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LC/MS QUANTIFICATION OF 52 CARBAMATES: A FULLY AUTOMATED PROTOCOL

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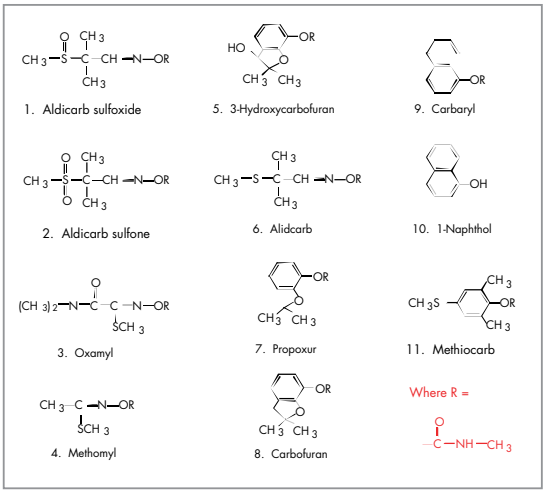
Introduction

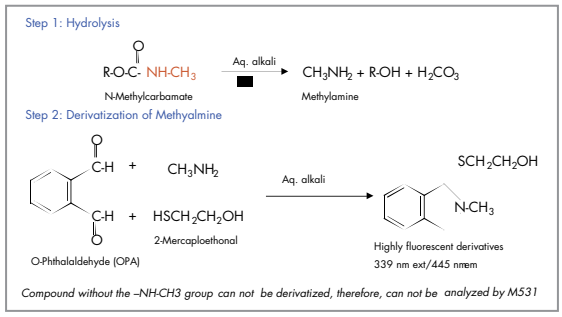
- Carbamate, thiocarbamate, and urea-based pesticides are commonly used as agricultural pesticides.
  - They have demonstrated toxicological effects. Recently, they have been implicated as endocrine disruptors.
  - The field run-off water transports these analytes into the soil, ground water, and tributaries
  - Waste treatment does not remove ALL pesticides before discharge into the tributaries
  - These are the sources of YOUR drinking water
- There is a strong need for analytical methods to screen and quantify carbamates in raw agricultural commodities, drinking and surface water and soil.
- The manufacturing waste of the carbamates must be characterized prior to disposal.
- US EPA Regulations
  - Regulates pesticide use
  - Requires routine monitoring of drinking water and raw source water, soil and waste matrices
  - Includes the required routine EPA Method 531 (M531) for drinking and raw source water, 8318 is for soil and waste matrices

Current Status



The Current EPA Method (M531.1) for Carbamate Analysis in Waste Water and Drinking Water

- M531.1 method utilizes HPLC with post column derivatization fluorescence detection (PCFD).
- The method includes 11 compounds, which all contain the N-methyl group which is crucial for the derivatization.
- The method uses a ternary gradient for the LC separation.
- The injection volume is 400 µL.
- Currently, EPA regulates two compounds: Carbofuran (40 ppb action limit) and Oxamyl (200 ppb action limit).



Why is N-Methyl Group Crucial?

The fluorescence detection used by M531 requires post column derivatization of the carbamate. The N-methyl group is required for the derivatization in order to form the highly fluorescent derivatives.

The Advantages and Limitations of Post Column Fluorescence Methods

- Advantages
  - Specific for N-methyl carbamates
  - < 1 ppb detection limits
  - Validated (EPA & Std Methods) and routinely used
  - Moderate equipment costs
- Limitations
  - Not all carbamate manufacturing precursors and degradation products are N-methyl structures
  - Requires baseline resolution for quantification
  - Some LC system complexity; requires dual post column derivatization with fluorescence

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Our Project Goal

To develop an LC/MS method for simultaneous detection of 52 carbamates in complex matrices

