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A FULLY AUTOMATIC MULTI-ANALYTE QUANTIFICATION PROTOCOL FOR CARBAMATES - A COMPARISON OF LC/MS VS LC/MS/MS

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INTRODUCTION

- In the late 1960s, several major environmental issues emerged, accelerating the concern for environmental protection. In 1998, the EPA published the first Drinking Water Contaminant Candidate List (DWCCL). The DWCCL listed analytes from various classes. Among them, carbamates, thiocarbamates, and phenylureas such as oxamyl, carbofuran, diuron, and linuron comprise some of the basic structures. In recent years, these "short-life" carbamate pesticides, in addition to organophosphorus pesticides, have replaced most of the "long-life" organochlorine pesticides, which were prohibited due to their high toxicity and slow degradation rate. The increasing use of carbamate pesticides in agriculture resulted in demands for sensitive and specific analytical methods for these compounds because "carbamate pesticides affect the nervous system by disrupting an enzyme that regulates acetylcholine, a neurotransmitter"
- The use of carbamates as agrochemicals to improve crop yields is not without tradeoffs. For example, they can leach into the groundwater and local tributaries, which are sources of drinking water supply. The wastewater effluent from Public Owned Treatment Work (POTW) also empties into the tributaries. Therefore, these matrices need to be tested to ensure drinking water quality.
- Liquid Chromatography (LC) is the preferred separation technique for carbamates, thiocarbamates and phenylurea because most of these compounds are polar and thermally labile. In Gas Chromatography (GC) analysis, these compounds either show signs of thermal decomposition or fail to elute from the column.
- Currently, the US EPA recommends different methods depending on the matrix and the target analytes. For example, the EPA Office of Water recommends Method 531.27 for carbamates in ground and drinking water, and Method 5328 for phenylureas in drinking water. The EPA Office of Solid Waste (OSW) recommends Method 83189 for carbamates in soil, water and waste matrices.
- When analyzing an unknown sample from a complex matrix using the approved EPA methods, chemists have to decide which analytical method to use. Without knowing the actual sample content, this decision can be difficult. If the target analytes fall into more than one method, multiple analyses become necessary. To address these problems, various jurisdictions have begun promoting LC/MS and LC/MS/MS methods in lieu of conventional LC or GC methods.
- Multi-analyte LC/MS and LC/MS/MS, in addition to GC/MS, are the methods for the future because they do not require chromatographic resolution and they do not need post column derivatization. However, analysts often feel overwhelmed when choosing which analytical technique to use (LC/MS or LC/MS/MS). What are the pros and cons of each option? In addition, sometimes chromatographers feel intimidated by the demanding nature of MS method development.
- To address these concerns, we have developed a simple automatic quantification protocol for carbamates, thiocarbamates, and phenylureas. Thirty-eight target analytes were used for this project to demonstrate the wide applicability of this protocol. This protocol can be easily adapted by either LC/MS or LC/MS/MS. The quantification results of both techniques are compared.

Our Project Goal

To develop a simple multi-analyte quantification protocol for 38 carbamates, thiocarbamates, and phenylureas in complex matrices.

Requirements of this protocol include:

- Direct injection with NO sample cleanup.
- Direct MS or MS/MS detection with NO post column derivatization.
- Complete automation covering optimization, sample analysis, quantification and report generation.
- Tested for Milford, Massachusetts wastewater and drinking water

Carbamate Analogs			Thio-Carbamate	Urea Analogs
(M531 Mix)				
Aldicarb sulfoxide	Aminocarb	Formatamate	Diallate	Bramacil
Aldicarb Sulfone	Benomyl	Metolcarb	EPTC	Chloroxuron
Aldicarb	Bendiocarb	Mexacarbate	Molinate	Diuron
Carbaryl	Carbendazim	Propachlor	Tillam	Fenuron
Carbofuran	Cycloate	Promecarb	Vernolate	Fluometuron
3OH-Carbofuran	Eserine	Methiocarb		Linuron
Methomyl	Propoxur	Thiodicarb		Monuron
1-Naphthol				Neburon
Oxamyl				Siduron
				Tebuthiuron

- Suitable for post column derivitization Fluorescence Detection
- Not suitable for the post column derivitization Fluorescence Detection

Table 1. List of the 38 Target Analytes.

