Carbamates - Analysis by API LC/MS

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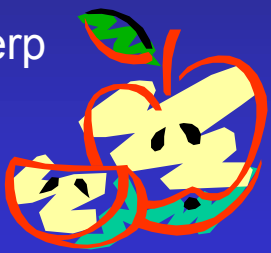
LC/MS
Waters Corporation



Introduction to carbamate analysis by Atmospheric Pressure Ionization (API) LC/MS.

Appropriate Chromatographic Conditions

HPLC: Waters Alliance™ System
Column: Waters Symmetry™ C_{18}, 1.0 x150 mm
Temperature: 35°C
Injection Volume: 10µL
Mobile Phase:
A: 10% Methanol/10 mM NH_4Ac
B: 90% Methanol/10 mM NH_4Ac

Flow Rate: 75 µL/min

Gradient:

Time	%A	%B	Curve
Initial	90	10	
10	10	90	6
12	10	90	6

Note that the chromatographic conditions are different from the HPLC post-column derivatization method .

A Symmetry C_{18} with a 1.0 mm ID (inner diameter) was used.

An ammonium acetate buffer was added to the mobile phase in order to create better ionization for the mass spectrometer.

Optimum flow rate for the column dimension was 75 µL/min.

The gradient program for the HPLC post-column derivatization method is fairly intricate, because fluorescence and UV detection are 2-dimensional. For good quantitation, each peak must be baseline resolved from one another. Mass Spec, on the other hand, adds the additional dimension of mass, allowing the mass spectrometer to "resolve" co-eluting compounds on the basis of their molecular weight. For this reason, the simpler and faster gradient, shown here as used.

Note. Later work with spiked matrix used a 2.1 mm x 150 mm column. By simply scaling up the flow rate, the same chromatography could be obtained without the excessive delay times incurred with the microbore column.

Mass Spectrometer Conditions

Instrument: Waters ZMD ZSpray™ Mass Detector

Interface: Positive Electrospray (ESI^+)

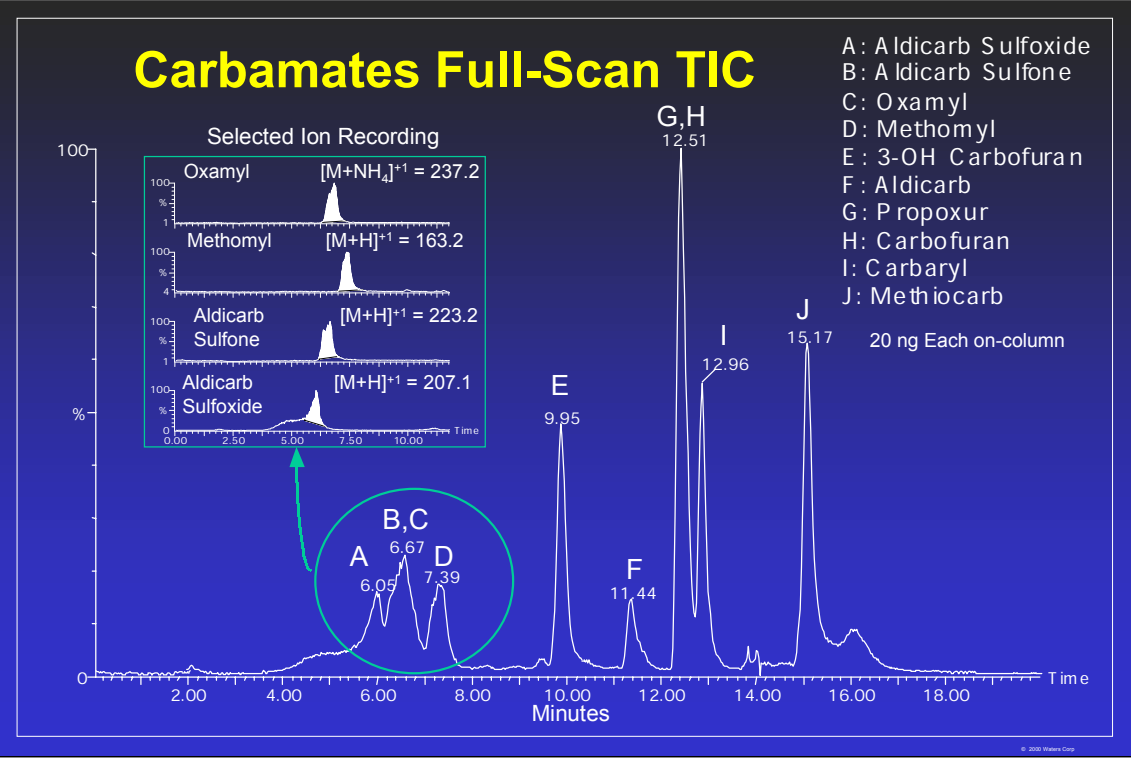
Scan Function: Multiple Selected-Ion Recording (SIR):

Group	Time mins.	Compound	Mass	Cone Voltage	Dwell Time
1	0-9	Aldicarb Sulfoxide	207.1	18V	0.5 secs
		Aldicarb Sulfone	223.2	25V	0.5 secs
		Oxamyl	237.2	10V	0.5 secs
		Methomyl	163.2	15V	0.5 secs
2	9-11	3-OH Carbofuran	238.2	15V	1.5 secs
3	10.5-12.5	Aldicarb	208.2	8V	1.5 secs
4	11.5-14	Propoxur	210.2	18V	0.4 secs
		Carbofuran	222.2	22V	0.4 secs
		Carbaryl	202.2	18V	0.4 secs
5	14-20	Methiocarb	226.2	19V	0.6 sec

MassLynx ™ Software

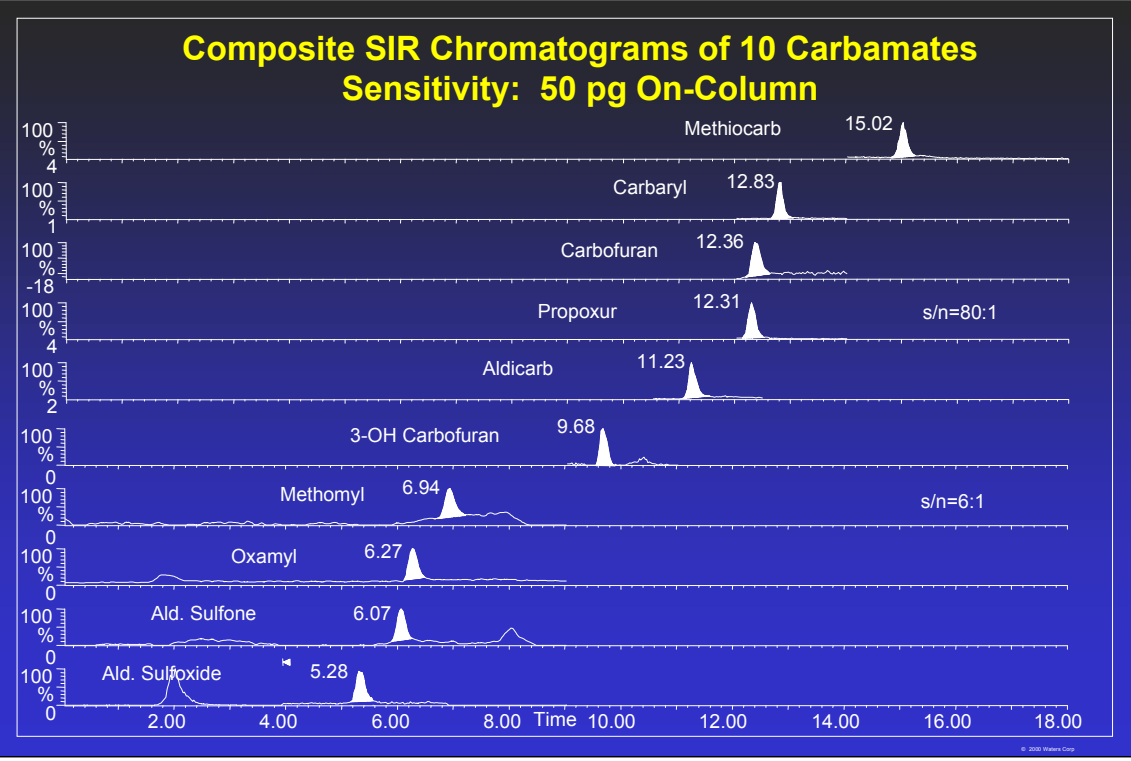
The ZMD mass detector can easily switch between positive and negative ion monitoring. However, all of the analytes in this case worked best using the electrospray interface in the positive ion mode. The ZMD operates in full scan or selected ion recording (SIR) acquisitions modes.