- Require NO post column derivatization
- Desire an automatic protocol

More Carbamates and Their Degradation Products Need to Be Detected

- For carbamate compounds, the degradation products may be equally or more harmful than the parent analytes.
- The US EPA has increased awareness of this problem and is looking for new, novel methods to address the "screening" of these analytes at the action limits.
  - Some analytes do not have established action limits
- Waters Industrial Application Group has been asked by EPA Office of Solid Waste (OSW) to develop a single LC/MS screening method for carbamate pesticides, their manufacturing precursors and degradation products.
  - Includes 52 analytes
  - To replace 6 current methods: 8141A, 8270C (GC/MS), 8313, 8321A (LC/PDA/TS-MS*)
  - Summarized in "Carbamates Method Evaluation Report" from SAIC** to OSW dated Aug 20, 1998

*TS-MS: Thermospray MS
**SAIC: Science Applications International Corporation

The 52 Carbamates Requested by US EPA

LC/MS Is the Method of Choice

- Most of these 52 compounds are either extremely polar or ionic. GC would not be suitable for them. HPLC is the natural method of choice for the separation.
- UV is not a suitable detector for the 52 carbamate analysis. Of the 32 compounds that can not be analyzed by the PCFD, 22 of them have either no or very weak UV absorbance.
- For either UV or Fluorescence detection, all analytes need to be baseline resolved for proper quantification (even for semi-quantitative analysis).
- The single quadruple MS detector offers the ability to analyze much larger range of compounds, especially with Electrospray (ESI) and APCI with sufficient sensitivity and high selectivity.
- MS detection is highly selective
  - With its ability of multi-channel detection, there is no need for baseline resolution for the 52 analytes
  - Interference for analytes in complex matrix is much less compared with UV or PCFD
- MS offers structure confirmation should there be a need.

Carbamate Analogs			Thio-Carbamate	Urea Analogs	Complexes and Chelators
(M531 Mix)	Aminocarb	Ethyl Carbamate	Butylate	Bramacil	Ferbam (Fe+3 DMDTC)
Aldicarb sulfoxide	Barbamate	Formatamate	Diallate	Chloroxuron	Metam Na (MMDTC anion)
Aldicarb Sulfone	Benomyl	Metolcarb	EPTC	Diuron	DMDTC (Anion)
Aldicarb	Bendiocarb	Mexacarbate	Molinate	Fenuron	OH MMDTC (Anion)
Carbaryl	Carbendazim	Propachlor	Tillam	Fluometuron	Ziram (Zn-2 DMDTC)
Carbofuran	Carbosulfuan	Promecarb	Triallate	Linuron	
3OH-Carbofuran	Chlorpropham	Propham	Vernolate	Monuron	
Methiocarb	Cycloate	Prosulfocarb		Neburon	
Methomyl	Eserine	Thiodicarb		Siduron	
1-Naphthol	Eserine Salicylate			Tebuthiuron	
Oxamyl					
Propoxur					

- Suitable for the EPA M531.1 (20)
- Not suitable for the EPA M531.1 (32)

The compounds in red have the N-methyl group, and therefore can be derivatized and detected by fluorescence detection (M531). There are 20 of them.
The compounds in gray do not have the N-methyl group, and therefore can not be derivatized and detected by the fluorescence detection. They have to be determined by other types of detectors. There are 32 compounds that can not be detected via the current EPA Method M531.1.

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Waters® Alliance® HT System (includes the 2795 Separations Module/2996 PDA Detector/Micromass® ZQ™ Mass Detector)

LC Conditions

- Column: Waters Symmetry® C_8 2.1 x 150 mm, 3.5mL, 40 °C
- Flow Rate: 0.3 mL/min
- Sample Temp: 5° C
- Mobile Phase:
  - A: 10 mM NH4OAc in Water, pH 5.0
  - B: 10 mM NH4OAc 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

