Bhupinder Singh¹; David S. Jensen²; Andrew J. Miles²; Andrew E. Dadson²; Matthew R. Linford¹

¹ Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT-84602, USA
² Diamond Analytics, 1260 S 1600 W, Orem, UT-84058, USA

Introduction

The Diamond Analytics Flare core-shell column is made by depositing alternating layers of polyallyamine (PAAm) and nanodiamond on solid carbon core particles.¹,² The final PAAm layer is reacted with 1,2-epoxyoctadecane and cross linked with 1,2,7,8-diepoxyoctane to produce a mixed-mode weak anion exchange (WAX)/C₁₈ column.² These diverse functionalities impart unique selectivity to the column. A number of classes of compounds have been successfully analyzed on the column, including the separation of critical pairs of acidic herbicides.³

The present work is focused on understanding the retention mechanism of acids (acidic herbicides) as a function of their pKₐ values on the Flare column at low pH. In particular, gradient elution was used to separate 2,4-D (2,4-dichlorophenoxyacetic acid), MCPA (2-methyl-4-chlorophenoxyacetic acid), 2,4-DB (4-(2,4-dichlorophenoxybutyric acid), MCPB (4-(4-chloro-2-methylphenoxy) butanoic acid), dichlorprop, mecoprop and dicamba (see Figure 1). Interestingly, at high pH the elution order of the analytes is reversed.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>Cl</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>MCPA</td>
<td>CH₃</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>Dichlorprop</td>
<td>Cl</td>
<td>CH₃</td>
<td>-</td>
</tr>
<tr>
<td>Mecoprop</td>
<td>CH₃</td>
<td>CH₃</td>
<td>-</td>
</tr>
<tr>
<td>2,4-DB</td>
<td>-</td>
<td>-</td>
<td>Cl</td>
</tr>
<tr>
<td>MCPB</td>
<td>-</td>
<td>-</td>
<td>CH₃</td>
</tr>
</tbody>
</table>

*Figure 1.* Structures of various acidic herbicides. Dicamba is on the upper right. The X, Y, and Z groups in the table define the other analytes.

Experimental

All analytes were purchased from Sigma-Aldrich (St. Louis, MO).
**Chromatograph:** Agilent 1290 Infinity Binary LC, DAD, ChemStation software

**Sample:** 2 mg/mL of 2,4-D, MCPA, 2,4-DB, MCPP, dichlorprop, mecoprop and/or dicamba in a mixture of 1:1 H₂O:acetonitrile (ACN)

**Column:** Flare Mixed-Mode Column (4.6 × 3.3 mm, 4.0 µm)

**Injection volume:** 2.0 µL

**Temperature:** 60 °C

**Flow rate:** 1.0 mL/min

**Detection:** UV detection at 280 nm

**Mobile Phase:**
- A: 1.5 % formic acid in H₂O, pH 2.0
- B: 1.5 % formic acid in ACN

**Elution:** Gradient
- 40 to 60 % B in 6 minutes

---

### Table 1

<table>
<thead>
<tr>
<th>Analytes</th>
<th>pKₐ</th>
<th>[A⁻]/[HA]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCPB</td>
<td>4.5</td>
<td>0.003</td>
</tr>
<tr>
<td>2,4-DB</td>
<td>4.1</td>
<td>0.008</td>
</tr>
<tr>
<td>Mecoprop</td>
<td>3.86</td>
<td>0.014</td>
</tr>
<tr>
<td>MCPA</td>
<td>3.73</td>
<td>0.019</td>
</tr>
<tr>
<td>Dichlorprop</td>
<td>3.0</td>
<td>0.100</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2.87</td>
<td>0.134</td>
</tr>
<tr>
<td>Dicamba</td>
<td>1.87</td>
<td>1.349</td>
</tr>
</tbody>
</table>

*Table 1.* pKₐ values of acidic herbicide analytes and an estimate of the ratio of deprotonated to protonated analyte ([A⁻]/[HA]) in the pH 2.0 mobile phase.

---

### Results and Discussion

We first discuss the charge/protonation state of the Flare column and the acidic herbicide analytes at pH 2.0. The state of the column is simple to understand. At this low pH the amino groups on the Flare stationary phase are mostly protonated so the Flare column is strongly positively charged. To help us understand the protonation state of the acidic analytes, we use the Henderson-Hasselbach (H.-H.) equation. A description of the acid-base chemistry that leads to the H.-H. equation and a derivation of the equation itself are given in a Diamond Analytics app note on this topic. The H.-H. equation is:

\[
pH = pK_a + \log ([A^-]/[HA])
\]

where the pH is that of the mobile phase, the pKₐ is that of the analyte, [A⁻] is the concentration of the deprotonated analyte, and [HA] is the concentration of the protonated analyte. Of course the H.-H. equation, as presented here, applies more accurately to aqueous solutions. Nevertheless, it should help us estimate the degree of analyte deprotonation in the water/ACN mobile phase and thus analyte retention.

Table 1 gives the pKₐ values of the acidic analytes considered herein and also the ratio of the deprotonated to protonated form of these analytes at the mobile phase pH as given by the H.-H. equation. The least acidic herbicides, i.e., MCPB and 2,4-DB, are almost entirely protonated at pH 2.0. In contrast, the most acidic herbicides, i.e., dichlorprop, 2,4-D, and dicamba have significant amounts of the analyte in the deprotonated form.

The seven acidic herbicide analytes under consideration here were injected on the Flare column. As shown in Figure 2, the retention of these analytes is strongly influenced by their pKₐ values. That is, less acidic analytes with higher pKₐ values show less retention – a smaller fraction of these analytes is deprotonated – they are mostly neutral so they interact less with the positively charged stationary phase. For example, MCPB has the highest pKₐ value (4.5) of the analytes considered herein and elutes first. In contrast, the more acidic analytes with lower
$pK_a$ values show greater retention – a larger fraction of these analytes is deprotonated so they interact more with the positively charged stationary phase. For example, dicamba has the lowest $pK_a$ value (1.87) of the analytes considered herein and elutes last. Note also that even though the stationary phase has C$_{18}$ chains, at lower pH it is charged so it will work less effectively in reversed phase mode.

**Figure 2.** Dependence of retention factor ($k$) on the $pK_a$ values of the analytes. (in order of decreasing retention : Dicamba, 2,4-D, Dichlorprop, MCPA, Mecoprop, 2,4-DB, MCPB.)

To illustrate that the retention mechanism of the Flare column is strongly pH dependant, dicamba, 2,4-D, and 2,4-DB were injected separately using a pH 12 mobile phase. We have previously shown that the Flare column acts in reversed phase mode (not ion exchange mode) under these conditions.$^5,6$ Accordingly, for these analytes we observed a reversal of the elution order that is in Figure 2. Dicamba was least retained (it is the most compact of the three analytes), followed by 2,4-D (it is a structural isomer of dicamba but more extended), followed by 2,4-DB (it has two more methylene units than 2,4-D).

**References**