The best sensitivity for carbamates is obtained running in SIR mode. The MassLynx software group function tells the mass spectrometer which ions to monitor. This slide shows the acquisition program used for quantitative analysis. A specific ion is monitored for each analyte over its retention time window. As each ion is monitored, the mass spectrometer uses the optimum cone voltage setting for that analyte, increasing sensitivity.



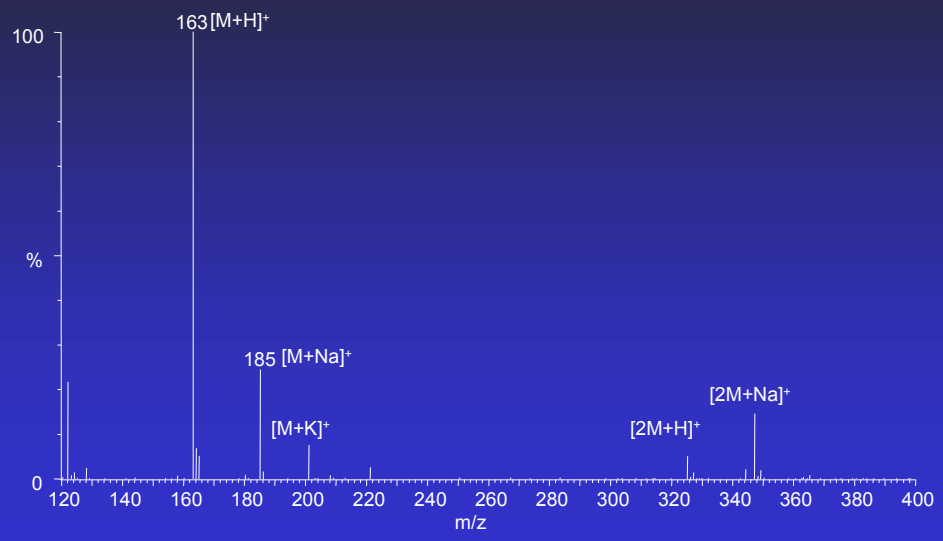
This is a good illustration of how the mass spectrometer can resolve co-eluting peaks. The large plot is the total ion chromatogram, which plots the sum of all ion signal in each scan versus time. It is roughly equivalent to a UV or fluorescence detector output. As can be seen, the early-eluting compounds are poorly resolved and co-elute. However, since each mass acts as an independent data channel, by plotting masses specific to each compound (inset), the mass spectrometer is able to extract four discrete peaks. Since the masses are distinct for each compound, they can be quantitated even though they co-elute.

Note: The poor peak shape of the first compound is a result of the fact that the sample was injected from a 100% organic solution. Subsequent work used standards/samples in more aqueous solutions, which improved peak shape of the early-eluting components.



This slide illustrates the sensitivity of the method. A mixture of all compounds (5 pg/µL) was injected and analyzed. Displayed are all the chromatograms of all the monitored ions. This represents 50 pg (<500fmol) of each compound on-column. Signal to noise varied from 6:1 for methomyl, to approximately 80:1 for propoxur

Background-subtracted Full-scan ESI+ Spectrum of Methomyl

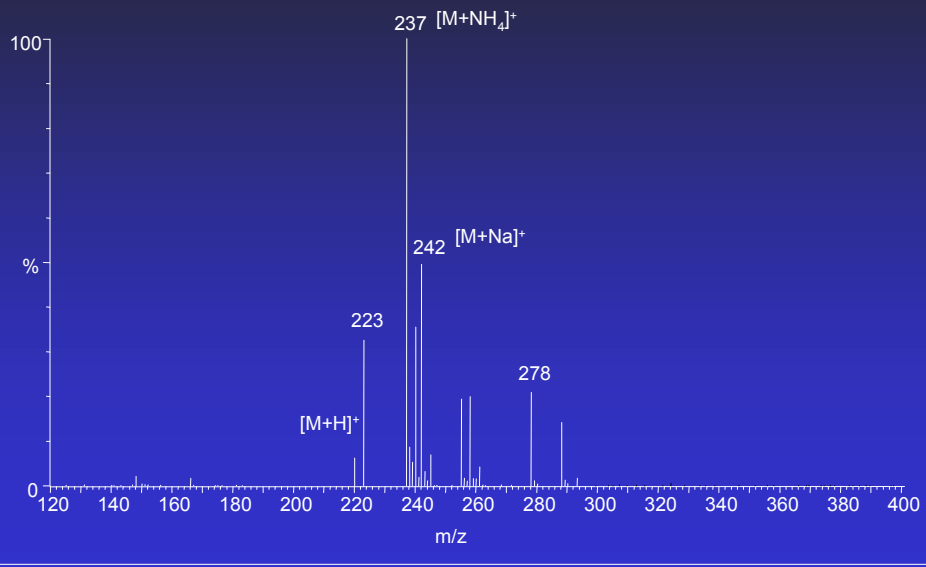


This slide shows the mass spectrum of methomyl acquired by the ZMD using the electrospray interface in positive ion mode. The most intense ion is the $[M+H]^+$ ion at 161. However, several other ions are seen, including the sodium and potassium adduct ions as well as the dimer and its adduct ion.

This is a good illustration of how instrument response is compound-dependent.

