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Exact-mass library for pesticides using a molecular-feature database

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An automated molecular-feature database (MFD) consisting of the exact monoisotopic mass of 100 compounds, at least one exact mass product ion for each compound, and chromatographic retention time were used to identify pesticides in food and water samples. The MFD software compiles a list of accurate mass ions, excludes noise, and compares them with the monoisotopic exact masses in the database. The screening criteria consisted of ±5 ppm accurate mass window, ±0.2 min retention time window, and a minimum 1000 counts (signal-to-noise (S/N) ratio of ~10:1). The limit of detection for 100 tested compounds varied from <0.01 mg/kg for 72% of the compounds to <0.1 mg/kg for 95% of the compounds. The MFD search was useful for rapid screening and identification of pesticides in food and water, as shown in actual samples. The combined use of accurate mass and chromatographic retention time eliminated false positives in the automated analysis. The major weakness of the MFD is matrix interferences and loss of mass accuracy. Strengths of the MFD include rapid screening of 100 compounds at sensitive levels compared with a manual approach and the ease of use of the library for any accurate mass spectrometer instrumentation capable of routine sub-5-ppm mass accuracy. Copyright © 2006 John Wiley & Sons, Ltd.

Recently, the use of reverse-search methods for gas chromatography/mass spectrometry (GC/MS) has made it possible to search the large National Institute for Standards and Testing (NIST) pesticide libraries in minutes[1] and has made screening quite simple for pesticides amenable to GC/MS. Unfortunately, similar reverse-search methods have not been available for liquid chromatography/mass spectrometry (LC/MS). Single quadrupole and triple quadrupole mass spectrometers do not operate in full scan mode for pesticide screening because of a lack of sensitivity.[2,3] Although libraries for LC/MS three-dimensional ion traps have been produced, they have not been popular due to difficulties in reproducibility of fragmentation and the need for authentic standard analysis for each instrument.[4–6] Recently, liquid chromatography/quadrupole/ion trap (LC/Q-trap with a linear ion trap) and automated library searching has been used for drugs in blood and urine, and the screening of 301 drugs has been reported.[7] There is considerable interest in this technique because of the combination of both multiple reaction monitoring (MRM) and full scan spectra being taken by what is called, by Applied Biosystems (Foster City, CA, USA), a data-dependent scan. However, with the LC/Q-trap (as with triple quadrupole instruments) there is a limitation on the number of ions that may be selected because of dwell times and ion windows.

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Another approach that uses full scan information is liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS), which is both sensitive and accurate.[8] The combination of accurate mass and sensitivity is needed for screening of pesticides by their empirical formula. For example, Thurman *et al*.[9] used an approach of TOF, ion trap, and the Merck Index database to identify pesticides in food and also degradation products, without the initial use of primary standards.[9–11] Bobeldijk *et al*. also used the Merck Index, the NIST library, and their own database to screen water pollutants.[12] The methods in these examples rely on manually searching the databases, compound by compound. Recently, however, Laks *et al*. have reported[13,14] the use of mass accuracy of 30 ppm and database analysis to identify ~600 drugs in blood and urine without the use of primary standards, using only the protonated molecule, MH^+. They used an automated data explorer program and LC/TOFMS. In spite of the progress that has been made, the ability to perform true library analysis is still a problem to be solved for LC/MS and for the rapid analysis of environmental samples.

The problems to be overcome include the acquisition of reproducible spectra and ion ratios, the lack of routine programs for the rapid screening of samples rather than manual checking of data, and the need for some estimate of the probability of the correct identification. Our study contributes to the solution of these problems by using an

automated molecular-feature database (MFD) that allows the user to screen and to identify 100 pesticides in food and water extracts using positive ionization LC/TOFMS and the monoisotopic exact mass of MH^+, and at least one product ion, and retention time of the compound. The advantages and limitations of the approach are discussed as well as the reliability (match probability) of a library search using accurate mass.

EXPERIMENTAL

Chemicals, solvents and chromatography

HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used for the analyses. Analytical reagent-grade standards were purchased from both Sigma (St. Louis, MO, USA) and Chem Service (West Chester, PA, USA). The separation of the different species from the whole fruit extracts was carried out using an HPLC system, consisting of vacuum degasser, autosampler and a binary pump (Agilent Series 1100; Agilent Technologies, Palo Alto, CA, USA), equipped with a reversed-phase C_8 analytical column (150 mm × 4.6 mm, 5 μm particle size; Zorbax Eclipse XDB-C8). The column temperature was maintained at 25°C. Mobile phases A and B were water with 0.1% formic acid and acetonitrile, respectively. A binary gradient elution was made as follows: isocratic conditions for 5 min at 10% of solvent B, then a linear gradient from 10 to 100% of solvent B, from 5 to 30 min. The flow rate used was kept at 0.6 mL min^{-1} and 50 μL of sample extract were injected in each study.

Time-of-flight mass spectrometry

The HPLC system was interfaced to an Agilent MSD TOF mass spectrometer equipped with an electrospray ionization (ESI) interface operating in positive ion mode, using the following operation parameters: capillary voltage, 4000 V; nebulizer pressure, 40 psig; drying gas, 9 L min^{-1}; gas temperature, 300°C; fragmentor voltage, 190 V and 230 V; skimmer voltage, 60 V; octapole DC 1, 37.5 V; octapole RF, 250 V. LC/MS accurate mass spectra were recorded across the range *m/z* 50–1000. The instrument provided a typical resolving power (FWHM) of 9500 ± 500 (*m/z* 922.0098). The full scan data recorded was processed with Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany) with accurate mass application-specific additions from Agilent MSD TOF software.

Accurate mass measurements of each peak from the total ion chromatograms were obtained using a dual-nebulizer ESI source with an automated calibrant delivery system, which introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution containing the internal reference compounds: purine and hexakis(1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine, respectively (MH^+ ions at *m/z* 121.0509 and 922.0098).

