Screening in Routine Pesticide Residue Analysis - How Useful is ToF?

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INTRODUCTION

Governmental laboratories responsible for pesticide residue analysis have the duty to monitor whether or not food samples from the local market comply with legislation to ensure consumer protection. 502 entries of pesticide residue definitions are listed in the MRL-database of the Commission. Beyond that, a general MRL of 0.01 mg/kg is set for all pesticides not listed in the annexes of EC 396/2005. The Pesticide Manual (Editor CDS Tomlin, BCPC) lists more than 1500 pesticides, but not all of them are relevant in terms of residues in food and feed. Therefore, effort is put in establishing priority lists, focusing on important pesticides which are currently in use and relevant in terms of residue occurrence. One of these activities is the "Check your scope" tool of the EU-RL-SRM.

But criminal activities also exist in the area of agriculture and every now and then illegal substances are found, more or less by chance. These, if at all on a priority list, are attributed with low priority, because under "normal" circumstances they are not to be expected.

SCREENING AN OPTION?

How can we deal with this? In recent years developments in analytical instrumentation have been very promising and, in combination with powerful multi-residue methods (MRM) like QuEChERS, which cover a broad polarity range, multi-target screening seems to be an option.

Mixtures of about 50 pesticides were used for spiking 10 different QuEChERS-extracts at 1, 5 and 10 ppb-levels, respectively, to evaluate the usefulness of LC-ToF and GCxGC-ToF as routine screening methods for pesticide residue analysis.

LC-ToF

UPLC: Acquity, Waters, Column: Acquity UPLC BEH C18, 1.7 µm 2.1 x 100 mm, Column temp. 40°C. Mobile phase: A: 1 mmol NH_4formiate + 0.002% formic acid in water, B: 1 mmol NH_4formiate + 0.002% formic acid in MeOH, Gradient: multi step gradient 5 – 90% B in 12 min. Flow rate: flow gradient 0.2 - 0.4 mL/min. Injection: 3 µL **MS**: micrOTOF-Q (Bruker Daltonik). ESI(+), m/z 50-600. Calibration: sodium formate/ acetate clusters injected at the beginning of each chromatographic run. Target Analysis parameters were: retention time tolerance 0.3 min, extracted ion chromatogram 5 mDa, mSigma threshold 250.

Multi-Target Screening list: 704 entries with retention times and exact masses for pesticides, degradation products and adducts.

The following mixture was chosen for validation experiments:

3-Hydroxy-Carbofuran, Acetamiprid, Acibenzolar-S-methyl, Aminocarb, Ancymidol, Bendiocarb, Bensultap, Carbaryl, Carbendazim, Carbofuran, Clothianidin, Difenoconazole, Diflubenzuron, Dinotefuran, Dioxacarb, Ethirimole, Ethoxyquin, Fenhexamid, Fenobucarb, Fenothiocarb, Fenpropimorph, Flonicamid, Fluazuron, Flufenoxuron, Flutolanil, Furathiocarb, Imazalil, Imidacloprid, Isoprocarb, Landrin, Linuron, Malaoxon, Methiocarb, Methoxyfenozide, Metolcarb, Mevinphos, Monolinuron, Nitenpyram, Ofurace, Orbencarb, Phosmet, Phosmet-oxon, Phosphamidone, Phoxim, Prochloraz, Promecarb, Prometon, Propamocarb, Propanil, Propoxur, Spiroxamine, Tebuconazole, Tebufenozide, Thiabendazole, Thiacloprid, Thiamethoxam, Thiobencarb, Thiophanate-methyl

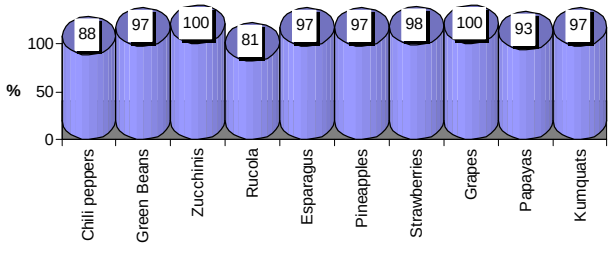


Fig. 1: UPLC-ToF, pesticide identification at the 0.01 ppm level for different matrices in %

GCxGC-ToF

GC: Agilent 7890 with Gerstel MPS and PTV, Columns: 1st Dimension: 30 m DB5-MS, 0.25 mm ID, 0.25 µm film, 2nd Dimension: 2m VF-17m, 0.1 mm ID, 0.1 µm film, injection: 3 µL solvent vent mode, 1st oven 80°C to 300°C, modulation period 4 sec, hot pulse time 0.6 sec, cool time 1.4 sec.