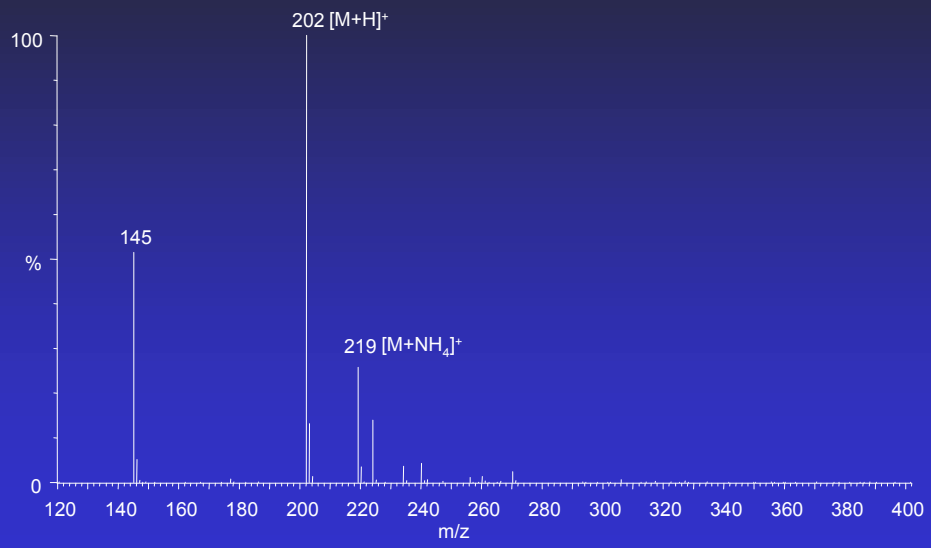
Note: These slides are from infusion of individual carbamate standards at concentrations of approximately 20 ppm. Dimerization is not uncommon when infusing relatively high concentrations of sample.

Background-subtracted Full-scan ESI$^+$ Spectrum of Oxamyl



For Oxamyl, the expected protonated molecular ion at m/z 200 is miniscule compared to the adduct ions. Clearly this compound is very ionophoric, and will scavenge any cations in solution. For this reason the mobile phase contained 10 mM ammonium acetate in order to provide a constant level of ammonium cations and the ammonium adduct at m/z 237 was monitored in subsequent quantitative analyses.

Background-subtracted Full-scan ESI$^+$ Spectrum of Carbaryl



This slide shows the resulting ESI spectrum of Carbaryl. This compound gave a simpler spectrum where the protonated molecular ion at m/z 202 displayed the greatest intensity. This ion would be monitored for quantitative studies.

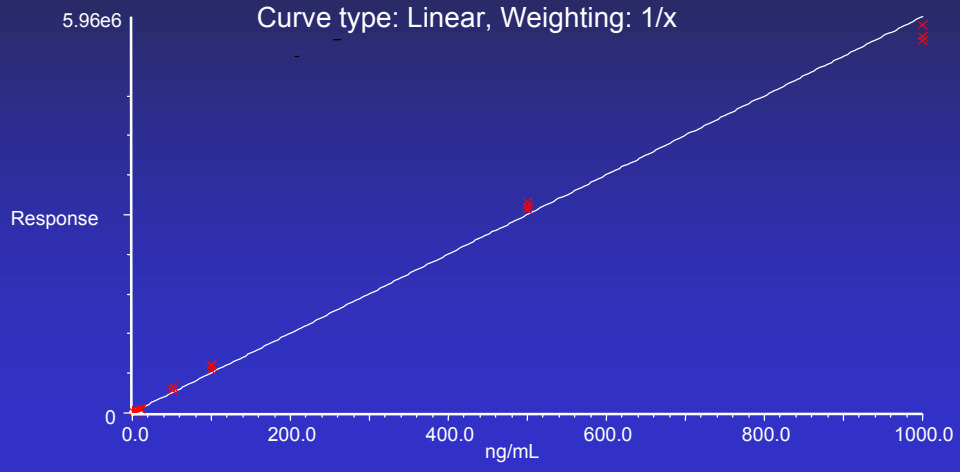
Quantitation of Carbamates via LC/MS

- Once the retention times and mass spectral behavior of carbamates is known under appropriate chromatographic conditions, we are ready to quantitate.
- Must still achieve acceptable limits of detection and quantitation to insure reliable pesticide screening.
- Must still achieve the same limits of detection and quantitation with actual samples in matrix.

In order to quantitate, we must first evaluate the sensitivity, linearity, accuracy, and precision of the method using standards. Then we can run actual samples.

Methomyl Calibration Curve; 5-1000 ng/mL

Compound 4 name: methomyl (m/z 163)
Coefficient of Determination: 0.995876
Response type: External Std, Area
Curve type: Linear, Weighting: 1/x



Here is a representative calibration curve for methomyl.

Linearity and Sensitivity

Linearity was assessed from triplicate analysis of a series of calibration standards (5-1000 ng/mL). Instrumental LOD and LOQ were defined as 3X and 10X the standard deviation of the calculated concentrations, respectively. LOD and LOQ were determined from 5 replicate injections of a standard mixture (50 pg each analyte on-column).

	Coefficient of determination	LOD*	LOQ*
Aldicarb Sulfoxide	0.9969	8	26
Aldicarb Sulfone	0.9982	18	61
Oxamyl	0.9990	7	22
Methomyl	0.9959	16	55
3-OH Carbofuran	0.9970	4	14
Aldicarb	0.9963	2	5
Propoxur	0.9967	17	55
Carbofuran	0.9981	9	30
Carbaryl	0.9994	3	11
Methiocarb	0.9995	4	14

* pg on-column

Linearity was determined over a 200-fold concentration range with very good results.

Note: Unlike UV detection, the mass spectrometer is inherently non-linear. The degree f non-linearity is compound dependent and also varies with analyte concentration range and matrix effects. This as an inherent behavior of API ionization, and is not instrument specific. For this reason, weighting is often used. This is NOT CHEATING! Also note that, because linear regression used a weighting factor, the familiar term "R^2" is replaced with statistically equivalent "coefficient of determination."

The lowest standard was injected in replicate (n=5) in order to determine the instrumental limits or detection (LOD) and quantitation (LOQ). This slide lists instrumental sensitivity limits calculated for the amount of material injected on-column.

Precision and Accuracy

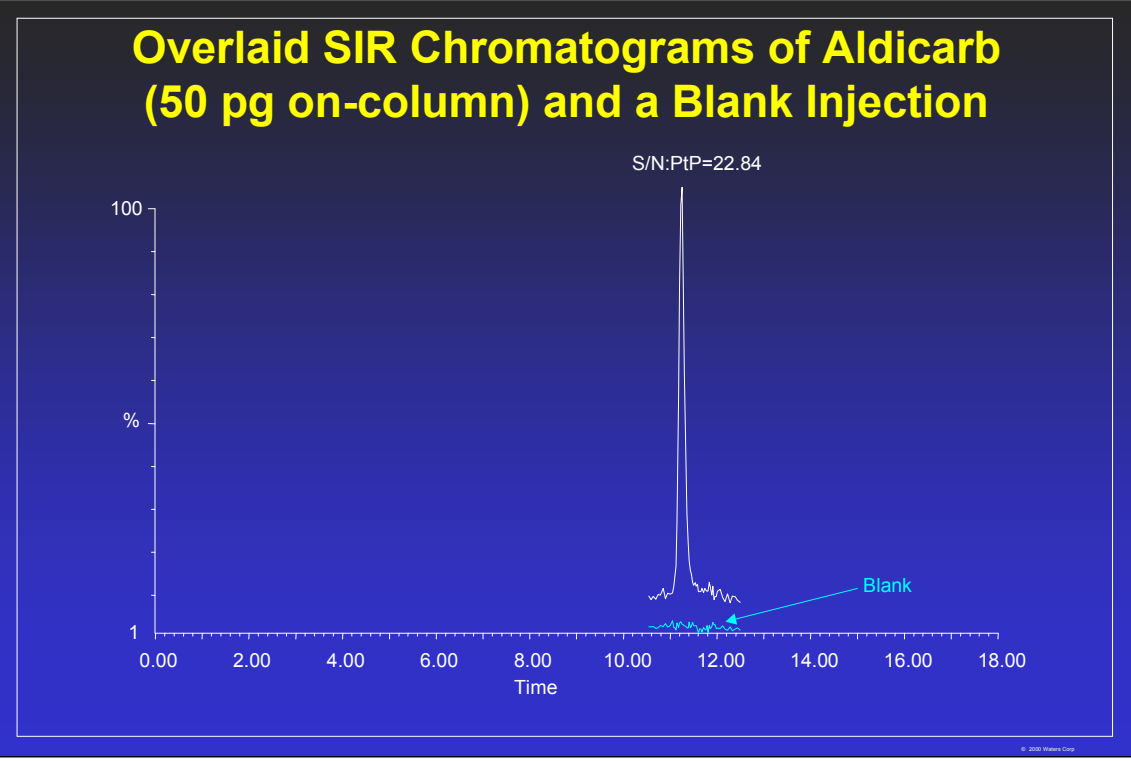
Five replicate injections of a 5 ng/mL standard solution were made. Precision is defined as the percent coefficient of variation of the calculated concentrations. Accuracy is defined as the percent difference from theoretical of the mean calculated concentration.