Sample preparation

A 15-g portion of food sample previously homogenized was weighed in a 200-mL PTFE centrifuge tube. Then, 15 mL of acetonitrile were added and the tube was vigorously shaken for 1 min. After this time, 1.5 g of NaCl and 4 g of $MgSO_4$ were added and the shaking process was repeated for 1 min. The extract was then centrifuged (3700 rpm) for 1 min. A 5-mL aliquot of the supernatant (acetonitrile phase) was taken with a pipette and transferred to a 15-mL graduated centrifuge tube, containing 250 mg of PSA (propylamino solid-phase extraction cartridge, Supelco) and 750 mg of $MgSO_4$. The tube was shaken energetically for 20 s. After this, the extract was centrifuged again (3700 rpm) for 1 min. Finally, an extract containing 1 g of sample mL^{-1} in 100% acetonitrile was obtained.[15] The extract was then evaporated near to dryness and reconstituted to the initial mobile phase composition. Prior to analysis, the extract was passed through a 0.45 μm PTFE filter (Millex FG, Millipore).

The water sample (100 mL) was passed through an Oasis cartridge and eluted with 2 mL ethyl acetate. The solvent was evaporated to near dryness and re-dissolved in 0.3 mL of mobile phase for LC/TOFMS analysis.

Pesticide database

Theoretical monoisotopic exact masses of compounds based on their molecular formulae were calculated using an Excel spreadsheet and put into csv format (comma separated values) for use by the instrument software of the LC/TOFMS system. The LC/TOFMS instrument software at the completion of the sample run then searches the csv file automatically and generates a report on the number of compounds that were screened as positive in the database. Search criteria include ppm mass tolerance (5 ppm), retention time window (0.2 min), and minimum signal (1000 counts or a signal to noise (S/N) ratio of ~10:1) or other variables chosen by the operator including adducts and neutral loss fragments. A minimum of one accurate mass product ion is also included in the database for identification.

The limits of detection (LODs) were determined in food matrices (lettuce, tomato, pepper, melon and apple). The LOD was based on the appearance of the correct accurate mass of X + 1 and X + 2 isotopic signatures at 3-ppm accuracy, where X is the monoisotopic mass of the MH^+ ion. The LOD for water samples was based on the sample pre-concentration from a 100-mL water sample to a 300-μL final sample volume or 300-fold concentration.

RESULTS AND DISCUSSION

Creation of the library database

The algorithm of the database search is called a molecular-feature extractor, which finds all ions that represent real compounds eliminating all ions that are background noise or noise spikes. The search software then compares the ions found with all the specified adducts in the compound database. The user specifies the adducts sought (proton, NH_4^+, Na^+, etc.). The molecular-feature approach is more suited to large databases because of the ease of operation and the speed by which the search is done compared with the extracted ion approach, which is much slower. The software is similar to other algorithms such as CODA (Advanced Chemistry Development, Toronto, Canada) or AMDIS (NIST, Washington DC, USA).

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In contrast, a reverse-search database extracts each ion of interest in the database from the sample file and compares the accurate mass with the database, which is a time-consuming process (about 10–100 times longer), but this is how one typically analyzes the data by hand.

The creation of the database library involves three steps: selection of the pesticide and its product ion, calculation of the exact masses of the molecules, and the creation of the csv file. A description of each molecule and its retention time may be included but is not necessary for database operation. The csv file is then the database that is searched. The process involves the selection of a method file that uses the LC/TOFMS sample data file, which has already been acquired on the instrument, and the actual processing of the work list to generate the MFD (molecular formula database) search and data report. Typically, on the most complex food sample (the pepper extract of ~2500 peaks, Fig. 1), a database of 100 compounds is searched in 2 to 5 min including production of the printed report.

The csv file may be formed by use of a spreadsheet supplied by the instrument software that calculates the exact mass of the molecule of interest. One enters the number of carbon, hydrogen, oxygen, nitrogen, chlorine, or sulfur atoms and the spreadsheet calculates the exact mass of the neutral compound. The specified adduct (H^+, NH_4^+, etc.) is then used for the accurate mass comparison of the MFD search with an error of ±5 ppm (set by the user).

An example printout from the MFD is shown in Fig. 2. The report contains formula, compound, exact mass of the neutral molecule, error in mDa and error in ppm, retention time error in minutes, and a description (i.e. triflumizol, fungicide). The mass spectrum of the compound shows the MH^+ and the isotope signature of the compound, which is useful for a quick check and partial confirmation of the formula, especially since most pesticides (about 70% of the 100 compound database) show an interesting X + 2 ion from a halogen or sulfur atom.

The data for this database was obtained by measuring the LODs, the product ions, and the retention times for the 100 pesticides shown in Table 1. These compounds consist of the major pesticides that are used in Europe and in the USA to treat crops of fruits and vegetables and those compounds commonly reported in water quality studies.[16] They include a mixture of insecticides (organophosphates, carbamates, and imidazols), herbicides (triazines and phenylureas), and fungicides (imidazoles and thiazoles).

The LOD was determined at a S/N ratio of 3:1 and categorized at various levels for the EU regulation of 0.01 for baby food, 0.05, 0.1, and 0.5 mg/kg for various food levels, depending on pesticide and crop type. The LOD was equal to or less than 0.01 mg/kg for 72 compounds and less than or equal to 0.05 for 86 compounds (86%). The 0.05 mg/kg LOD is also a critical one for food monitoring of banned substances or controlled compounds. The LOD for 95% of the compounds was equal to or less than 0.1 mg/kg for food and less than 0.3 mg/L for water samples, which is less than the EPA drinking water regulations for the majority of pesticides.[17] Only four compounds were found to be insensitive, with LODs of 0.5 ppm for food and 2 mg/L for water (Table 1).

