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The development and inter-laboratory verification of LC–MS libraries for organic chemicals of environmental concern☆

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ABSTRACT

The development, verification, and comparison study between LC–MS libraries for two manufacturers' instruments and a verified protocol are discussed. Compounds in the libraries are among those considered by the U.S. EPA Office of Water as threats to drinking water including pesticides, drugs of abuse, and pharmaceuticals. The LC–MS library protocol was verified through an inter-laboratory study that involved Federal, State, and private laboratories. The results demonstrated that the libraries are transferable between the same manufacturer's product line, and have applicability between manufacturers. Although ion abundance ratios within mass spectra were shown to be different between the manufacturers' instruments, the NIST search engine match probability was at 96% or greater for 64 out of 67 compounds evaluated.

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1. Introduction

Gas chromatography coupled with mass spectrometry (GC–MS) is one of the best techniques for identifying unknown compounds in environmental samples. A major reason for its utility is the searchable libraries of mass spectra that have been compiled using electron impact ionization. These libraries are essentially instrument independent, so whichever brand of GC–MS is used, a compound can theoretically be tentatively identified, if it is included in the mass spectral libraries. This is made possible by the use of standard 70-eV electron impact ionization using a standardized tuning procedure as described elsewhere [1]. Libraries of mass spectra, such as the NIST [2] library, have automatic searching routines which list the top possibilities.

The more recently introduced liquid chromatography–mass spectrometry (LC–MS) has advantages over GC–MS for organic compounds that are thermally labile, polar, or non-volatile. Derivatization of polar analytes and solvent extraction of drinking water are not required prior to analysis, both of which greatly increase the analysis time. Water samples can be analyzed directly after filtration through a syringe-driven disposable filter to remove debris that can clog the LC injector, tubing, or column.

Additionally, highly-polar or low-volatility organic compounds do not traverse GC columns or do so over such a long time that discrete gas chromatographic peaks may not be observed. Thermally unstable compounds are often degraded in the GC inlet or later in a hot GC column. HPLC separations are generally accomplished at room temperature, so thermal stability of the analyte is usually not an issue. Eluting analytes are then ionized to produce spectra via electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or atmospheric pressure photoionization (APPI).

However, LC–MS has not had the benefit of searchable libraries that contain reproducible spectra for several reasons. First, the pressure in the LC–MS ion source (no greater than 1 atm) is higher relative to GC–MS because of the need to convert liquid to gas in the interface between the HPLC and the MS. Ions created at atmospheric pressure undergo ion-molecule collisions which alter the ion distribution depending on their residence time in the source and other factors. On the other hand, electron impact (EI) ionization that is typical of GC–MS systems operates at low gas pressure, which prevents ion-molecule collisions regardless of the ion source

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design. The fragmentation process is reproducible due to standard tuning criteria and the use of a standard 70 eV. There are many treatises written on the mechanisms that produce ions in EI ionization [3].

In atmospheric ionization sources (ESI, APCI, or APPI), multiple ion-molecule collisions remove energy from precursor ions, which then lack sufficient internal energy to fragment. This "soft ionization" generally provides mass spectra lacking product ions. A product ion due to loss of a water or carbon dioxide molecule can appear from some compounds. In addition to the precursor ion, adduct ions are often observed depending on the ionization environment due to the use of solvents and modifiers to optimize chromatography and sensitivity. These simple spectra, while they are indicative of the molecular weight, do not present the diagnostic power of the EI ionization spectra with its rich fragmentation pattern. A spectrum with a precursor ion and a few adduct ions is certainly not unique to a certain compound. Therefore, a library of such spectra would provide little discrimination among analytes.

To provide multiple product ions from analytes, the energy of collisions must be increased sufficiently to break bonds within the precursor ion. Single MS-stage instruments can use in-source collision-induced dissociation (CID), but the presence of various solvent, additive, and contaminant molecules can cause variation in the product ion spectra, and not all ions observed may originate from the analyte. Instruments, such as triple quadrupole mass spectrometers and ion traps, can focus the ion of interest and energize this species to effect further fragmentation free of extraneous ions. Both the ion trap and triple quadrupole mass spectrometers generate fragmentation by applying a voltage or energy to the ionized species and simultaneously add a collision gas to cause reactive collisions resulting in diagnostic ions. These product ions are related to the structure of the protonated or deprotonated molecule and could thus be used for diagnostic purposes. In principle, compilation of mass spectral libraries for each type of ionization and for both ion trap and triple quadrupole instruments should be feasible.

To provide reproducible product ion spectra for a library, voltage and collision gas pressures must be reproducible for individual instruments and for similar instruments that use the library. These requirements were not met by early ion traps and triple quadrupole mass spectrometers, and compilation of mass spectral libraries was not practical. However, an attempt was made to standardize conditions in triple quadrupole mass spectrometers. By using the kinetics of a well-defined reaction, Martinez [4] attempted to standardize conditions to generate reproducible spectra. Martinez's method was not valid for ion traps and little support was forthcoming from the analytical community for this attempt to standardize spectra. Consequently, the mass spectral library idea floundered.