	Mean (ng/mL)	S.D.	% C.V.	% Diff.
Aldicarb Sulfoxide	7.48	0.259	3.5	49.6
Aldicarb Sulfone	6.32	0.606	9.6	26.4
Oxamyl	6.68	0.217	3.2	33.6
Methomyl*	5.50	0.548	10.0	10.0
3-OH Carbofuran	6.80	0.141	2.1	36.0
Aldicarb	7.36	0.055	0.7	47.2
Propoxur	4.90	0.552	11.3	-2.0
Carbofuran*	3.98	0.299	7.5	-20.5
Carbaryl	6.26	0.114	1.8	25.2
Methiocarb	5.70	0.141	2.5	14.0

*n=4

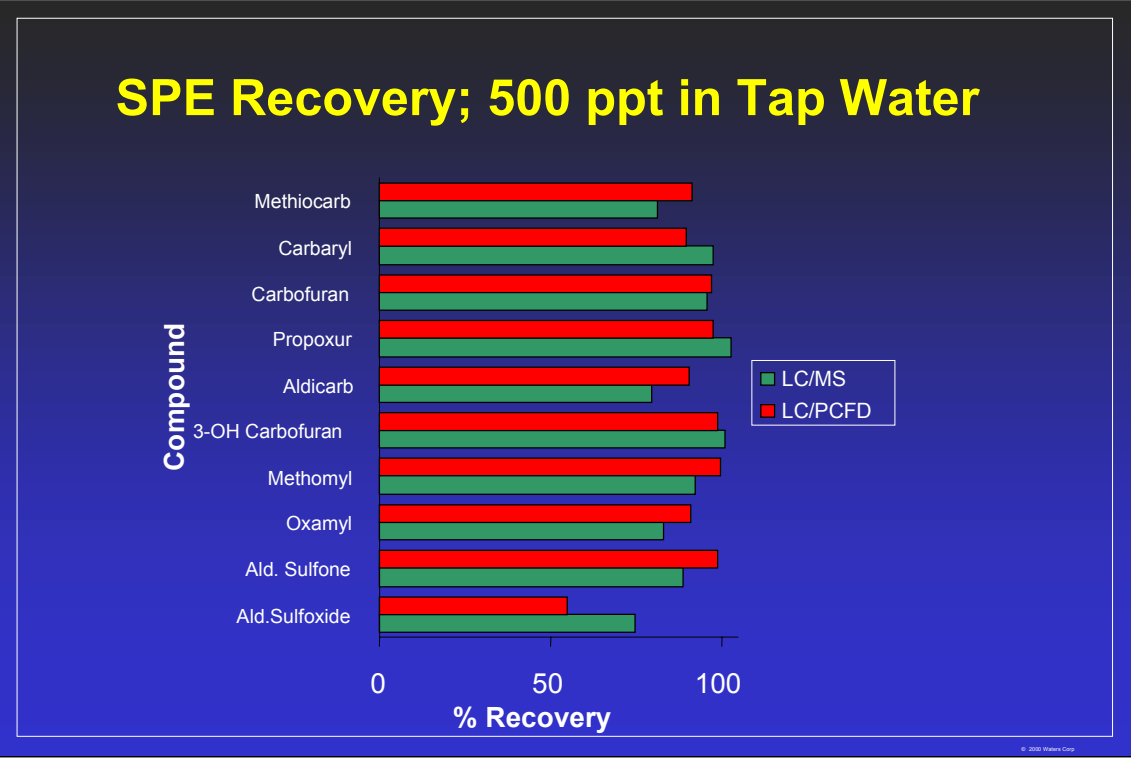
Linearity is nice so is sensitivity, but the bottom line is accuracy and precision. This slide presents the accuracy and precision results from replicate injections of the lowest standard. Precision (defined as % C.V.) and accuracy (defined as the % difference from theoretical of the mean) are quite good.

Note: These values are probably higher than what people are used to seeing with LC/UV methods, but it should be borne in mind that we are talking about very low amounts (<500 fmol) on-column; levels that are unobtainable by UV detection. Obviously, precision and accuracy values are much better for higher concentrations. This slide shows "worst case" data. Also, remember that unlike a UV detector, LC/MS is a very dynamic process. When working at the ragged edge of concentration, precision and accuracy often fall off. Still, by LC/MC standards, these results are very respectable.



This Selected -Ion Recording chromatogram of Aldicarb represents 50 pg (<500fmol) on column. Signal to noise is 22.84 to 1.

Based on standards, we have demonstrated that our method provides adequate sensitivity, linearity, precision, and accuracy. Unfortunately, analytical chemists are not paid to analyze standards. We have to look at real samples, which have a tendency to be messy. A better test of this method would be to apply it to the analysis of real samples. Let's take a look at the results of some actual samples.



The first matrix we looked at was water. Milford drinking water was spiked with a carbamate mixture at 500 ppt, and extracted and concentrated by SPE cleanup using Oasis HLB cartridges. The sample was analyzed by both traditional post-column derivatization fluorescence detection (red) as well as by LC/MS (green), and the recoveries were compared.

As can be seen, there is good agreement between the two methods. The differences for aldicarb sulfoxide are believed to be due to sample degradation in the time interval between the two analyses.

