

STRATEGIES FOR SCREENING OF EMERGING CONTAMINANTS USING ToF/MS AND NOVEL DATA MINING TOOLS

Waters

THE SCIENCE OF WHAT'S POSSIBLE™

Authors: Tim Jenkins*, Gordon Kearney, Peter Hancock, Keith Worrall
Affiliations: Waters Corporation, MS Technologies, Atlas Park, Simonsway, Manchester M22 5PP

INTRODUCTION

"There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. There are things we don't know we don't know."

- Donald Rumsfeld (Former US Secretary of Defence)

Identification and monitoring of emerging contaminants in the environment 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 two 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.

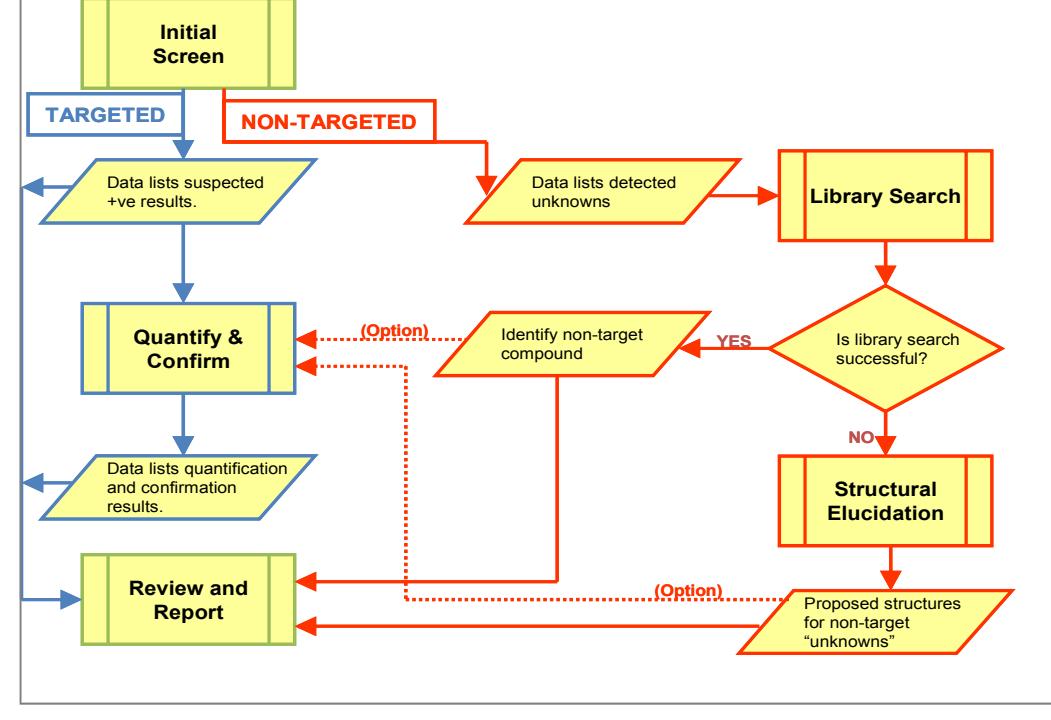


Figure 1. Possible integrated workflows for screening, quantitation and confirmation experiments

TARGETED STRATEGY

The strategy adopted for targeted screening using oaToF/MS is described below;

- For each target compound characteristic ions that can be used for detection are selected.
- Extract 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.
- The sample may then undergo quantitation and confirmatory analysis. (With ToF/MS it is possible to carry out screening, quantitation and confirmation on the same instrument).

