

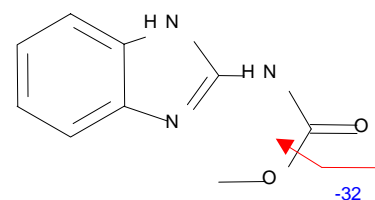
## Application of LC/Electrospray Ion Trap Mass Spectrometry for Identification and Quantification of Pesticides in Complex Matrices

### Introduction

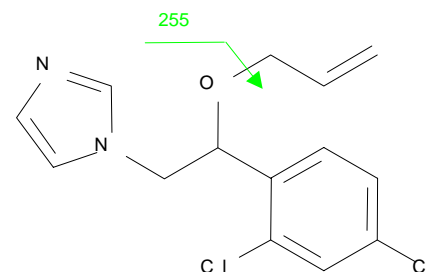
The simple monitoring of particular molecular ions during an LC/MS run is not sufficient for an unambiguous identification and quantification of pesticides in complex matrices, as for example in extracts of vegetables. One reason for this is that the limited dynamic range of any analysis system will not allow quantification of trace amounts in the presence of excess amounts of other components. Furthermore, coeluting compounds which form ions of the same  $m/z$ -value as the target compound will lead to wrong quantitation results.

Therefore, for quantitation of pesticides in complex matrices a method is needed that eliminates the influence of matrix compounds. Such a method can utilize the MS/MS capability of an ion trap system: during the LC-run all scans are performed in a so called „MS/MS-mode“. This means that after the accumulation step of the ion trap all ions which do not have a predefined  $m/z$ -value are ejected. Only ions of the (known)  $m/z$ -value of the target substance remain in the trap. In a second step these ions are fragmented and at last the fragment ions are scanned. If compound specific fragment ions are formed, an unambiguous distinction from matrix compounds and hence a quantification becomes possible. In cases where more than one fragment ion is formed, the high scanning speed of an ion trap system furthermore allows to scan **all fragments** so that loss of ion intensity and hence sensitivity is avoided (this is not possible to the same extent for triple quadrupole systems).

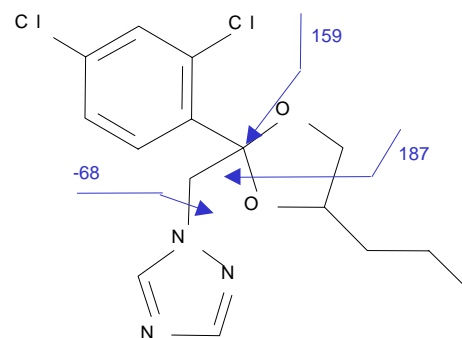
We describe an LC/ESI-MS/MS method with an ion trap mass spectrometer that allows for fast, selective and sensitive quantification of three pesticides (carbendazim, imazalil, propiconazole, **fig. 2**) in extracts from carrots and green beans. An additional LC-ESI-MS/MS method was developed to quantify a fourth pesticide (aldicarb, **fig. 2**) within the same extracts, which because of its chemical lability cannot be analyzed with the same method as the other pesticides.