Also desirable would be HPLC mass spectral libraries for single-stage quadrupole instruments, which are the workhorses for environmental analyses. Unfortunately, these instruments are not effective at generating product ions. An attempt was made to add a repeller to the ion sources of single quadrupole systems that could break apart protonated molecules [5], which were effective at generating product ions. However, this was not reproducible from instrument to instrument.

There have been direct efforts to generate EI ionization spectra under LC conditions. The particle beam LC–MS interface [6] removed most of the solvent in the interface before solvated ions entered the ion source and struck heated surfaces. The desolvated molecules were then ionized by 70-eV electrons to provide EI-searchable mass spectra. This worked well for certain compounds [7], but was not universally adopted because of problems with thermal degradation and low volatility of compounds.

Another effort is the recent work by Granot and Amirav [8] to generate LC–MS spectra with EI ionization in supersonic molecular beams. This method shows some promise, but it is too early to predict its commercial application. Cappiello and Palma [9] interfaced a nanoscale LC to a direct electron ionization system to examine small to medium molecular weight molecules of different polarities. This technique shows some promise for those compounds that might have matrix problems when introduced through API interfaces.

Only recently have the electronics of mass spectrometers become stable enough that reproducible voltages and pressures provide reproducible CID spectra, at least on a single instrument. This stability is important in the collision region of a triple quadrupole mass spectrometer or the source region of a single quadrupole mass spectrometer through CID.

Therefore, it should be possible to collect spectra from an individual mass spectrometer and expect that these spectra will form a standardized library that the user can search during subsequent analyses. In fact, there should be two such libraries. The first would be generated from triple stage quadrupoles (LC–MS/MS), in which a single ion is focused, presumably the protonated molecule, in the first quadrupole and then sent into the second quadrupole or the collision cell, which contains an inert gas, such as argon, where the ion would undergo energetic collisions to produce product ions, which would be scanned in the third quadrupole and then detected. The other library from LC–MS spectra would be produced by some kind of device (repeller, cone, etc.) in the ion source that is effective at generating product ions. There would be no discrimination of the ions, so every ion in the source at the time of fragmentation would add to this spectrum. The first library described above would be "purer" than the latter because of the fact that interference ions could be present in the source as the voltages were applied to fragment the ion of interest.

Some attempts to compile searchable LC–MS and LC–MS/MS libraries with modern instruments have shown promise [10–15] while others encountered difficulties that precluded their use [16]. Encouraged by the success of Gergov et al. [11] in developing libraries for drugs, we attempted to create LC–MS and LC–MS/MS libraries for chemicals that could cause harm and disrupt distribution in a drinking water system. The ability to quickly and accurately identify a large number of organic compounds has become an important goal in this effort. LC–MS library technology is not only potentially useful for drinking water but also to identify or characterize agents that could be used in a terrorist incident, to monitor food safety, and to screen product quality.

LC–MS and LC–MS/MS libraries have been compiled for identification of chemicals that might pose a threat to drinking water. The Chicago Regional Laboratory (CRL) of the U.S. Environmental Protection Agency initially developed these libraries based on compounds that were potential threats to our nation's water supply. To validate the library protocol [17], other laboratories were recruited to verify that they could identify the chemicals in drinking water by comparing library mass spectra of standards with mass spectra from simulated unknowns obtained using the same solvents, methods, and instrument make as used by the CRL. In addition, the US EPA Office of Research and Development-Las Vegas Laboratory was recruited to test the library protocol with an instrument from a different manufacturer to determine if the library might have more general application.

2. Experimental

2.1. Instrumentation

The LC–MS Library System Protocol was developed using a Waters Corporation Quattro Premier™ triple quad (Milford, MA) with the ZSpray™ dual orthogonal sampling interface with Waters MassLynx™ 4.0 software. Other models used by the other labs

during the validation were ZQ™ single quad and Quattro Micro™ triple quad. However, to test the applicability of the protocol across different makes, a Thermo Electron Corporation Finnigan TSQ Quantum Ultra AM™ triple quadrupole mass spectrometer (San Jose, CA) was tested in this study. MS and MS/MS library-searchable spectra were generated for comparison with the CRL libraries.

2.2. Library development

The first list of target compounds included in the library project was supplied by the Water Security Division of the US EPA Office of Water. These compounds of concern are toxic substances and are readily available. The target list was divided into two groups, base/neutral and acidic compounds. The first library protocol addressed the base/neutral compounds.

Most of the compounds in Table 1 were obtained as neat standards, generously provided by the US EPA Office of Pesticide Programs (OPP) National Pesticide Standards Repository. The others were purchased from Aldrich Chemical Company (Milwaukee, WI), Cambridge Isotope Laboratories (Andover, MA), and Cerilliant (Round Rock, TX). The standards were diluted using a 50:50 water:acetonitrile mixture to an approximate concentration of 400 ppm (parts per million).

