Poster Note PN-19

Pesticide Target Screening with GC-APCI coupled to high-resolution QTOF-MS

Introduction

Pesticides are frequently found as contaminations in food and environmental matrices. They commonly are analyzed by GC-MS or LC-MS. Both are complementary, "orthogonal" methods, comprising in total >1100 known pesticides. While they overlap in scope, each method alone covers exclusively a certain range of pesticides: GC-MS is more common for semi-volatile compounds, LC-MS is favorable for polar and thermo-labile pesticides.

Full scan accurate mass screening with atmospheric pressure inlet LC-MS has become increasingly popular in recent years. It is able to cover hundreds of target compounds in a single run and additionally enables the identification of unknowns and retrospective analysis. Target compounds are identified by their retention time, mass accuracy and isotope pattern. Reliability of identification is improved by using diagnostic ions generated by broadband CID alternating with full scan data acquisition. Diagnostic ions are valuable in complex matrices as they support the differentiation of target analytes signals from the matrix background.
Here we describe for the first time the application of bbCID data acquisition for pesticide target screening by coupling a GC to an atmospheric pressure chemical ionization source (GC-APCI) and a high resolution QTOF-MS.

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Keywords	Instrumentation and Software
GC-APCI	impact II
QTOF-MS	456-GC with PAL Combi-xt Autoinjector coupled to
Pesticide	GC-APCI II source
Screening	DataAnalysis 4.3
Accurate mass	TASQ 1.0

Methods

For this GC-APCI-MS screening study a mix of 60 representative pesticides was chosen with regard to their relevance to routine screening. The mix was diluted in dichloromethane to appropriate concentrations for the generation of calibration curves between 0.01 ng/ml (0.01 pg/µl) and 1000 ng/ml. Samples were prepared by spiking 10 pg and 50 pg of the pesticide mix into 1 ml QuEChERS extracts of orange, peach and tomato.
For all analyses 2 µl of each sample were injected into the GC. GC-MS analysis was performed using a 450-GC with PAL Combi-xt Autoinjector coupled with a GC-APCI II