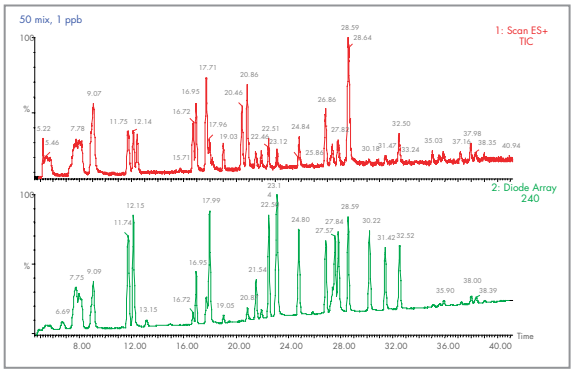
- Injection Volume: 50 µL

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

A Fully Automated LC/MS Protocol

Full Scan TIC of 52 Carbamates

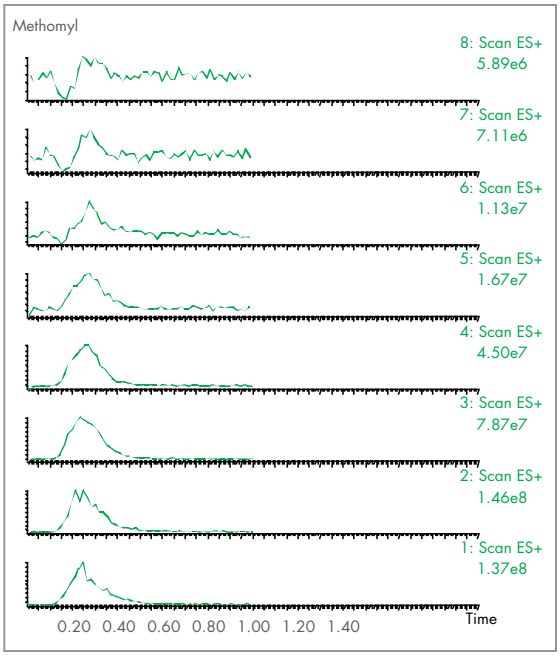


The very first step for this project was to develop a HPLC method to separate the 52 analytes. Since the intention was to use MS as a detector, there was no need for baseline resolution. This significantly cuts down the method development time and the run time.

- Automation was accomplished by the QuanLynx™ Application Manager, a MassLynx™ Software option composed of QuanOptimize™ and QuanLynx Browser.
  - QuanOptimize handles all the experiment runs and data collection.
  - QuanLynx will perform the post run processing of the raw data and allow user to view the analytical results.
- Once the LC conditions for the 50 analytes were developed, we only needed to infuse one analyte into the ZQ Mass Detector (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).
- 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 full scan injection for each of the standards with multiple cone voltages (52 injections)
  - Set up an SIR MS acquisition method based on the optimum cone voltage for each compound
  - Run the quantification analysis using the SIR method it created
  - Create a quantification method based on the LC/MS result
  - Perform quantification and create a final report which can be viewed in the QuanLynx browser

MS Conditions and Methods by QuanOptimize

Full Scan Optimization with Standard



- 8 full scan traces of Methomyl from one injection.
- This is the first step of the fully automated protocol: Optimization. This was done by flow injection analysis. The MS scan range was 100 ([MW +/- 50] Da).
- The cone voltage optimization range was defined by user, which was then divided into no more than 8 mini-steps by QuanOptimize.
- The full scan peaks were integrated by QuanOptimize and the optimum cone voltage was chosen based on peak area.
- For 52 analytes, the optimization was finished in less than two hours.
- Table 1 shows the optimization results for all 52 analytes via QuanOptimize. The numbers in red were the m/z values that were chosen by the author via manual optimization.

