Multicomponent biopesticide test items: Analytical challenges and opportunities (OECD 106, OECD 307, OECD 221)

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Introduction

- Biopesticides are often composed of complex mixtures of substances.
- The ID of these components may not be well defined, and the composition of a test item may change over time (e.g. degradation during a study).
- This can present analytical challenges as it may not be clear what to measure.
- Typical LC/MS methods using multiple reaction monitoring (MRM) on triple quadrupole instruments are most suited to analysis of well-defined mixtures.
- As an alternative approach, high resolution-accurate mass spectrometry (HRAM) can be used for both identification and quantitation of multiple components in complex mixtures.
- The example presented here demonstrates this technique for the analysis of an algal polysaccharide composed of a mixture of sugars.
- The test item was used to perform OECD 106 (adsorption/desorption), OECD 307 (transformation in soil), and OECD 221 (*Lemna sp*. growth inhibition) tests.

Methodology

Material and test conditions

- Mass spectrometry was performed using a ThermoFisher Q-Exactive instrument coupled to a Dionex Ultimate 3000 UHPLC system.
- Spectra were recorded in negative ion mode at 70,000 resolution in the range m/z 800-2200. Compounds of interest were separated from matrix interferences on a C18 column.
- Due to the instability of the test item, samples were frozen immediately after extraction and thawed individually a few minutes before analysis by LC/MS.

Identification of components of interest

- To identify optimal conditions for analysis of the components of interest, a solution of the test item was infused directly into the mass spectrometer using a syringe pump.
- Different mobile phases and additives were introduced into the flow via a T-junction.
- Various adducts and charge states were detected depending on the conditions used.
- The best results were obtained when using ammonium acetate as a mobile phase additive, where multiple components corresponding to polysaccharides were detected as triply charged ions ($[M-3H]^{3-}$).
- In total, over 20 distinct polysaccharides were identified with a mixture of isotopomers detected for each. The $^{13}C_3$ isotopomers were the most intense, so were selected for use in subsequent method development.

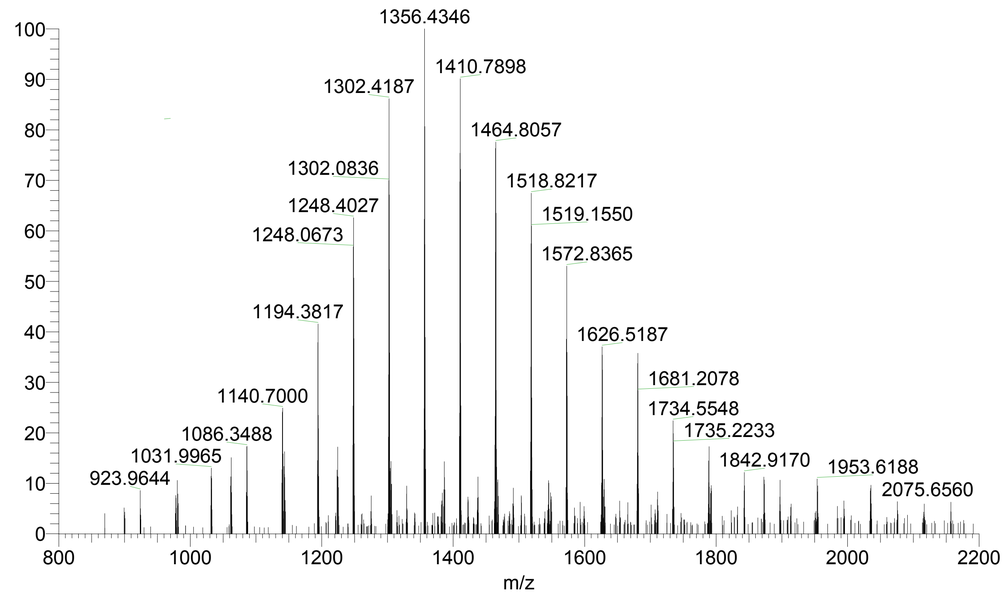

Figure 1: Full scan mass spectrum showing the range of ions observed for the test item

Quantitation of the test item

- To quantify the test item, the five most intense peaks were selected as marker compounds.
- Calibration curves were plotted between 0.05-10 µg/mL with linearity (r^2) >0.999 and the bias of each individual point <10%.
- While only these selected peaks were used for quantitation, an advantage of using HRAM full scan mass spectra is that the data could subsequently be re-analysed if necessary.
- For example, the pattern of the test item was compared between the start and end of studies to monitor for any differences in the rate of degradation of individual components.

