# Comparison of two approaches for GC-MS based screening of pesticides in food/feed: GCxGC-hsTOF-MS vs GC-hrTOF-MS

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**Abstract**
For untargeted screening of pesticides and contaminants in food and feed full scan mass spectrometry is required. Quadrupole, ion trap or time-of-flight (TOF) mass spectrometers can all fulfil this requirement. TOF-MS instruments are more sensitive (typically by one order of magnitude) and are therefore the preferred option. A comparison has been made between high resolution and low resolution TOF and low resolution TOF coupled to a two dimensional GC system.
The best results with respect to selectivity and sensitivity is obtained with two dimensional gas chromatography low resolution- high speed TOF MS.

**Introduction**
Full scan mass spectrometric detection is a prerequisite for the comprehensive screening of GC amenable pesticides and contaminants. Quadrupole, ion trap or time-of-flight (TOF) mass spectrometers can all fulfil this requirement. TOF-MS instruments are more sensitive and are therefore the preferred option. Standard GC with nominal mass resolution MS lacks adequate selectivity for automated detection and identification of high numbers of target analytes at low ng/mL concentrations in complex sample extracts. Two approaches to improve selectivity include the use of comprehensive two-dimensional GC combined with TOF high speed MS (GCxGC-hsTOF-MS) to provide enhanced chromatographic resolution [1] and the use of GC with high resolution TOF-MS (GC-hrTOF-MS) to provide enhanced mass resolution [2]. In theory, a combination of these two approaches would be even more powerful, but this requires hrTOF-MS detectors with high acquisition rates (≥100 Hz), and such instruments do not exist yet. Numerous literature references are available on the use of GCxGC-hsTOF-MS and GC-hrTOF-MS for pesticide residues analysis. However, the results have been obtained using different samples, analytes and instrumental conditions, so it is not possible to make a valid comparison with respect to the selectivity and quantitative performance of the two approaches. In the work reported here the relative performance of GCxGC-hsTOF-MS (LECO, Pegasus III, nominal mass) and GC-hrTOF-MS (Waters, GCT premier, ~7000 resolution) are compared using the same pesticides and contaminants in common extracts of Beef Chilli baby food and cereal-based mixed animal feed.

**Materials and methods**
Samples were extracted using ethyl acetate. After clean-up by gelpermeation chromatography (GPC) and dispersive SPE (PSA), the extracts were spiked with 100 pesticides and indicator PCBs at concentrations equivalent to 10 to 100 µg/kg expressed on sample. Different matrix concentrations (0.2, 1 and 5 g/ml for baby food and 0.2 and 1 g/ml for feed) were prepared to allow an evaluation of possible saturation effects due to high amounts of matrix, see table 1.

Table 1: Matrix and analyte concentration in baby food and animal feed sample extracts

| | Matrix in extract [g/ml] | Analyte in extract [ng/ml] | Matrix on-column [mg] | Analyte on-coulmn [pg] |
|---|---|---|---|---|
| Baby food | 0.2 | 2 – 20 | 1 | 10 – 100 |
| | 1 | 10 – 100 | 5 | 50 – 500 |
| | 5 | 10 – 100 | 25 | 50 – 500 |
| Animal feed | 0.2 | 2 – 20 | 1 | 10 – 100 |
| | 1 | 10 – 100 | 5 | 50 – 500 |

**Results and discussion**

The extracts were analysed firstly using GC-hsTOF-MS (LECO) to demonstrate the need for increased selectivity with these complex extracts. The same extracts were subsequently analysed by GCxGC-hsTOF-MS and GC-hrTOF-MS. The injection volume was in all cases 5 µl. The data was evaluated with emphasis on retention times in the 1D chromatogram where target analytes co-eluted with intense matrix peaks. Deviations in the exact mass measurement were observed with GC-hrTOF-MS resulting in the occasional non-detection of pesticides when using narrow mass (± 25 mDa) windows to enhance selectivity. The detection of pesticides and contaminants using GCxGC-hsTOF-MS was affected less by the matrix. An example is given in figure 1A; Malathion in animal feed is spiked at 10 ng/gram and analysed at GC-hsTOF-MS. The TIC chromatogram shows intense signals where malathion co-elutes with matrix compounds; even the extracted ion chromatogram (173 m/z) clearly shows interferences. The software peak deconvolution algorithm was not capable to generate a "clean" mass spectrum of the pesticide. As expected malathion could not be detected and confirmed due to the lack of selectivity and mass resolution using a GC-hsTOF-MS.