EXPERIMENTAL

LC Conditions

- Column: Waters Symmetry® C_8 2.1 x 150 mm, 3.5 µm, 40 °C
- Flow Rate: 0.3 mL/min
- Sample Temp: 5 °C
- Mobile Phase:

  A: 10 mM NH_4OAc in Water, pH 5.0

  B:10 mM NH_4OAc in Acetonitrile

- Gradient:

Time	A%	B%	Flow	Curve
0.00	95.0	5.0	0.3	1
40.0	30.0	70.0	0.3	6
50.0	0.0	100	0.3	1
64.0	95	5	0.3	1

- Injection Volume: 50 µL

MS Conditions

- Ionization: ESI+
- Capillary Voltage: 3.5 kv
- Source Temperature: 140 °C
- Desolvation Temperature: 350 °C
- Desolvation Gas Flow (L/Hr): 650
- Cone Gas Flow (L/Hr): 0
- LM Resolution: 14.5
- HM Resolution: 14.5
- Ion Energy: 1.5
- Dwell Time(s): 0.02
- Inter Channel Delay(s): 0.02
- Inter scan Delay(s): 0.02

The LC/MS System was Waters® Alliance® HT/Micromass® ZQ™ 2000

The LC/MS/MS System was Waters Alliance HT/Micromass Quattro micro™

Automatic Protocol by QuanOptimize™

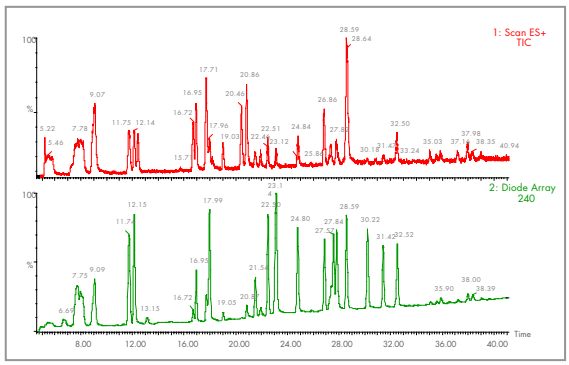
- QuanOptimize is an integral part of the QuanLynx™ Application Manager, a MassLynx™ Software option. QuanLynx is composed of QuanOptimize and QuanLynx Browser.
  - QuanOptimize handles all the experiment runs and data collection.
  - QuanLynx performs the post run processing of the raw data and allows users to view the analytical results.
- With any LC-Mass Spectrometry method development, the very first step would be to develop a LC method. Once the LC condition for the target analytes was determined, only one analyte needs to be infused into the mass spectrometer (T with the LC mobile phase at the proper flow rate, 0.3 ml/min) to optimize the tune page parameters (everything except the cone voltages for parent ions and collision energies for daughter ions).
- We then provided the necessary method files and sample lists to QuanOptimize to set up the run (MS tune file, sample list, LC method, and quantification method template).

QuanOptimize would then perform the following tasks:

- Run a MS scan injection with multiple cone voltages for each of the standards (38 injections).
- For MS/MS analysis, a second injection for each standard will be made with the optimum cone voltage based on the first injection, and multiple collision energies will be applied for optimization.
- For MS analysis, an SIR MS acquisition method will be set up based on the optimum cone voltage for each compound.
- For MS/MS analysis, an MRM MS/MS acquisition method will be set up based on the optimum cone voltage and collision energy for each compound.
- Run the quantification analysis using either the SIR method or the MRM method that was created.
- Create a quantification processing method based on the LC/MS or LC/MS/MS result.
- Perform quantification and generate a report which can be reviewed in QuanLynx browser.