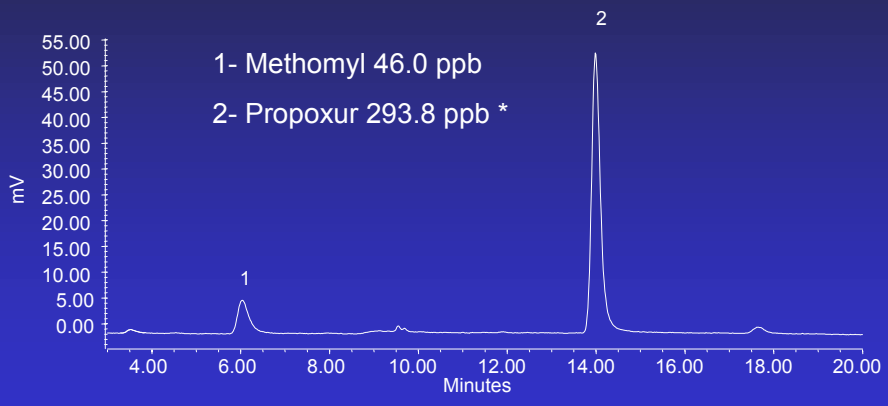
Carbamates LC/MS

A comparison of results obtained from 500 ng/L spiked drinking water samples. Each extract was analyzed by LC/MS and also by LC with post-column derivatization/fluorescence detection (PCFD). [% recovery (% RSD)]

Compound	LC/MS	LC/PCFD
aldicarb sulfoxide	74.8 (19)	54.7 (0.5)
aldicarb sulfone	88.7 (16)	98.7 (4.0)
oxamyl	83.2 (18)	90.8 (7.0)
methomyl	92.3 (8.0)	99.9 (6.4)
3-hydroxycarbofuran	101 (8.6)	98.7 (2.3)
aldicarb	79.4 (9.3)	90.7 (9.3)
propoxur	103 (13)	97.5 (5.6)
carbofuran	95.6 (7.5)	97.2 (4.7)
carbaryl	97.7 (14)	89.6 (2.2)
methiocarb	81.2 (14)	91.6 (2.2)

Here is a comparison of the actual results.

LC/PCFD Analysis of Harvested Bell Pepper Extract # 00329

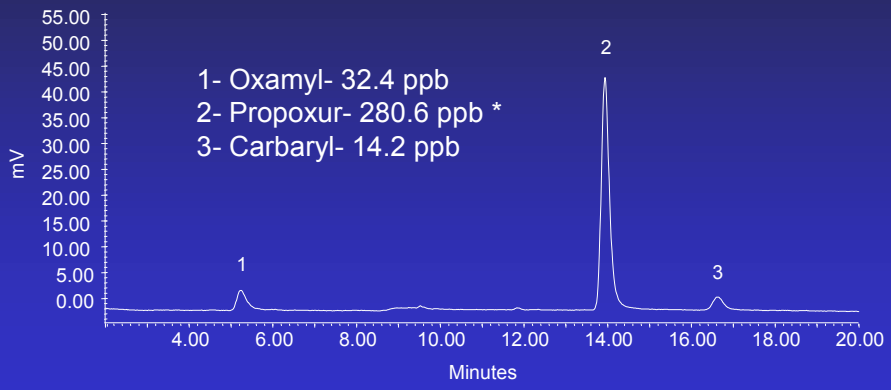


* spiked in sample

Bell peppers are more complex than water. We were provided with several extracts from peppers with incurred (and therefore unknown) levels of carbamates except for propoxur, which was spiked into all the samples.

The samples were analyzed by the traditional post-column derivatization fluorescence detection method. Methomyl was detected in this sample.

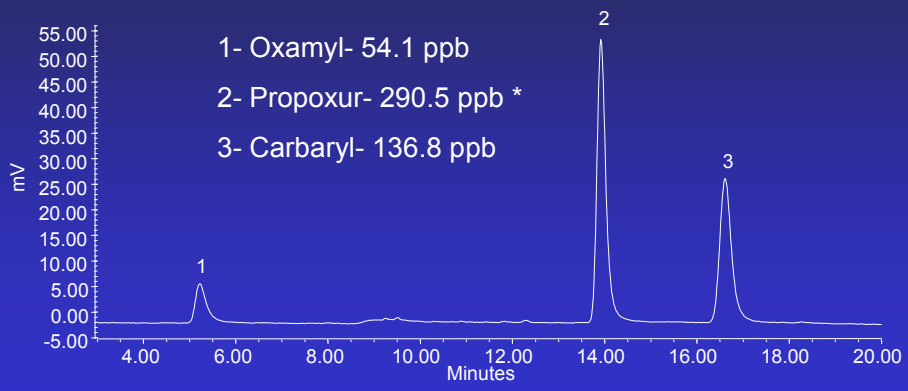
LC/PCFD Analysis of Harvested Bell Pepper Extract # 00336



* spiked in sample

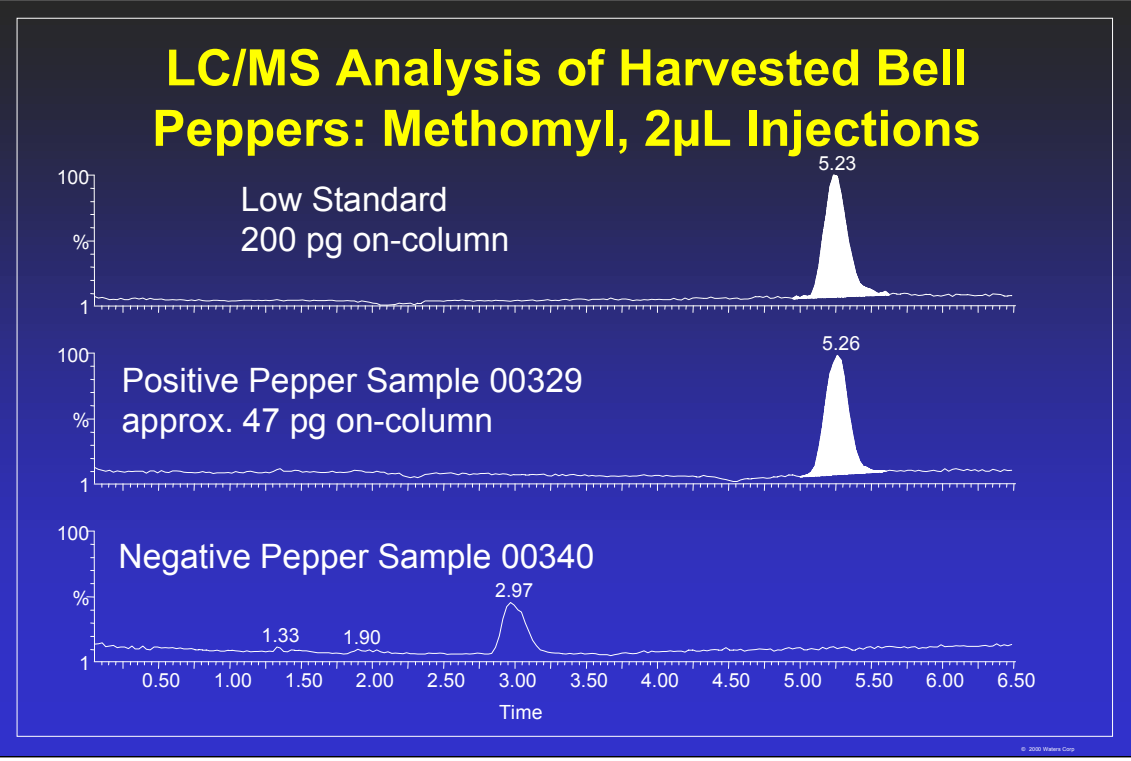
Here is a chromatogram of a different sample where oxamyl and carbaryl were detected.