The same animal feed sample was analysed using a GC-hrTOF-MS to improve mass resolution. Identical chromatographic conditions results of course in the same co-elution of matrix and malathion. As shown in figure 1B, the extracted ion chromatogram of 173.0805 m/z ± 25 mDa didn't show a chromatographic peak. Opening the mass window to ± 50 mDa the malathion signal becomes visible and malathion could be detected but not confirmed due to shifting of the accurate mass.
In nominal mass spectrometry confirmation of, in this example for malathion, is based on retention time and comparison of obtained mass spectra with reference spectra from libraries. Using high resolution mass spectrometry the exact masses are available, so comparison is based on retention time and the ion ratio of 3 structure related masses. At the spike concentration level (10 ng/gram) malathion could not be confirmed in animal feed using GC-hrTOF-MS.

By using GCxGC-hsTOF-MS it was able to confirm malathion in the same animal feed sample, see figure 1C. The extracted ion chromatogram (173 m/z) is "cleaner" compared to the chromatograms obtained using GC-hsTOF-MS or GC-hrTOF-MS. Peak deconvolution results in clean mass spectra, as a result automatic detection and confirmation was possible.

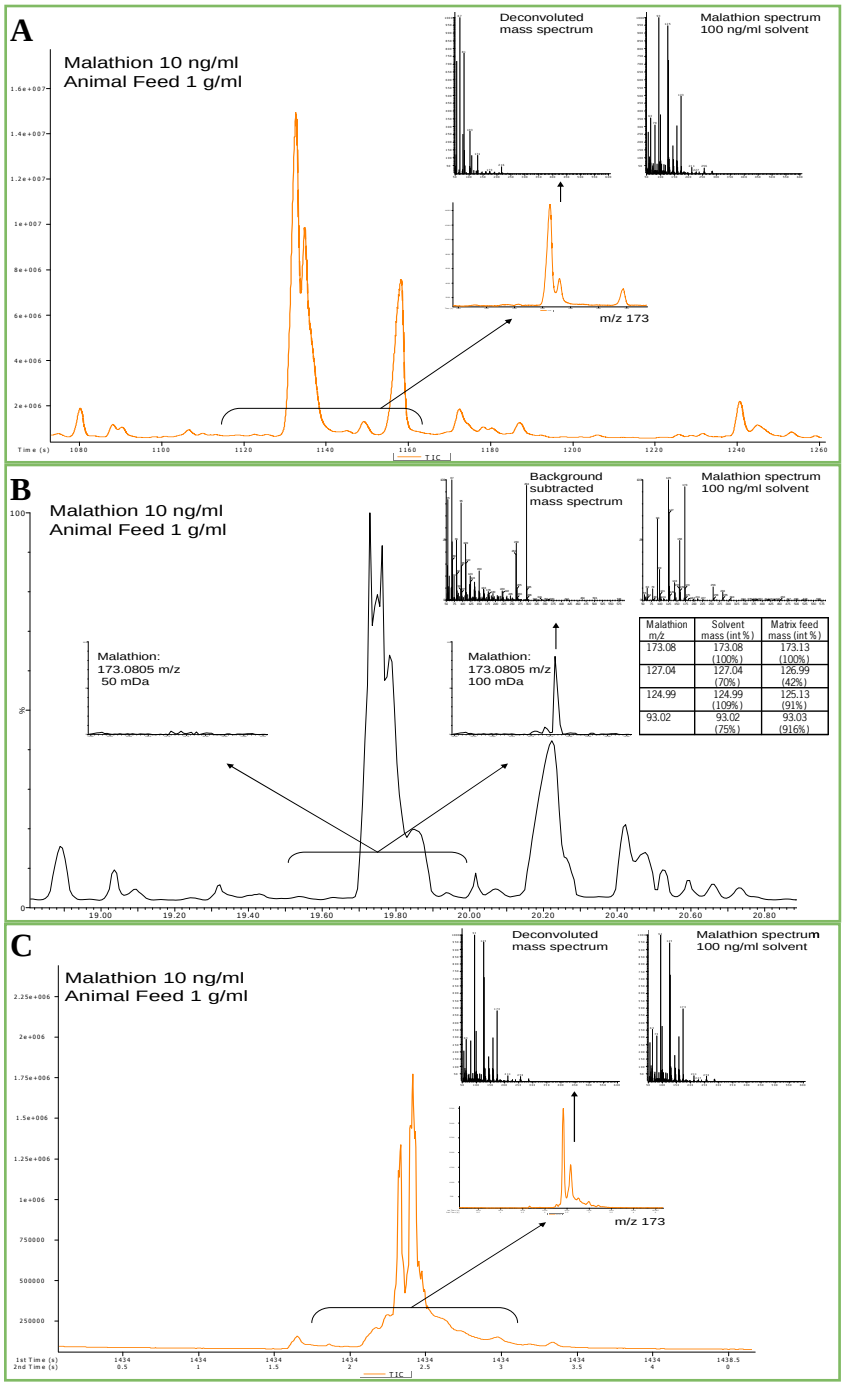


Figure 1: **A)** GC-hsTOF-MS chromatogram of malathion in animal feed at 10 ng/g, and the deconvoluted mass spectra obtained for malathion in the sample and in solvent. **B)** GC-hrTOFMS chromatogram of same animal feed sample with m/z 173.0805 ± 25 and 50 mDa windows. Mass spectra are background subtracted. **C)** GCxGC-hsTOF-MS chromatogram of the animal feed sample and the deconvoluted mass spectra for malathion in feed and solvent.