To acquire library mass spectra, the CRL infused standard solutions into a 'T' junction where they combined with mobile phase (5 mM ammonium bicarbonate in 50:50 water:acetonitrile, pH 10) before entering the mass spectrometer. Infusion was used to obtain optimal cone and collision energies for a compound to produce substantial fragmentation while maintaining at least 10% abundance of the precursor ion. After these settings were obtained, LC–MS analysis (25 ng of material on column) was undertaken to acquire retention time data and to verify that the cone and collision energies during infusion provided similar fragmentation when the standard eluted from the column. The amount of material injected was used to make sure that the concentration levels provided enough ion statistics to provide quality spectra for identification with different library searching techniques.

For MS scanning (single quadrupole), the electrospray source conditions were as follows: capillary voltage: 3.5 kV; extractor: 2 V; RF lens voltage: 0.2 V; source temperature: 120 °C; desolvation temperature: 300 °C; desolvation gas flow: 500 L h^{-1}; cone gas flow: 50 L h^{-1}. The analyzer section was maintained as follows: entrance: 50 V; exit: 50 V; collision: 2 V; multiplier: 650 V. These were the optimal settings used at the CRL, but optimal settings may vary slightly from instrument to instrument. The optimal cone voltage was different for each compound; these values were tabulated (for MS and MS/MS) and are listed in Table 1 together with collision energies for each compound.

For MS/MS scanning (triple quadrupole), the electrospray source conditions were the same as for the MS scanning mode. The analyzer settings for the MS/MS scanning mode were as follows: entrance: −1 V; exit: 2 V; collision: variable (see Table 1); multiplier: 650 V.

The solvent gradient under which MS and MS/MS data were recorded was as follows: 95:5 (H_2O:100 mM NH_4HCO_3, pH 10) at time = 0; hold for 2 min; 95:5 (acetonitrile:100 mM NH_4HCO_3) at time = 20.0 min; hold for 2 min; back to original conditions at time = 30.0 min. The flow rate was 0.3 mL min^{-1}. The column temperature was 30 °C and the sample compartment was held at 15 °C.

The diagnostic precursor and product ions with relative abundances exceeding 5% are listed in Table 1. They represent spectra taken both under source CID (MS) and MS/MS conditions using the collision cell.

The instruments were tuned and calibrated according to the procedures given by the manufacturer. The initial protocol followed by the volunteer labs is given in the following sections.

2.3. Tentative identification of an unknown

2.3.1. LC conditions and settings

The LC conditions were set to screen water samples and were not optimized for chromatographic separation. The Waters Alliance® 2695 HPLC with an XBridge™ C18, 2.1 mm × 150-mm column packed with 3.5-μm diameter particles, was used during the study. Any column capable of performing at high pH with adequate separation of these analytes may be used. The library protocol was not based on retention time of the analytes but on matching of spectra. The injection volume was 100 μL of a filtered water sample if possible. The elution gradient and other conditions were described earlier.

2.3.2. MS method file conditions and settings

To acquire MS and MS/MS spectra, the mass spectrometer was tuned using the conditions specified earlier (see Section 2.2). The MS method file, made up of one or more individual MS scanning functions, was created to detect compounds of interest at specific retention times and cone voltage settings. For example, a cone voltage of 35 V is the optimal value for aldicarb sulfone, buprofezin, carbofuran, and three other compounds in Table 1 to acquire product ion mass spectra most similar to those in the library, while a cone voltage of 75 V is optimal for 2-aminobenzimidazole, cyprodinil, and thiabendazole. The combination of several such MS scanning functions, each with a different cone voltage, is best suited to screen for multiple compounds in a sample. This screening approach is used to maximize the number of compounds screened simultaneously.

To ensure mass spectra were acquired for water samples at the optimal or nearly optimal cone voltage for each compound in the library, the cone voltage was cycled through six values: 15, 30, 45, 60, 75, and 90 V during acquisition. A 0.3-s scan was acquired for each voltage separated by a 0.1-s interscan delay. The total cycle time was 2.4 s and HPLC chromatographic peak widths were typically 20–40 s.

With in-source CID, co-eluting compounds can yield composite mass spectra containing product ions from multiple precursor ions, and good library matches are not likely. MS/MS is then necessary to isolate individual precursor ions before product ions are produced by CID to provide clean product ion spectra. Library-matchable product-ion spectra are then provided by the enhanced sensitivity and selectivity of MS/MS. For each unknown, MS/MS scanning methods require user input of the optimal cone voltage, collision energy, and precursor ion m/z such as shown in Table 1 into a menu.

Similar retention times for a tentatively identified compound and the standard provide an orthogonal measure to strengthen tentative identifications made using the library.

3. Results and discussion

3.1. Library searching

After full scan spectra at various voltages have been recorded for each compound, these spectra were searched against the MS library as described by the Masslynx™ or NIST library search manual. When >70% probability scores were obtained or when the operator thought a match was possible, the cone voltages from the library were compared with those for the acquired product ion spectra, and a tentative identification was made when they were consistent. The evidence for a somewhat doubtful, tentative identification of a compound could be enhanced by acquiring product ion spectra at the optimal cone voltage (and collision energy for MS/MS) for the compound from Table 1 to provide the strongest mass spectral evidence for the tentative identification. If the product ion spectrum is a match in the MS/MS library

Table 1
Library compounds.

