The application of LC-UFMSMS to the analysis of pesticide residues in negative ion ESI

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Introduction: Analysis using Nexera-LCMS-8040

Glyphosate is a widely used herbicide and residues in food and water for human consumption are of regulatory interest. The LCMS analysis of residual glyphosate and its metabolite AMPA in agricultural specimens is limited primarily by the impact of the polarity of these compounds on chromatography and sample preparation. We describe here the analysis of FMOC derivatised analytes using a reversed-phase separation and multiple ion MRM detection in negative ion ESI.

Method

Food samples were spiked with glyphosate and AMPA and extracted and reconstituted to give extracts equivalent to 0.001, 0.0025, 0.005, 0.01, 0.1 and 1 ppm. Extracts were derivatised with FMOC reagent using standard protocols (Figure 2, method details available upon request but not reported here). Derivatised extracts were delivered to the analyst as blind specimens. Analysis was completed on a Nexera LC-30-LCMS-8040 (Shimadzu, Kyoto, Japan) equipped with a Shimpack XR-ODS III (75mm x 2.1, 1.7µm particle) column. The sample was injected with a Sil-30AC autosampler and injection volume ranged in size from 1 to 10 µL. Analytical conditions were the same as those used for the LCMS-8030 analysis of herbicides (see right).

Figure 1: Organisations contributing to the blind analysis of FMOC samples

Discussion

The FMOC derivatives enable the use of a reversed-phase separation rather than a HILIC or other separation strategy (Figure 3). This approach allows the use of a more robust mobile phase to support negative ion ESI and the quantitation of glyphosate and AMPA in food extracts. The method sensitivity exceeded current regulatory requirements and so provided the capacity to incorporate miniaturisation of the sample preparation steps or the use of smaller specimens.

Conclusion

The UFSweeper™ equipped LCMS-8040 allowed detection of 1 pg of FMOC-glyphosate and 2.5 pg of FMOC-AMPA on column with signal to noise ratios of > 5:1 and > 6:1 respectively (Figure 4). The method was linear over the range of 1 – 1000 pg on column for both analytes with correlation coefficients of r^2 = 0.9999 for both the entire concentration range and also for the range 1-50 pg (Figure 5).

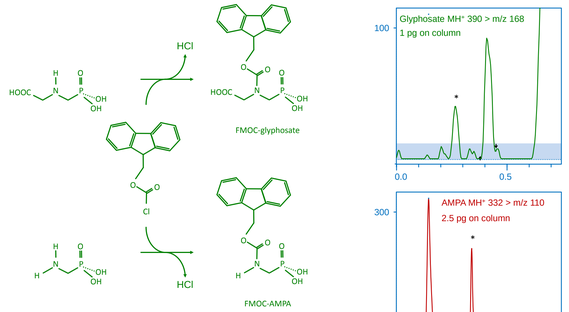

Figure 2: FMOC derivatisation of Glyphosate and its metabolite AMPA

Figure 4 (above): S/N for AMPA and glyphosate. (peaks from the matrix)*

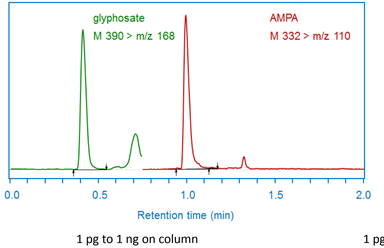

Figure 3 (left): The separation of FMOC derivatives of AMPA and glyphosate.

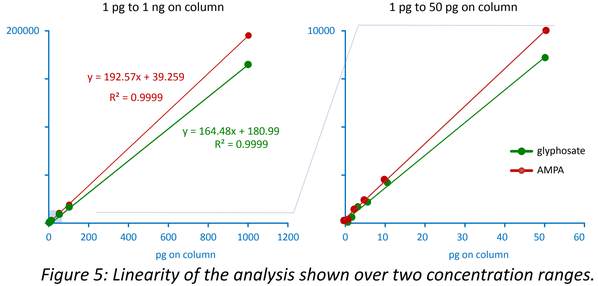

Figure 5: Linearity of the analysis shown over two concentration ranges.

Introduction : Analysis using Nexera-LCMS-8030

Phenoxy and carboxylate herbicides and related compounds are good targets for negative ion ESI. Sensitivity can be hindered by mixed chemistry (e.g. picrolam) that supports zwitter-ionic character as well as early elution from reversed-phase columns. We describe the analysis of a range of herbicides by negative ion ESI using the Shimadzu LCMS-8030 ion source.