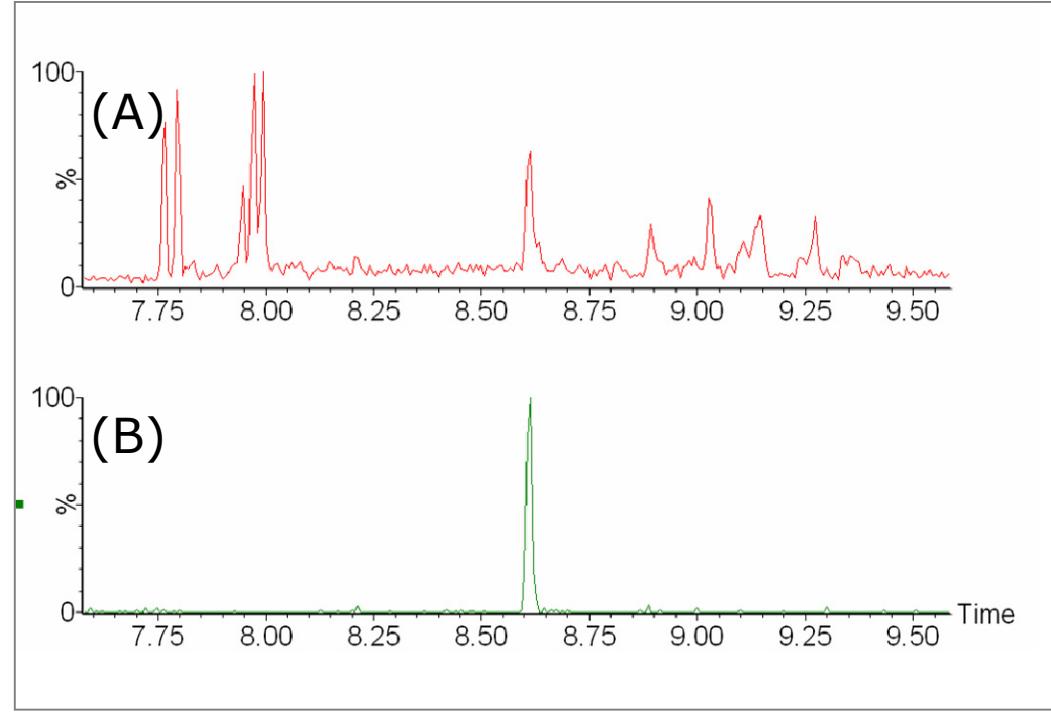


Figure 2. The improved selectivity offered by exact mass extracted ion chromatograms (XICs)

Figure 2 shows vinclozolin (0.01 mg/kg) in cucumber, a relatively simple matrix. The nominal mass chromatogram (1 Da, m/z 212), illustrated in Figure 2(A), contains a number of intense peaks which could interfere with automatic integration. In the exact mass chromatogram, Figure 2(B), (20 mDa, m/z 212.0034) vinclozolin has no interference.

Improving selectivity can lead to an increase in the signal-to-noise (S/N) ratio which could be the difference between detecting the compound and not detecting it. The mean difference between the nominal and exact mass S/N was typically a factor of five for all residues.

Example of a Typical GC/MS Screening Experiment

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

II^o Objective: Quantitative and confirmatory analysis

Sample Preparation: QuEChERS type extraction (dispersive solid phase extraction) at 1g/mL matrix equivalent conc.

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 Rx-5ms (Restek)

A further benefit of ToF/MS is illustrated in Figure 3. Using a quadrupole, increasing the number of ions/residues will decrease the overall sensitivity (peak area and S/N). With exact mass ToF/MS increasing the number of monitored ions does not decrease the sensitivity, as can be seen by the two peak areas of 7.0, when moving from one ion acquired to four ions acquired. Therefore, if there is interference for one mass, processing can be moved to a different mass without re-injection. The number of confirmation ions/residues can be increased without effect.