Base Peak Chromatogram and Extracted Ion Chromatograms of Analytes

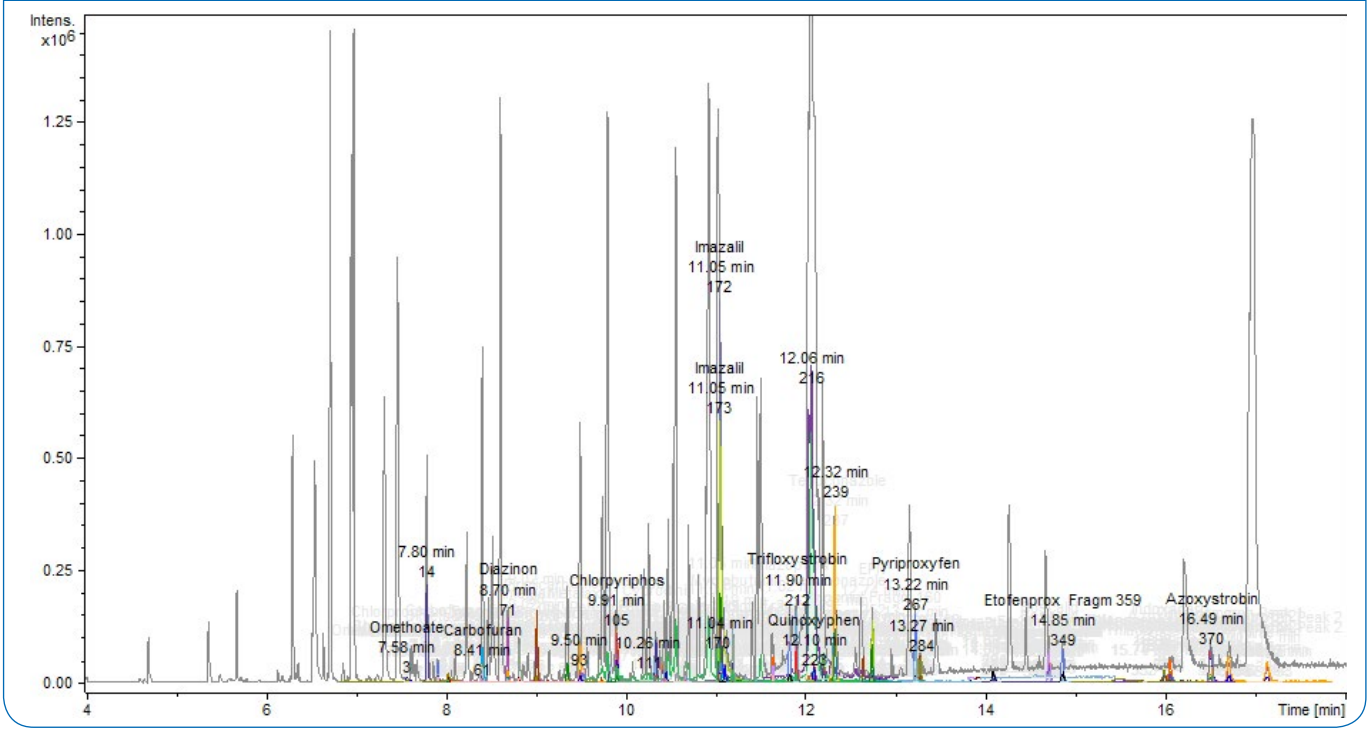

Figure 1: Base Peak (grey BPC) and Extracted Ion Chromatograms (EIC) of 48 pesticides in peach QuEChERS extract (50 ng/ml each): BPC shows a complex peak pattern of non-target matrix substances.

BPC and EICs at 13.2 min

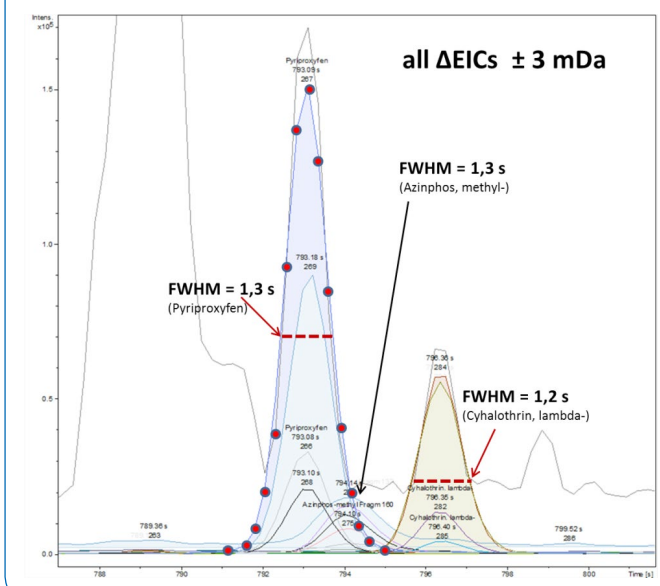

Figure 2: Excerpt of BPC (grey) and EICs at 13.2 min, EIC peaks with symmetric and sharp peak shapes with typical FWHM of 1.2 to 1.4 s. Peak shapes are well described when using scan rates ≥8 Hz.

source to an impact II QTOF mass spectrometer (all Bruker Daltonics). The GC was operated with a 30 m Rxi-5ms capillary column (0.25 mm ID, 0.25 µm film thickness), operated at 1.2 ml/min constant helium flow and a GC oven temperature program at 70°C (1 min) - 25°C/min - 180°C - 15°C/min - 300°C (8.1 min). Pulsed splitless injection was at 280°C (40 psi for 0.25 min, 1 min splittless time).

MS Data were acquired from 50 - 1000 m/z in alternating full scan and bbCID acquisition mode at 8 spectra per second operated in the positive ionization mode.
All spectra were calibrated using PFTBA as external calibration gas injected automatically into the APCI source at the beginning of each MS run. Data were evaluated using DataAnalysis (Vers. 4.3) and TASQ (Vers. 1.0) software for target analysis and quantification (Bruker Daltonics). TASQ data processing criteria are: ΔRT 0.7 min, EIC width ±3 mDa of $M^{\cdot+}$, $[M+H]^+$, $[M+H+2]^+$ and fragment ions.

