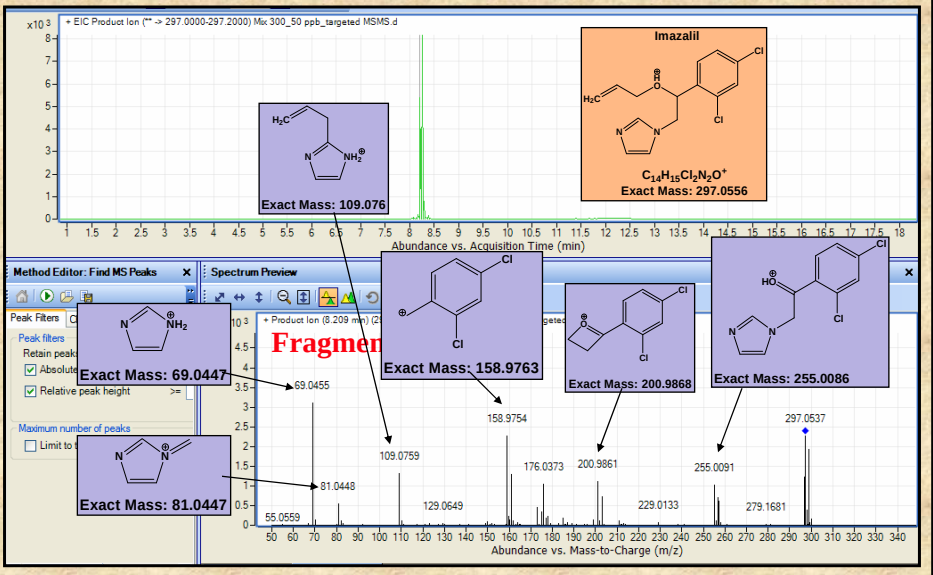


Analysis of Surface Water Samples

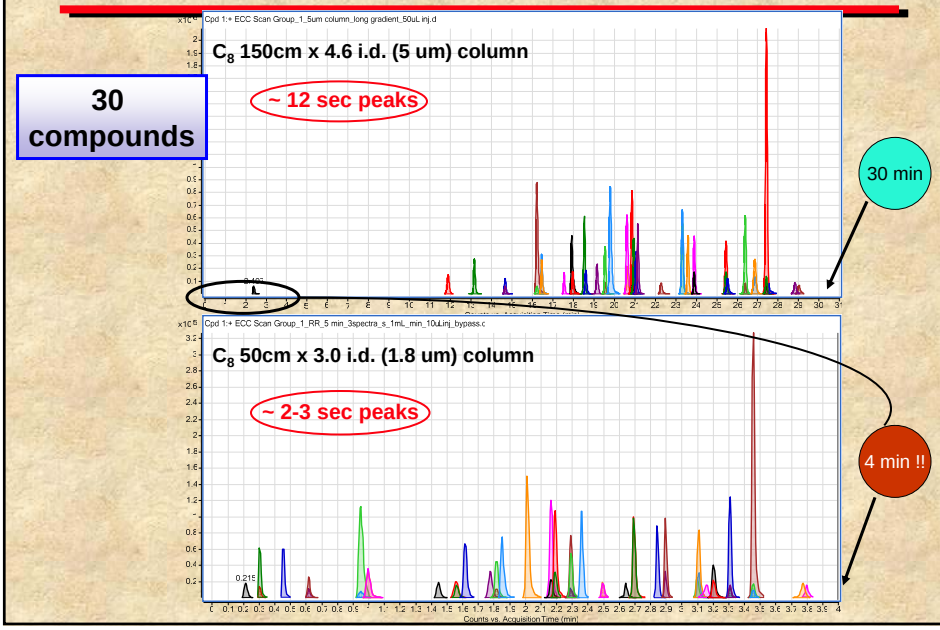


Imazalil by Q-TOF-MS

Imazalil confirmation by MS/MS



Rapid Resolution Chromatography



Conclusions

LC/TOF-MS offers the following advantages for environmental analysis…

- Full spectral sensitivity.
- High resolution generates selectivity for complex matrix samples and simplifies sample handling.
- Routine accurate mass measurement capability verifies elemental compositions of pesticides and confirm identities of degradation products.
- The isotope pattern recognition and mass defect of A+1 and A+2 peaks can be measured and it is very useful for id of unknowns.

Acknowledgments

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- **Jerry Zweigenbaum (from Agilent) for providing standards and instrument support.**