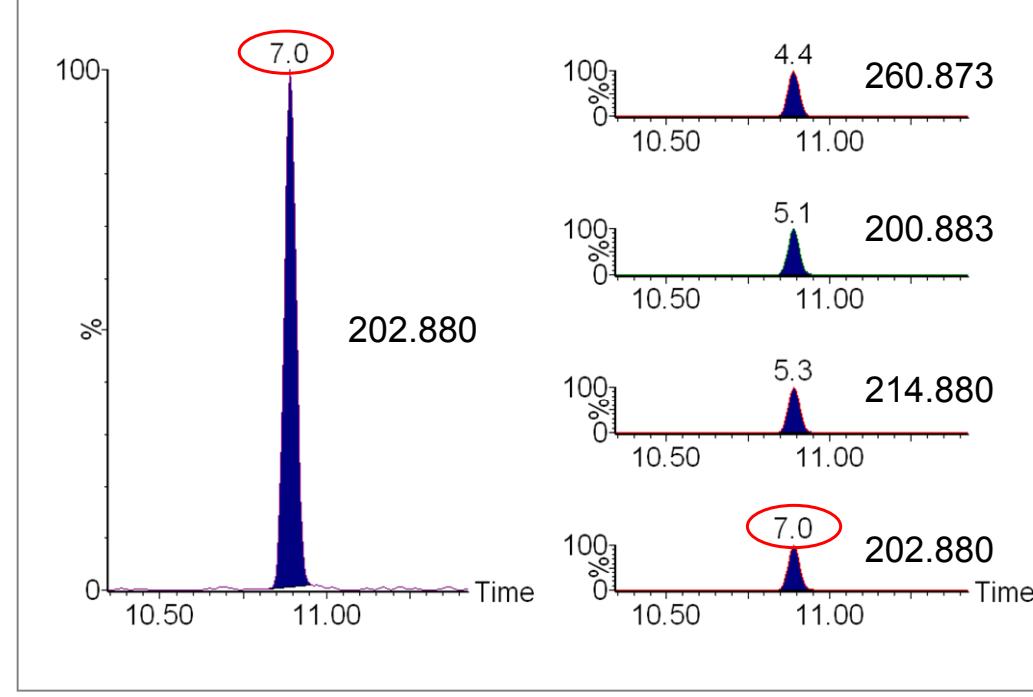


Figure 3. XICs of Tecnazene at 0.01 mg/kg in pear. The intensity of the 202.880 peak remains the same regardless of how many ions are monitored.

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.

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

Data is processed automatically using a dedicated software package developed for detection and quantitation of target compounds (TargetLynx, Waters Corporation). An example of the TargetLynx browser window is shown in Figure 4.

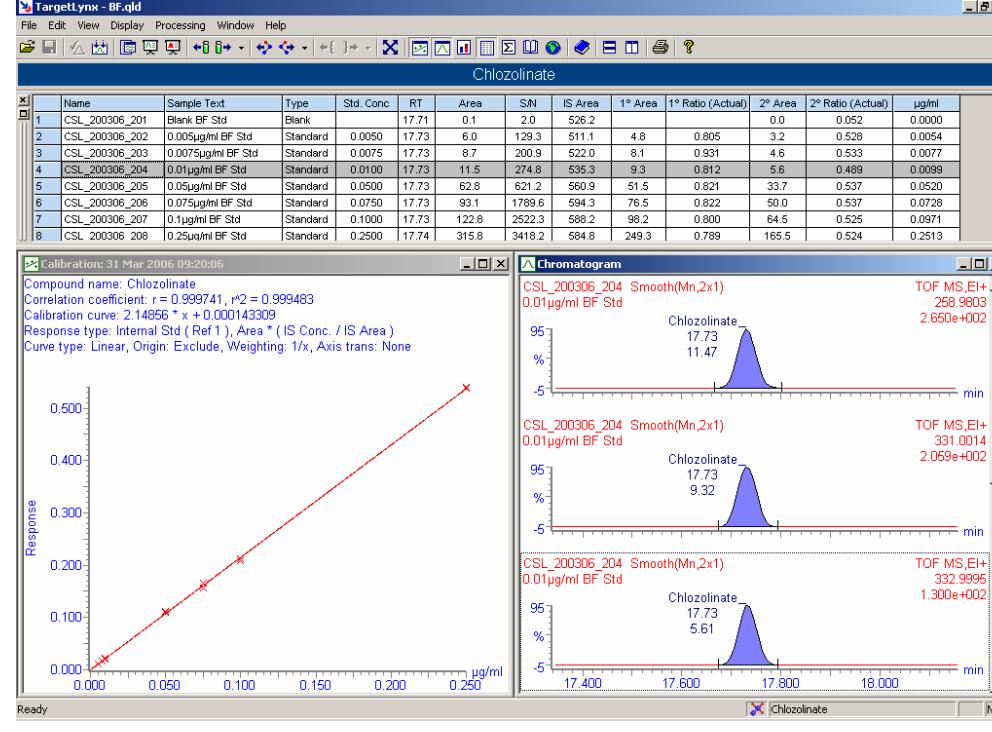


Figure 4. TargetLynx browser showing three XICs, calibration curve and peak data for Chlozolate.

