Multi-targeting Pesticides

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This article looks at how to simultaneously identify a wide range of selected pesticides with a high degree of specificity.

The usefulness of liquid chromatography mass spectrometry mass spectrometry (LC–MS–MS) methods for the unambiguous identification and quantification of pesticides in complex matrix samples is well known. Triple quadrupole systems have proven to be useful for this task because of their high specificity in MS–MS mode and their low detection limits. However, working in MS–MS mode makes any MS system blind to other compounds of interest.

Therefore, it is difficult to develop methods for simultaneous analysis of high numbers of pesticides. Thus, other ways of achieving specificity are of interest, such as the high mass accuracy and mass resolution of an electrospray ionization time-of-flight (ESI-TOF) system. It can generate high specificity without limiting the number of simultaneously observed target compounds (i.e., multi-targeting).[1,2]

Molecular Formula Generation: The Isotopic Pattern Filter

With high mass accuracy alone, confident molecular formulae cannot be generated for the most part. An isotopic abundance pattern filter needs to be applied to reduce the number of molecular formula candidates if, for example, the presence of a putative compound has to be confirmed.[3]

Using an actual ESI-TOF MS and a sophisticated software solution, ESI-TOF MS is a key to both simultaneous screening for multiple targets and sum formula confirmation. This is because the mass accuracy is apparently independent of peak intensity so it is possible to generate extracted ion traces with a window down to a few mDa, allowing for extreme selectivity and simple and fast identification.

Additionally, the conserved correct isotopic pattern makes it possible to reduce the number of possible hits within a given mass interval by at least one order of magnitude. SigmaFit software strongly helps to find the correct elemental composition (Figure 1). For a similar confidence with mass accuracy alone, 50 ppb would be required, based on the unambiguous formula generation from reserpine; a 609 Da molecule.

Experimental

Different matrix samples, spiked at various levels with a commercial pesticide standard (Ehrenstorfer, Pesticide Mix 34)

Figure 1: The reduction of possible formulae for the confident determination of the elemental composition of an LC–MS peak by using isotopic pattern information (SigmaFit).

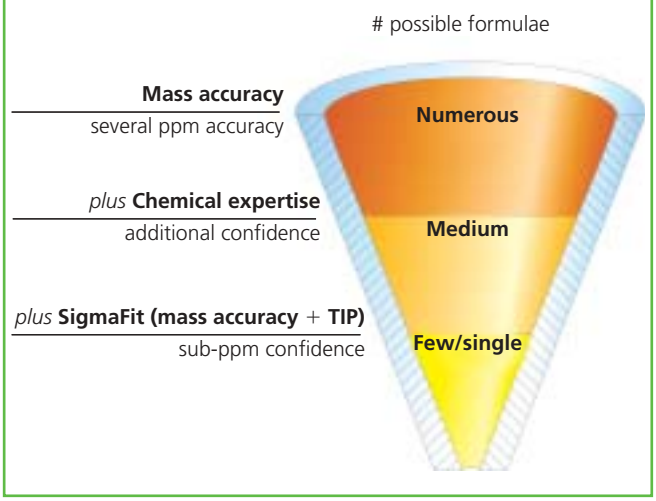

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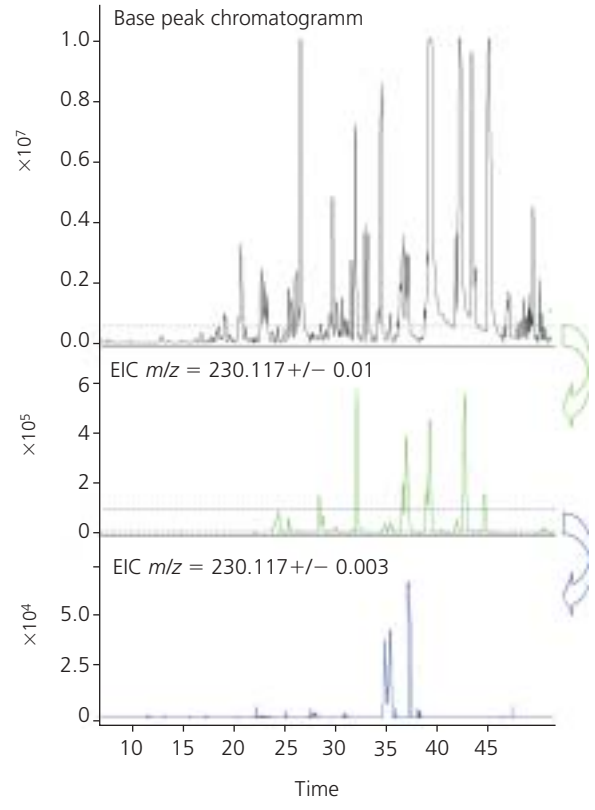
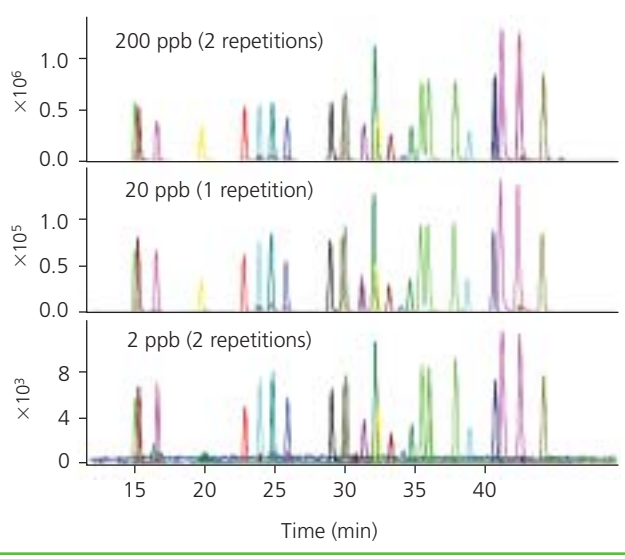
Figure 2: Base peak chromatogram (top) of a chamomille extract spiked with pesticides on the level of 10 ppb. Mass traces of *m/z* = 320.117 (corresponding to $[M+H]^+$ of the spiked sebutylazine/propazine/terbutylazine ($C_9H_{16}ClN$)) are shown with an accuracy of 10 mDa (middle) and 3 mDa (bottom).