Table 1: Analytical results for 48 Pesticides sorted according to LOQ

Analyte	RT [min]	LOD [ng/mL]	Working range	R^2 Linearity	Quant m/z [Da]	Ion type	Δm/z [ppm]	Δm/z [mDa]
Pyriproxyfen	13.20	0.1	0.5-500	0.9985	322.1438	M+H	0.61	0.20
Quinoxyphen	12.08	0.5	0.5-500	0.9961	308.0040	M+H	0.81	0.25
Azoxystrobin	16.47	0.5	1-500	0.9987	404.1241	M+H	0.63	0.25
EPN	12.72	0.5	1-500	0.9979	324.0454	M+H	0.55	0.18
Myclobutanil	11.18	0.5	1-500	0.9988	289.1215	M+H	0.62	0.18
Trifloxystrobin	11.88	0.5	1-500	0.9994	409.1370	M+H	0.55	0.22
Chlorpyriphos	9.89	0.5	1-1000	0.9938	351.9306	M+H+2	0.61	0.22
Pendimethalin	10.30	0.5	1-1000	0.9974	212.0666	Fragment	0.41	0.09
Metalaxyl	9.48	0.5	5-500	0.9970	280.1543	M+H	0.54	0.15
Pirimicarb	9.00	0.5	5-500	0.9967	239.1503	M+H	0.53	0.13
Triazophos	11.80	0.5	5-500	0.9985	314.0723	M+H	0.69	0.22
Boscalid	14.68	1	5-500	0.9991	343.0399	M+H	1.13	0.39
Cyhalothrin. lambda-	13.24	1	5-500	0.9962	225.0289	Fragment	0.61	0.14
Cyprodinil	10.32	1	5-500	0.9965	226.1339	M+H	0.97	0.22
Diazinon	8.68	1	5-500	0.9995	305.1083	M+H	0.44	0.14
Fludioxonil	11.01	1	5-500	0.9938	248.0392	M^+	0.48	0.12
Propargite	12.28	1	5-500	0.9971	350.1546	M^+	0.54	0.22
Chlorpyriphos-methyl	9.34	1	5-1000	0.9936	323.8993	M+H+2	0.52	0.17
Dimethomorph Peak 1	16.67	1	5-1000	0.9949	388.1310	M+H	1.16	0.45
Dimethomorph Peak 2	17.09	1	5-1000	0.9974	388.1310	M+H	1.25	0.48
Ethion	11.62	1	5-1000	0.9975	384.9949	M+H	0.52	0.20
Indoxacarb	16.02	1	5-1000	0.9979	528.0780	M+H	0.67	0.36
Penconazole	10.40	1	5-1000	0.9971	284.0716	M+H	0.42	0.12
Phosmet	12.71	1	5-1000	0.9973	160.0393	Fragment	0.69	0.11
Profenophos	11.09	1	5-1000	0.9972	374.9402	M+H+2	0.96	0.36
Tebuconazole	12.31	1	5-1000	0.9968	308.1524	M+H	0.44	0.13
Tolyfluanid	10.44	5	5-1000	0.9898	237.9655	Fragment	0.51	0.12
Kresoxim-methyl	11.18	5	5-500	0.9885	206.0812	Fragment	1.86	0.38
Etofenprox	14.83	5	10-500	0.9993	359.2006	Fragment	0.44	0.16
Carbofuran	8.40	5	10-1000	0.9915	222.1125	M+H	0.79	0.17
Difenoconazole Peak 1	15.96	5	10-1000	0.9958	406.0720	M+H	0.57	0.23
Difenoconazole Peak 2	16.03	5	10-1000	0.9932	406.0720	M+H	0.57	0.23
Fenhexamid	12.18	5	10-1000	0.9982	302.0709	M+H	0.79	0.24
Prochloraz	14.06	5	10-1000	0.9959	376.0381	M+H	0.41	0.16
Pyrimethanil	8.81	1	50-500	0.9972	200.1182	M+H	0.69	0.14
Carbendazim	10.30	10	50-500	0.9883	191.0689	M^+	1.18	0.23
Chlorpropham	7.91	10	50-500	0.9955	213.0551	M^+	0.92	0.20
Bifenthrin	12.63	1	50-1000	0.9906	181.1012	Fragment	0.68	0.12
Azinphos-methyl	13.22	5	50-1000	0.9985	132.0444	Fragment	1.01	0.13
Dimethoate	8.39	5	50-1000	0.9973	230.0069	M+H	0.62	0.14
Carbaryl	9.51	10	50-1000	0.9948	145.0648	Fragment	0.82	0.12
Cypermethrin I	14.54	10	50-1000	0.9971	416.0815	M+H	1.07	0.44
Cypermethrin II	14.62	10	50-1000	0.9940	416.0815	M+H	0.77	0.32
Cypermethrin III	14.69	10	50-1000	0.9927	416.0815	M+H	0.73	0.30
Imazalil	11.07	10	50-1000	0.9958	297.0556	M+H	0.54	0.16
Linuron	9.81	10	50-1000	0.9935	249.0192	M+H	0.53	0.13
Thiacloprid	15.70	10	50-1000	0.9969	253.0309	M+H	0.33	0.08
Triadimenol I	10.55	10	50-1000	0.9805	296.1160	M+H	0.76	0.21
Triadimenol II	10.65	10	50-1000	0.9982	296.1160	M+H	0.67	0.20
Monocrotophos	8.03	50	50-1000	0.9957	193.0260	Fragment	0.33	0.06
Omethoate	7.59	50	50-1000	0.9675	214.0297	M+H	0.52	0.11
Thiabendazole	10.80	50	50-1000	0.9928	202.0433	M+H	0.64	0.13
Acetamiprid	12.67	<50	50-1000	0.9977	126.0105	Fragment	0.50	0.06