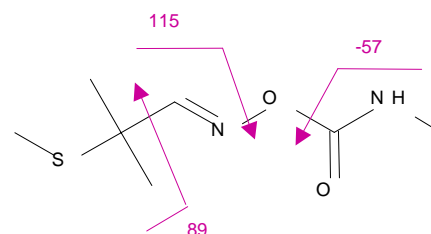
Carbendazim



Imazalil



Propiconazole

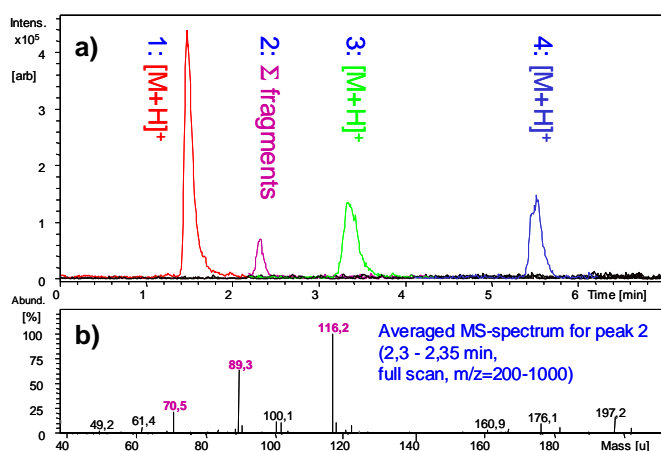


Aldicarb

**Fig. 1:** Structures of the pesticides investigated in this work, explanation of main fragments of MS/MS spectra shown in fig. 2.

## Preliminary Experiments

The simultaneous quantitation of all four pesticides is not possible. For imazalil the use of a buffer system is essential to avoid tailing and to obtain a peak form that allows for integration; under these conditions aldicarb does not form a sodium adduct, but gets protonated. The protonated form is less stable, so that only fragment ions but no  $[M+H]^+$  ions can be detected. A well-defined MS/MS experiment and hence a specific detection and quantitation of aldicarb is therefore prevented.



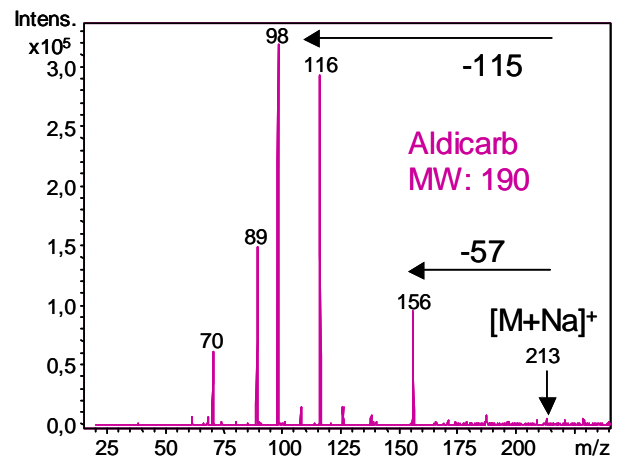
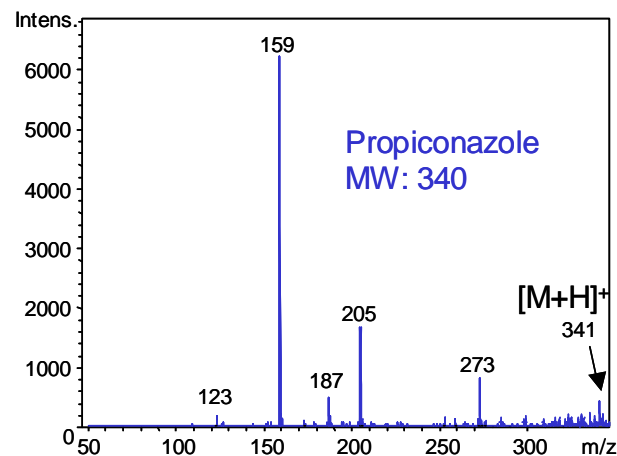
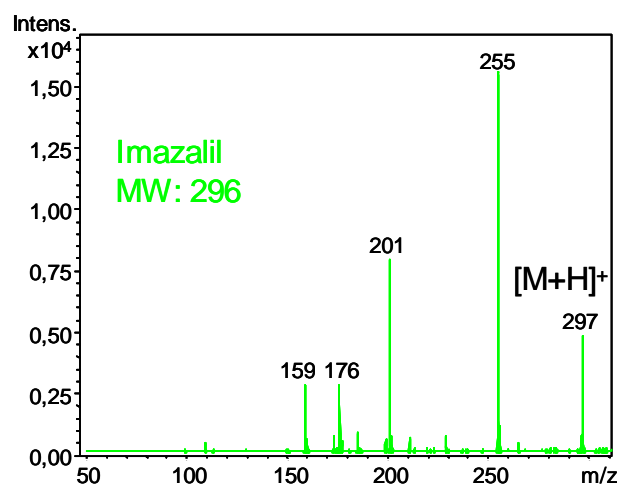
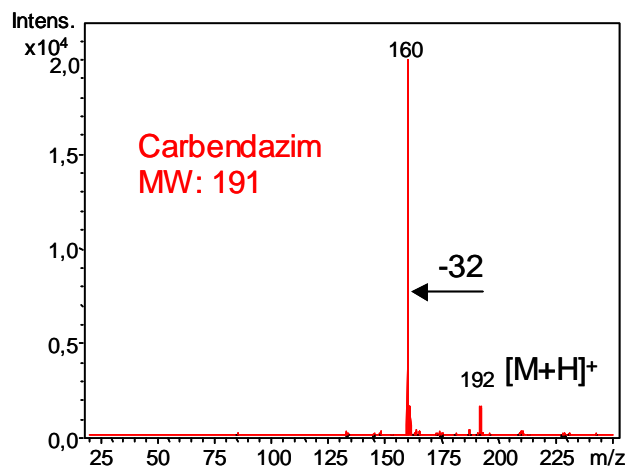
- 1: Carbendazim: ion trace m/z = 192
- 2: Aldicarb: sum trace m/z = 70 + 89 + 116
- 3: Imazalil: ion trace m/z = 297
- 4: Propiconazole: ion trace m/z = 341