Compound	CAS number	Nominal mass (g/mol)	Cone MS[a] (V)	Cone MS/MS (V)	Collision MS/MS (eV)	Precursor > MS/MS product ions (*m*/*z* units)
2-Aminobenzimidazole	934-32-7	133	75	48	32	134 > 92, 80
3-Hydroxy carbofuran	16655-82-6	237	40	30	9	220 > 163, 135
Acetamipirid	135410-20-7	222	46	25	14	223 > 126
Acetochlor	34256-82-1	269	32	28	11	270 > 224, 148
Acibenzolar-s-methyl[b]	135158-54-2	210	60	37	23	211 > 168, 136, 91
Aconitine	302-27-2	645	88	60	43	646 > 586, 105
Alachlor	15972-60-8	269	34	22	13	270 > 238, 162
Alanine[b]	56-41-7	89	32	19	12	90 > 44
Aldicarb	116-06-3	190	15	10	4	208 > 191, 116
Aldicarb sulfone	1646-88-4	222	35	25	8	223 > 166, 148, 76
Aldicarb sulfoxide	1646-87-3	206	25	20	5	207 > 132, 89
Allethrin	584-79-2	302	34	20	9	303 > 151, 135
Ametryn	834-12-8	227	58	35	20	228 > 186, 96
Amitraz	33089-61-1	293	34	22	11	294 > 253, 163
ANTU	86-88-4	202	46	27	15	203 > 186, 144
Atrazine	1912-24-9	215	55	38	19	216 > 174, 96, 79
Atropine	51-55-8	289	58	40	23	290 > 124, 93
Azinphos-methyl	86-50-0	317	25	18	6	318 > 261, 160
Azoxystrobin	131860-33-8	403	35	25	11	404 > 372
Bentazon[b]	25057-89-0	240	−55	−35	−24	239 > 197, 132
Bromoxynil[c]	1689-84-5	275	−53			276 > 79, 81, 185, 274, 123
Buprofezin	69327-76-0	305	35	22	13	306 > 201, 116
Butylate	2008-41-5	217	49	24	16	218 > 190, 162, 156, 100, 89
Carbaryl	63-25-2	201	25	20	5	202 > 145
Carbendazim	10605-21-7	191	40	30	15	192 > 160
Carbofuran	1563-66-2	221	35	25	11	222 > 165, 123
Chloramben[b]	133-90-4	205	−31	−20	−8	204 > 160
Chlorimuron-ethyl	90982-32-4	414	42	28	13	415 > 369, 213, 186
Chlorobenzilate[b,c]	510-15-6	324	−22			323 > 295, 249
Chlorsulfuron	64902-72-3	357	38	29	13	358 > 167, 141
Clethodim	99129-21-2	359	42	23	14	360 > 268, 164
Clodinafop-propargyl[c]	105512-06-9	349	46			350 > 266, 268, 91, 238, 269
Clomazone	81777-89-1	239	46	26	14	240 > 125, 128
Colchicine	64-86-8	399	72	41	29	400 > 358, 310
Cotinine	486-56-6	176	54	35	20	177 > 146, 98, 80
Coumarin[b]	91-64-5	146	55	35	20	147 > 103, 91
Cyanazine	21725-46-2	240	55	36	21	241 > 214, 104, 96
Cyclanilide	113136-77-9	273	−38	−24	−13	272 > 228, 192, 160
Cycloheximide	66-81-9	281	45	30	16	282 > 264, 246
Cyprodinil	121552-61-2	225	75	48	28	226 > 210, 108, 93
Cyromazine	66215-27-8	166	58	34	21	167 > 125, 85
Daminozide	1596-84-5	160	32	20	11	161 > 143, 115, 101
DDVP[b]	62-73-7	220	44	32	15	221 > 145, 127, 109
Desethyl atrazine	19988-24-0	169	48	32	16	170 > 128, 86
Desisopropyl atrazine	1007-28-9	173	57	32	22	174 > 132, 104, 96
Diazinon	333-41-5	304	50	30	18	305 > 169, 153
Dicrotophos	141-66-2	237	35	25	9	238 > 193, 112
Digitoxin	71-63-6	764.4	31	22	9	783 > 748, 636
Digoxin[b]	20830-75-5	780.4	33	22	10	782 > 652, 97
Diphacinone	82-66-6	340	45	30	14	341 > 323, 263, 235
Diuron	330-54-1	232	40	30	14	233 > 72
Dodine[b]	2439-10-3	287	67	40	23	228 > 186, 85, 71
Emetine, HCl[b]	483-18-1	480	140	55	37	481 > 436, 246, 165
EPTC	759-94-4	189	40	22	12	190 > 162, 128, 89, 86
Ethiofencarb	56729-20-5	225	25	18	7	226 > 169, 164, 107
Ethion[b]	563-12-2	384	30	20	8	385 > 215, 199
Ethoprophos	13194-48-4	242	42	27	13	243 > 215, 173, 131
Fenitrothion[b,c]	122-14-5	277		32	17	278 > 246, 125
Fensulfothion	115-90-2	308	50	35	18	309 > 281, 253, 157
Fenthion[b]	55-38-9	278	55	30	16	279 > 247, 169
Formothion	2540-82-1	257	34	25	14	279 > 116, 88, 118, 231, 145
Heroin	561-27-3	369	97	44	36	370 > 328, 165
Hexazinone	51235-04-2	252	38	28	13	253 > 171, 85
Imazalil	35554-44-0	296	55	35	21	297 > 255, 159, 109
Imazamethabenz-methyl	81405-85-8	288	52	33	19	289 > 257, 229, 86
Imazaquin	81335-37-7	311	60	36	24	312 > 270, 267, 252, 199, 86
Imazethapyr	81335-77-5	289	58	35	23	290 > 248, 245, 230, 177, 86
Imidacloprid	13826-41-3	255	40	29	14	256 > 209, 175, 84
Isofenphos	25311-71-1	345	18	10	4	346 > 287, 245
Isoxaflutole	141112-29-0	359	41	30	11	360 > 251
Kresoxim-methyl	143390-89-0	313	30	20	7	314 > 282, 267, 206, 116
LAMPA	40158-98-3	323	60	34	25	324 > 281, 223
LSD	50-37-3	323	59	34	24	324 > 281, 223
Malathion	121-75-5	330	30	22	8	331 > 285, 127
Mesotrione	104206-82-8	339	−29	−16	−8	338 > 291