Product ions for each of the pesticides were also determined at a low and a medium fragmentor voltage (190 and 230 V, respectively). About 95% of the compounds gave a product ion (see Table 2) which comes from the

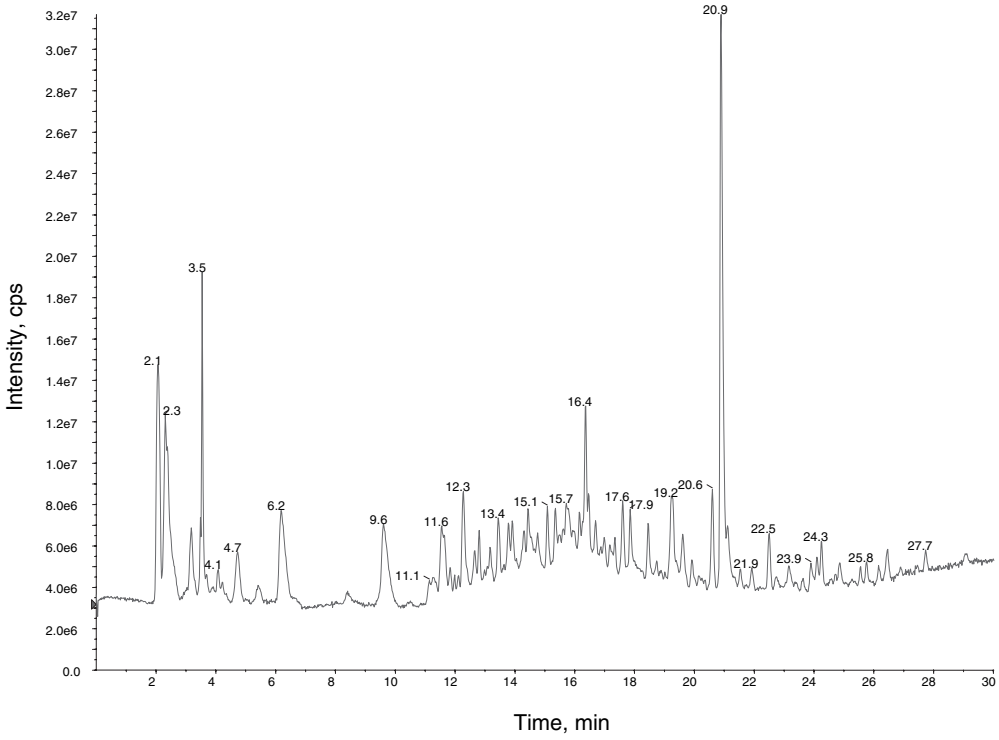

Figure 1. Blank pepper sample showing complexity of the sample with ~2500 accurate mass peaks detected at an S/N ratio of 10:1.

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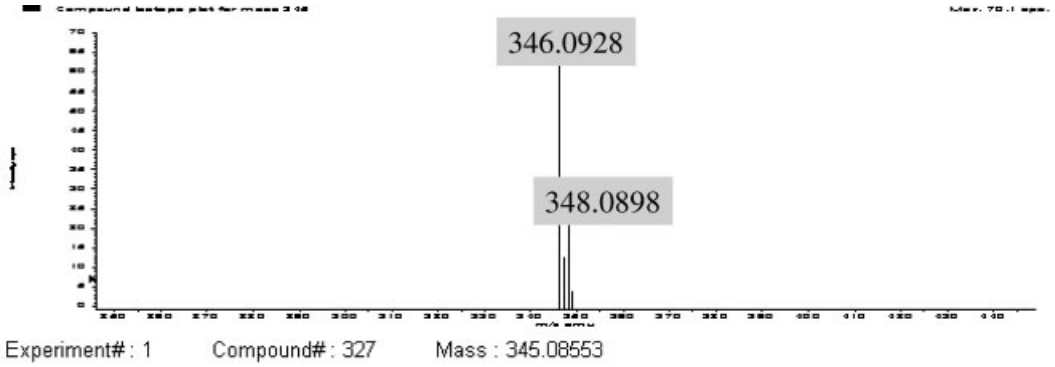

Mass Value = 345.08553							
#	Formula	Compound	Mass	Error (mDa)	* Error (ppm)	Ret. Time Error	Description
1	C15H15N3OClF3	Triflumizol	345.08557	-0.04	-0.1	-0.02	Fungicide

Figure 2. Example of a report from an MFD search; mass of neutral is reported in the printout and MH^+ is shown with the X + 2 ion cluster.

Table 1. Limits of detection for pesticides in food and water samples with retention time and accurate mass

Compound	Ret. time	Formula	Exact mass of molecule	LOD food mg/kg	LOD water μg/L
Atrazine	21.1	C8H14N5Cl	215.0938	0.005	0.015
Azoxystrobin	24.0	C22H17N3O5	403.1168	0.005	0.015
Benalaxyl	26.8	C20H23NO3	325.1678	0.005	0.015
Buprofezin	27.2	C16H23N3OS	305.1562	0.005	0.015
Cyanazine	22.0	C9H13N6Cl	240.0890	0.005	0.015
Diazinon	27.6	C12H21N2O3PS	304.1010	0.005	0.015
Difenconazole	26.4	C19H17Cl2N3O3	405.0647	0.005	0.015
Isomer	26.6	C19H17Cl2N3O3	405.0647	0.005	0.015
Difenoxuron	21.3	C16H18N2O3	286.1317	0.005	0.015
Dimethomorph	22.2	C21H22NO4Cl	387.1237	0.005	0.015
Fenamiphos	23.9	C13H22NO3PS	303.1058	0.005	0.015
Imazalil	18.0	C14H14N2OCl2	296.0483	0.005	0.015
Imazapyr	20.0	C13H15N3O3	261.1113	0.005	0.015
Imazaquin	20.0	C17H17N3O3	311.1270	0.005	0.015
Irgarol	21.2	C11H19N5S	253.1361	0.005	0.015
Irgarol metabolite	17.0	C8H15N5S	213.1048	0.005	0.015
Isoproturon	21.3	C12H18N2O	206.1419	0.005	0.015
Mebendazole	18.2	C16H13N3O3	295.0957	0.005	0.015
Metolachlor	25.6	C15H22NO2Cl	283.1339	0.005	0.015
Metribuzin	15.0	C8H14N4OS	214.0888	0.005	0.015
Nicosulfuron	17.0	C15H18N6O6S	410.1009	0.005	0.015
Prochloraz	23.0	C15H16Cl3N3O2	375.0308	0.005	0.015
Prometon	16.6	C10H19N5O	225.1590	0.005	0.015
Prometryn	19.0	C10H19N5S	241.1361	0.005	0.015
Propazine	23.0	C9H16N5Cl	229.1094	0.005	0.015
Propiconazole	25.9	C15H17Cl2N3O2	341.0698	0.005	0.015
Isomer	26.1	C15H17Cl2N3O2	341.0698	0.005	0.015
Simazine	18.8	C7H12N5Cl	201.0781	0.005	0.015
Spinosyn A	20.9	C41H65NO10	731.4608	0.005	0.015
Spinosyn D	21.9	C42H67NO10	745.4765	0.005	0.015
Spiroxamine	19.6	C18H35NO2	297.2668	0.005	0.015
Isomer	19.7	C18H35NO2	297.2668	0.005	0.015
Terbuthylazine	23.4	C9H16N5Cl	229.1094	0.005	0.015
Terbutyrn	20.4	C10H19N5S	241.1361	0.005	0.015
Triflumazole	25.9	C15H15ClF3N3O	345.0856	0.005	0.015
Acetamiprid	16.3	C10H11N4Cl	222.0672	0.01	0.03
Acetochlor	23.0	C14H20NO2Cl	269.1183	0.01	0.03
Alachlor	23.0	C14H20NO2Cl	269.1183	0.01	0.03
Bensultap	21.4	C17H21NO4S4	431.0353	0.01	0.03
Bromuconazole	23.8	C13H12N3OCl2Br	374.9541	0.01	0.03
Carbaryl	21.3	C12H11NO2	201.0790	0.01	0.03

