

# Multi-Target Screening of up to 650 Pesticides in a Single LC/MS Run by Exact Mass Ion Traces

● IMSC 2009, Poster PWA 34

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## Introduction

Conventional multitarget screening methods for monitoring of contaminants like pesticides in food are usually based on triple quadrupole mass spectrometers (LC/QqQ-MS).

### Advantages of LC/QqQ-MS:

- + high specificity/selectivity
- + low detection limits
- + quantification in complex matrix samples

### Limitations:

- number of parallel MRM transitions
- standards required for MRM method development
- detection limits sacrificed by increasing target numbers
- targeted detection => system blind for anything unexpected
- No retrospective data analysis for unexpected compounds possible

Emerging **alternative technique**: screening with modern **Time-of-Flight Mass Spectrometers, LC/(Qq-)TOF-MS:**

### Advantages compared to LC/QqQ-MS:

- + **"unlimited" number of targets** (> 1000 pesticides known, roughly half of them suitable for LC/MS analysis)
- + **retrospective analysis, evaluation of unknowns**
- + detection limits independent of target compound number
- + **standard availability no essential prerequisite**
- + additional statistical processing/profiling is possible

**Technical keyfeatures** of ESI-TOF-MS systems, enabling for multitarget screening:

- **mass accuracy, high mass resolution** allowing for EIC traces with narrow mass windows (high resolution EIC, hrEIC, Fig. 1)
- **selectivity**
- **true isotope pattern** (TIP™, SigmaFit™) => **confirmation**
- **stability of mass accuracy** over time, MS signal intensity and *m/z* range
- **MS/MS ability** (ISCID, MRM) => **additional confirmation** (ISCID: In-Source Collision-Induced-Dissociation)

## Intention of this work

• **Buildup of a comprehensive database for ESI-TOF-MS based screening:** detailed knowledge about **retention time, adduct and in-source-fragment formation** helps to avoid wrong assignments and false positive results, if complex real life samples shall be screened for hundreds of pesticides simultaneously. Additional knowledge about **fragmentation under ESI-TOF-MS conditions** provides for additional result confirmation in case of ambiguous findings.

A large set of **~ 650 individual pesticide standards** was run and evaluated under ESI conditions in both polarities. Two **different LC gradients** were used, which were optimized for best compound distribution over the complete chromatogram:  
(A) optimized for **high throughput**, complete pesticide elution after 5 min, cycle time 10 min  
(B) optimized for **enhanced separation** of isobaric pesticides, complete pesticide elution after 15 min, cycle time 20 min

**Information collected for each individual pesticide:** sum formula, name, CAS RN, retention time, observed adducts ([M+H]<sup>+</sup>, [M+NH4]<sup>+</sup>, [M+Na]<sup>+</sup>, [M+HCOO]<sup>-</sup>, [M+Cl]<sup>-</sup>...), fragments of significant intensity both under fulvician and ISCID conditions, fulvician and ISCID spectrum.  
• **Practicability test of such a database for pesticide screening in different real life samples:** The database was applied to **seven different food extract samples**, which previously had been evaluated with conventional techniques (GC/MS, GC/TOF-MS, LC/MS/MS) on QqQ systems) in the **CVUA Stuttgart**.

## Methods

**HPLC:** Ultimate 3000 Rapid Separation LC ("RSLC", Dionex)

**Column:** Accellam RSLC C18 2.1 x100 mm, 2.2 µm (Dionex)

**Mobile phase:** A= H<sub>2</sub>O/MeOH 90/10 (1mM NH<sub>4</sub>format, 0.002% HCOOH)

B= MeOH (1mM NH<sub>4</sub>format, 0.002% HCOOH).

**Gradient:** Multistep gradients: (A) 99-0.1% A @14 min, cycle time 20 min

(B) 95 - 0.1% A @4 min, cycle time 10 min

**Flow rate:** (A) flow gradient 0.2-0.48 ml/min, (B) 0.48 ml/min.

**Injection:** 1 µl

**Samples:** ~ 650 individual pesticide standards (~500 µg/µl), multi compound mix of all 650 pesticides (~100 µg/µl)

**Extract samples:** lamb's lettuce, parsley, sweet pepper (2x), endive, strawberry, pomelo (QueChERS extraction method).