LC/PCFD Analysis of Harvested Bell Pepper Extract # 00340

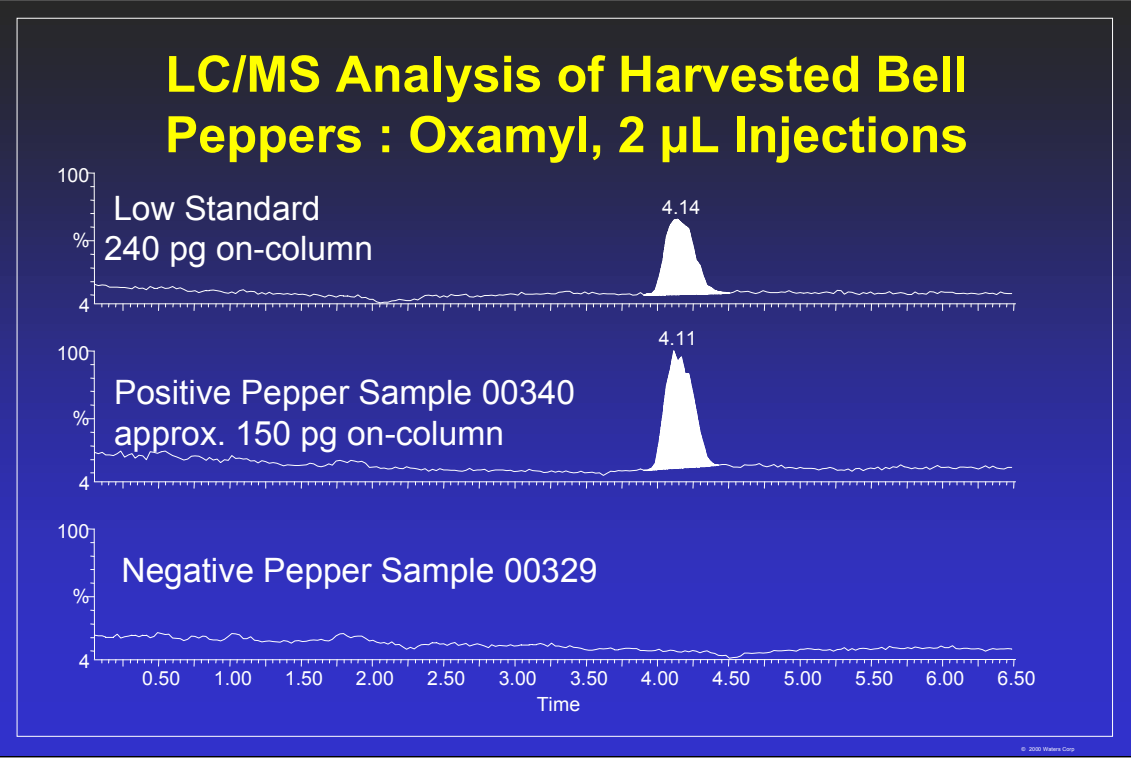


* spiked in sample

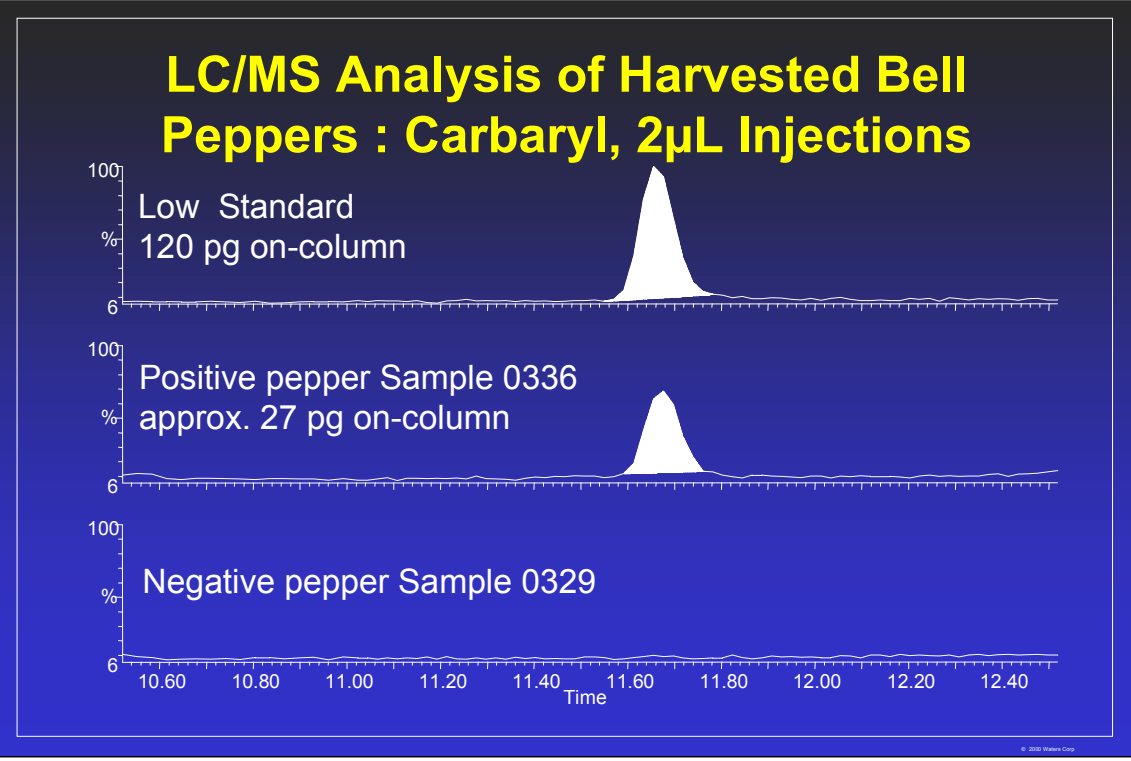
Detection of carbamates in another sample of bell pepper.



The same samples were also analyzed by LC/MS. Quantitation was done on the basis of a 3-point standard. This slide compares the methomyl chromatogram from the low standard (top) with chromatograms from positive (middle) and negative (bottom) pepper extract. As may be seen from the extracts, the selectivity provided by LC/MS results in extremely clean baselines.



These chromatograms compare a standard, positive, and negative sample for oxamyl.



One last slide showing carbaryl response.

Harvested Bell Pepper Samples

Sample ID	Methomyl HPLC (ppb)	Methomyl LC/MS (ppb)	Oxamyl HPLC (ppb)	Oxamyl LC/MS (ppb)	Carbaryl HPLC (ppb)	Carbaryl LC/MS (ppb)	Propoxur HPLC (ppb)	Propoxur LC/MS (ppb)
329	46	46.5	-	-	-	-	293.8	276.5
330	411.5	342.5	-	-	-	-	319.7	323.5
332	32.5	40.5	-	-	-	-	298.6	241.0
336	-	-	32.4	48.0	14.2	13.5	280.6	263.5
340	-	-	54.1	76.0	136.7	154.5	290.5	341.5

These samples had incurred carbamate levels except for propoxur, which was spiked into all the samples, and so the actual concentration was unknown, outside of the LC/MS determination. As an additional check, the samples were analyzed via the traditional post-column derivatization fluorescence method, and the mean determination of both methods were compared. As may be seen, there is good agreement between the two methodologies.