MS: LECO Pegasus® 4D with "consumable-free" modulation system used in the 3-dimensional mode using a scan rate of 100 scans/min. Our own MS-spectra library has currently more than 750 GC-MS spectra.

The following mixture was chosen for validation experiments:

Alachlor, Benalaxyl, Bromophos, Bromophos-ethyl, Butralin, Carbophenothion, Chlorfenprop-methyl, Chlorfenvinphos, Chloropropylate, Cyhalofop-butyl, p,p-DDD, p,p-DDE, p,p-DDT, Diazinon, Dichlobenil, Diethofencarb, Diphenylamine, Ethion, Fenamidone, Fenazaquin, Fenpropathrin, Fenson, Fenvalerate, Fluazifop-P-Butyl, Formothion, Furalaxyl, Hexazinon, Lindan, Mecarbam, Methoxychlor, Nitrothal-isopropyl, Oxadixyl, Pentachloroaniline, Pentachloroanisol, Phenthoate, Picoxystrobin, Piperonylbutoxide, Propachlor, Propham, Prothiophos, Spiromesifen, Sulfotep, Tecnazene, Tolclofos-methyl, Trifluralin

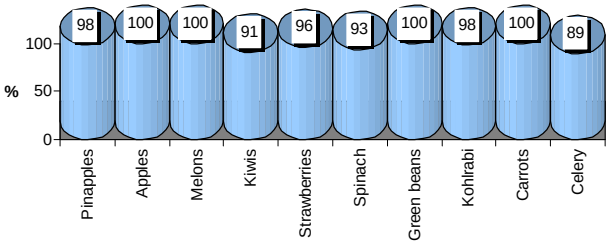
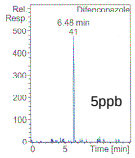
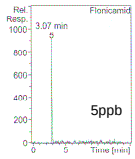
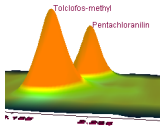


Fig. 2: GCxGC-ToF, pesticide identification at the 0.01 ppm level for different matrices in %







5ppb

COMPLIANCE WITH AQC-GUIDELINES

Document No. Sanco/10684/2009, No. 54 Qualitative Screening Methods states:

- a false negative rate of 5 % is accepted
- positives have to be confirmed
- basic validation with 10 different matrices from each group in duplicate and on-going validation

RESULTS

A screening reporting level of 0.01 ppm can be achieved for most pesticides in fruit and vegetables for both instruments. About 50 % of the spiked analytes were found at the 0.001 ppm-level with the LC-ToF and at the 0.005 ppm-level with the GCxGC-ToF.

Participation in the 1st EU Screening-Proficiency Test 2009

Matrix: orange, 44 participants, 48 hr. to report results. 230 possible analytes, thereof 47 spiked; 13 between 1 – 2 ppm, 17 between 0.1 – 1 ppm, 17 between 0.05 – 0.1 ppm. 44 reported, thereof 39 analytes LC-amenable: after 12 min we knew that 37 of the 39 analytes were positive.

SUMMARY

With the use of these instruments our laboratory follows two strategies: a) to confirm residue findings detected in the routine multi-residue procedure and b) to check for the presence of pesticides and metabolites not included in the above methods due to capacity limitations or other reasons. Our screening scope also includes pesticides with a potential for illegal use based on information from various sources. We consider both approaches (selective monitoring and semi-selective screening) as an extremely valuable combination, the only unsolved problem being false negatives in low concentration ranges. A screening reporting level of 0.01 ppm can be achieved for most pesticides in fruit and vegetables.

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