**Fig. 3:**

a) LC/ESI-MS chromatogram of pesticide mix (standard solutions): with buffered eluent all compounds are detectable as  $[M+H]^+$ , except for aldicarb.

b) The averaged MS spectrum for peak 2 allows for identification of aldicarb:  $[M+H]^+$  gives the same fragments as  $[M+Na]^+$  (Fig. 2), of course except for those which contain sodium ( $m/z = 98, 156$ ).

**Fig. 2:** MS/MS spectra, obtained from direct infusion experiments. 4  $\mu$ l/min of pesticide solutions (Carbendazim 463  $\mu$ g/ $\mu$ l, Imazalil 52  $\mu$ g/ $\mu$ l, Propiconazole 67  $\mu$ g/ $\mu$ l, Aldicarb 40  $\mu$ g/ $\mu$ l) were combined with an LC flow of 0.2 ml/min 50:50 H<sub>2</sub>O/MeOH.



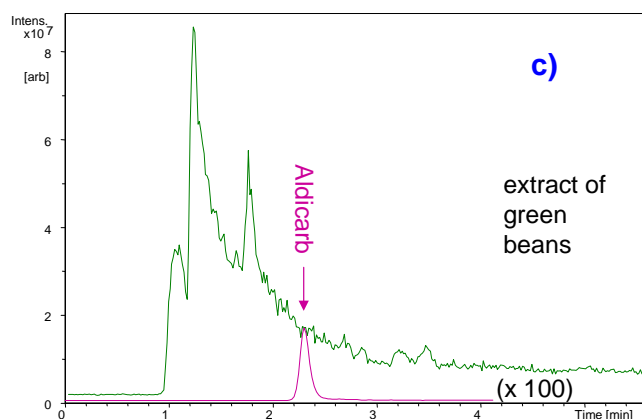
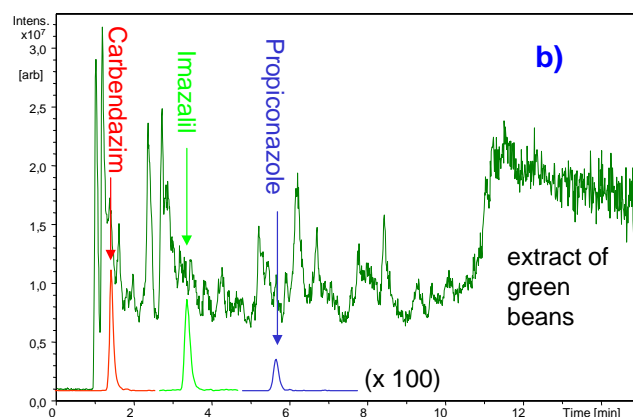
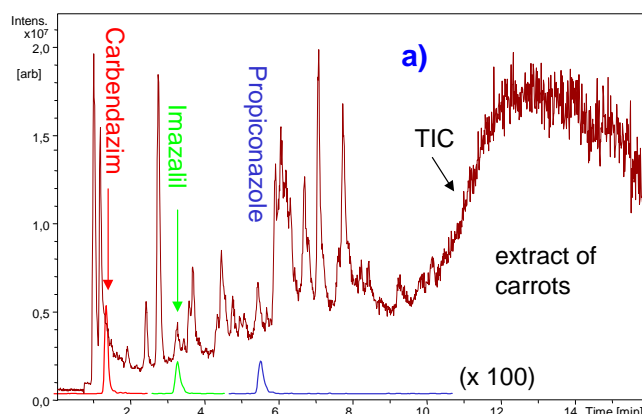
## Experimental Conditions

Matrix extracts from carrots and green beans were prepared following common test procedures for the analysis of pesticides. Blank matrix extracts were fortified with mixtures of pesticide standard solutions to get fortified levels of 5.0 / 20.0 / 50.0 pg/ $\mu$ l of each compound (example 1). An additional series of samples was prepared from the green bean extract. These samples were spiked with 0.1 / 0.5 / 2.5 pg/ $\mu$ l of each pesticide but without aldicarb (example 2). The calibration curves obtained this way were used to quantify test-samples with known concentrations (quantitation with external calibration).

HPLC conditions:	
<b>Instrument:</b>	HP 1100 HPLC system (with vacuum degasser, binary pump, autosampler, thermostatic column holder, variable wavelength detector, Agilent)