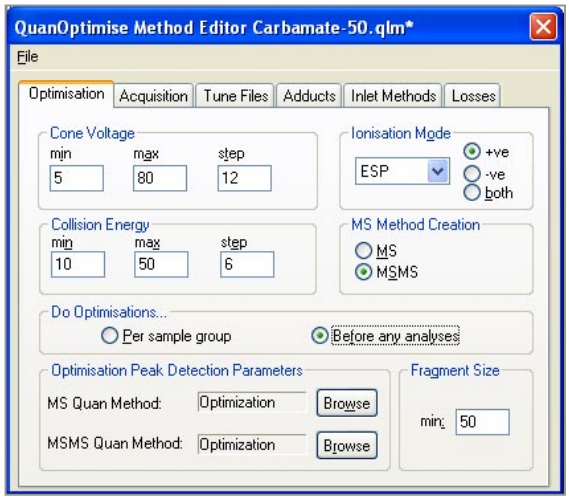
OPTIMIZATION

Full Scan TIC of the Carbamates

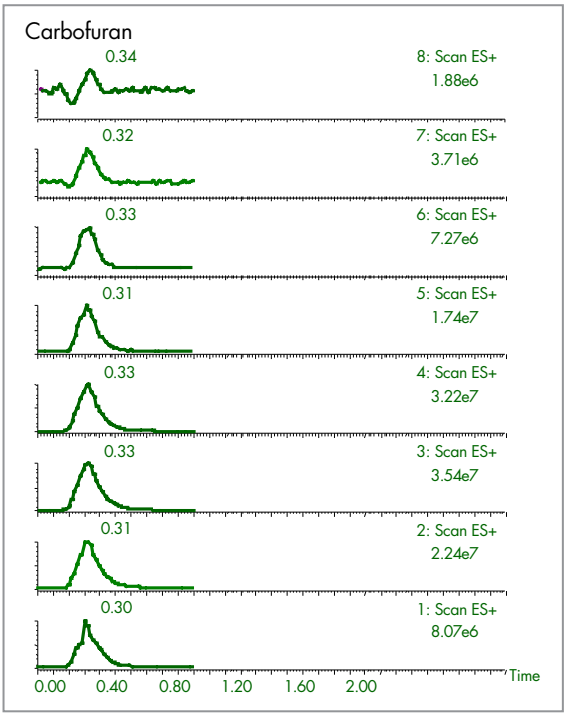


The very first step for this project was to develop a HPLC method to separate the 38 analytes. Since the intention was to use MS as a detector, baseline resolution was not necessary. This significantly reduced the LC method development time.

Optimization Set Up

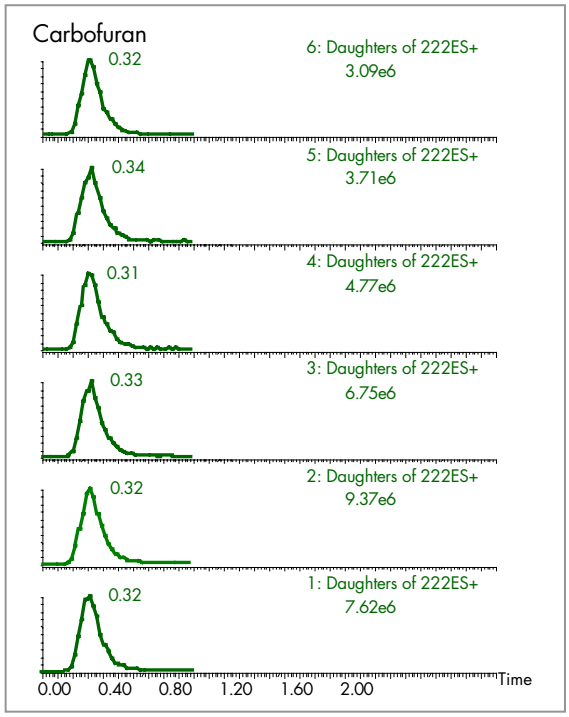


MS Optimization



- 8 MS full scan traces of carbofuran from one injection.
- This is the first step of the fully automated protocol: Optimization. This was done via flow injection analysis. The MS scan range was [MW + 50] Da.
- The cone voltage optimization range was defined by user, which was then divided into 8 mini-steps by QuanOptimize.
- The full scan peaks were integrated by QuanOptimize and the optimum cone voltage was chosen based on peak area.
- Shown in Table 2 are the optimization results for all 38 analytes via QuanOptimize. The numbers in red were the m/z values from manual optimization.

MS/MS Optimization



- 6 MS/MS daughter scan traces of carbofuran.
- A second injection of the standard solution was necessary.
- The cone voltage was automatically set at an optimum value based on the first injection.
- The collision energy optimization range was defined by the user. The maximum number of steps allowed was 8. In this example, there were 6 steps.
- The peaks were also integrated by QuanOptimize and the optimum daughter ion and its collision energy was determined based on peak area.
- Shown in Table 3 are the MS/MS optimization results for all 38 analytes via QuanOptimize.