Table 1 (*Continued*)

Compound	CAS number	Nominal mass (g/mol)	Cone MS[a] (V)	Cone MS/MS (V)	Collision MS/MS (eV)	Precursor > MS/MS product ions (*m*/*z* units)
Metalaxyl	57837-19-1	279	40	28	13	280 > 248, 220, 192
Methamidophos	10265-92-6	141	50	35	18	143 > 125, 113, 95
Methiocarb	2032-65-7	225	30	22	8	226 > 169, 121
Methomyl	16752-77-5	162	22	16	6	163 > 122, 106, 88
Methoprene	40596-69-8	310	22	15	6	311 > 279, 237, 219, 191
Metolachlor	51218-45-2	283	36	22	12	284 > 252
Metsulfuron-methyl	74223-64-6	381	35	23	11	382 > 167, 141
Mevinphos	7786-34-7	224	27	20	7	225 > 193, 127, 99
Molinate	2212-67-1	187	37	26	13	188 > 126, 98, 83
Monocrotophos	6923-22-4	223	30	21	8	224 > 193, 98
Naled[b]	300-76-5	378	24	23	8	379 > 127
Napropamide	15299-99-7	271	45	28	14	272 > 199, 171, 129, 74
Naptalam	132-66-1	291	−38	−23	−12	290 > 246
Nicotine	54-11-5	162	50	35	18	163 > 132, 130, 117, 106
Oxamyl	23135-22-0	219	20	14	6	237 > 220, 90, 72
Permethrin[b]	52645-53-1	390	32	24	8	408 > 355, 183
Phorate[b]	298-02-2	260	20	18	6	261 > 75
Phosalone[b]	2310-17-0	367	36	25	9	368 > 322, 182
Phosmet[b]	732-11-6	317	30	20	7	318 > 160
Pirimicarb	23103-98-2	238	42	28	14	239 > 182, 72
Pirimiphos-methyl	29232-93-7	305	60	42	24	306 > 164, 136, 108, 95
Prometon	1610-18-0	225	56	35	20	226 > 184, 142
Prometryn	7287-19-6	241	56	36	21	242 > 200, 158
Propachlor	1918-16-7	211	41	26	14	212 > 170, 152
Propamocarb	24579-73-5	188	39	26	14	189 > 144, 102
Propoxur	114-26-1	209	28	20	7	210 > 168, 153, 111
Prosulfuron	94125-34-5	419	45	30	15	420 > 167, 141
Pyridaben	96489-71-3	364	34	25	11	365 > 309, 147
Pyridaphenthion	119-12-0	340	50	37	18	341 > 313, 205, 189
Quinine	56-54-2	324	73	38	28	325 > 160, 81
Resmethrin[b]	10453-86-8	338	42	25	15	339 > 321, 293, 171, 143, 121, 91
Sethoxydim	74051-80-2	327	45	25	15	328 > 282, 220, 180, 178
Simazine	122-34-9	201	55	40	20	202 > 174, 132, 124, 104, 96
Simetryn	1014-70-6	213	60	40	21	214 > 186, 144, 124, 96
Spiroxamine	118134-30-8	297	51	32	17	298 > 144, 100
Strychnine	57-24-9	334	95	66	44	335 > 184, 156, 144, 129
Tebuconazole	107534-96-3	307	53	34	20	308 > 165, 151, 125
Tebufenpyrad	119168-77-3	333	70	48	26	334 > 171, 145, 117
Temephos	3383-96-8	466	62	35	22	467 > 419, 405, 357, 249, 155, 125
Terbumeton	33693-04-8	225	45	33	16	226 > 170, 114
Terbuthylazine	5915-41-3	229	45	31	14	230 > 174
Thiabendazole	148-79-8	201	75	44	29	202 > 175, 131, 92
Thiamethoxam	153719-23-4	291	33	20	10	292, 246, 211, 210, 132
Thifensufuron-methyl	79277-27-3	387	38	26	11	388 > 167, 141
Thiram	137-26-8	240	21	12	7	241 > 196, 120, 88
Tralkoxydim	87820-88-0	329	44	25	15	330 > 284, 164, 138, 122
Triadimefon	43121-43-3	293	45	28	16	294 > 225, 197
Tri-allate[b]	2303-17-5	303	41	26	15	304 > 262, 143, 128, 86
Triasulfuron	82097-50-5	401	42	28	15	402 > 219, 167, 141
Trichlorfon[b]	52-68-6	256	39	26	12	257 > 221, 127
Trifloxystrobin[b]	141517-21-7	408	40	24	13	409 > 206, 186, 116
Trinexapac-ethyl	95266-40-3	252	39	25	13	253 > 207, 185, 69
Triticonazole	131983-72-7	317	36	25	12	318 > 70
Warfarin	81-81-2	308	42	25	13	309 > 251, 163