Method

Herbicide standards were spiked into food samples (grains or fruits), extracted using a Quechers regime and supplied as blind samples for analysis. Chromatography was carried out on a Nexera LC-30-LCMS-8030 (Shimadzu, Kyoto, Japan) equipped with a Shimpack XR-ODS III (75mm x 2.1, 1.7µm particle) column at a flow rate of 0.4mL/min and a mobile phase of 90% 10mM aqueous ammonium acetate (A): 10% 10mM ammonium acetate in methanol (B). The initial solvent composition was held from 0 to 0.2 min then ramped to 95% solvent B at 10min (hold 2 min). MRM was optimised automatically and parameters (protonated molecular ion, selected fragment ion, Q1 pre-rod bias voltage, optimised collision potential difference and Q3 pre-rod bias) are shown in Table 1. The drying line was 280 °C, N_2 nebulising gas 3L/min, heater block 400 °C, the drying gas at 15L/min and the CID gas was argon at 230 kPa. Injections of 1 to 10 µL equivalent to 1 to 1000 pg on column were made.

	Parent	Fragment	Q1	CE (V)	Q3
1. clopyralid	189.8	146.0	19	12	16
2. picloram	238.8	194.9	25	10	13
3. dicamba	218.8	175.0	23	8	11
4. fluoxypyr	252.8	195.0	26	12	13
5. bromoxynil	275.8	166.9	13	34	16
6. MCPA	198.9	141.1	21	14	14
7. 2,4-D	218.8	161.0	22	14	10
8. triclopyr	253.8	196.0	12	12	13
9. 2,4,5-T	252.8	194.9	26	12	23
10. fluazifop	326.0	254.0	16	14	17
11. quizalofop	342.9	271.0	17	14	18
12. haloxyfop	359.9	287.9	17	14	13
13. glyphosate	390.0	168.0	19	12	16
		150.0	19	12	16
14. AMPA	332.0	110.0	16	7	10

Table 1 : Negative ion MRM parameters for test compounds.

Discussion and Results

The LCMS-8030 allowed detection of herbicides extracted from food products (Figure 6). Linearity was demonstrable over three orders of magnitude and MRM ion ratios were also stable over this range. Negative ion sensitivity was < 2 pg on column for all compounds studied with signal to noise of 3:1 or greater. (Figure 7) Sensitivity was improved by the inclusion of an in-line filter to supress background contaminants from the water (data not shown). Sensitivity for pure standards was improved primarily because of the absence of matrix interferences.

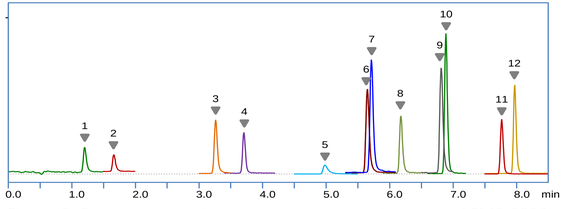

Figure 6: Chromatogram for phenoxy herbicides in food on LCMS-8030.

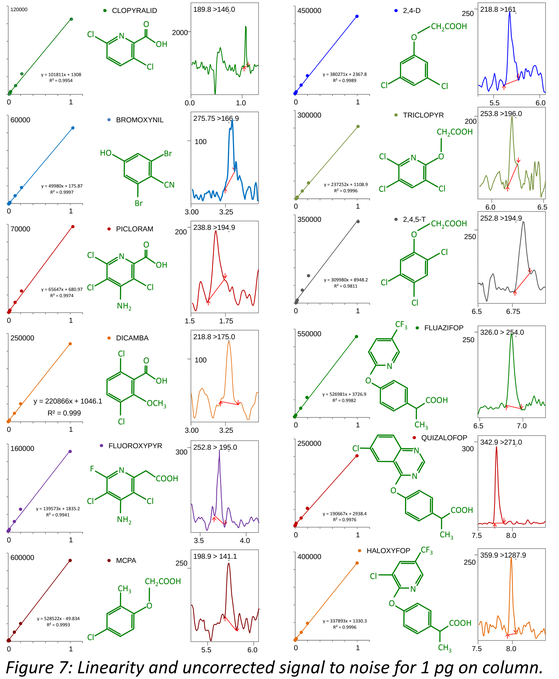

Figure 7: Linearity and uncorrected signal to noise for 1 pg on column.