	Name	Formula	M+H	M+NH4	m/z	Cone V
1	Aldicarb	C7H14N2O2S	191	208	207.95	CV 5
2	Aldicarbsulfoxide	C7H14N2O3S	207	224	206.92	CV 17
3	Aminocarb	C11H16N2O2	209	226	209.03	CV 17
4	Aldicarbsulfone	C7H14N2O4S	223	240	222.87	CV 17
5	Bendiocarb	C11H13NO4	224	241	223.92	CV 17
6	Benomyl 192	C14H18N4O3	291	308	191.93	CV 17
7	Bromacil	C9H13N2O2Br	262	279	262.8	CV 17
8	Carbaryl	C12H11NO2	202	219	201.93	CV 17
9	Carbendazim	C9H9N3O2	192	209	191.93	CV 17
10	Carbofuran	C12H15NO3	222	239	221.98	CV 17
11	3OH Carbofuran	C12H15NO4	238	255	237.93	CV 17
12	Chloroxuron	C15H15N2O2Cl	291	308	290.88	CV 29
13	Cycloate	C11H21NOS	216	233	215.96	CV 17
14	Dillate	C10H17NOSCl2	270	287	269.85	CV 29
15	Diruon	C9H10N2OCl2	233	250	232.81	CV 17
16	EPTC	C9H19NOS	190	207	189.97	CV 17
17	Eserine	C15H21N3O2	276	293	275.98	CV 17
18	Fenuron	C9H12N2O	165	182	165	CV 17
19	Fluometuron	C10H11N2OF3	233	250	232.93	CV 29
20	Formatamate	C11H15N3O2	222	239	221.87	CV 17
21	Linuron	C9H10N2O2Cl2	249	266	248.82	CV 17
22	Methiocarb	C11H15NO2S	226	243	225.91	CV 17
23	Methomyl	C5H10N2O2S	163	180	162.93	CV 5
24	Metolcarb	C9H11NO2	166	183	165.96	CV 17
25	Mexacarbate	C12H18N2O2	223	240	223.03	CV 17
26	Molinate	C9H17NOS	188	205	188	CV 17
27	Monuron	C9H11N2OCl	199	216	198.93	CV 17
28	1-Napthol	C10H8O	145	162	145.09	CV 41
29	Neburon	C12H16N2OCl2	275	292	274.87	CV 29
30	Oxamyl	C7H13N3O3S	220	237	236.93	CV 5
31	Promecarb	C12H17NO2	208	225	207.99	CV 17
32	Propachlor	C11H14NOCl	212	229	211.95	CV 17
33	Propoxur	C11H15NO3	210	227	209.93	CV 53
34	Siduron	C14H20N2O	233	250	233.06	CV 29
35	Tebuthiuron	C9H16N4OS	229	246	228.97	CV 17
36	Thiodicarb	C10H18N4O4S3	355	372	354.88	CV 53
37	Tillam	C10H21NOS	204	221	203.94	CV 17
38	Verolate	C10H21NOS	204	221	203.99	CV 17

Table 2. MS Parameters.