	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	Barbamate	C11H9NO2Cl2	258	275	257.81	CV 17
6	Bendiocarb	C11H13NO4	224	241	223.92	CV 17
7	Benomyl 192	C14H18N4O3	291	308	191.93	CV 17
8	Bromacil	C9H13N2O2Br	262	279	262.8	CV 17
9	Butylate	C11H23NOS	218	235	217.94	CV 17
10	Carbaryl	C12H11NO2	202	219	201.93	CV 17
11	Carbendazim	C9H9N3O2	192	209	191.93	CV 17
12	Carbofuran	C12H15NO3	222	239	221.98	CV 17
13	3OH Carbofuran	C12H15NO4	238	255	237.93	CV 17
14	Carbosulfan	C19H32N2O3S	369	386	386.27	CV 17
15	Chloroxuron	C15H15N2O2Cl	291	308	290.88	CV 29
16	Chlorpropham	C10H12NO2Cl	214	231	213.89	CV 17
17	Cycloate	C11H21NOS	216	233	215.96	CV 17
18	Diallate	C10H17NOSCl2	270	287	269.85	CV 29
19	Diruon	C9H10N2OCl2	233	250	232.81	CV 17
20	EPTC	C9H19NOS	190	207	189.97	CV 17
21	Eserine	C15H21N3O2	276	293	275.98	CV 17
22	Eserine Salicylate	C22H27N3O5	414	431	413.67	CV 29
23	Ethyl Carbamate	C3H7NO2	90	107	89.88	CV 17
24	Fenuron	C9H12N2O	165	182	165	CV 17
25	Ferbam	C9H18N3S6Fe	417	434	417.31	CV 29
26	Fluometuron	C10H11N2OF3	233	250	232.93	CV 29
27	Formatamate	C11H15N3O2	222	239	221.87	CV 17
28	Linuron	C9H10N2O2Cl2	249	266	248.82	CV 17
29	Metam Na	C2H4NS2Na	130	147	129.96	CV 41
30	Methiocarb	C11H15NO2S	226	243	225.91	CV 17
31	Methomyl	C5H10N2O2S	163	180	162.93	CV 5
32	Metolcarb	C9H11NO2	166	183	165.96	CV 17
33	Mexacarbate	C12H18N2O2	223	240	223.03	CV 17
34	Molinate	C9H17NOS	188	205	188	CV 17
35	Monuron	C9H11N2OCl	199	216	198.93	CV 17
36	1-Napthol	C10H8O	145	162	145.09	CV 41
37	Neburon	C12H16N2OCl2	275	292	274.87	CV 29
38	Oxamyl	C7H13N3O3S	220	237	236.93	CV 5
39	Promecarb	C12H17NO2	208	225	207.99	CV 17
40	Propachlor	C11H14NOCl	212	229	211.95	CV 17
41	Propham	C10H13NO2	180	197	179.69	CV 17
42	Propoxur	C11H15NO3	210	227	209.93	CV 53
43	Prosulfocarb		252		252	CV 17
44	Siduron	C14H20N2O	233	250	233.06	CV 29
45	Tebuthiuron	C9H16N4OS	229	246	228.97	CV 17
46	Thiodicarb	C10H18N4O4S3	355	372	354.88	CV 53
47	Tillam	C10H21NOS	204	221	203.94	CV 17
48	Trialliate	C10H16NSOCl3	304	321	303.79	CV 17
49	Verolate	C10H21NOS	204	221	203.99	CV 17
50	Ziram	C6H12N2S4Zn	305	322	304.82	CV 29
51	DMDTC		129	146	129.07	CV 41
52	OH-DMDTC		145	162	161.9	CV 5