[a] For full scan (single quadrupole) MS analysis, the collision energy was maintained at 2 eV and the collision gas (argon) remained off.
[b] These compounds have not been verified in interlaboratory studies.
[c] Those compounds in this table that *only* have MS settings are in the MS library *only*; and those compounds that *only* have MS/MS settings are *only* in the MS/MS library.

there is a high probability that the unknown has been identified.

3.2. Inter-laboratory verification of protocol and libraries

Thirteen solutions containing a total of 129 organic compounds included in the CRL libraries were prepared and distributed by CRL to the six participating laboratories. Each unknown sample contained between 9 and 11 analytes. Each participating lab received 6 or 7 unknown solutions that they were required to characterize. The unknown solutions were mixed considering retention time so the compounds would not co-elute. The samples were allotted so that a total of three laboratories received each individual chemical. The concentration of each analyte was 20 times (at a minimum) the noise level found at CRL. The laboratories did not know what compounds were contained in the solutions they received. Each laboratory was required to identify the constituents in the solutions they received using LC–MS Library System Protocol Version 1.2 created by CRL [17].

Identification of an analyte was required by at least two out of three laboratories that received it for the library spectra to be considered verified. Any less than two correct identifications would require further work on the spectra in the library or consultation with the participating labs depending on possible reasons for the misidentification. Compounds that were not correctly identified would be listed as not verified in the library until they were satisfactorily identified in blind samples by at least two out of three laboratories.

Table 2
Results of inter-laboratory verification.

Legend reason codes	Definitions
A	Found by participating lab
B	Found at CRL, oversight by participating lab
C	Found at CRL, masked by high background noise level
D	Not found at CRL, participating lab did not follow protocol
F	Not found at CRL, background noise level high

Compound	Lab result one reason	Lab result two reason	Lab result three reason
Acibenzolar-s-methyl	C	F	A
Bentazon	A (not confirmed by MS/MS)	A	C
Chloramben	C	F	C
Chlorobenzilate	B	A	F
Coumarin	B	A	F
DDVP	F	A	F
Digoxin	B	C	A
Dodine	F	A	F
Emetin, HCl	A	F	B
Ethion	C	A	F
Fenthion	F	F	C
Naled	C	F	A
Permethrin	A	B	F
Phorate	F	F	A
Phosmet	A	B	F
Trifloxystrobin	A	D	B
Alanine	F	C	C
Fenitrothion	F	F	F
Phosalone	C	C	F
Resmethrin	F	F	F
Tri-allate	C	F	F
Trichlorfon	F	F	F

The results using Waters instrumentation verified 107 out of 129 compounds contained in the library as shown in Table 1. The compounds with the letter "b" were not verified through the inter-laboratory process. The reasons are discussed here and tabulated in Table 2.

LAMPA, which is iso-LSD, has the same mass spectrum as LSD and cannot be distinguished by this protocol. LAMPA is not psychoactive, but like LSD, it is classified as a Schedule I drug under the Controlled Substance Act of 1970. Because LSD is prepared from ergot alkaloids with isomeric configuration at the C-8 position, both LSD and LAMPA are present in most illicit drug preparations. An LC–MS library cannot distinguish between LSD and LAMPA since they are sterioisomers. Therefore, if LAMPA or LSD is identified in a sample, it was verified to be reported as LAMPA/LSD.

Dicrotophos, used as a spiking compound, degraded to monocrotophos and was identified by two participating labs as monocrotophos in the sample.

Fenitrothion was identified by MS/MS only. The compound provided insufficient ion abundance in MS full scans to be tentatively identified in the MS single quadrupole portion of the protocol.

Bromoxynil, chlorobenzilate, clodinafop-propargyl, and formothion were tentatively identified by MS only. No MS/MS spectra are in the library for these compounds due to poor MS/MS sensitivity.

The reasons the 22 compounds were not verified, after CRL reviewed all the data received from the participating labs, are provided in Table 2. Contributing factors may be poor sensitivity due to poor chromatography or matrix interferences caused by elevated chromatograph baselines. It is also believed that some compounds may have decomposed in the water samples before being analyzed by the participating labs. Bentazon was found by two labs, but one lab was not able to confirm its presence by MS/MS. There were no false positives reported by the participating laboratories.

