EFFECTIVE STRATEGIES FOR THE SCREENING OF CONTAMINANTS IN FOOD USING TOF/MS AND NOVEL DATA MINING TOOLS

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INTRODUCTION

Identification and monitoring of contaminants in the food chain is of major importance with an ever expanding number of compounds under consideration. Sensitive, accurate screening places stringent demands upon analytical instrumentation and associated software and successful screening strategies must address both.

Here we describe GC and UPLC oa-ToF/MS solutions based on complimentary approaches...

- **TARGETED SCREENING involves detecting specific ions and calculating ion ratios. Post-target screening is also possible by adding compounds (ions) to the method after analysis, extending the number of residues monitored without compromising sensitivity.**
- **NON-TARGET SCREENING uses automatic peak detection, spectral deconvolution, and searching against experimental or theoretical spectral libraries with further confirmation of identified compounds by accurate mass scoring and isotope pattern fitting.**

ANALYTICAL WORKFLOWS

Many analytical strategies involve three distinct facets...

- **Screening:** removes negative samples from the workflow
- **Quantitation:** accurately determines analyte levels
- **Confirmation:** establishes confidence in assigned identities

Typically these will involve more than one analysis. Screening experiments should be designed to avoid false negatives and minimize false positive rates. ToF/MS can assist this process by (a) providing sufficiently sensitive detection to avoid false negatives; and (b) providing sufficient selectivity to avoid false positive rates. Following screening, positive samples will require quantitation and confirmatory analysis. Possible ways of integrating these workflows to provide full characterization of complex samples are shown in *Figure 1*. We will show that targeted screening can utilize many of the experimental and software techniques applied to quantitative analysis whereas non-target screening requires the use of alternative approaches involving spectral signatures and deconvolution software.

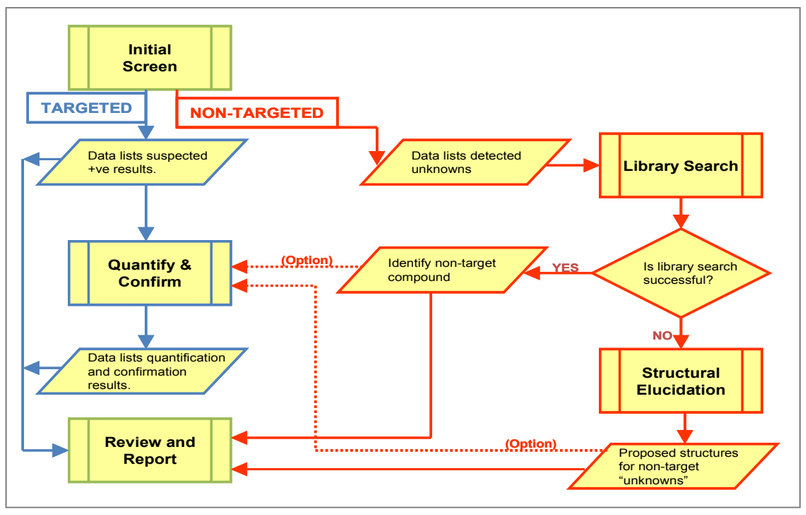

Figure 1. Possible integrated workflows for screening, quantitation and confirmation experiments

TARGETED STRATEGY

The strategy adopted for targeted screening using oa-ToF/MS is...

- For each target compound characteristic ions are selected.
- Extracted ion chromatograms (XICs) are constructed with narrow mass windows (typically 0.02-0.05 Da).
- If a peak is detected in the primary XIC then the compound is suspected to be present.
- If the peak response exceeds a threshold level then the sample is a presumptive positive.
- Additional XICs and ion ratios may be monitored to increase specificity (reduce false positive rate) or provide confirmation of identity if appropriate criteria can be met.
- It may then undergo quantitation and confirmatory analysis.

Example of a typical GC/MS screening experiment

Objective: Targeted screening of >100 pesticide residues in fruit, vegetables and baby food at levels <0.01 mg/kg.

