ENVIRONMENTAL SCREENING OF PESTICIDES: A NEW SCIENTIFIC INFORMATION SYSTEM TO ENABLE ROUTINE ACCURATE MASS NON-TARGETED SCREENING

G. Bondoux1,2, J.M. Joumier1, A. Gledhill2, D. Roberts2, J. Burgess

1 Waters Corporation, St Quentin en Yvelines, France.
2 Waters Corporation, Manchester, UK.
3 Waters Corporation, Milford, MA, USA.

INTRODUCTION
Recent advances in instrumentation technology have led to major gains in sensitivity and linearity, enabling high resolution MS analysers eg TOF instruments, to be used for low level food safety and environmental analyses. Regarding food safety, the SANCO Document 12495/2011 [ref 1], concerning method validation and quality control for pesticide residue analysis, now includes the use of high resolution instruments. The same document also defines screening detection limits as corresponding to a maximum false negative rate of 5 %. To avoid costly confirmation analysis, the percentage of false positive should be kept to a minimum. Although addressing the food safety methods, the SANCO guidelines are universally recognised as references in other application fields, including environment.

Screening requires a combination of several key attributes to be successful: chromatography, MS Resolution, sensitivity and software. A good chromatographic separation simplifies the work of the MS detector due to less interferences and more intense peaks. It also makes it possible to separate isomers that cannot be distinguished by MS alone, whatever the resolution. One also should not forget that the retention time is a mandatory confirmation point in the SANCO document. The same document recommends a minimum resolution of 20 000 and a mass accuracy better than 5 ppm.

Each injection of a real sample leads to tens of thousands of MS data points (exact mass/retention time). A single chemical compound will lead to multiple identification points corresponding to the parent ion, the isotopes and adducts.

Consequently, the general approach with high resolution instruments, which is to rely on the exact mass and retention time to identify the presence of residues amongst a library, leads to an unacceptable number of false positives when working with real samples (Ref 2 and Ref 3).

As positive samples need to be confirmed, the cost of it is important, limiting the interest of the approach.

The most advanced group have developed their own software solution to overcome that difficulty: the exact mass information is exported to external software tools, in order to analyse the presence of adducts, fragments and consequently filter the number of possible candidates (Ref 4).

Recently a new scientific information system was introduced. This new system streamlines the workflow and utilises all the available data to ensure that all non-compliant samples are detected, whilst getting a low false positive rate.

The LC/MS method was developed on an ACQUITY I-Class UPLC / XEVO G2-S QToF from Waters operated in positive electrospray ionisation mode. Instrument control, MS data acquisition and processing were through the recently introduced software, UNIFI.

All data were acquired in MS2 mode. In this mode, the collision cell switches very quickly from low collision energy for obtaining the molecular weight information of the compounds, to high collision energy, for the fragmentation information. This provides full unbiased, accurate mass information on both precursors and products in a single injection and is easily achievable with the fast acquisition speeds of TOF-MS (30 spectra per second), even with UPLC peak widths.

Data were searched against a subset of 578 LC-amenable pesticides extracted from the Scientific Library within UNIFI. Molecular formulae, fragment ions and retention times were included for identification and confirmation. The list of the compounds found in the sample based on the exact mass information was rationalized automatically by using the isotopic pattern, fragments and retention time. Identified compounds can be then quantified against calibration curves from standards. Further investigation for compounds that are not present in the library can be done using structural elucidation tools including elemental composition, isotopic pattern matching and halogen filtering.

The method was used for various water samples. Fig 2 shows the chromatogram for metolachlor in a surface water sample. The sample was screened against the 578 compound database and returned 14 positive identifications.

RESULTS AND DISCUSSION

A major difficulty with using high resolution instruments (Tofs or Orbitraps) for pesticide screening is data interpretation. When running a screening method, false positive occur when compounds are not sufficiently confirmed. False negative (missed compounds) can be caused by a lack of sensitivity, or by the difficulty to find the compound in a complex matrix.

The first important step after data acquisition is the detection of the MS peaks. The new UNIFI software used a sophisticated 3D method to detect the peak apex, and identify the real MS peaks, their isotopes and adducts. The next step is to organise the data. UNIFI software uses components as a way of simplifying and organising complex data into groups which relate to the same chemical entity. Each component contains a wealth of analytical information: mass, retention time, isotopic, fragments (MS2 data), adducts... This approach is key to a) rationalize automatically the list of the compounds found in the sample based on the exact mass information and thus reduce the number of false positives.

INSTRUMENTS AND METHODS

Analyte specific information that can be used for confirmation purposes, such as molecular structure and fragmentation pathway can be routinely generated from the accurate mass (≤3 ppm) spectral data acquired within a single injection. Processing simultaneously acquired accurate mass precursor and fragment ion data through the new scientific information system has enabled the pesticide residues to be confirmed with confidence and very low false positive rates. Figure 3 illustrates the MS spectra generated for metolachlor. Structural elucidation information for all the pesticides correctly identified has been generated.

The most advanced group have developed their own software solutions to enable routine accurate mass non-targeted screening.

CONCLUSIONS

Enhancements in both hardware and software technology have contributed to the development of a robust TOF based solution for residue screening.

The detection, identification and quantification of pesticides in complex matrices can routinely be achieved at relevant concentrations.

Structural elucidation information for all the identified pesticides was generated by collecting simultaneous acquisition of accurate mass precursor and accurate mass fragment ions.

Processing simultaneously acquired accurate mass precursor and fragment ion data through a new scientific information system, enabled pesticide residues to be confirmed with confidence and very low false positive rates.

Figure 2: The UPLC-XEVO G2-S QToF system. The new QToF instrument embeds the stepwave transfer optics for unmatched sensitivity

Figure 3: The top right hand panel show the list of the identified compounds, with the identification elements (adducts, exact mass, retention time ...)