(*Continues*)

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Table 1. (Continued)

Compound	Ret. time	Formula	Exact mass of molecule	LOD food mg/kg	LOD water µg/L
Carbendazim	6.2	C9H9N3O2	191.0695	0.01	0.03
Carbofuran	20.4	C12H15NO3	221.1052	0.01	0.03
Cartap	3.1	C7H15N3O2S2	237.0606	0.01	0.03
Chlorfenvinphos	26.5	C12H14Cl3O4P	357.9695	0.01	0.03
Cyproconazole	23.4	C15H18N3OCl	291.1138	0.01	0.03
Cyromazine	2.9	C6H10N6	166.0967	0.01	0.03
Deethylatrazine	15.3	C6H10N5Cl	187.0625	0.01	0.03
Deisopropylatrazine	12.1	C5H8N5Cl	173.0468	0.01	0.03
Dichlorvos	20.0	C4H7Cl2O4P	219.9459	0.01	0.03
Dimethenamide	24.0	C12H18NO2SCl	275.0747	0.01	0.03
Dimethoate	16.3	C5H12NO3PS2	228.9996	0.01	0.03
Diuron	21.0	C9H10N2OCl2	232.0170	0.01	0.03
Ethiofencarb	21.8	C11H15NO2S	225.0823	0.01	0.03
Fenuron	15.7	C9H12N2O	164.0950	0.01	0.03
Imidacloprid	15.7	C9H10N5O2Cl	255.0523	0.01	0.03
Lenacil	19.2	C13H18N2O2	234.1368	0.01	0.03
Malathion 1	22.5	C10H19O6PS2	330.0361	0.01	0.03
Malathion 2	16.9	C10H19O6PS2	330.0361	0.01	0.03
Metalaxyl	21.2	C15H21NO4	279.1471	0.01	0.03
Methiocarb	23.5	C11H15NO2S	225.0823	0.01	0.03
Methomyl	12.1	C5H10N2O2S	162.0463	0.01	0.03
Monuron	18.7	C9H11ClN2O	198.0560	0.01	0.03
Nitenpyram	11.9	C11H15ClN4O2	270.0884	0.01	0.03
Oxadixyl	19.1	C14H18N2O4	278.1267	0.01	0.03
Profenfos	28.6	C11H15BrClO3PS	371.9351	0.01	0.03
Promecarb	24.0	C12H17NO2	205.1341	0.01	0.03
Propachlor	25.6	C11H14NOCl	211.0764	0.01	0.03
Prosulfocarb	29.0	C14H21NOS	251.1344	0.01	0.03
Thiabendazole	3.7	C10H7N3S	201.0361	0.01	0.03
Thiacloprid	17.7	C10H9N4SCl	252.0236	0.01	0.03
Thiocyclam	4.5	C5H11NS3	181.0054	0.01	0.03
Aldicarb	18.5	C7H14N2O2S	190.0776	0.05	0.15
Aldicarb sulfoxide	6.0	C7H14N2O3S	206.0725	0.05	0.15
Bendiocarb	20.6	C11H13NO4	223.0845	0.05	0.15
Chlorotoluron	20.4	C10H13N2OCl	212.0716	0.05	0.15
Flufenacet	25.0	C14H13N3O2SF4	363.0665	0.05	0.15
Hydroxyatrazine	11.5	C8H15N5O	197.1277	0.05	0.15
Lufenuron	28.6	C17H8N2O3Cl2F8	509.9784	0.05	0.15
Metamytron	14.0	C10H10N4O	202.0855	0.05	0.15
Methidathion	24.1	C6H11N2O4PS3	301.9619	0.05	0.15
Methiocarb sulfone	17.4	C11H15NO4S	257.0722	0.05	0.15
Molinate	24.8	C9H17NOS	187.1031	0.05	0.15
Parathion ethyl	27.3	C10H14NO5PS	291.0330	0.05	0.15
Propanil	17.0	C9H9NOCl2	217.0061	0.05	0.15
Triclocarban	27.5	C13H9Cl3N2O	313.9780	0.05	0.15
Aldicarb sulfone	11.4	C7H14N2O4S	222.0674	0.1	0.30
Bromacil	18.5	C9H13N2O2Br	260.0160	0.1	0.30
Bromuconazole	23.8	C13H12N3OCl2Br	374.9541	0.1	0.30
Butylate	15.0	C11H23NOS	217.1500	0.1	0.30
Diflubenzuron	25.0	C14H9N2O2ClF2	310.0321	0.1	0.30
Flufenoxuron	29.2	C21H11N2O3ClF6	488.0362	0.1	0.30
Fluroxypyr	18.8	C7H5N2O3Cl2F	253.9661	0.1	0.30
Hexaflumuron	27.2	C16H8N2O3Cl2F6	459.9816	0.1	0.30
Imazalil degradate	14.6	C11H10N2OCl2	256.0170	0.1	0.30
Iprodione	25.4	C13H13N3O3Cl2	329.0334	0.1	0.30
Pendimethalin	25.0	C13H19N3O4	281.1376	0.1	0.30
Teflubenzuron	27.6	C14H6N2O2Cl2F4	379.9742	0.1	0.30
Captan	24.4	C9H8Cl3NO2S	298.9341	0.5	2.0
Chloropyrifos methyl	28.2	C7H7Cl3NO3PS	320.8950	0.5	2.0
Spiromesifen	23.0	C23H30O4	370.2144	0.5	2.0
Thiosultap	3.2	C5H13NO6S4	310.9626	0.5	2.0

