COGENT 2.0
Diamond Hydride™

Analysis of Herbicides and Metabolites

Glufosinate
N-Acetylglufosinate
Glufosinate Propanoic Acid
Diquat
Paraquat

Extended Application Note

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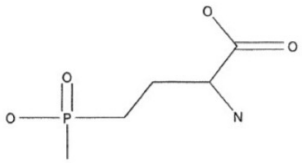
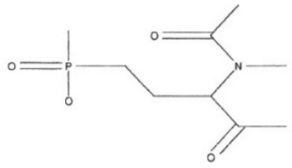
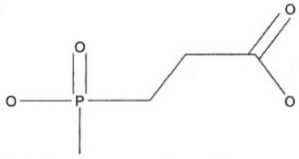


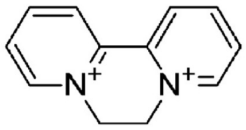
Glufosinate



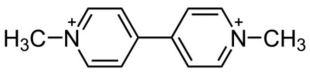
N-acetyl Glufosinate



Glufosinate propanoic acid



Diquat



Paraquat

Figure 1

Introduction

The use of herbicides can be a controversial issue. Although they can be effective in preventing weeds from destroying agricultural crops, there are a number of possible adverse effects. First, the weeds themselves may develop resistance to the herbicides over time. The introduction of herbicides with an alternative mode of action such as glufosinate was spurred by such resistance in the case of glyphosate. Second, the toxicity of these compounds is not limited to plants but has also been demonstrated in the case of animals and humans. This may become an issue where the herbicides end up in soil and waters.

Two primary metabolites of glufosinate are N-acetylglufosinate and glufosinate propanoic acid. Due to the potentially toxic nature of these herbicides, analysis of their metabolites in a variety of sample matrices may be required. However, all three compounds are quite polar and therefore difficult to retain with conventional reversed phase chromatography. Ion-pairing agents may be used to increase retention, but these additives are not amenable to LC-MS.

Other notable herbicides include diquat and paraquat. These two compounds are permanently charged cationic amines and can be problematic in chromatographic separations. The structures of all five compounds are shown in **Figure 1**.

Experimental

Materials

Glufosinate (1720.64 ppm), N-acetylglufosinate (639.2 ppm), and glufosinate propanoic acid (1302.5 ppm) stock solutions were diluted 1:100 with a diluent of 4:1 DI H_2O: methanol. For a separate mixture, stock solutions of paraquat 2432.4 (ppm) and diquat 2037.9 (ppm) were diluted 1:100 with the same diluent. Paraquat and diquat were stored in plastic vials as they bind tightly to glass.

Instrumentation

Detection was achieved using an ESI – POS – Agilent 6210 MSD TOF mass spectrometer. The mobile phase solvents were A: DI H_2O/ 0.1% formic acid and B: acetonitrile/ 0.1% formic acid. In the case of the glufonsinate and metabolites, negative ion mode was also investigated in which case the mobile phase solvents were A: 10mM ammonium acetate and B: 95% Acetonitrile/ 5% DI H_2O/ 10 mM ammonium acetate (v/v).

The gradients for each analysis were as follows:

Glufosinate & Metabolites		**Glufosinate & Metabolites**		**Diquat & Paraquat**	
Negative Ion Mode		**Positive Ion Mode**		**Positive Ion Mode**	
Time (min)	%B	Time (min)	%B	Time (min)	%B
0	90	0	80	0	90
1	90	1	80	1	90
1.2	5	1.5	5	1.2	5
5	5	5	5	5	5
6	90	6	80	6	90