Confirmation/Screening

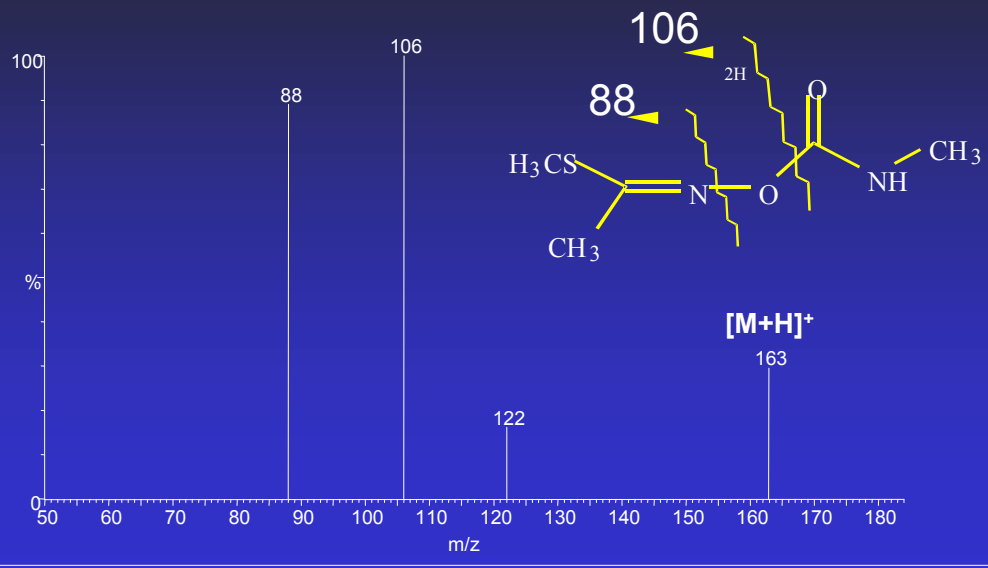
- "Three-ion rule" used to eliminate false positives
- In this example, "In-Source CID" was used to generate fragment ions from the analyte (Methomyl)
- Relative abundance of pseudomolecular/fragment ion ratios were compared for standard and spiked spinach extracts (n=10)

The "3-ion rule" is a convention which arose from the early days of GC/MS. In order to provide confirmation and avoid false positives, three structurally significant ions are monitored by SIR for each compound. Identification is considered confirmed if all three ions have the same retention time/chromatographic profile and their relative intensities match those of an authentic standard. Unfortunately, atmospheric-pressure ionization spectra typically do not yield abundant fragment ions as is the case for electron-impact

ionization used in GC/MS. However, fragmentation can be induced by increasing the voltage on the sampling cone in a process known as "in-source Collisionally Induced Dissociation."

Note: CID fragmentation is extremely compound and tune-dependent; CID spectra are not nearly as reproducible as electron-impact spectra. Also, bear in mind that as sampling cone voltage is increased to induce fragmentation, very often absolute signal will decrease, meaning that the analyst will have to strike a balance between overall sensitivity and confirmation.

CID Fragmentation of Methomyl



This is an abbreviated spectrum of methomyl under CID conditions. As may be seen, several structurally significant fragment ions are observed. The insert shows the proposed identity of these fragments.

Note: The ion at m/z 122 cannot be explained by a simple bond cleavage and probably arises from rearrangement during the CID process. Because of the unknown identity of this ion, it was not considered in the following slides.

Methomyl Confirmation in Spinach

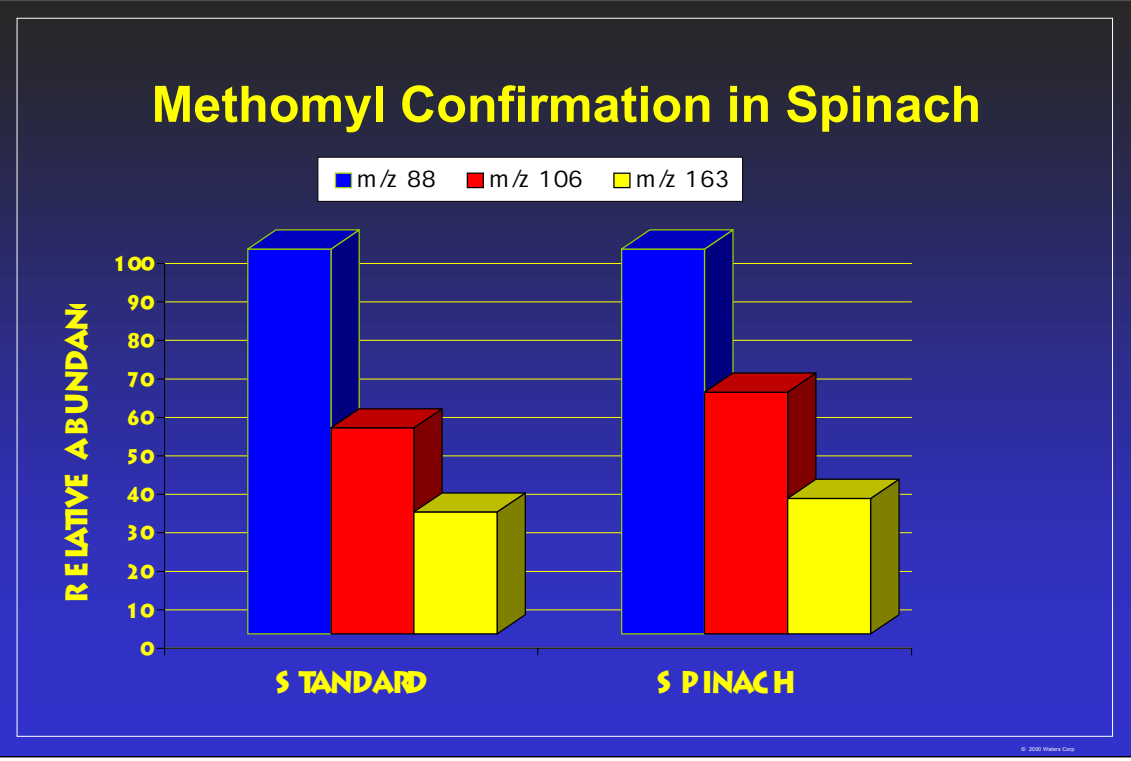
	Standard			Spinach		
Ion m/z	88 Da	106 Da	163 Da	88 Da	106 Da	163 Da
% Relative Abundance	100	51	34	100	55	34
	100	56	36	100	62	36
	100	54	34	100	55	30
	100	51	32	100	65	35
	100	55	31	100	63	36
	100	52	30	100	65	37
	100	54	30	100	69	38
	100	54	30	100	71	37
	100	54	30	100	72	38
	100	55	30	100	51	31
Mean	100	53.6	31.7	100	62.8	35.2
S.D.	-	1.71	2.21	0	7.16	2.78
% C.V.	-	3.2	7.0	0	11.4	7.9

In a separate experiment, both spiked spinach extract and a solvent standard were injected 10 times, and mass spectra were obtained under CID conditions.

This table compares the methomyl ion ratios for the two samples. Ion ratios are normalized relative to the most intense ion at m/z 88.