<b>Column:</b>	SAM OD-5-100 C <sub>18</sub> 5 $\mu$ m, 2.1 x 50mm (SMT)
<b>Flow rate:</b>	0,3 ml/min
<b>Temperature:</b>	40°C
<b>1) Analysis of carbendazim, imazalil and propiconazole:</b>	
<b>Mobile phase:</b>	A: water (10 mM NH <sub>4</sub> OAc + 0.1% (v/v) formic acid), B: acetonitrile
<b>Gradient:</b>	0 – 7 min: gradient from 30% B to 90% B (v/v) 7 – 10 min: gradient from 90% B to 100% B (v/v) > 10 min isocratic 100% B
<b>Injected vol.</b>	5 $\mu$ l
<b>2) Analysis of aldicarb:</b>	
<b>Additional sample treatment:</b>	To every sample a small amount of NaCl-solution was added (1 drop of 10 mM NaCl in water added to 500 $\mu$ l sample)
<b>Mobile phase:</b>	A: water, B: acetonitrile
<b>Gradient:</b>	0 – 25 min: gradient from 30% B to 100% B (v/v) > 25 min isocratic 100% B
<b>Injected vol.</b>	10 $\mu$ l
<b>MS conditions:</b>	
<b>Instrument:</b>	Esquire-LC Ion Trap LC/MS <sup>(n)</sup> system (Bruker Daltonik GmbH)
<b>Mode:</b>	ESI positive
<b>Dry gas:</b>	(N <sub>2</sub> ) 10 l/min
<b>MS/MS:</b>	CID (He)
<b>Scan:</b>	m/z = 40 - 500
<b>Nebulizer:</b>	22 psi
<b>Dry temp.:</b>	300°C
<b>Target analysis:</b>	
<b>1) Analysis of carbendazim, imazalil and propiconazole:</b>	
0,0 - 2,5 min:	MS/MS 192: [M+H] of carbendazim; quantitation: ion trace m/z = 160
2,5 - 4,5 min:	MS/MS 297: [M+H] of imazalil; quantitation: sum trace m/z = 255+201+176+159
> 4,5 min:	MS/MS 342: [M+H] of propiconazole; quantitation: sum trace m/z = 205+159
<b>2) Analysis of aldicarb:</b>	
MS/MS 213: [M+Na] of aldicarb; quantitation: sum trace m/z=156+116+98+89	