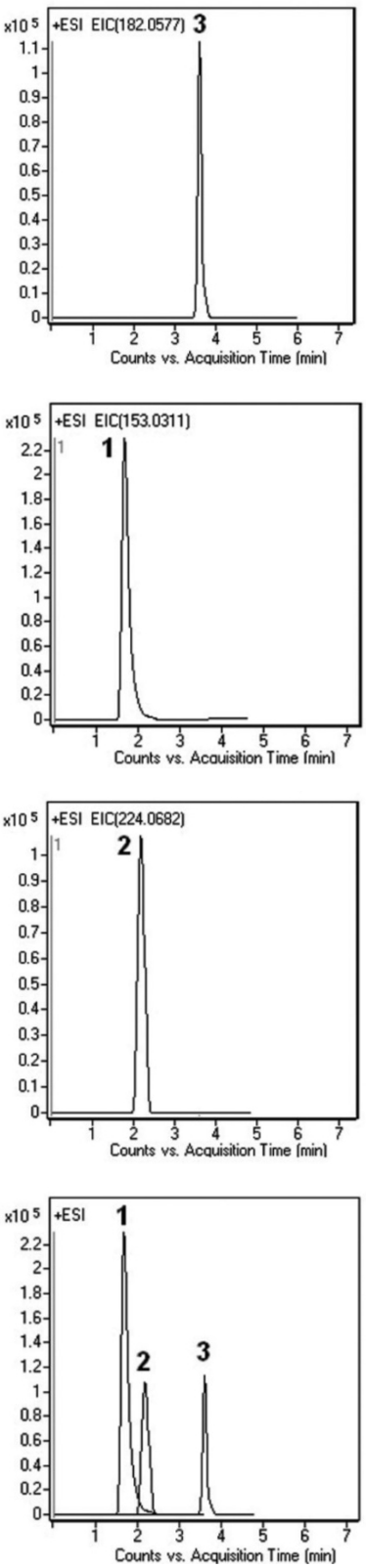


Figure 3

Sample Preparation

The Diamond Hydride 2.o™ 2.1 x 50 mm column was used for glufosinate and metabolites in negative mode as well as for the diquat/paraquat separation. For glufosinate and metabolites in positive mode, the Diamond Hydride™ 4µm 2.1 x 150 mm column was used instead. In all cases, the flow rate was 0.4 mL/min.

Results and Discussion

For glufosinate and metabolites, negative ion mode was first investigated. The data is shown in **Figure 2**.

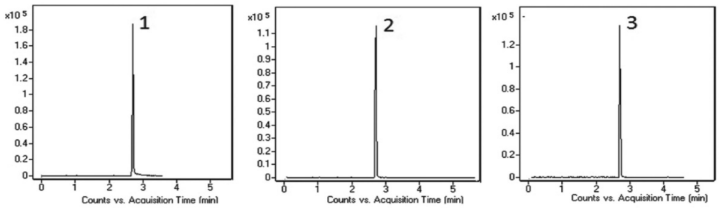


Figure 2

The use of ammonium acetate in the mobile phase (suitable for analyses in negative ion mode) produced analyte peaks of excellent efficiency and as well as symmetry. Each analyte peak can be isolated with the use of an extracted ion chromatogram (EIC). Although the peaks maynot be chromatographically separated, specificity can be obtained with the EICs. Still, analysts may require chromatographic separation if they are using another detection mode such as ELSD or CAD. To this end, we also wanted to demonstrate that this could also be achieved under suitable conditions.

The analytes contain both positively and negatively ionizable moieties and therefore should be readily detectable using either positive or negative ion mode. A formic acid additive was chosen for studies conducted in positive mode. **Figure 3** shows the separation obtained under these conditions.

Here the tradeoff is in terms of efficiency and peak shape. Although the peaks are not quite as sharp and symmetrical as with the negative ion method, chromatographic separation is obtained. Every analyst may have different analysis goals and may find one of these methods more useful than the other. For LC-MS analyses, the sharper peaks lead to greater sensitivity which may be a priority in many applications. The chromatographic separation may not be necessary at all. Nonetheless, it is important to demonstrate that the Diamond Hydride™ column can not only retain these polar compounds, it can separate them as well.

Paraquat and diquat are permanently charged cationic compounds and would therefore be best analyzed in positive mode. Due to their strongly cationic nature, these compounds should be stored in plastic vials to avoid electrostatic adsorption with silanols on the surface of glass vials. In terms of chromatography, the compounds may have a tendency to tail for the same reason; the cationic groups bind with residual silanols present in the analytical column. However, columns based on TYPE-C Silica™ are virtually free of silanols and therefore these problems can be avoided. This is illustrated in **Figure 4**, where the two compounds are separated with excellent peak shapes.

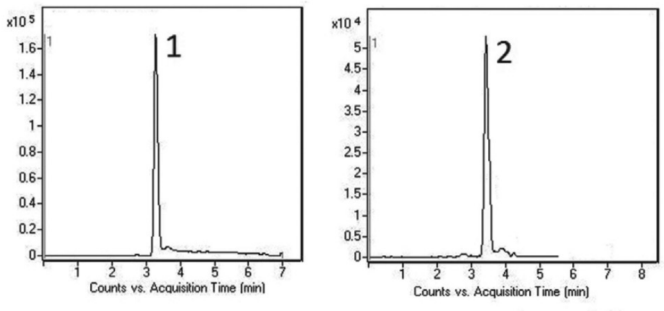


Figure 4

Conclusion

The Diamond Hydride™ column has shown excellent suitability for analysis of these types of compounds. The retention and peak shapes are excellent, which can be obtained without ion-pair agents. The use of LC-MS is particularly important for these compounds since they may need to be analyzed in complex matrices such as crops, groundwater, and soil.