Standards were obtained from LGC Standards (UK), ~150 standards and extract samples were kindly provided by CVUA Stuttgart (Germany)

**TOF-MS:** micrOTOF™ orthogonal ESI-TOF-MS (Bruker Daltonik GmbH).

**Calibration:** external, sodium formate/acetate mixed clusters injected at the beginning of each chromatographic run.

**Mass accuracy specs:** 5 ppm for external calibration.

**Ionization:** ESI(+), ESI(-) Scan range: 30-1000 m/z.

**Scan mode:** ISCID: alternating acquisition of fulvician- and ISCID spectra.

**Data Evaluation:** TargetAnalysis™ 1.2 (Bruker Daltonik GmbH), using a retention time/ sum formula database. Compound detection on hrEICs, confirmation and result rating based on retention time (optional, no prerequisite), mass accuracy and isotope pattern (SigmaFit™).

Rating parameters set here: low/high level boundaries 0.05/0.1 min, 3 ppm/ 5 ppm, sigma 0.5/0.1 (= good/bad isotope pattern match). For MS data acquired in ISCID mode additional result confirmation is performed based on qualifier ions, which optionally can be specified in the TA database. A potential result will be removed from the result list, if a given qualifier ion can not be detected in the corresponding ISCID spectrum. Data processing can be done in automated way after end of acquisition, typical processing times are <1 min.

**Database example part:**

m/z	ESI(+)	RT	formula	name	CAS	Qualifier 1	Qualifier 2
226	1339	7.59	C14H15NO	Cyprodinil	121550-61-2		
406	07	19.73	C11H17ClN2O3	Difenconazole Peak 1	119446-68-3	251.002497	337.029276
406	07	19.73	C11H17ClN2O3	Difenconazole Peak 2	119446-68-3	251.002497	337.029276
302	09	31.19	C8H11N2O4P3	Metolachlor	990-37-8	85.039639	145.006626
319	09	36.81	C9H11N2O4P3	Metolachlor (NH4)	990-37-8	85.039639	145.006626
143	08	25.82	C14H14N2O	Oxamyl	990-37-8	85.039639	145.006626
220	07	20.38	C7H11N3O3S	Oxamyl Fragment 145	23135-22-0	72.04439	
72	04	30.04	C3H7NO	Oxamyl Fragment 72	23135-22-0	72.04439	
90	06	36.65	C3H7NO2	Oxamyl Fragment 90	23135-22-0	72.04439	
237	10	10.87	C7H11N3O3S	Oxamyl (NH4)	23135-22-0	72.04439	
296	11	03.11	C14H18ClN2O2	Treatment	55219-65-3	318.039756	
318	09	17.76	C14H18ClN2O2	Treatment (Na)	55219-65-3	318.039756	

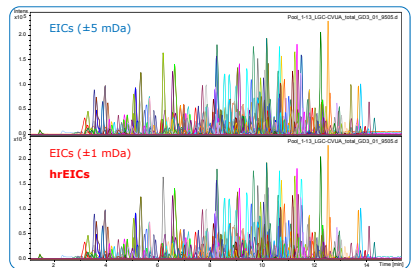


Fig. 1: Multi compound standard of 650 pesticides (100µg/µl), gradient (A): Compound EICs different mass windows (5, 1 mDa). Even for 1 mDa-hrEICs the chromatogram is unchanged! => mass accuracy is stable over full MS intensity range!

## Results

### Multicomponent standard (Fig. 1 – 3):

• **Average mass accuracy** for the 650 compound standard for three consecutive runs: **1.17, 1.32, 1.25 ppm** (~±0.7 mDa, obvious outliers due to unresolved MS signals from coeluting compounds are excluded), > **90%** of the signals have mass accuracy better than **3 ppm**.

• Masses are stable over the time of every chromatographic run. Mass shifts between calibration spectra from start/end of a chromatogram are << 1 mDa. **Just one external calibration (with a calibrant spectrum within deadline of the LC run) is sufficient to handle the complete m/z-, intensity- and time range of each analysis.** This setup even works for the gradient (B) with high flowrate and short deadline. No additional lockmass calibrant is necessary, thus avoiding additional interferences and suppression effects.

• For the vast majority of pesticides **good ISCID spectra** can be obtained, allowing for additional confirmation in case of ambiguous findings (bad mass accuracy or sigma ratings due to background interference). The intensity of ISCID fragments often is comparable or even higher than the intensity of the precursor ion, so that ISCID confirmation works well even at low intensity levels.