	Name	Formula	M+H	Transition	CV	CE
1	Aldicarb	C7H14N2O2S	191	208.24 > 116.07	13	5
2	Aldicarbsulfoxide	C7H14N2O3S	207	207.23 > 132.04	21	5
3	Aminocarb	C11H16N2O2	209	209.24 > 152.10	21	12
4	Aldicarbsulfone	C7H14N2O4S	223	223.20 > 86.02	21	12
5	Bendiocarb	C11H13NO4	224	224.21 > 109.02	21	19
6	Benomyl	C14H18N4O3	291	192.20 > 160.04	29	19
7	Bromacil	C9H13N2O2Br	262	263.11 > 206.96	21	12
8	Carbaryl	C12H11NO2	202	202.24 > 145.05	21	12
9	Carbendazim	C9H9N3O2	192	192.22 > 160.04	29	19
10	Carbofuran	C12H15NO3	222	222.24 > 165.03	21	12
11	3OH Carbofuran	C12H15NO4	238	238.24 > 163.03	21	12
12	Chloroxuron	C15H15N2O2Cl	291	291.18 > 71.99	29	19
13	Cycloate	C11H21NOS	216	216.25 > 83.03	21	19
14	Dillate	C10H17NOSCl2	270	270.11 > 86.00	21	19
15	Diruon	C9H10N2OCl2	233	233.14 > 71.97	29	19
16	EPTC	C9H19NOS	190	190.28 > 86.00	21	12
17	Eserine	C15H21N3O2	276	276.24 > 162.05	21	19
18	Fenuron	C9H12N2O	165	165.20 > 71.97	21	12
19	Fluometuron	C10H11N2OF3	233	233.17 > 71.95	29	19
20	Formatamate	C11H15N3O2	222	222.19 > 165.11	13	12
21	Linuron	C9H10N2O2Cl2	249	249.11 > 181.96	29	12
22	Methiocarb	C11H15NO2S	226	226.19 > 169.02	21	12
23	Methomyl	C5H10N2O2S	163	163.17 > 87.96	13	5
24	Metolarb	C9H11NO2	166	166.20 > 109.05	21	12
25	Mexacarbate	C12H18N2O2	223	223.24 > 166.09	29	12
26	Molinate	C9H17NOS	188	188.25 > 126.09	21	12
27	Monuron	C9H11N2OCl	199	199.18 > 71.95	21	12
28	1-Napthol	C10H8O	145	145.22 > 82.26	29	26
29	Neburon	C12H16N2OCl2	275	275.12 > 88.07	29	19
30	Oxamyl	C7H13N3O3S	220	237.17 > 71.97	13	12
31	Promecarb	C12H17NO2	208	208.24 > 151.09	21	12
32	Propachlor	C11H14NOCl	212	212.17 > 169.99	29	12
33	Propoxur	C11H15NO3	210	210.24 > 111.01	21	12
34	Siduron	C14H20N2O	233	233.27 > 137.07	29	19
35	Tebuthiuron	C9H16N4OS	229	229.24 > 172.08	29	19
36	Thiodicarb	C10H18N4O4S3	355	355.14 > 87.98	21	12
37	Tillam	C10H21NOS	204	204.25 > 128.11	21	12
38	Verolate	C10H21NOS	204	204.27 > 128.09	21	12

Table 3. MS/MS Parameters.

LC/MS Quantification

- Only one injection required for each standard for optimization.
- Lower cost for system purchase and maintenance.
- Lower demands for previous MS operation experience.

LC/MS/MS Quantification

- Two injections required for each standard for optimization.
- For the 38 compounds, can be up to 40 times more sensitive than the LC/MS.
- Previous MS experience can be very helpful.
- Higher cost for system purchase and maintenance.
- Less background noise with complex matrices.

QUANTIFICATION

Matrix Spikes—LC/MS

- The Milford drinking water was collected from the tap in Milford.
- The Public Owned Treatment Work (POTW) effluent wastewater was collected from a local wastewater treatment plant. The wastewater effluent was sampled prior to its discharge into the Charles River.

Matrix Spikes—LC/MS/MS

- Each matrix spike contained all of the 38 target analytes.
- For LC/MS, the matrix was spiked at two levels: 2 ppb and 20 ppb.
- For LC/MS/MS, the matrix was spiked at two levels: 0.2 ppb and 2 ppb.

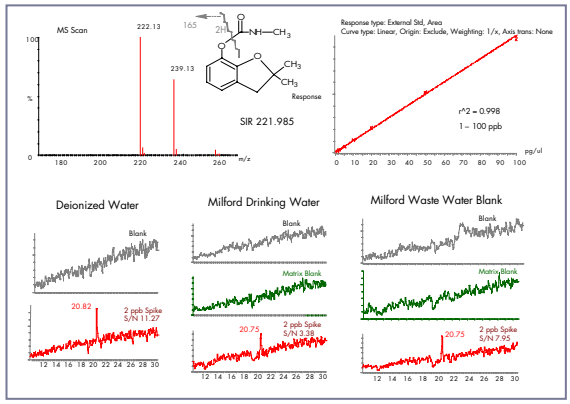


Figure 1. LC/MS for Carbofuran in Milford Drinking Water and Milford Wastewater.