were analysed using an Agilent 1100 LC system (Agilent Technologies, Waldbronn, Germany) interfaced with a micrOTOF ESI-TOF MS. An RP-HPLC column (3 µm particles, 2.1 × 125 mm Hypersil ODS C18 material, 0.2 mL/min flow-rate) with an acetonitrile/water (1 mM NH_4OAc) gradient (5–55% acetonitrile in 45 min) was applied for separation.

The micrOTOF was equipped with an orthogonal ESI source and operated in positive mode. Calibration was performed externally prior to a sample series with a sodium formate solution, and additionally internally for each chromatogram by injecting the calibrant at the beginning and at the end of each run via a six-port divert valve equipped with a 100 µL loop.

Figure 3: Five repetitions at three concentrations of a pesticide standard, containing 28 pesticides.



Selective Recovery of Three Pesticide Isoforms From a Plant Extract

The selectivity of the method based on accurate mass traces is demonstrated for three azine isoforms spiked in chamomile (Figure 2). A window of 10 mDa gives a dozen peaks, whereas a selectivity of a mass trace defined to a window of 3 mDa is sufficient for unequivocal identification. Retention time is required for the attribution of the individual isomers.

Detection limits well below 2 ppb have been determined, both for standards (Figure 3) and for spiking this amount in various matrices (Figure 4 for lettuce extract).

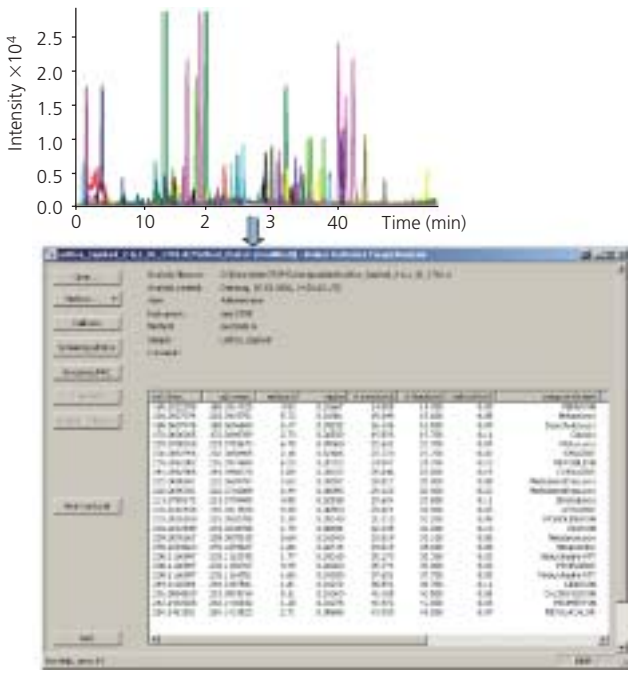
A basic requirement for reliable detection of compounds with such narrow mass traces is the mass stability for a wide dynamic range over the entire peak. As Figure 3 shows, this mass stability is given for at least four orders of magnitude.

Multi-target Compound Analysis of Pesticides

Automated target detection for the pesticide standard mix spiked to a lettuce extract can be achieved by automated peak detection on the extracted ion chromatograms (EICs) expected for the $[M+H]^+$ ions of each compound in a database. This database contains the names, sum formulas, exact masses and retention times for about 230 pesticides.

As shown previously, a mass window of 3 mDa allows for selective detection of the compounds, if present. Based on accurate mass and known retention times the compounds

Figure 4: Mass traces of 28 pesticides spiked to an extract of lettuce on the level of 2ppb (top) and resulting search in a database, based on accurate mass, SigmaFit and retention time.



present in the sample are identified. For each identification candidate the theoretical isotope abundance pattern is compared with the experimental one. The derived SigmaFit values serve either for confirmation of the putative compounds or prevent false positive identifications by combination of accurate mass with the true isotopic pattern for the highest confidence in compound identification. Figure 4 shows the EICs and database search results for the spiked lettuce extract.

Conclusion

Screening of several hundreds of possible pesticides is easily feasible with one single ESI-TOF MS run. Based on accurate mass, a high selectivity is achieved. Even over a dynamic range of four orders of magnitude, the mass traces could be defined within 0.003 Da. With sensitivity in the sub-ppm-range, pesticides can be readily characterized on the level of policy requirements in a variety of matrices.

Efficient multi-target screening using this system was also reported elsewhere: Toxicological drug screening in urine was based on accurate mass, SigmaFit isotopic pattern analysis and automated database search. In automatic runs, correct SigmaFit values and accurate masses were achieved over a wide dynamic range, while a mean mass error was only 2.51 ppm.[4]

Compared with classical approaches by triple quadrupole instruments, an ESI-TOF MS solution allows a high number of targets to be screened within one LC run and without the loss of sensitivity; it allows identification of unknown peaks based on accurate mass and isotopic pattern information from the software while data can be archived and reprocessed later for additional compounds. Moreover, data can be profiled for further statistical evaluation.

References

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