## Quantitation Results

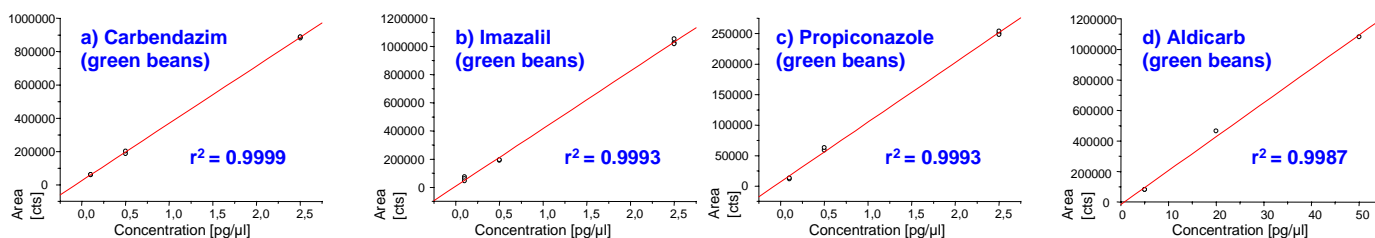
Whereas from the chromatograms obtained in full scan mode (fig.4, TIC) due to the excess of matrix none of the compounds could be detected as [M+H] or [M+Na] respectively, the target analysis (fig.4) allows to obtain clear and integrable peaks for each pesticide. Their peaks are shown amplified by a factor of 100.



- Total Ion Current (full scan: m/z = 50 - 600)
- Ion trace of m/z = 160 (MS/MS of m/z = 192)
- Sum trace of m/z = 255+201+176+159 (MS/MS of m/z = 297)
- Sum trace of m/z = 205+159 (MS/MS of m/z = 342)
- Sum trace of m/z = 156+116+98+89 (MS/MS of m/z = 213)

**Fig. 4:** LC/ESI-MS/MS chromatograms of spiked extracts:

- a) Carrots, target analysis of carbendazim, imazalil and propiconazole.
- b) Green beans, target analysis of carbendazim, imazalil and propiconazole.
- c) Green beans, target analysis of aldicarb



**Fig. 5:** Calibration curve examples (green bean extract): **5a-c)** Carbendazim, imazalil and propiconazole (conc. 0.1 - 2.5 pg/μl; 2 or 3 injections for each level) **5d)** Aldicarb (conc. 5 - 50 pg/μl, 2 injections for lowest level).

Good linearities were found for all substances in both matrices and for both concentration ranges (fig. 5, table 2 and 3). The limit of quantitation is determined to be in the range of only a few picogram (absolute amount) or in the range of 0.2 pg/μl in case of carbendazim, imazalil and propiconazole. Aldicarb was not examined in the lower concentration range, but at least a limit of about 5 pg/μl is achievable.