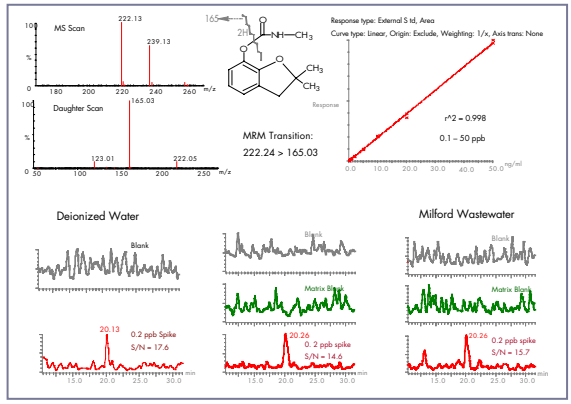


Figure 2. LC/MS/MS for Carbofuran in Milford Drinking Water and Wastewater.

	Name	M+H	M+NH4	Tr	LOD (ppb)	r2	Drinking* Recovery%	Waste* Recovery%
1	Aldicarb	191	208	16.71	2.81	0.981	130	113
2	Aldicarbsulfoxide	207	224	5.56	3.66	0.978	109	112
3	Aminocarb	209	226	17.71	0.353	0.996	108	99.7
4	Aldicarbsulfone	223	240	7.4	0.721	0.997	113	105
5	Bendiocarb	224	241	20.86	3.68	0.994	73.5	63.5
6	Benomyl 192	291	308	12.14	1.29	0.899	120	144
7	Bromacil	262	279	17.86	19.35	0.972	94.5	95.2
8	Carbaryl	202	219	21.94	1.47	0.996	92.5	85
9	Carbendazim	192	209	12.14	0.134	0.898	120	144
10	Carbofuran	222	239	20.86	2.26	0.996	104	101
11	3OH Carbofuran	238	255	12.14	2.17	0.993	90.2	79
12	Chloroxuron	291	308	28.56	1.32	0.996	109	72.7
13	Cycloate	216	233	35.49	1.8	0.975	121	127
14	Dillate	270	287	38.4	13.7	0.994	129	119
15	Diruon	233	250	23.12	0.888	0.998	120	102
16	EPTC	190	207	32.33	5.12	0.995	82.5	96.7
17	Eserine	276	293	9.07	0.0912	0.957	132	129
18	Fenuron	165	182	11.75	0.566	0.996	119	107
19	Fluometuron	233	250	22.51	0.673	0.996	114	104
20	Formatamate	222	239	20.86	1.29	0.997	100	90.5
21	Linuron	249	266	28.71	2.5	0.996	93.5	95.2
22	Methiocarb	226	243	27.22	4.78	0.986	83	112
23	Methomyl	163	180	8.17	0.41	0.998	114	109
24	Metolcarb	166	183	11.71	2.23	0.991	108	105
25	Mexacarbate	223	240	28.62	0.319	0.997	119	112
26	Molinate	188	205	27.95	2.24	0.992	121	128
27	Monuron	199	216	17.96	2.29	0.996	111	112
28	1-Napthol	145	162	21.86	1.12	0.994	104	99.5
29	Neburon	275	292	32.48	1.47	0.992	112	118
30	Oxamyl	220	237	7.78	1.91	0.989	94	82.8
31	Promecarb	208	225	28.65	1.23	0.991	105	111
32	Propachlor	212	229	24.84	0.806	0.995	114	122
33	Propoxur	210	227	20.46	1.2	0.999	128	143
34	Siduron	233	250	27	0.64	0.997	118	109
35	Tebuthiuron	229	246	16.93	1.16	0.992	113	108
36	Thiodicarb	355	372	20.8	1.09	0.994	113	110
37	Tillam	204	221	35.86	16	0.887	126	97.2
38	Verolate	204	221	35.86	5.68	0.993	110	121

Table 4. MS Quantification Results.

*The recoveries for the Milford drinking water and wastewater were based on 20 ppb spikes.

