LC/MS Application Note

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Fast Analysis of Phenoxyacetic and Phenoxypropionic Herbicides in Vegetable Matrix by Negative Ion Electrospray LC/MS/MS

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Introduction

Phenoxy acids are used as herbicides in agricultural and domestic areas. These compounds have to be detected at trace level to ensure a safe drinking water and food supply and verify appropriate remediation of these pollutants from contaminated sites.

Among the analytical methods developed, LC/UV exhibits poor sensitivity and inadequate selectivity for complex vegetable matrices. Negative ion ESI LC/MS has exceptionally high sensitivity for these herbicides and, with the added selectivity of MS/MS, provides accurate analyses even for the most complex sample matrices.

A highly sensitive and fast method is described below for high throughput identification and quantitation four common herbicides in vegetable extracts (Figure 1).

Instrumentation

- Varian ProStar 410 AutoSampler
- Varian ProStar 210 Solvent Delivery Modules
- Varian 1200L LC/MS with ESI source

Materials and Reagents

- Standard solutions: pesticides were provided by the GIRPA group, France.
- ASE®, 300 (Dionex)
- Florisil (Carlo Erba)
- Hydromatrix bulk material (Varian Part No. 198003)
- All other chemicals are reagent grade or HPLC grade.

Compound Structures

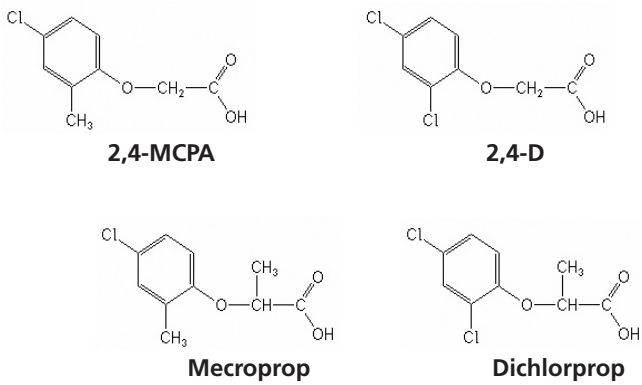

Figure 1. Chemical structure of studied herbicides.

Sample Preparation and Extraction

Liquid/solid extraction is performed using the Varian Sample Preparation Products' Hydromatrix (25g) and matrix (25g onion). Two grams of Florisil is added to the homogeneous mixture followed by Accelerated Solvent Extraction (ASE) with ethyl acetate (100 mL, 100 bar, 50 °C). After 100 µL of n-dodecane is added to the extract, the solution is evaporated to dryness and the residue is dissolved with 25 mL of acetonitrile. The final solution is then spiked with 5 ppb of herbicides.

HPLC Conditions

Column	Polaris C18A 3 µm, 150 x 2 mm ID (Varian Part No. A2001150x020)			
Mixer	250 µL static mixer			
Solvent A	water			
Solvent B	Acetonitrile			
LC Program	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	80	20	0.25
	5:00	00	100	0.25
Injection Volume	20 µL			
Injection Solvent	water/acetonitrile (50:50)			

MS Parameters

Ionization Mode	ESI negative
Collision Gas	1.4 mTorr Argon
API Drying Gas	19 psi at 250 °C (N_2)
API Nebulizing Gas	52 psi air
Scan Time	1.0 sec
SIM Width	0.7 amu
Needle	3500V
Shield	600V
Capillary	30V
Detector	1900V
SIM mode	deprotonated molecules $[M-H]^-$

MS/MS Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)	Retention Time (min)
2,4-MCPA	199	141	14	4.46
2,4-D	219	161	18	4.56
Mecroprop	213	141	12	4.77
Dichlorprop	233	161	18	4.84

Results and Discussion

The MS/MS conditions shown above are determined with the Automated MS/MS Breakdown software (also see Varian LC/MS Application Note 11 and 12).

The LC method uses a fast gradient program for a complete analysis in less than ten minutes. Due to the structural similarity of the acidic herbicides, incomplete resolution is observed under faster separation conditions.

The relative selectivity of LC/MS (SIM mode) and LC/MS/MS (MRM mode) are directly compared using spiked matrix samples. For pure standards, the sensitivity in the SIM mode is excellent, but even at the highest spiked concentration of 5 ppb, matrix interferences are strong. In Figure 2, SIM plots for all four herbicides shows extra peaks and baseline instability. For mecoprop, an interfering peak clearly overlaps with the analyte. As with a LC/UV detector, reliable quantitation is not possible with SIM, especially at lower concentrations.