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Table 2. Products ions for pesticides in database

Compound	Exact mass of product ions	Formula of product ion
Atrazine	174.0541	C5 H9 Cl N5
Acetamiprid	126.0105	C6 H5 Cl N
Acetochlor	148.1121	C10 H14 N
Alachlor	162.1277	C11 H16 N
Aldicarb	116.0528	C5 H10 N S
Aldicarb	89.0419	C4 H9 S
Aldicarb sulfone	76.0393	C2 H6 N O2
Aldicarb sulfoxide	76.0393	C2 H6 N O2
Azoxystrobin	372.0979	C21 H14 N3 O4
Benalaxyl	208.1332	C12 H18 N O2
Bendiocarb	109.0284	C6 H5 O2
Bensultap	290.0338	C11 H16 N O2 S3
Bromucanoazole	158.9763	C7 H5 Cl2
Buprofezin	201.1056	C9 H17 N2 O S
Butylate	162.0947	C7 H16 N O S
Captan	235.9698	C8 H8 Cl2 N O S
Carbaryl	145.0648	C10 H9 O
Carbendazim	160.0505	C8 H6 N3 O
Carbofuran	165.091	C10 H13 O2
Carbofuran 2	123.0446	C7 H7 O2
Cartap 1	104.9827	C3 H5 S2
Cartap 2	150.0406	C5 H12 N S2
Chlorfenvinphos	98.9845	H4 O4 P
Chlorpyrifos methyl	124.9821	C2 H6 O2 P S
Cyanazine	214.0854	C8 H13 Cl N5
Cyproconazole	70.04	C2 H4 N3
Cyromazine	108.0556	C5 H6 N3
Deethylatrazine	146.0228	C3 H5 Cl N5
Deisopropylatrazine	146.0028	C3 H5 Cl N5
Diazinon	169.0794	C8 H13 N2 S
Dichlorvos	109.0049	C2 H6 O3 P
Difeconazole	251.0025	C13 H9 Cl2 O
Difenoxuron	123.0441	C7 H7 O2
Diflubenzuron	141.0146	C7 H3 F2 O
Dimethenamide	244.0557	C11 H15 Cl N O S
Dimethoate	124.9821	C2 H6 O2 P S
Dimethomorph	301.0626	C17 H14 Cl O3
Diuron	72.0444	C3 H6 N O
Ethiofencarb	107.0491	C7 H7 O2
Ethiofencarb	164.0706	C9 H10 N O2
Fenamiphos	276.0818	C11 H19 N O3 P S
Fenuron	72.0444	C3 H6 N O
Flufenacet 1	194.0976	C11 H13 F N O
Flufenacet 2	152.0506	C8 H7 F N O
Flufenoxuron	158.0412	C7 H6 F2 N O
Fluroxpyr	208.9679	C6 H4 Cl2 F N2 O
Hexaflumuron	158.0412	C7 H6 F2 N O
Hydroxyatrazine	156.088	C5 H10 N5 O
Imazalil	158.9763	C7 H5 Cl2
Imazapyr	234.1237	C12 H16 N3 O2
Imazaquin	284.1394	C16 H18 N3 O2
Imidacloprid	175.0978	C9 H11 N4
Iprodione	244.9879	C9 H7 Cl2 N2 O2
Irgarol	198.0808	C7 H12 N5 S
Irgarol metabolite	158.0495	C4 H8 N5 S
Isoproturon	165.1022	C9 H13 N2 O
Lenacil	153.0659	C7 H9 N2 O2
Lufenuron	158.0412	C7 H6 F2 N O
Malathion	285.0015	C8 H14 O5 P S2
Mebendazole	264.0768	C15 H10 N3 O2
Metalaxyl	220.1332	C13 H18 N O2
Metamitron	175.0978	C9 H11 N4
Methidathion 1	85.0396	C3 H5 N2 O
Methidathion 2	145.0066	C4 H5 N2 O2 S
Methiocarb	169.0682	C9 H13 O S
Methiocarb sulfone	201.058	C9 H13 O3 S
Methomyl	106.0321	C3 H8 N O S
Metolachlor	252.115	C14 H19 Cl N O
Metolcarb	94.0413	C6 H6 O
Metribuzin	187.1012	C7 H15 N4 S
Molinate	126.0913	C7 H12 N O
Monuron	72.0444	C3 H6 N O
Nicosulfuron	182.056	C7 H8 N3 O3
Nitenpyram	225.1027	C11 H16 Cl N3
Oxadixyl	133.0886	C9 H11 N
Parathion Ethyl	235.9777	C6 H7 N O5 P S
Pendimethalin	194.056	C8 H8 N3 O3
Prochloraz	308.0006	C12 H13 Cl3 N O2
Prochloraz	265.9537	C9 H7 Cl3 N O2
Profenofos	344.9111	C9 H12 Br Cl O3 P S
Promecarb	151.1117	C10 H15 O
Promecarb	109.0653	C7 H9 O
Prometon	184.1193	C7 H14 N5 O
Prometryn	200.0964	C7 H14 N5 S
Propachlor	170.0367	C8 H9 Cl N O
Propanil	161.9872	C6 H6 Cl2 N
Propiconazole	158.9763	C7 H5 Cl2
Prosulfocarb	91.0542	C7 H7
Simazine	132.0323	C4 H7 Cl N3
Spinosdad A	544.3633	C32 H50 N O6
Spinosdad D	558.3789	C33 H52 N O6
Spiromesifen	255.138	C17 H20 O2
Spiroxamine	144.1383	C8 H18 N O
Teflubenzuron	158.0412	C7 H6 F2 N O
Terbuthylazine	174.0541	C5 H9 Cl N5
Terbutyrn	186.0808	C6 H12 N5 S
Thiabendazole	175.0324	C9 H7 N2 S
Thiacloprid	126.0105	C6 H5 Cl N
Thiocyclam	136.9548	C3 H5 S3
Thiosultap	232.013	C5 H14 N O3 S3
Triclocarban	127.0183	C6 H6 Cl N
Triflumizole	278.0554	C2 H12 Cl F3 N O
Flumeturon	72.0444	C3 H6 N O