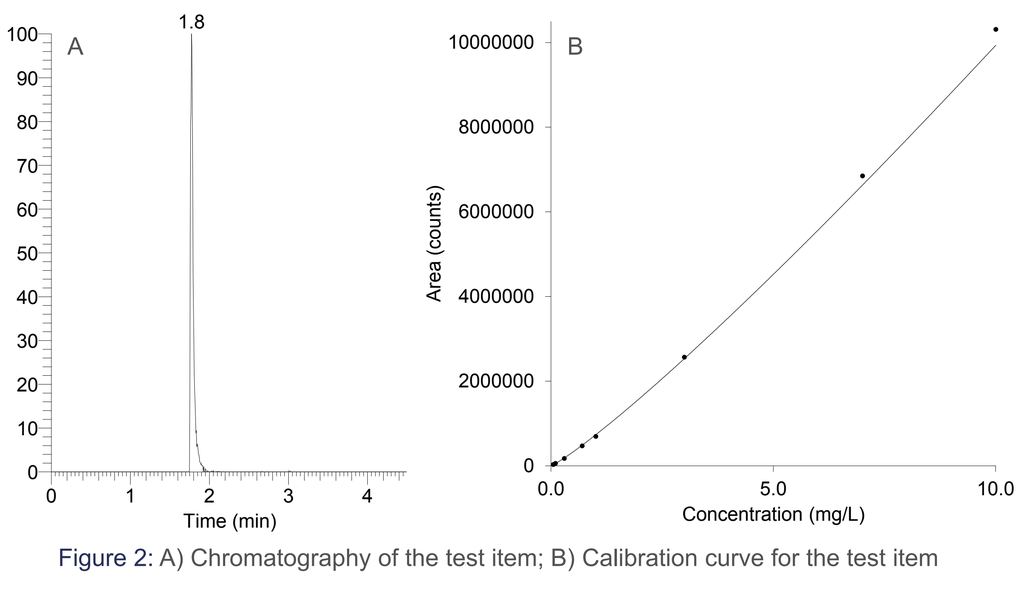

Figure 2: A) Chromatography of the test item; B) Calibration curve for the test item

Results & Discussion

OECD 106 – adsorption/desorption

- Preliminary tests showed that the test item was stable in aqueous $CaCl_2$ solutions, but very unstable in contact with soil. Sterilising the soils slowed the degradation rate, but did not eliminate it.
- The main test was carried out using small amounts of soil and short adsorption times to find an optimum balance between adsorption and degradation.
- Method development, optimization, and validation (SANCO/825/00) took approximately six months, while the main test duration was only 10 minutes.

	Soil			
Parameter	1	2	3	4
K_F	0.693	0.002	0.643	0.020
K_{FOC}	22.0	0.2	16.0	1.1

Table 1: Values obtained in the OECD 106 test

OECD 307 – transformation in soil

- An OECD 307 test was carried out using small soil aliquots (1.5 g) treated with a high concentration of the test item (2.5 mg).
- This allowed determination of DT_{50} values of 0.6-12 hours, and DT_{90} values of 11-39 hours for four soils.
- Interrogation of the full scan mass spectra showed that there was no change in the pattern of the test item during the test.