Results

The purpose of this study was to evaluate the applicability of GC-APCI-QTOF-MS for pesticide target screening using the concepts performed in LC-MS, i.e. mass spectra were acquired using full and alternating bbCID scan.

(1) In a first step a fast GC program for the set of 60 pesticides was optimized.

(2) 48 of the 60 pesticides were assigned by retention time and accurate monoisotopic masses of the ions (which is an acceptable result because of the varieties of pesticide properties as polarity, volatility, etc.).

Typical BPC plus EICs of 50 ng pesticides in 1 mL peach matrix sample are shown in fig.1. Most GC peaks are very sharp, e.g. 79% show Full Width at Half peak Maximum (FWHM) below 2 s and 95% below 3 s FWHM. Therefore fast scan rates ≥8 Hz are very important to generate enough MS data points and describe peaks accurately and reproducibly. Although some GC peaks are overlaying, they are separated by mass. An excerpt of EICs at RT 13.2 min is shown in fig. 2 where MS data points are drawn for the $[M+H]^+$ ion (322.1442 Da) of Pyriproxyfen.

(3) In the third step master list files for TASQ import were created for all target pesticides containing retention time, accurate monoisotopic mass of quantification and diagnostic ions, type of ion, concentration of calibration standards. A summary of TASQ quantification results is shown in table 1. Typical LODs are between 0.1 and 50 ng/ml for GC peaks with low response. Linear range of quantification typically was 2 orders of magnitude or higher between 1 - 1000 ng/ml, while some analytes with very good GC response show 0.5 - 500 ng/ml. Good R^2 linearity values with >0.99 are significant for nearly all 48 pesticides.