	Name	MRM Transition	Tr	LOD (ppb)	r2	Drinking* Recovery%	Waste* Recovery%
1	Aldicarb*	208.24 > 116.07	16.71	0.995	0.13	92.5	110
2	Aldicarbsulfoxide*	207.23 > 132.04	5.56	0.995	0.221	100	87.5
3	Aminocarb	209.24 > 152.10	17.71	0.996	0.0993	112	112
4	Aldicarbsulfone*	223.20 > 86.02	7.4	0.992	0.512	92.5	92.5
5	Bendiocarb	224.21 > 109.02	20.86	0.993	0.584	70.0	95.0
6	Benomyl	192.20 > 160.04	12.14	0.997	0.157	102	112
7	Bromacil	263.11 > 206.96	17.86	0.981	0.534	60.0	77.5
8	Carbaryl*	202.24 > 145.05	21.94	0.994	0.197	95.0	92.5
9	Carbendazim	192.22 > 160.04	12.14	0.997	0.152	102	115
10	Carbofuran*	222.24 > 165.03	20.86	0.998	0.0954	87.5	85
11	3OH Carbofuran*	238.24 > 163.03	12.14	0.996	0.848	82.5	77.5
12	Chloroxuron	291.18 > 71.99	28.56	0.994	0.365	105	102
13	Cycloate	216.25 > 83.03	35.49	0.954	0.621	125	87.5
14	Dillate	270.11 > 86.00	38.4	0.979	2.64	137***	91.0***
15	Diruon	233.14 > 71.97	23.12	0.994	0.91	105	120
16	EPTC	190.28 > 86.00	32.33	0.938	0.318	100	122
17	Eserine	276.24 > 162.05	9.07	0.992	0.275	170	142
18	Fenuron	165.20 > 71.97	11.75	0.995	0.0988	120	97.5
19	Fluometuron	233.17 > 71.95	22.51	0.994	0.0785	92.5	100
20	Formatamate	222.19 > 165.11	20.86	0.995	0.307	82.5	100
21	Linuron	249.11 > 181.96	28.71	0.936	0.142	180	108
22	Methiocarb*	226.19 > 169.02	27.22	0.995	0.603	100	87.5
23	Methomyl*	163.17 > 87.96	8.17	0.996	0.250	115	112.5
24	Metolarb	166.20 > 109.05	11.71	0.993	0.450	70.0	97.5
25	Mexacarbate	223.24 > 166.09	28.62	0.925	0.150	110	115
26	Molinate	188.25 > 126.09	27.95	0.993	1.70	82.5	82.5
27	Monuron	199.18 > 71.95	17.96	0.993	0.281	97.5	108
28	1-Napthol*	145.22 > 82.26	21.86	0.937	1.71	108***	69.8***
29	Neburon	275.12 > 88.07	32.48	0.992	0.419	128	118
30	Oxamyl	237.17 > 71.97	7.78	0.996	0.436	75.0	92.5
31	Promecarb	208.24 > 151.09	28.65	0.997	0.283	100	97.5
32	Propachlor	212.17 > 169.99	24.84	0.995	0.152	90.0	125
33	Propoxur*	210.24 > 111.01	20.46	0.996	0.241	112	102
34	Siduron	233.27 > 137.07	27	0.991	0.114	100	130
35	Tebuthiuron	229.24 > 172.08	16.93	0.998	0.0894	110	110
36	Thiodicarb	355.14 > 87.98	20.8	0.991	0.695	140	108
37	Tillam	204.25 > 128.11	35.86	0.992	0.421	80	75
38	Verolate	204.27 > 128.09	35.86	0.976	0.75	92.5	100

Table 5. MS/MS Quantification Results.

*Compound monitored by EPA Method 531.2 (M531 Mixture)
**All Recoveries calculated based on 2 ppb spike except marked
***Recoveries calculated based on 20 ppb spike

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CONCLUSION

- We have developed an automatic quantification protocol for simultaneous detection of 38 carbamates, thiocarbamates and phenylureas.
  - Easily adapted to LC/MS and LC/MS/MS systems
  - Enhances capability to analyze much wider range of analytes (compared to fluorescence detection and UV detection)
  - Minimizes method development time
    - Do not require baseline resolution for the LC separation
    - Fully automated LC/MS or LC/MS/MS optimization
  - Does not require post column derivatization
  - Sufficient sensitivity to accommodate the EPA requirement
    - LC/MS was capable of detecting low ppb levels at 50 µL injection volume (less than the 400 µL indicated in EPA M531)
    - LC/MS/MS was capable of detecting ppt levels at 50 µL injection volume
  - High selectivity to accommodate complex matrices
    - Method applied to wastewater and drinking water with direct injection
    - Recoveries were within the EPA regulated range without sample cleaning

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