### Food extract samples (Table 1, 2, Fig. 4 – 6):

• The ESI-TOF-MS findings are almost identical with those resulting from conventional techniques (GC/MS plus LC/QqQ-MS/MS). Only two GC/MS compounds, which do not have a significant response under ESI conditions are missed (pentachloranilin, chlorthalonil) as well as Fenbutatin-oxil, which needs a specific (LC) method.

• The results cover a wide range of compounds and concentrations. Especially at low concentrations background interferences can cause higher mass errors or bad isotope patterns resulting in "++" ratings. In these cases the simultaneous acquisition of ISCID spectra has proven to be a good tool for alternative compound confirmation. Almost all findings could successfully be confirmed with help of their corresponding qualifier ions, only for cyprodinil no qualifier ion could be defined.

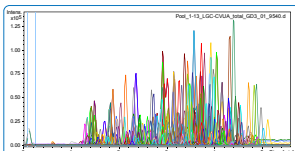


Fig. 2: Multi compound standard of 650 pesticides, gradient (B): overlaid compound EICs.

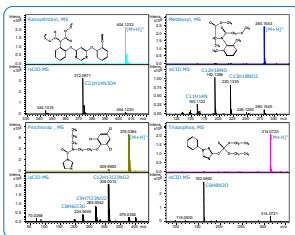


Fig. 3: Fulvician and ISCID example spectra for selected pesticide standards

### Table 1: TargetAnalysis results for extract samples:

Sample	Pesticide	Retention Time (min)	Mass Accuracy (ppm)	Isotope Pattern (SigmaFit)	ISCID (SigmaFit)	Qualifier Ion
lamb's lettuce:	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
parsley:	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
sweet pepper (1):	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
sweet pepper (2):	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
endive:	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
strawberry:	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
pomelo:	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min
	Chlorpyrifos	11.23	1.1	++	++	Chlorpyrifos 12.66 min

Result ratings: ++ very good, + good, - bad, -+ bigger deviation for RT, mass or sigma, result needs additional confirmation

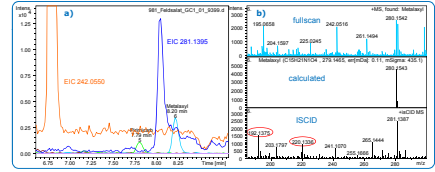


Fig. 4: Chromatogram and Metaxaly spectra for lamb's lettuce sample: background masses at m/z 251 and 242 interfere with the compound isotope pattern of metaxaly and pirimicarb, leading to bad sigma ratings in the TA result table. The ISCID spectrum confirms the presence of Metaxaly.

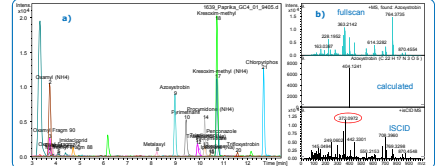


Fig. 5: Chromatogram and Azoxytrobin spectra for sweet pepper sample (2): (a) compound EICs traces, (b) the ISCID spectrum confirms the presence of Azoxytrobin.

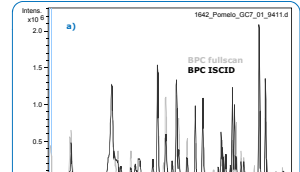


Fig. 6: Pomelo extract: BasePeakChromatogram, giving an impression about matrix load (a) and detected compound EIC traces (b).

## Conclusions

- A database for **~650 pesticides** with retention times and detailed ionization and ISCID fragmentation behavior is available
- The database is **successfully applied** for fast and fully automated screening for hundreds of target compounds ("unlimited" number) in various food extract samples and with good sensitivity
- Screening results are comparable to those of conventional screening techniques.
- Qualifier ions can be obtained in **ISCID mode** within the same LC run and serve for **supplementary confirmation** in case of isobaric interferences.
- Additional potential: **retrospective analysis, evaluation of unknowns or unexpected effects.**



**ESI-(Q-)TOF-MS technology is a promising new technique for multi-target screening applications**

### Acknowledgment

Many thanks to Ellen Scherbaum and Eberhard Schüle (CVUA Stuttgart, Germany) for providing standards and food extracts samples with their findings!