(*Continues*)

fragmentation of the MH^+ ion although the product ions varied in signal intensity. All product ions were verified by accurate mass measurement and structure to have originated from the MH^+ ion of the parent molecule. Retention time matching was also used with an error of ±0.01 min via a deconvolution software program (called Molecular Hunter). The database was created with the MH^+ ion, the product ion, and the retention time of the standard using a spreadsheet in csv format (comma separated value).

Library search of pesticides in fruits and vegetables

Table 3 shows the results of a library search of six fruit and vegetable samples from a local grocery store (apple, pear, tomato, potato, pepper, cucumber) and one commercial brand of olive oil for the 100 pesticides in the database. The MFD search found between 617 and 2681 accurate mass peaks in the sample chromatograms (Table 3). The least complicated sample matrix was tomato with 617 peaks, and the apple and pepper were the most complex samples with

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Table 3. Screened pesticides in food and water samples using the MFD

Sample	Peaks screened	Pesticide matches <5 ppm	Pesticides identified LC/TOF MS	Error mDa	Error ppm	Ret. time error (min)	FALSE Neg.	FALSE Pos.
Apple	2681	12	Imazalil				1	0
			Imazalil degradate	−1	−3.9	−0.08		
			Iprodione	0.22	0.7	−0.12		
			Fluqinconazole	0.05	0.1			
			Difenoconazole	−0.74	−1.8			
Olive oil	1678	10	Terbuthylazine	0.06	0.2	0.09	0	0
			(Deisopropylatrazine)					1
Pepper	2402	41	Imazalil	0.3	1	0.07	0	
			Diazinon	0.11	0.3	0.12		
			Buprofezin	1	3.3	0.1		
Tomato	617	8	Buprofezin				3	
			Carbendazim					
			Thiophanate methyl					
Cucumber	1619	17	Thiabendazole	0.2	1	0.01	0	0
			Malathion isomer 1	0.04	0.1	0.05		
			Malathion isomer 2	0.22	0.7	−0.03		
			Malathion oxon	0.25	0.8	0.08		
			Imazalil	−0.1	0.3	0.05		
Pear	1209	14	Imazalil				1	0
			Carbendazim	−0.21	−1.1	0.05		
			Imazalil Degradate	0.51	2	0.03		
			Phosmet	0.31	1	0.04		
Potato	1150	11	None				0	0
Surface water Kansas USA	1271	25	Atrazine				1	
			Prometon	0.02	0.1	−0.02		
			Hydroxyatrazine	−0.35	−1.8	−0.04		
			Deisopropylatrazine	−0.15	−0.9	−0.05		
			Simazine	−0.44	−2.2	−0.04		
			Diuron	0.1	0.4	−0.04		
			Propazine	−0.18	−0.8	−0.01		
			Dimethenamide	−0.17	−0.6	−0.04		
			Metolachlor	0.07	0.2	−0.04		
			(Propachlor)					1
Surface water Kansas USA	1710	25	Atrazine				2	
			Terbuthylazine					
			Deisopropylatrazine	−0.41	−2.4	−0.5		
			Simazine	−0.33	−1.7	−0.05		
			Diuron	−0.52	−2.3	−0.04		
			Propazine	−0.35	−1.5	−0.01		
			Dimethenamide	−0.03	−0.1	−0.04		
			Metolachlor	0.1	0.3	−0.04		
			Bromacil	−0.56	−2.2	0.02		
			Diazinon	0.34	1.1	−0.13		
			Deethylatrazine	−0.35	−1.9	−0.08		
			Absolute Means	0.30	1.18	0.07		
			Absolute Std Dev	0.25	0.97	0.09		

~2500 peaks each. The sensitivity of the MFD search was set at a S/N ratio of 10:1. The quantity of peaks found approximately doubles on decreasing the S/N ratio from 20:1 to 10:1. The value of 10:1 is chosen in order to obtain good X + 1 and X + 2 isotope signatures of the compound with the maximum instrument sensitivity.

The accuracy window of the MFD search is set at 5 ppm in order to be well within the mass accuracy of the LC/TOFMS system. The number of pesticides found in the 5-ppm mass window of these samples varied from 8 to 41 compounds. The only criterion to be included in this match was that the MH^+ ion was within 5 ppm of the database value. Thus, as an example, the pepper sample, which contained 2402 peaks, had only 41 of these peaks that met the 5-ppm accuracy window (Table 3).

Of these 41 peaks, only three formulae were identified based on the correct isotope signature, and the correct retention-time match. They were the compounds imazalil, diazinon, and buprofezin. The identification was checked not only in the printout of the automated database match, but also by manual confirmation of the data file.

The confirmation of the empirical formula varied from no detections in the potato sample to one pesticide in olive oil, three pesticides in pepper and tomato, and five pesticides in the cucumber and apple. The compound most commonly identified by the screen in the fruit and vegetable samples was imazalil, which is a post-harvest fungicide used for transport and storage of fruits and vegetables before sale. Other compounds included organophosphate insecticides, such as diazinon, phosmet and malathion, and the oxon of

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malathion, a pesticide degradation product. The insect growth regulator buprofezin was found in a tomato and pepper sample.