Table 1: The MS Parameters

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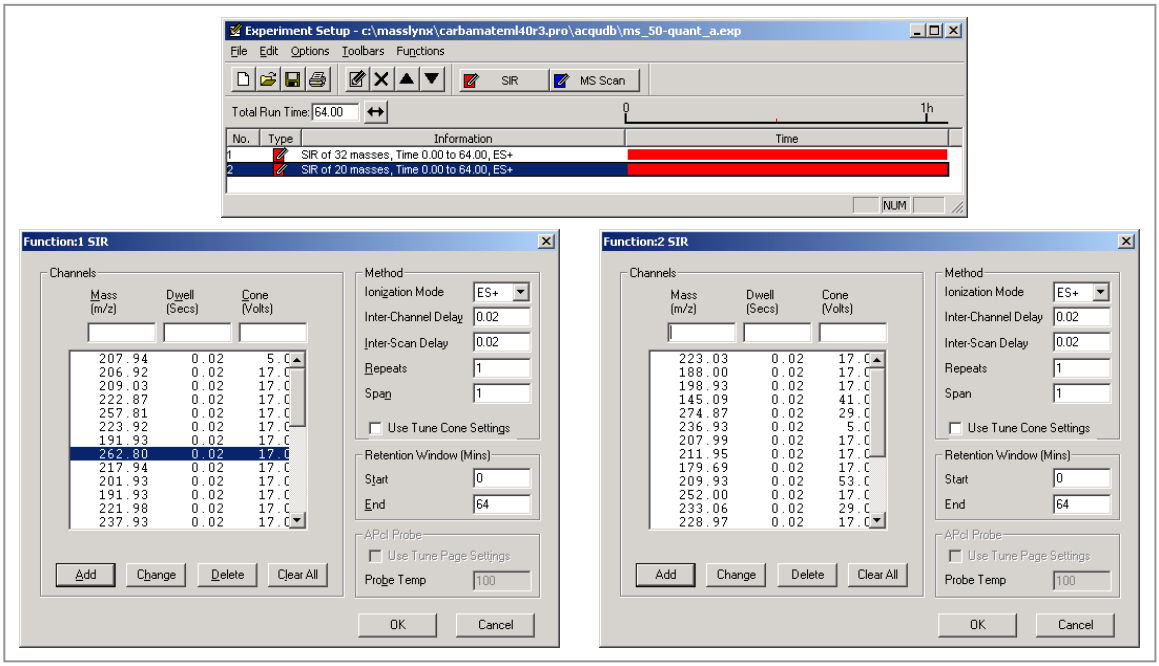
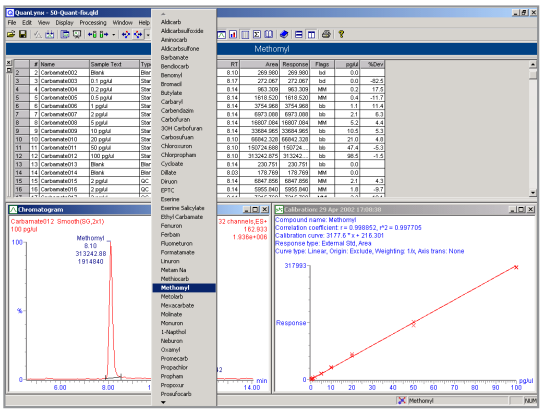
SIR MS Method



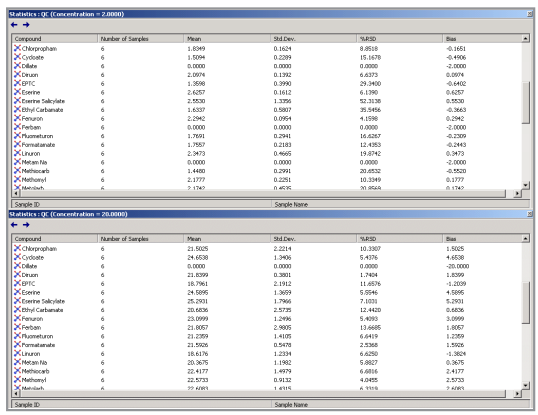
Shown here is the SIR MS method that QuanOptimize created for the quantification analysis. The maximum channels per function in MassLynx is limited to 32, therefore, QuanOptimize created a method with two functions to accommodate 52 analytes. All the exact m/z value and cone voltage for each analyte was shown in Table 1. Once QuanOptimize finished all injections, it created a quantification method and processed the whole sample list. The report for the whole analysis can be viewed from the QuanLynx browser shown below.