3.3. Library protocol modifications and library searching for the Thermo Finnigan instrument

A Thermo Finnigan instrument was used to compile similar LC–MS and LC–MS/MS libraries. The instrument was tuned according to the manufacturer's specifications and for maximum sensitivity before spectral acquisition. Other than this initial optimization process, similar procedures as noted above were followed to develop a standardized library that could be compared with the Waters/CRL libraries. Infusing each compound into the ESI source allowed for maximizing the signal by tuning the gas flows and voltages, while observing the $[M+H]^+$ or $[M-H]^-$ ion. As with the Waters instrument, each standard was infused into a 'T' junction, where it combined with the mobile phase before entering the mass spectrometer. The electrospray source conditions for both MS and MS/MS scanning were as follows: spray voltage, 4000 V; sheath gas pressure, 40 units; auxiliary gas pressure, 10 units; and capillary temperature, 250 °C. During this process, source CID voltages (for LC–MS spectra) and collision energies (for LC–MS/MS spectra) were manipulated for each compound to generate fragmentation while maintaining at least 10% abundance of the precursor ion. Once these settings were obtained, LC–MS analysis of 25 ng of material on-column was performed to acquire retention time data and to verify that similar product ion spectra were obtained with the source CID voltages and collision energies used during infusion. The collision gas (argon) was kept at 1.5 mTorr and the collision energy was increased to reduce the precursor ion to 10% of the resulting base peak.

A subset of the 129 unknown compounds sent to the US EPA Office of Research and Development Laboratory in Las Vegas was analyzed for independent confirmation on instrumentation from a manufacturer other than that used in the inter-laboratory study. MS spectra were acquired with a Thermo Finnigan TSQ Quantum Ultra AM™ triple quadrupole mass spectrometer at collision energies of 10, 20, 30, 40, 50, 60, and 76 V (maximum). Each spectrum at each voltage was examined to determine the highest probability match and the instrument provided excellent matches with the Waters/CRL LC–MS library.

The 67-compound subset of the unknown standards was then tested against this library. The probability indicated the confidence that the unknown spectrum matched that particular compound's spectrum in the CRL library. The matches demonstrated that the LC–MS libraries are transferable between the Waters and Thermo Finnigan instruments even though the ion ratios within spectra were often different between the instruments. Even so, the NIST search engine probability match factor was high and correctly identified the simulated unknowns as shown in Table 3. The MS cone/source CID voltages were compared between the voltage under which the Waters/CRL library was developed and the voltage that resulted in the greatest match factor using the Thermo Finnigan NIST search and are presented in Table 3.

Based on the NIST searching algorithm the probabilities of finding each simulated unknown in the Waters/CRL library was 96% or greater except for metsulfuron-methyl, monocrotophos, and phosmet, which had probabilites of 83, 94, and 90%, respectively. Examples of these searches are given in Fig. 1 for propachlor and Fig. 2 for metsulfuron-methyl.

As can be seen, the algorithm ranks the occurrence of ions greater than the ion abundance. Since each spectrum is searched against the whole library, one can see from the search results in Fig. 1 that for propachlor, there is very little probability that the compound is anything other than propachlor; the next highest probability is 0.98% for terbumeton. The search results for metsulfuron-methyl are more tentative as the forward and reverse search results are poor, but the probability that the compound is metsulfuron-methyl is still at 83% with the next highest proba-

Table 3
LC–MS library match probability of spectra acquired by the Thermo Finnigan instrument against the Waters/CRL LC–MS library.

Compound	MS cone/source CID (V)		NIST probability
	Waters/CRL library	Thermo Finnigan	
2-Aminobenzimidazole	75	60	97
3-Hydroxy carbofuran	40	50	99
Acetamipirid	46	40	99
Acetochlor	32	50	99
Alachlor	34	50	98
Aldicarb	15	20	99
Ametryn	58	60	99
Amitraz	34	40	97
Atropine	58	60	96
Azinphos-methyl	25	40	99
Azoxystrobin	35	50	99
Buprofezin	35	40	99
Carbaryl	25	40	99
Carbofuran	35	40	99
Clodinafop-propargyl	46	50	98
Clomazone	46	60	99
Colchicine	72	76	97
Cyanazine	55	76	98
Cycloheximide	45	60	99
Cyprodinil	75	60	99
Diazinon	50	40	99
Dicrotophos	35	40	99
Ethiofencarb	25	40	99
Ethoprophos	42	40	99
Fensulfothion	50	76	98
Hexazinone	38	60	98
Imazalil	55	50	98
Imazamethabenz-methyl	52	60	99
Imidacloprid	40	50	99
Isofenphos	18	20	99
Kresoxim-methyl	30	20	99
Malathion	30	30	98
Methiocarb	30	30	99
Methomyl	22	40	99
Metolachlor	36	40	99
Metsulfuron-methyl	35	30	83
Mevinphos	27	30	98
Monocrotophos	30	40	94
Naled	24	30	98
Napropamide	45	60	99
Nicotine	50	40	98
Oxamyl	20	30	97
Phosmet	30	30	90
Pirimicarb	42	40	98
Pirimiphos-methyl	60	60	99
Prometon	56	60	99
Prometryn	56	60	99
Propachlor	41	50	99
Propamocarb	39	30	99
Propoxur	28	50	99
Prosulfuron	45	76	99
Pyridaben	34	40	99
Pyridaphenthion	50	60	98
Quinine	73	60	96
Sethoxydim	45	40	97
Simetryn	60	50	99
Spiroxamine	51	50	99
Tebuconazole	53	50	98
Tebufenpyrad	70	76	98
Temephos	62	60	99
Terbumeton	45	40	99
Thiamethoxam	33	50	99
Triadimefon	45	30	98
Triasulfuron	42	50	98
Trichlorfon	39	30	97
Trifloxystrobin	40	40	99
Warfarin	42	30	99