The tomato sample caused the most problems for the screening database, and in some ways, it was the most useful sample analyzed. It gave three false negatives (i.e. screened negative but contained buprofezin, thiophanate methyl, and carbendazim). The cause of the false negatives was the high concentration (greater than 1 mg/kg) of the pesticides present in the tomato, which skewed the accurate mass outside the 5-ppm window due to partial saturation of the detector. This problem may be corrected by using the X + 1 ^{13}C isotope of the pesticide for screening, thus keeping the mass accuracy within the 5-ppm accuracy window. The same problem of false negative occurred with the pear sample for imazalil. This was also corrected by the addition of the X + 1 isotope of imazalil. Thus, the database has now been corrected for all of the major high-use compounds by the addition of the ^{13}C isotope of the MH^+ ion. This correction is done in a spreadsheet involving the subtraction of 12.00000 for one ^{12}C atom and the addition of 13.00335 for the accurate mass of the ^{13}C atom. This correction does not take into account other X + 1 isotopes (i.e. ^{2}H or ^{15}N), so it is a close approximation (within 0.5 mDa) meant to find extremely high concentrations of parent pesticide only, but it does easily stay within a mass accuracy of 5 ppm. An additional error of approximately 0.1 to 1 ppm is added by using the ^{13}C isotope only and this is dependent on the number of carbon, hydrogen, and nitrogen atoms in the compound.

The accuracy of all confirmed samples had an absolute-value average of 0.3 mDa or ~1.2 ppm and a standard deviation of 0.25 mDa and ~1.0 ppm (Table 3). The absolute-value average for retention-time match was 0.07 min with a standard deviation of 0.09 min. Thus, the windows chosen for the database search are chosen with enough margin of error to find 99% of the samples based on two standard deviations of the mean for mass accuracy and retention time. These data show the most serious deficiency of the database search, which is the occurrence of false negatives. Therefore, the addition of ^{13}C atoms is an important step in the correct operation of the database search. The occurrence of false positives is easier to deal with, and the solution involves the use of an authentic standard and at least one product ion to identify the compounds.[a]

Library search of water samples

The MFD search was tested for extracts of two water samples taken during the Spring runoff[16] in the state of Kansas (USA). This time of year is important for the runoff of herbicides from corn, wheat, and sorghum that are treated with herbicide prior to planting in April and May.[16] The MFD search found 1271 peaks in the first sample; of those, 25 peaks fell within the 5-ppm accuracy window and nine formulae were confirmed by accurate mass, isotopic ratio, and retention-time match. This sample contained also one false negative, atrazine, because of the extremely high concentration of this compound (5 mg/kg in the methanolic extract), which skewed the mass accuracy. This problem was corrected, in the same manner as for the food samples, by the addition of the ^{13}C peak for atrazine at *m/z* 217.1041.

A false positive occurred for the compound propachlor at *m/z* 212.0836, which also has the same empirical formula and the same exact mass as a product ion of metolachlor. The X + 2 isotopic signature is, of course, correct because propachlor and the metolachlor product ion have the same formula. The retention time was not available for propachlor but it was available for metolachlor. A simple fragmentation study of metolachlor at higher fragmentor voltages did show that the *m/z* 212.0836 ion originated from metolachlor as a low-abundance product ion.

The second water sample contained 1710 peaks of which 25 were screened under the 5-ppm mass accuracy window. Of these, 11 formulae were confirmed. There were two false negatives caused by high concentrations, atrazine and terbuthylazine. Both these compounds were detected by the use of the ^{13}C isotope (error within 2 ppm). Terbuthylazine was used in this sample as an internal standard. The solid-phase extraction, which multiplied the concentration in water by 300-fold, created the over saturation for this compound and the loss of mass accuracy. Manual screening of the large peaks was used to detect these false negatives.

These experiments indicate that the MFD search with monoisotopic mass only (and X + 2 confirmation) is useful for data exploration and is a quick way to search for many compounds of interest. The MFD was useful to find compounds such as fluqinconazole and difenoconazole in apples, and hydroxyatrazine, prometon, and diuron in water samples, which were not seen by the first manual inspection of the sample file.

Probability of library match

The concept of confirmation by accurate mass has been addressed by Stolker *et al.*[18] as part of the EU program for banned substances in animal products. The identification point system is used to find target compounds and for accurate mass the requirement is a retention-time match and two ions at accurate mass, for a total of 2.0 identification points for each ion and a total of 4 identification points, as well as correct ion ratios. Another requirement is that the resolving power be 10 000 for each of the ions (at 10% valley or 20 000 FWHM). The requirement of resolving power of 20 000 (FWHM) for the low mass-to-charge ions found in pesticides (less than *m/z* 300) is not achievable by current LC/TOFMS instrumentation, based on instrument manufacturer information. However, using this as a guide for match quality, it is possible to calculate approximate probabilities of an error of the pesticide database.

Examining the data in Table 1, it was noted that there are nine sets of compounds that have the same molecular formula and thus the same exact mass. This is approximately 18% of the pesticides examined in this study. Furthermore, the number of product ions that have the same exact mass was six sets or 12% of the total ions. These product ions are often diagnostic of a class or set of compounds and are useful for identification of degradation products of

[a]Author's note: At the time of proof correction, recent advances in the TOF software version 2.0.1 now allow for correction of saturation so that entering the ^{13}C masses are not required to remain within 5-ppm accuracy.

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Table 4. Approximate number of possible formulae that can be calculated using common elements of the pesticides: C, H, N, O, S, P, F, Br, Cl, and Na

Mass accuracy (error in ppm)	Number of elemental formulae possible at mass error 1–10 ppm				
10	5	100	300	1000	10000
5	3	50	150	500	5000
2	2	20	60	200	2000
1	1	10	30	100	1000
	m/z 100	*m/z* 200	*m/z* 300	*m/z* 400	*m/z* 1000
			Mass range		

pesticides in food and water samples.[19,20] Combining these probabilities for the possibility that a pesticide might have the same exact mass MH^+ ion and the same product ion (ion comes from in-source CID fragmentation) gives a 2.2% chance, or approximately two compounds in the database: $0.18 \times 0.12 = 0.022$.

There are two hidden assumptions in this calculation. The first is that these probabilities are independent and they are not because of similar structures in nearly every set. The second is that the compounds would have the same exact retention time, which of course they do not. For example, see the separation of isomers of three compounds in Table 1. This represents the maximum probability of an error (the worst case scenario). Thus, a library match with the exact mass and the exact mass product ion results in a match of ~0.98 based on these assumptions (1–0.022 = 0.978 or ~0.98).