Using the same spiked matrix extract, the increased selectivity of MS/MS is readily apparent. The MRM chromatograms in Figure 3 are interference free. The baseline is clear from any false positive peaks and no cross talk effect is observed when similar product ions are monitored in the same segment time (Table 1 and Figure 3). With the elimination of these matrix interferences, quantitation with high confidence is possible.

The MRM method has excellent linearity for all the herbicides from 0.1 to 5 ppb as shown for dichlorprop in Figure 4. The developed MRM method is also very sensitive with a LOD of 0.1 ppb for all the pesticides under the described instrument conditions.

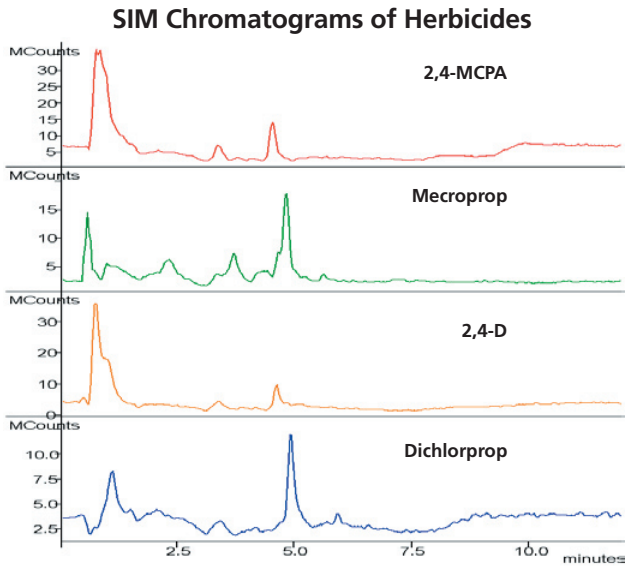

Figure 2. SIM chromatograms show baseline instability and interfering peaks (negative ESI, onion matrix, 5 ppb).

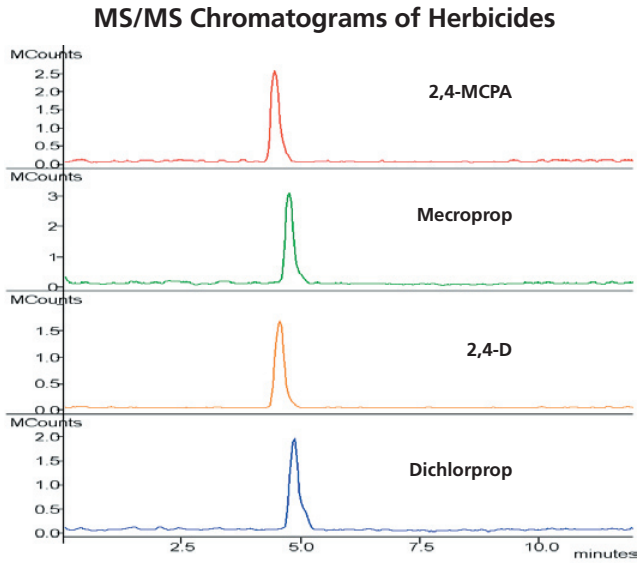

Figure 3. MS/MS selectivity eliminates interfering peaks observed in SIM mode (negative ESI, onion matrix, 5 ppb).

Example of a Calibration Curve for Dichlorprop

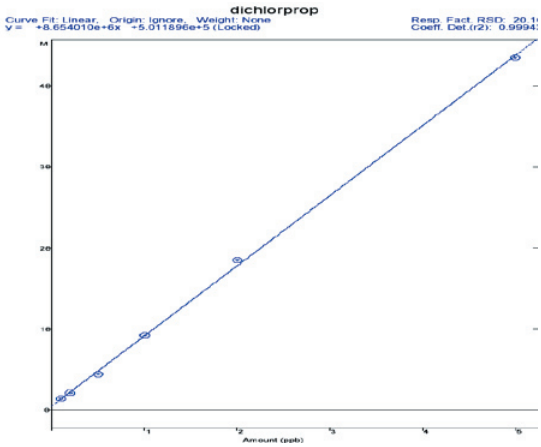

Figure 4. Calibration curve for dichlorprop at 0.1 to 5 ppb shows a correlation coefficient of 0.9994 (external standard).

Conclusion

This 1200L LC/MS/MS method is simple, fast, and highly sensitive. The method can quantitatively analyze pesticides at sub-ppb levels and maintain accuracy even with complex matrices. The 1200L LC/MS/MS system is recommended when sensitivity and selectivity are required at very low concentration.

These data represent typical results.
For further information, contact your local Varian Sales Office.