Report in QuanLynx Browser



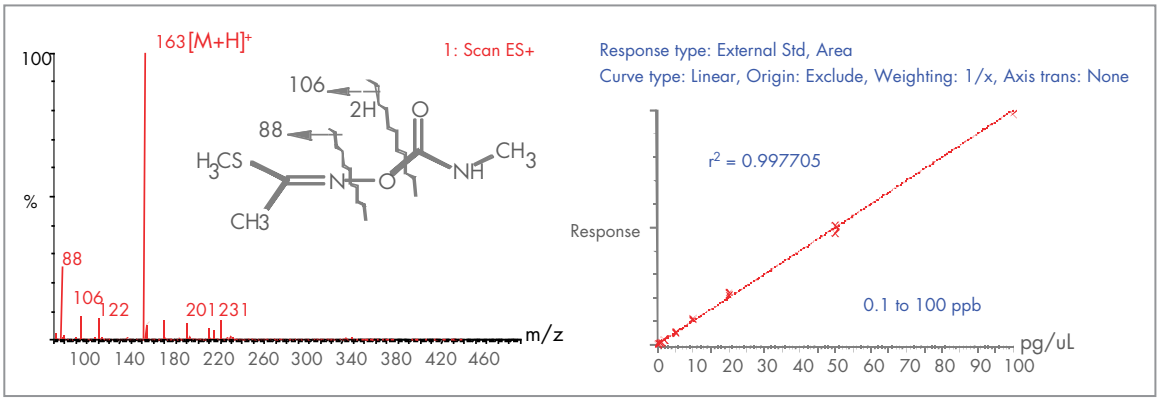
In the QuanLynx browser, the results are shown as one compound per page. Each page shows the result table, the corresponding chromatogram, and the calibration curve. In our analysis, there were 52 analytes, therefore, there were 52 pages in this report.

Statistics for QC

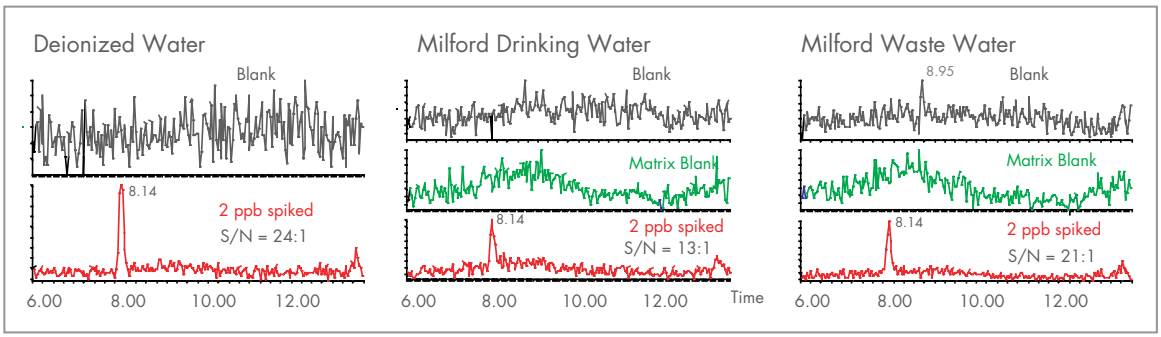


The statistic results for each analyte at each QC level can also be viewed from the QuanLynx Browser.

Results and Discussion
Standard Spiked into Real Matrices



Methomyl: In QuanOptimize analysis, each analyte was analyzed twice: once as a standard for full scan optimization, once in sample mixture for SIR quantification. Shown here on the left is the full scan spectrum for Methomyl. Shown here on the right is the calibration curve of Methomyl.
The quantification results for each analyte via QuanOptimize are shown on Table 2.



The analysis sequence for this work was: Blank, series concentration of standards (low to high), blank, low level QC (2 ppb), blank, high level QC (20 ppb), blank, drinking water matrix blank, 2 ppb spiked in drinking water, 20 ppb spiked in drinking water, blank, waste water blank, 2 ppb spiked in waste water, 20 ppb spiked in waste water.