Sample preparation: QuEChERS type extraction (dispersive solid phase extraction) at 1g/mL matrix equivalent concentration

GC/MS Conditions:
Waters GCT Premier
Electron Ionization (EI+) mode
Spectra acquired over m/z range 50 to 500
Resolution >7,000 resolution (FWHM)
Single point lock mass using Tris(trifluoromethyl)triazine
5 μL injection on cooled PTV in solvent vent mode
Column: 30m x 0.25mm id Rxi-5ms (Restek)

Figure 2 shows vinclozolin (0.01 mg/kg) in cucumber, a relatively simple matrix. The nominal mass XIC (**A**, 1 Da, m/z 212) contains a number of intense peaks which may interfere with automatic integration. In the exact mass XIC (**B**, 20 mDa, m/z 212.0034) vinclozolin has no interference. Improving the selectivity can lead to an increase in the S/N ratio. The mean difference between the nominal and exact mass S/N were typically a factor of five.

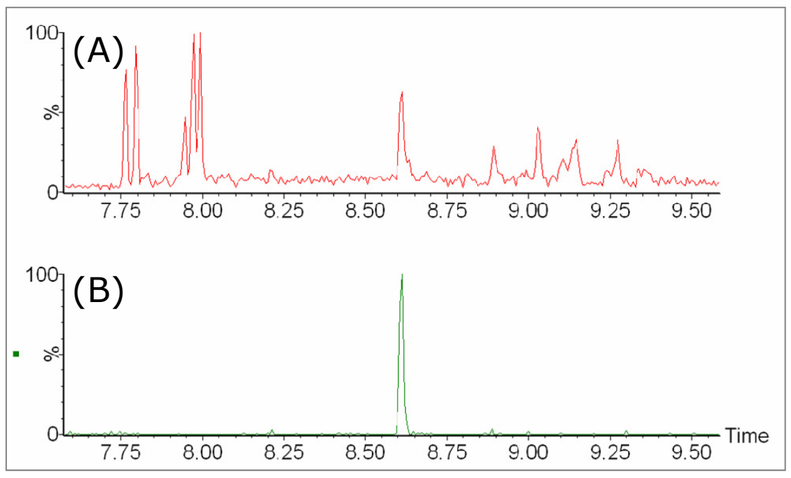

Figure 2. The improved selectivity offered by exact mass extracted ion chromatograms (XICs)

With ToF/MS the number of ions/residues can be increased without effect as all of the data is acquired all of the time. If there is interference on one mass, processing can be moved to a different mass without re-injection. This also allows post-targeting of compounds enabling the number of residues monitored to be increased without having to re-acquire the sample or compromise method performance.

Data is processed automatically using dedicated software developed for detection and quantitation of target compounds (TargetLynx). An example of the TargetLynx browser window is shown in *Figure 3*.

Figure 3. TargetLynx browser showing three XICs, calibration curve and peak data for Chlozolinate.

Using this approach, it was possible to detect >100 target residues at <0.01 mg/kg in several different food matrices.

The browser window illustrates that ToF/MS can also be used for quantitative analysis and enables confirmatory analysis using ion ratios. Whilst this strategy has been illustrated using GC-ToF/MS data the same approach can be applied using LC-ToF/MS.

NON-TARGETED STRATEGY

The strategy adopted for non-targeted screening is described below and is performed by a dedicated software package (ChromaLynx)...

- Detect component peaks in TIC chromatogram.
- Deconvolutes mass spectra of component peaks.
- Match against library of user or theoretical spectra.
- Screen results by fit factor to produce a list of candidates.
- Perform scoring with two tiers of mass accuracy for all ions.
- Use i-FIT™ software as further quality check.

Example of a Typical LC/MS Screening Experiment

Objective: Non-targeted screening of >100 veterinary drug residues in milk. MRLs typically 0.01-0.1 mg/L.

Sample Preparation: Dilute 50% with acetonitrile; isolate supernatant, dilute and filter, then inject.

UPLC/MS Conditions:
Waters LCT Premier XE
Electrospray Ionization (ES+) mode
Spectra acquired over m/z range 100 to 1000
Resolution >10,000 resolution (FWHM)
Single point lock mass using Leucine Enkephalin
Column: ACQUITY 1.7 μm BEH C_{18} 50mm x 2.1mm i.d.
Mobile phase: 0.1% formic acid / acetonitrile gradient

Figure 4 shows the ChromaLynx browser with several windows (A-E) open to illustrate its functionality. (A) TIC is complex which makes it difficult to determine analyte peaks of relevance. (B) XICs of the n most intense ions. (C) The component mass spectrum is deconvoluted (refined, background subtracted) and matched to the library. (D) If the match factor is above a certain level (user-defined) then a candidate is considered a good match, and assigned a √. Between this level and a secondary level is considered a tentative match and assigned a ?, and below this secondary level, a negative is marked by a X. (E) The formula from the library entry is used to run EleComp on the measured mass. If it is within user-defined limits (5ppm) the calculated composition will be displayed shaded green. Within a second limit (20ppm) shaded yellow, and outside the limits, the box is shaded red.