Next to malathion results of analysis for a few other compounds, which also co-elutes with matrix compounds, were compared to. After identification and quantification the LOQ has been calculated using a calibration curve in matrix, LOQ (figure 2A). Also the limit of automated detection (LOaD), which is the lowest concentration where confirmation criteria were met automatically was calculated.

Finally the linearity for each analyt/matrix for each technique was evaluated and expressed in a relative score. The total score per technique is shown in figure 2B.

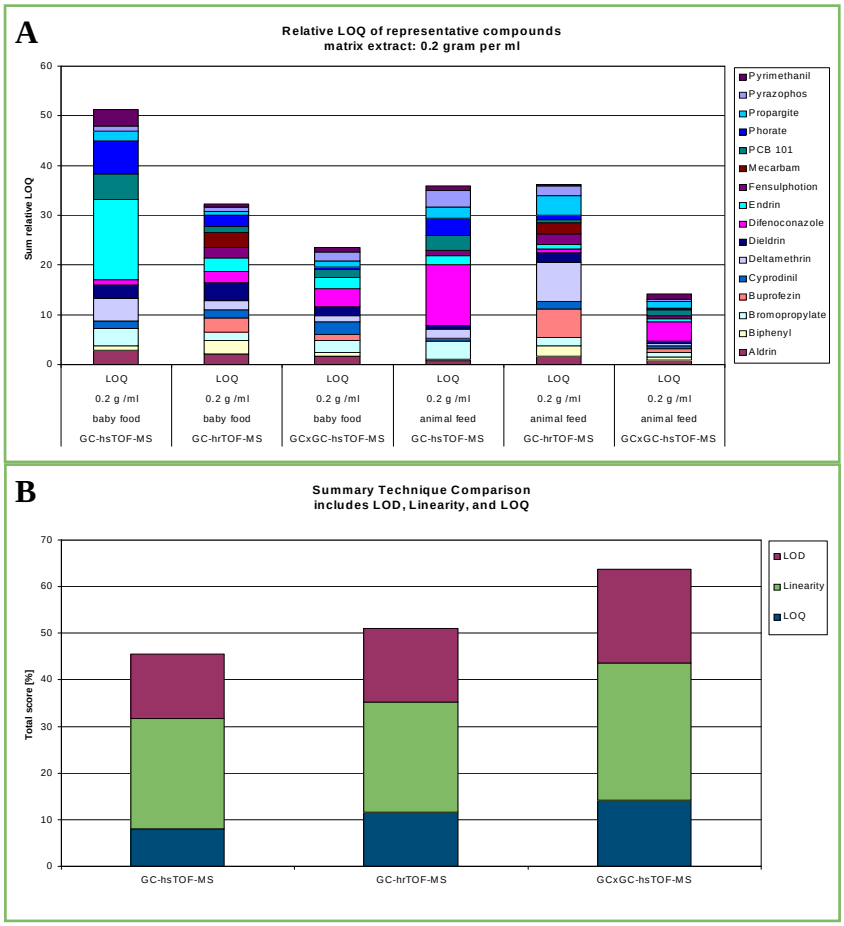


Figure 2: **A)** Relative LOQ for representative compounds which co-elute with matrix. **B)** Score per technique based on LOQ, linearity and the LOaD, the scores were summed.

## Conclusions

As expected, adverse effects of matrix on LOaD, LOQ and linearity were encountered using GC-hsTOF-MS with nominal mass for determination of pesticides in complex matrices. Lack of selectivity could not sufficiently been compensated using deconvolution. Better performance characteristics were obtained using GC-hrTOF-MS. The best performance was achieved using GCxGC-hsTOF-MS, although with this technique data processing was very time consuming.
However, currently available instruments and/or software still have inherent limitations so that the application of either approach to the routine screening of pesticides in complex samples is not straightforward.

## References

[1] M.K. van der Lee, G. van der Weg, W.A. Traag, H.G.J. Mol, J. Chromatogr. A, 1186 (2008) 325-339
[2] C.C. Leandro, P. Hancock, R.J. Fussell, B.J. Keely, J Chromatogr A. 1166 (2007) 152-62