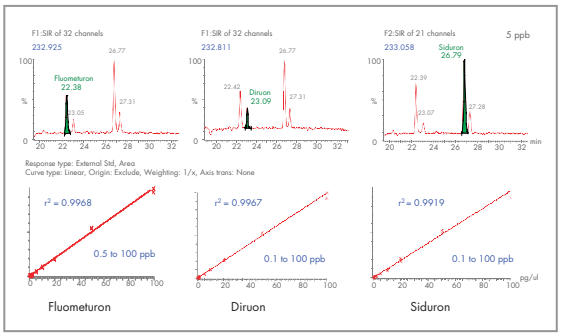
The drinking water was simply obtained from the tap in the building. The Milford waste water was obtained from the effluent of the Milford waste treatment plant.

The 2 ppb level shown is more than sufficient to accommodate the EPA requirement. However, with the S/N ratio above, there is room for even lower level.

Compounds with the Same m/z

- Among the 52 analytes, some of them have the same m/z value. For example, Diruon, Fluometuron and Siduron all show up at m/z 233 when optimized manually. As shown here, there are 4 distinct peaks at m/z 233. They are Diruon (t = 23.1 min.), Fluometruon (t = 22.4 min), and Siduron isomers (t = 26.8 and 27.3 min).
- Initially, we were concerned how QuanOptimize would distinguish all three compounds from one trace and accurately quantify them. As a result, QuanOptimize created 3 separate SIR channels for these three analytes. This made it possible to properly label and quantify each of the compounds automatically without them interfering with one another.

m/z 233



	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	Barbamate	258	275	7.71	3.7	0.946	117	94.5
6	Bendiocarb	224	241	20.86	3.68	0.994	73.5	63.5
7	Benomyl 192	291	308	12.14	1.29	0.899	120	144
8	Bromacil	262	279	17.86	19.35	0.972	94.5	95.2
9	Butylate	218	235	38.23	7.35	0.972	96.5	100
10	Carbaryl	202	219	21.94	1.47	0.996	92.5	85
11	Carbendazim	192	209	12.14	0.134	0.898	120	144
12	Carbofuran	222	239	20.86	2.26	0.996	104	101
13	3OH Carbofuran	238	255	12.14	2.17	0.993	90.2	79
14	Carbosulfan	369	386	45.19	No	No	No	No
15	Chloroxuron	291	308	28.56	1.32	0.996	109	72.7
16	Chlorpropham	214	231	24.75	3	0.997	113	107
17	Cycloate	216	233	35.49	1.8	0.975	121	127
18	Diallate	270	287	38.4	No	No	No	No
19	Diruon	233	250	23.12	0.888	0.998	120	102
20	EPTC	190	207	32.33	5.12	0.995	82.5	96.7
21	Eserine	276	293	9.07	0.0912	0.957	132	129
22	Eserine Salicylate	414	431	20.7	2.67	0.925	124	91.5
23	Ethyl Carbamate	90	107	7.73	5.72	0.995	92.2	93.2
24	Fenuron	165	182	11.75	0.566	0.996	119	107
25	Ferbam	417	434		5.56	0.955	97.2	95.2
26	Fluometuron	233	250	22.51	0.673	0.996	114	104
27	Formatamate	222	239	20.86	1.29	0.997	100	90.5
28	Linuron	249	266	28.71	2.5	0.996	93.5	95.2
29	Metam Na	130	147	8.07	10.6	0.968	100	99.8
30	Methiocarb	226	243	27.22	4.78	0.986	83	112
31	Methomyl	163	180	8.17	0.41	0.998	114	109
32	Metolcarb	166	183	11.71	2.23	0.991	108	105
33	Mexacarbate	223	240	28.62	0.319	0.997	119	112
34	Molinate	188	205	27.95	2.24	0.992	121	128
35	Monuron	199	216	17.96	2.29	0.996	111	112
36	1-Napthol	145	162	21.86	1.12	0.994	104	99.5
37	Neburon	275	292	32.48	1.47	0.992	112	118
38	Oxamyl	220	237	7.78	1.91	0.989	94	82.8
39	Promecarb	208	225	28.65	1.23	0.991	105	111
40	Propachlor	212	229	24.84	0.806	0.995	114	122
41	Propham	180	197		No	No	No	No
42	Propoxur	210	227	20.46	No	No	No	No
43	Prosufocarb	252		37.98	1.26	0.989	128	143
44	Siduron	233	250	27	0.64	0.997	118	109
45	Tebuthiuron	229	246	16.93	1.16	0.992	113	108
46	Thiodicarb	355	372	20.8	No	No	No	No
47	Tillam	204	221	35.86	16	0.887	126	97.2
48	Trialliate	304	321	19.04	7.28	0.991	124	124
49	Verolate	204	221	35.86	5.68	—	110	121
50	Ziram	305	322		No	No	No	No
51	DMDTC	129	146		41.7	0.88	113	128
52	OH-DMDTC	145	162		No	No	No	No