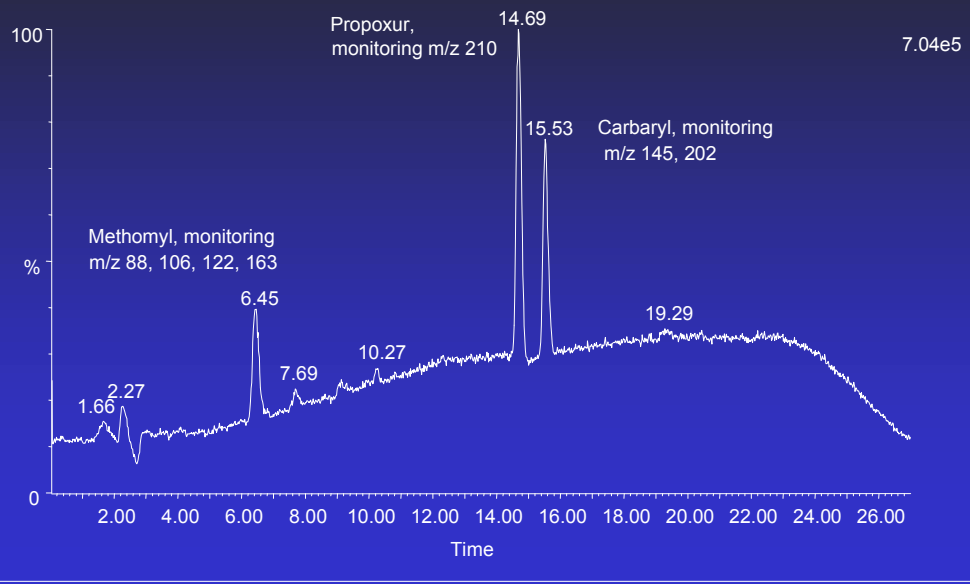
There is good agreement between the two data sets, indicating the utility of CID fragmentation for compound confirmation.

Note: More alert people may comment on the fact that the ion ratios expressed in this table differ from those in the previous slide, in which the ion at m/z 106 was the most intense ion. We believe that this is due to the fact that the data from both slides was acquired under slightly different conditions and/or on different days. While the table shows good INTR-assay reproducibility, whish is perfectly acceptable for confirmation of target analytes, some people might be tempted to conclude that CID spectra are well-suited to library matching. This is an assumption that other manufacturers are willing to foster. However, the utility of CID spectra for library-based identification requires excellent INTER-assay reproducibility, which front-end CID does not offer. This is a very important distinction which should be borne in mind when addressing claims of other manufacturers.



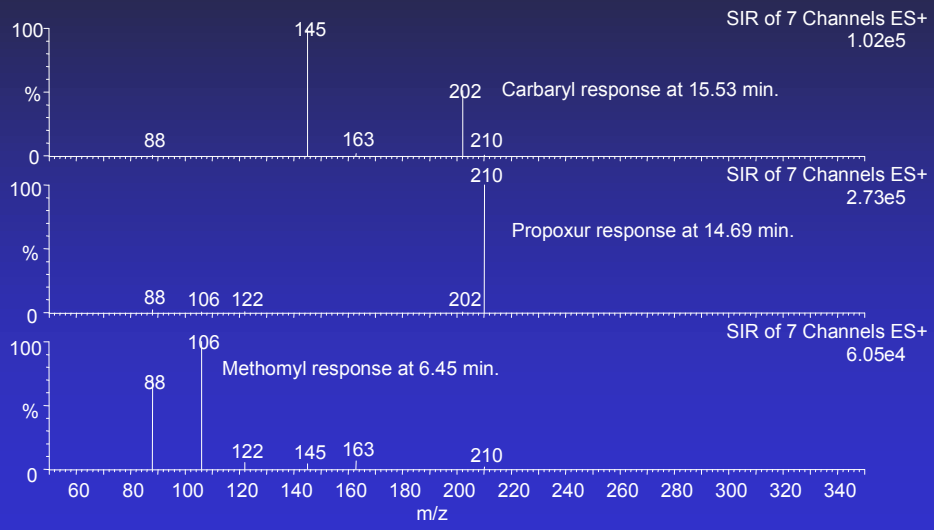
This is a more graphic representation of the results presented in the previous table. It is clear that compound identity can be confirmed even in the presence of a complex matrix.

Composite of a Three Component Standard
SIR of 7 Channels



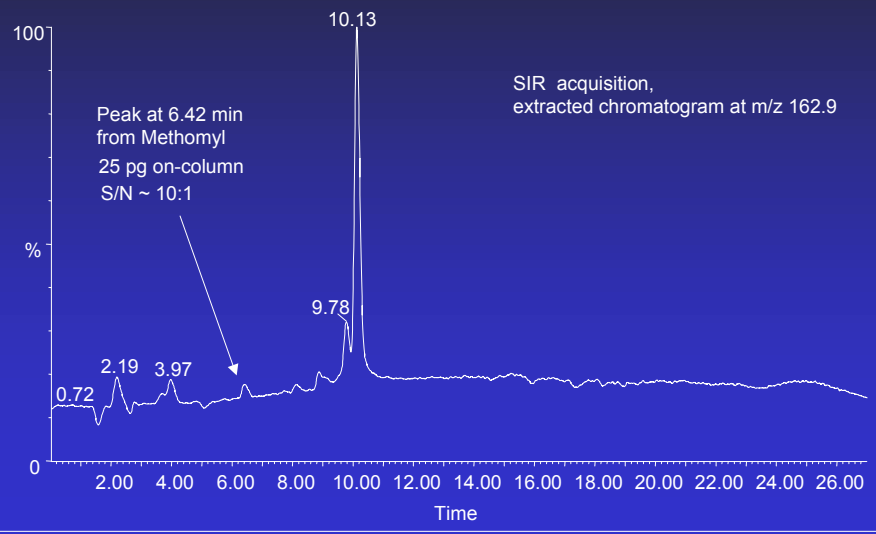
In a separate experiment, the chromatogram of three carbamates; methomyl, propoxur, and carbaryl, is displayed. They were analyzed by monitoring the seven fragment ions corresponding to the three compounds.

Characteristic Mass Spectral Behavior



Here is the corresponding mass spectra obtained from the three carbamates.

Detection of Methomyl in Orange Juice Extract, 8.5 ng/ml, 3 µl Injection



An orange juice extract was analyzed under the same conditions. Shown here is the extracted chromatogram at m/z 162.9 from the orange juice sample detecting methomyl at 8.5 ng/ml (25 pg on column) with excellent signal/noise ratio.

Conclusion

- LC/MS adds an additional dimension to on-line chromatographic analyses.
  - "Resolution" of co-eluting peaks
  - Detection of weak chromophores
- LC/MS enables you to perform qualitative and/or quantitative analyses.
  - SIR for sensitivity
  - CID for structural information

It should be pointed out that all of the preceding data was derived from a variety of experiments performed over several months in different laboratories. They represent 'real' results; no effort was made to produce "perfect" data. What is important is that, taken together, they all tell the same story, lab-to-lab, operator-to-operator, and day-to-day. Hopefully, the moral is clear:

First, LC/MS adds a third dimension to typical on-line separations. It allows you to "peek" under a chromatographic peak. As we saw, this allows the chemist a lot of leeway when it comes to methods development. As was shown, the mass spectrometer can 'resolve' co-eluting peaks, allowing significant reductions in run time. Response does not require a chromophoric or fluorescent group, so there is not need for derivatization, again, simplifying the analysis.

Second, LC/MS is unique in that it can provide very sensitive and specific quantitative information (when operated in the SIR mode), as well as qualitative, structural information (full-scan analysis provides molecular weight, CID provides structurally-significant fragment ions). Other analytical instruments are limited to one or the other.