**Table 1:** Detection limits of some pesticides (all in matrix)

<b>Aldicarb:</b>	1 ng abs. (APCI-MS)	[1]
	25 pg/μl (APCI-MS)	[2]
	<b>5 pg/μl (ESI-MS/MS)</b>	<b>this work</b>
<b>Carbendazim:</b>	3 pg/μl (APCI-MS)	[2]
	<b>0.2 pg/μl (ESI-MS/MS)</b>	<b>this work</b>

**Table 2:** Quantitation results and correlation coefficients  $r$  of calibration curves, **example 1:** Extract of carrots and green beans spiked with 5 - 50 pg/μl (all pesticides)

Sample:	Carbendazim			Imazalil			Propiconazole			Aldicarb		
	real conc. [pg/μl]	found [pg/μl]	% dev.	real conc. [pg/μl]	found [pg/μl]	% dev.	real conc. [pg/μl]	found [pg/μl]	% dev.	real conc. [pg/μl]	found [pg/μl]	% dev.
<b>Carrot:</b>												
1	7.5	6.9	-8.0	20	21	5.0	50	56.6	13.2	12.5	10	20
2	blank	n.d.	0	7.5	7.1	-5.3	10	9.7	-3	4	3	25
3	20	17.9	-10.5	10	8.7	-13.0	25	24.2	-3	7.5	6	20
4	10	10.8	8.0	55	56.6	2.9	blank	n.d.	0	blank	n.d.	0
<b>Corr. coeff.</b>	0.9982			0.9998			0.9998			0.9984		
<b>Green Beans:</b>												
1	40	43.4	8.5	12.5	12.3	-1.6	5	6.7	34	20	18	10
2	6	5.4	-10	blank	n.d.	0	15	14.7	-2	40	36	10
3	blank	n.d.	0	20	20.7	3.5	blank	n.d.	0	10	10	0
4	12.5	13.7	9.6	8	7.7	-3.8	blank	n.d.	0	8	7	12.5
<b>Corr. coeff.</b>	0.9997			0.9994			0.9991			0.9987		

**Table 3:** Quantitation results and correlation coefficients  $r$  of calibration curves, **example 2:** Extract of green beans spiked with 0.1 - 2.5 pg/μl (without aldicarb).

Sample:	Carbendazim			Imazalil			Propiconazole		
	real conc. [pg/μl]	found [pg/μl]	% dev.	real conc. [pg/μl]	found [pg/μl]	% dev.	real conc. [pg/μl]	found [pg/μl]	% dev.
1	0.8	0.7/0.7	-12.5	2.0	1.9/2.0	-5/0	1.0	1.3/0.9	30/-10
2	2.4	2.4/2.4	0	1.2	1.2/1.3	0/7.7	0.96	0.9/1.1	6.2/15
3	0.64	0.7/0.5	9.4/-22	0.64	0.5/0.6	-22/6.2	2.4	2.6/2.7	8.3/13
4	0.2	0.2/0.2	0	0.2	0.2/0.2	0	0.2	n.d.	-100
<b>Corr. coeff.</b>	0.9999			0.9993			0.9993		

- No „false positive“ results.
- Deviation between real and found concentrations in most cases lower than 5 - 10%, good quantitation results even for low concentration range.
- Larger deviations in most cases are found for lower concentrations, therefore not critical.

## Conclusion

This work clearly shows that quantification in complex matrices can not only be done with triple quadrupole mass spectrometers but also with ion trap systems.

LC/ESI-MS/MS analysis with an ion trap system allows to use one or even multiple compound-specific fragment ions simultaneously for quantitation (examples: aldicarb, imazalil). In this way, the fast, specific and sensitive quantification of pesticides even in complex matrices is possible.

Quantitation limits are only few pg absolute amounts (or 0.2 pg/ $\mu$ l for a 10  $\mu$ l injection) for carbendazim, imazalil and propiconazole. For aldicarb a quantitation limit of about 5 pg/ $\mu$ l was found.

## References

- [1] Niessen, W.M.A., Liquid Chromatography - Mass Spectrometry (1995) New York, ch. 12.
- [2] Barnes, K. A.; Fussell, R. J.; Startin, J. R.; Pegg, M. K.; Thorpe, S. A.; Reynolds, S. L., Rapid Commun. Mass Spectrom., 11 (1997) 117 - 123.

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