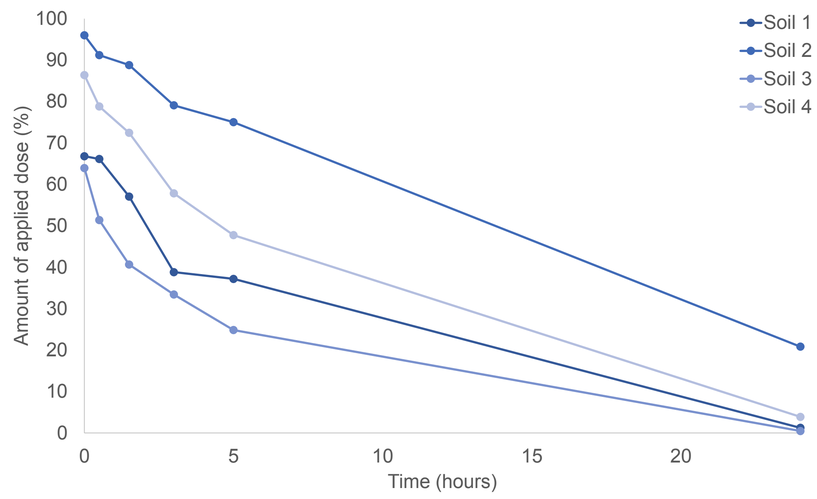

Figure 3: Degradation of the test item in the OECD 307 test

OECD 221 – *Lemna sp.* growth inhibition

- The impact of the test item on the growth of the freshwater aquatic plant *Lemna gibba* (duckweed) was investigated in a 7-day semi-static test.
- No toxic effects of the test item on *Lemna gibba* were observed.
- Mass spectrometry based quantitation of the test item throughout the test showed a decrease in the nominal concentration of 100 mg/L.
- A concurrent increase in the number of plants in the treated water compared to the control is consistent with the plants using the test item as an energy source.

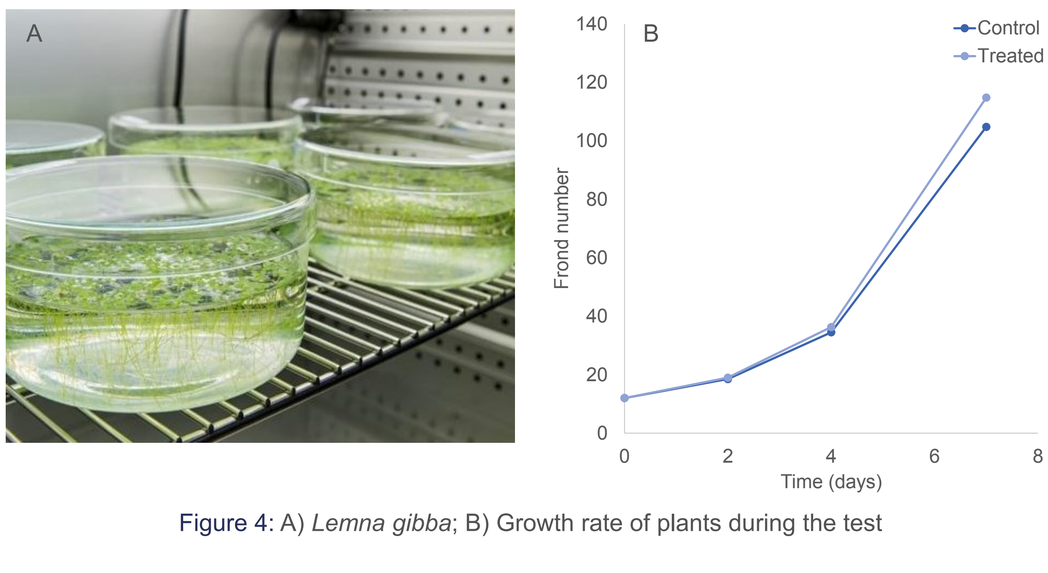

Figure 4: A) *Lemna gibba*; B) Growth rate of plants during the test

Conclusion

- An LC/MS method using HRAM detection was developed for the analysis of a complex algal polysaccharide.
- Quantitation was based on a limited number of marker compounds, while full scan spectra were used to check for changes in the make-up of the test item during tests.
- This method was used to support environmental fate and aquatic ecotoxicology tests following OECD 106, OECD 307, and OECD 221 guidelines.
- Other challenges included rapid degradation of the test item in contact with soil, and use of the test item as an energy source by plants.

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