This match may be improved by the use of a deconvolution program and re-examination of the assumption of retention time. The software is able to match the mass peak as a function of retention time with a reproducibility of ±0.02 min for the MH^+ ion and its product ion. The 100 pesticides are separated over a time window of 7 to 30 min with the majority of the compounds separating in a 10-min window. To be conservative, the 10-min window may be divided into 250 increments of ±0.02 min. Thus, the ±0.02-min window gives another level of error probability of approximately 0.004, or 4 chances in 1000 of overlapping ions, and the final probability match is lowered only by a slight amount, 0.98–0.004 = 0.98, that is probability of error of the database match is 0.98 or a match quality of 98%. The accurate mass library approach is quite useful for identification purposes, even without the use of standards, because the retention-time match is based on the overlapping of not only the two ions but also on the interferant having the same chromatographic behavior using a standard 5 micron C-8 or C-18 column with approximately 15 000 theoretical plates. Thus, chromatographic resolution is used for added match quality without the use of an authentic standard by separating the library ions from interferant ions. This makes this accurate mass library available for all instruments, although it may be necessary to use it in a more manual approach if software is not available.

The calculations of probability above do not consider the error in making the mass measurement and in assigning an empirical formula (i.e. the error of ±5 ppm of the search). The match probability is based on the exact mass being measured correctly against the database. Some skill in mass spectrometry is required here in order to use the X + 1 and X + 2 isotope information for the correct formula and to lower the probability of error from the 5 ppm of the search to the less than 2 ppm of instrument capability. To help in this manual process, the library program also gives the isotope table and the isotope abundances for each match, which is quite useful for correct formula identification. Most instrument manufacturers of accurate mass instruments provide software to help with isotope identification and matching.

Table 4 shows the number of compounds containing the common elements of C, H, N, O, S, P, Cl, Br, and Na, and the number of possible structures that may occur. This table gives us an approximation of the number of possible elemental formulae that are within the tolerance of the accurate mass that is measured. For example, between the masses of 200 and 300, there are between 50 and 150 possible elemental formulae that may occur at 5-ppm mass accuracy. Note that the number of possibilities increases exponentially with increasing mass. This number of possibilities may be reduced quite easily to a manageable number of two to three possibilities by examining the isotope cluster of the X + 1 and X + 2 ions. Both the accurate mass and the intensity of the isotope cluster may be used to simplify the number of correct formulae.

An example is shown for imazalil in Fig. 3, which was identified in the pepper extract (Table 2). Note that the accurate mass defect when going from the X peak to the X + 2 and the X + 4 peak was 1.997 mass units, which is characteristic of the addition of ^{37}Cl and is accounted for by the difference in mass between ^{35}Cl and ^{37}Cl. Also note that the abundances of the X + 2 and X + 4 isotopic patterns match a compound containing two Cl atoms. Likewise, the X + 1 isotope shows a positive mass defect of 1.0022 mass units for the ^{13}C isotope and a peak area consistent with 14 carbons. Thus, the isotopic pattern is a good match for imazalil with a formula of $C_{14}H_{14}N_2O_2Cl_2$. This analysis is a manual operation at this time, and the automation of this process is the last hurdle before the exact-mass database is totally automated.

CONCLUSIONS: WEAKNESS AND STRENGTHS OF THE EXACT-MASS LIBRARY

The major weakness of the MFD search was the occurrence of false negatives created by compounds present in high concentration. This problem is being dealt with by incorporating the X + 1 peak of MH^+ ions into the database or by

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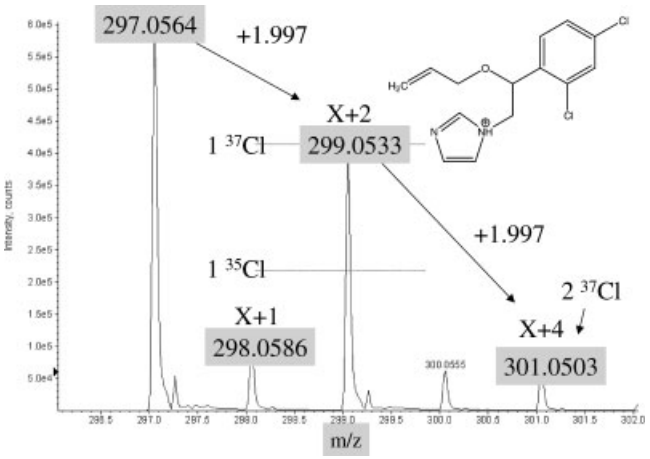

Figure 3. Isotopic pattern for imazalil showing the importance of accurate mass of the X + 1, X + 2, and X + 4 isotopes for identification, including peak height and accurate masses.

modification of the software so as not to saturate ion-intensity counts. If this is done, the accurate mass window may be used at the 5-ppm setting. See footnote [a] on page 3666 about correction of saturation with software version 2.0.1.

A second cause of false negatives is a mass interference that causes loss of mass accuracy. This problem was not detected in this study (by manual inspection), but it can occur depending on the matrix and the compounds being screened.

When the mass defect of the MH^+ ion is approximately +0.2 mDa there can be problems from the matrix because this value is similar to the mass defect of the food matrix, which consists of compounds containing C, H, N, and O. Most pesticides have a mass defect less than this value because of the addition of halogen atoms. There are a number of possible ways of solving this problem with the current instrumentation: increasing the resolution of the LC/TOFMS system, increasing chromatographic resolution, or reducing the number of possible formulae by use of isotope-matching software.

The strength of the MFD search is the speed of screening hundreds of pesticides in minutes, including both their protonated molecules and product ions. The use of classical fragmentation libraries with comparison with fragmentation patterns is probably not needed in LC/TOFMS using accurate mass because the match is based on presence of the ion rather than its signal intensity and its fragmentation pattern. The problem of instrument variation and matrix effects on fragmentation is partially eliminated with accurate mass. However, it may be necessary to monitor more than one product ion depending on the type of pesticide being determined. Pesticides without characteristic X + 2 isotopic patterns need at least two product ions.

It is the view of the authors that a large problem in LC/MS libraries is on the verge of being solved with the use of accurate mass databases for pesticide screening in food and water using the protonated molecule, a major product ion(s), isotopic matching, and chromatographic retention-time matching.

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