The browser window illustrates that ToF/MS can also be used for quantitative analysis and enables confirmatory analysis using ion ratio measurements. Whilst this strategy has been illustrated using GC-ToF/MS 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, Waters Corporation);

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

Figure 5 shows the Chromalynx browser with several windows (A-E) open to illustrate its functionality. The windows are now described in turn. (A) TIC is complex which makes it difficult to determine analyte peaks of relevance. (B) XICs of the n most intense ions (8 in this case) (C) The component mass spectrum from this chromatographic peak is deconvoluted (refined, background subtracted) and matched to the library.



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

(D) If the match factor is above a certain level (user-defined) then a candidate is considered a good match, and assigned a green tick. Below this level but above a secondary level is considered a tentative match and assigned a yellow (?), and below this 2nd level, a negative is marked by a red (X) – in this illustration, the 'exclude negatives' option has been enabled. (E) The formula from the library entry is used (+ user defined adducts) to run EleComp on the measured mass. If it is within certain limits (user defined, in this case 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 with no matching formula.

Example of a Typical LC/MS Screening Experiment

I^o 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
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 C₁₈ 50mm x 2.1mm id
Mobile phase: 0.1% formic acid / acetonitrile gradient

The LCT Premier produces spectra with both accurate mass and correct isotope ratios (See Figure 5). This offers the possibility of using isotope ratios to confirm identity.

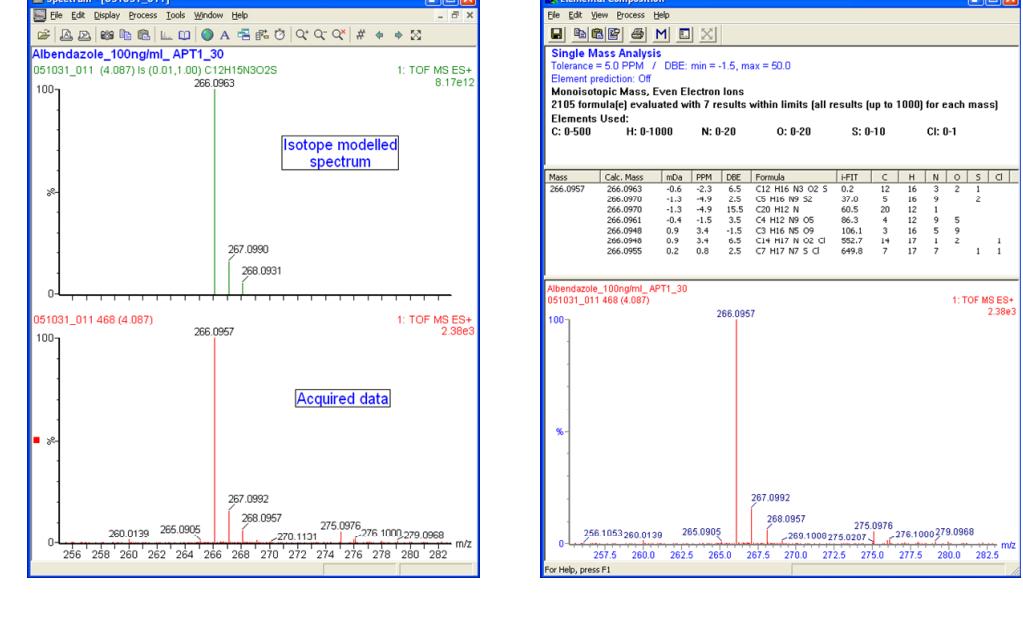


Figure 5. (A) Isotope modelled spectrum and acquired spectrum for Albendazole showing accuracy of exact mass and isotope patterns. (B) List of elemental compositions ranked by i-FIT showing correct elemental composition as lowest i-FIT score.

Taking this data into the Elemental composition 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.

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 generated or theoretical libraries can be used
- Exact mass scoring and isotope pattern fitting can be used to confirm identity.

Acknowledgements: The authors thank the following for collaborations resulting in the data presented in this poster; RIKILT Institute of Food Safety, Wageningen (Netherlands), CSL, York (UK), CVUA, Stuttgart (Germany)