Table 2: Quantification Results

The ions marked with red in [M+H] column and the [M+NH4] column were the ones that were chosen by the author as a result of manual optimization. In Table 1, the m/z values chosen by QuanOptimize were displayed. Most of them agreed with the manually picked ions.

The Limits Of Detection (LOD) reported here was calculated based on S/N = 3 with unsmoothed chromatograms.

The percent recovery was calculated based on 20 ppb standard spiked in to appropriate matrix. The EPA M531.1 method indicated that the acceptable recovery range is 70% to 130%. Compounds with recovery range outside the regulated number are considered to be affected by their matrices.

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How Does the LC/MS Method Do?

Carbamate Analogs			Thio-Carbamate	Urea Analogs	Complexes and Chelators
(M531 Mix)	Aminocarb	Ethyl Carbamate	Butylate	Bramacil	Ferbam (Fe+3 DMDTC)
Aldicarb sulfoxide	Barbamate	Formatamate	Dillate	Chloroxuron	Metam Na (MMDTC anion)
Aldicarb Sulfone	Benomyl	Metolcarb	EPTC	Diuron	MDTC (Anion)
Aldicarb	Bendiocarb	Mexacarbate	Molinate	Fenuron	OH MMDTC (Anion)
Carbaryl	Carbendazim	Propachlor	Tillam	Fluometuron	Xiram (Zn-2 DMDTC)
Carbofuran	Carbosulfuan	Propmecarb	Triallate	Linuron	
3OH-Carbofuran	Chlorpropham	Propham	Vernolate	Monuron	
Methiocarb	Cycloate	Prosulfocarb		Neburon	
Methomyl	Eserine	Thiodicarb		Siduron	
1-Naphthol	Eserine Salicylate			Tebuthiuron	
Oxamyl					
Propoxur					

- Suitable for both the EPA M531.1 and the LC/MS method (20)
- Suitable for ONLY the LC/MS methods (26)
- Not suitable for either (6)

It is important to realize that the LC/MS method developed in this presentation only utilized the reversed phase LC separation with positive electrospray MS detection. Some of these 6 compounds may be analyzed by APCI. Some of these ionic compounds may be analyzed via IC (ion chromatography)/ESI-MS.

Conclusion

- We have developed a LC/ESI-MS method for simultaneous detection of 46 carbamate and their waste constituents.
  - Enhance capability to analyze much wider range of analytes
  - Minimize method development time
  - Do not require baseline resolution for the LC separation
  - Fully automated LC/MS quantification protocol
  - Do not require post column derivatization
  - Sufficient sensitivity to accommodate the EPA requirement
  - Capable of detecting < 1 ppb at 50 µL injection volume (less than the 400 µL indicated in EPA M531)
  - Strong selectivity to handle complex matrices
  - Method applied to waste water and drinking water with direct injection
  - Recoveries were within the EPA regulated range without pre-cleaning sample prep
- This protocol will be submitted as a screening method to EPA and ASTM for validation

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