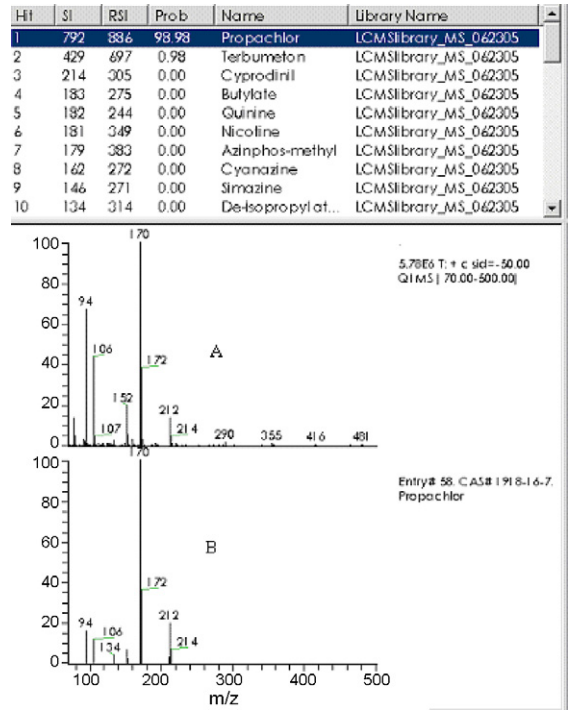

Fig. 1. LC–MS library search of propachlor acquired on a Thermo Finnigan instrument (A) compared to the library spectrum generated from a Waters instrument (B).

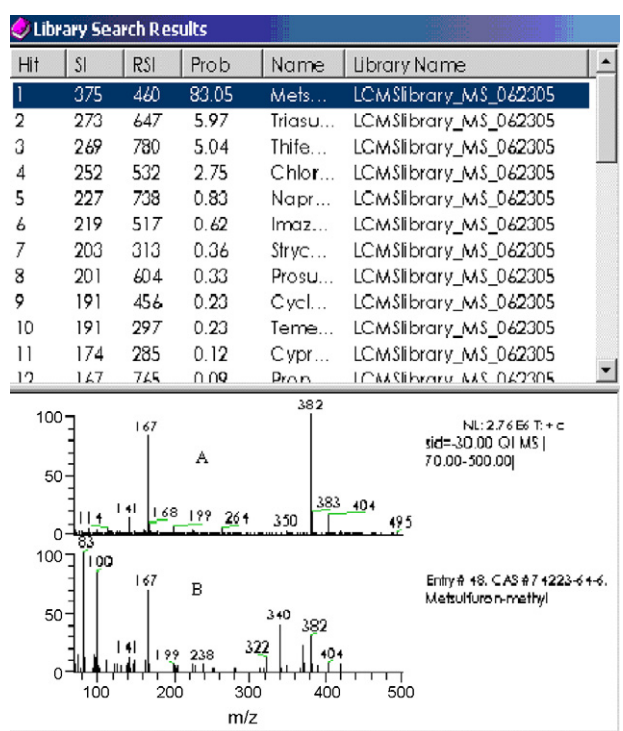

Fig. 2. LC–MS library search of metsulfuron-methyl acquired on a Thermo Finnigan instrument (A) compared to the library spectrum generated from a Waters instrument (B).

bility at 6%. On further review the Waters/CRL LC–MS library for metsulfuron-methyl most likely had interferences, which were not filtered out with the source CID LC–MS arrangement. Review of the Waters/CRL LC–MS/MS library (products of m/z 382) showed that ions at m/z 83, 100, and 340 were all absent; consequently, these ions were attributed to co-eluting impurities with the metsulfuron-methyl.

4. Conclusion

The Waters/CRL LC–MS library protocol was verified through an inter-laboratory study that involved Federal, State, and private laboratories. The results demonstrated that the libraries are transferable between the same manufacturer's product line, and have applicability between manufacturers. The ion ratios within a mass spectrum were different between two manufacturers' instruments, but the same product ions were usually observed. Despite the ion ratio differences, the NIST search engine match probability was 96% or greater for all of the compounds except for three. This work will be extended for the analysis of real world samples and the development of more sensitive MS/MS methods to enable low level analysis of select analytes. Through a cooperative research and development agreement (CRADA) between Waters Corporation and the US EPA Region 5 CRL, the libraries and protocol can be obtained from U.S. EPA Region 5 CRL free of charge.

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