Table 1 also shows mass accuracy data for each calibrant averaged over all concentrations: while mass accuracy for all calibrants is below 0.4 mDa (w/o Dimethomorph), 76% of all calibrants are below 1 ppm. Average mass accuracy over all calibration runs is 0.78 ppm and 0.88 ppm when the matrix sample runs are also included. Six pesticides show accuracies at ca. 1 ppm related to low response or noisy peaks, esp. for low concentration calibrant samples. Carbendiazim, Kresoxim and Boscalid are co-eluting with other pesticides. Following fig. 3 we observe only minor matrix effects in GC-APCI pesticide target screening, even if samples include substantial amounts of matrix compounds. These positive results are supported by excellent GC resolution separating analytes from matrix signals.
In summary this study has demonstrated the potential of pesticide screening with GC-APCI.

Comparison of Mass Accuracy of Calibrant and Matrix

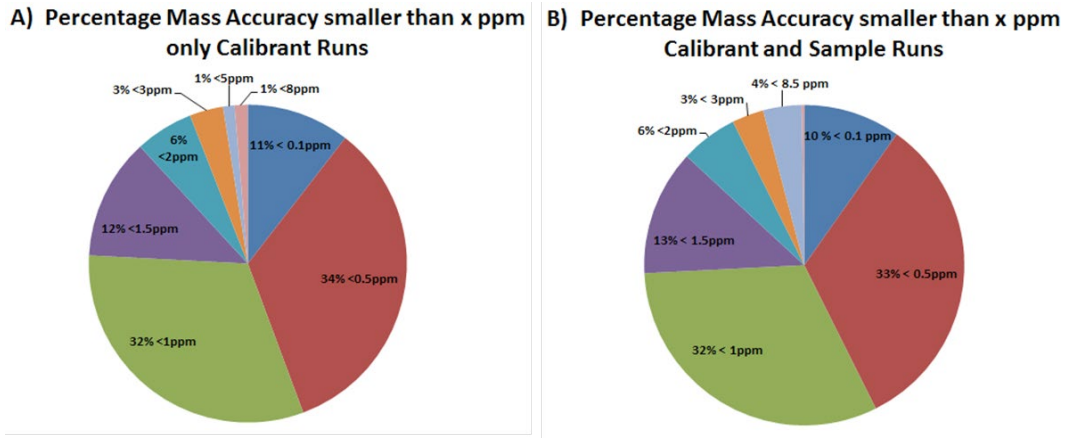

Figure 3: Comparison of mass accuracy over all EICs (±3 mDa): a) only for calibrant runs, b) for calibrant + matrix sample runs. For both groups nearly identical results are observed below 3 ppm (98% and 96%), even if matrix sample runs are included. For latter group a slightly increased % of mass deviations <8.5 ppm are observed (4% over 2% for only calibrant runs).

Calibration Curve of Indoxacarb

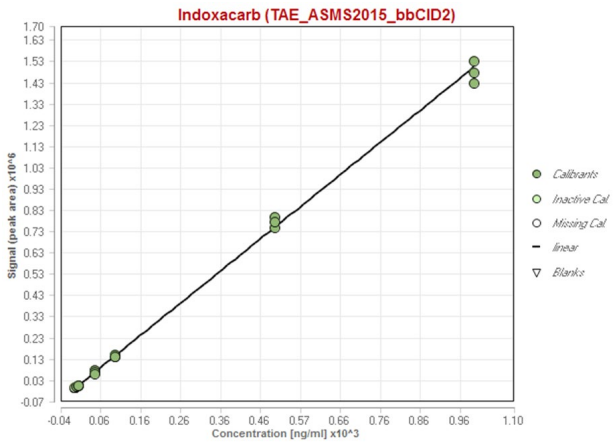

Figure 4: Representative calibration curve of Indoxacarb: LOQ 5 pg/µl, linearity 5–1000 pg/µl, R^2= 0.9979

Conclusion

- GC-APCI-QTOF-MS was applied for pesticide target screening with alternating full scan and bbCID MS data acquisition.
- Fast data acquisition ≥8 Hz is necessary for reliable quantitation of matrix samples
- Linearities are R^2 >0.99 between 1-1000 ng/ml
- All data were acquired with automated external PFTBA mass calibration
- Average mass accuracies of calibrant runs are <0.8 ppm including matrix sample runs <0.9 ppm or <0.4 mDa indicating only minor influence of matrix to pesticide screening results

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