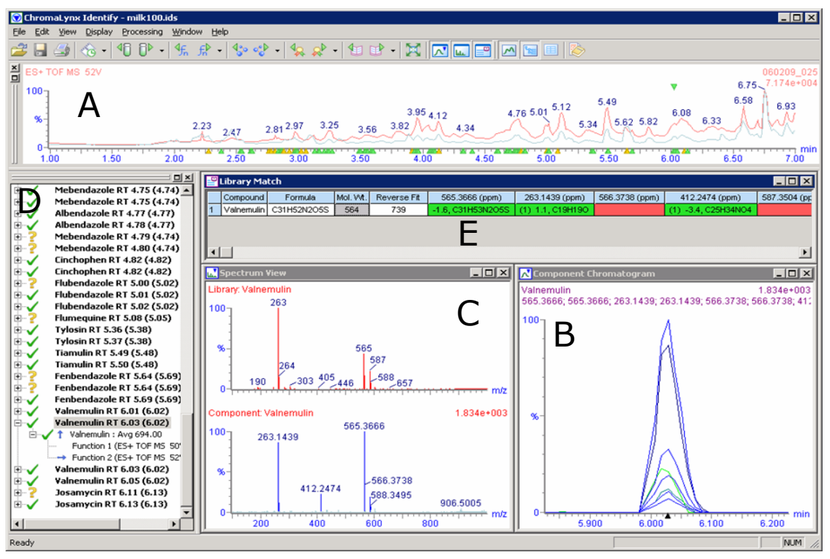

Figure 4. ChromaLynx browser window for non-target screening of veterinary drug compounds in milk.

The LCT Premier XE produces spectra with both accurate mass and correct isotope ratios. This offers the possibility of using isotope ratios to confirm identity.

Taking this data into the EleComp calculator shows several realistic compositional possibilities. Ranking these by i-FIT score puts the correct compound to the top of the list, even though it's not the closest match by mass accuracy (See *Figure 5*). The i-FIT approach offers potential as a confirmatory technique and various groups are investigating the use of exact-mass data as confirmation criteria. Currently only MS/MS ion ratios and high resolution (i.e. <10,000 at 10% peak height) measurements are considered confirmatory.

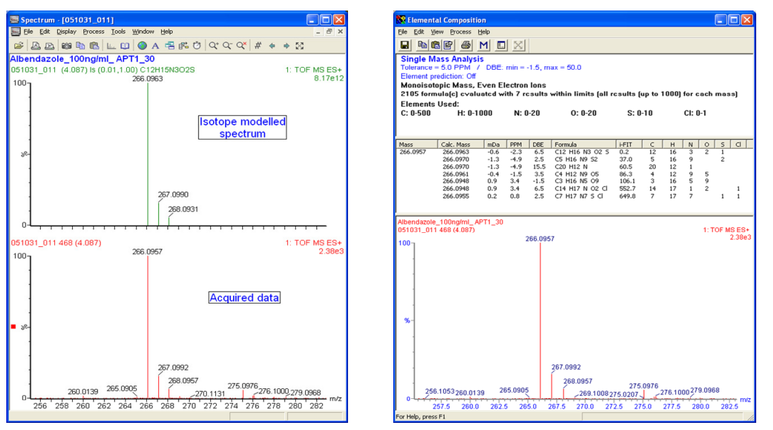

Figure 5. Isotope modelled and acquired spectra for Albendazole. List of elemental compositions ranked by i-Fit showing correct elemental composition as lowest i-Fit score.

CONCLUSIONS

High resolution, exact mass ToF/MS data offers selectivity and sensitivity benefits for screening and confirmatory analysis.

For Targeted screening...

- **Exact mass XICs provide low limits of detection.**
- **TargetLynx automates full screening & quantitation.**
- **Additional compounds can be added post-analysis.**
- **Old data can be mined to target new compounds.**

For Non-Targeted screening...

- **Deconvoluted components are identified by searching against libraries.**
- **Sensitivity in complex matrices is compromised compared to targeted approaches.**
- **Commercial, user or theoretical libraries can be used.**
- **Exact mass scoring and isotope pattern fitting can be used to confirm identity.**

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