Analytical Challenges of USEPA Method 535

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Method 535 Overview

- **Chloroacetanilide and acetamide herbicides are commonly used for controlling broadleaf and other annual weeds on commercial crops including corn and soybeans.**
- **Studies have found that the metabolites of these herbicides are more prevalent than the parent compounds themselves in ground and surface waters.**

Method 535 Overview (cont.)

- **USEPA Method 535 was developed for the determination of the oxanilic acid (OA) and ethanesulfonic acid (ESA) metabolites of the following herbicides:**
  - **Alachlor**
  - **Acetochlor**
  - **Metolachlor**
  - **Propachlor**
  - **Flufenacet**
  - **Dimethenamid**

A basic problem…

- **The method requires LC/MS/MS, and although it is a powerful and elegant tool, LC/MS/MS is not common in most environmental labs.**
- **Is there an alternate and more cost effective conventional approach to prescreen for these analytes?**

Methods and Materials

- **An Agilent 1100 Series LC/MS/MS with an Agilent 1200 Series LC with Diode Array Detector (DAD)**
  - **Column temperature controller**
  - **SL binary pump**
- **Varian 5000 LC with a Phenomenex TS-130 column heater and an HP 1040A DAD**
- **AccuStandard has all 12 acetamide herbicide degredates as neats so that we could easily experiment in various matrices and concentrations.**

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Initial Findings

- **The Agilent LC/MS/MS performed extremely well when following Method 535.**
- **However the method has some downsides**
  - **Preparation is cumbersome and time consuming.**
  - **Interferences from “High Concentrations” as outlined in Section 4 of the method can cause problems.**

Could there be a good pre-screening method?

- **We postulated that the conventional LC/UV may be able to prescreen these samples that may have higher concentrations.**
- **Using this quick screen would keep the LC/MS/MS detector clean and allow only properly diluted lower level samples for which it is best suited.**
- **This methodology could be applicable for "dirty" samples if the USEPA expands the scope of the method to apply to soil or wastewater.**

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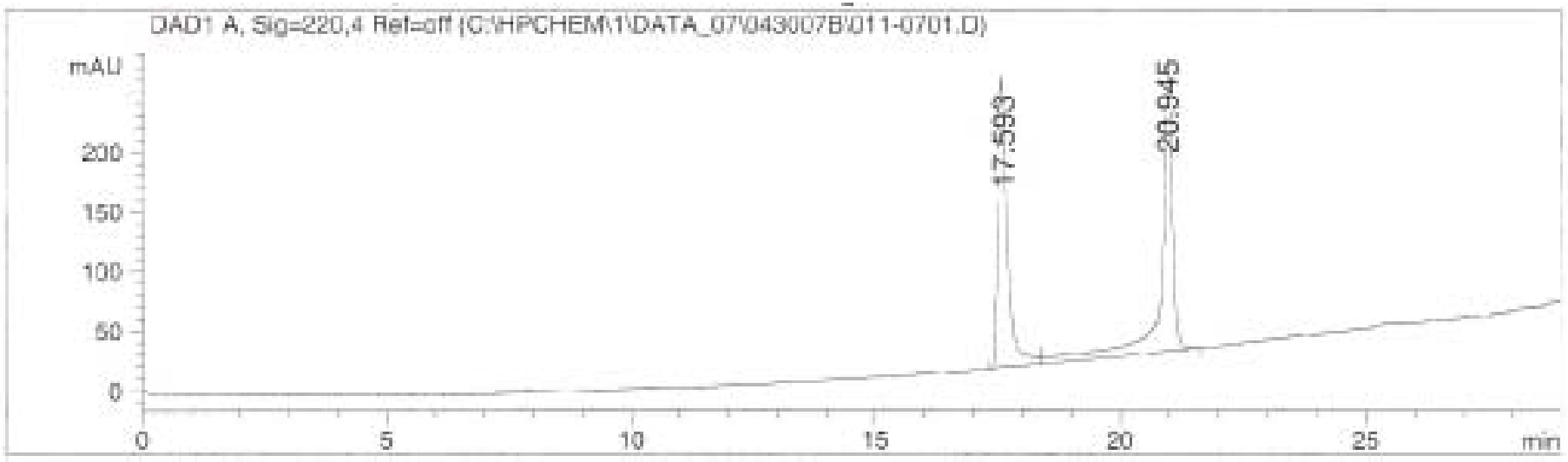
Experimental Methods

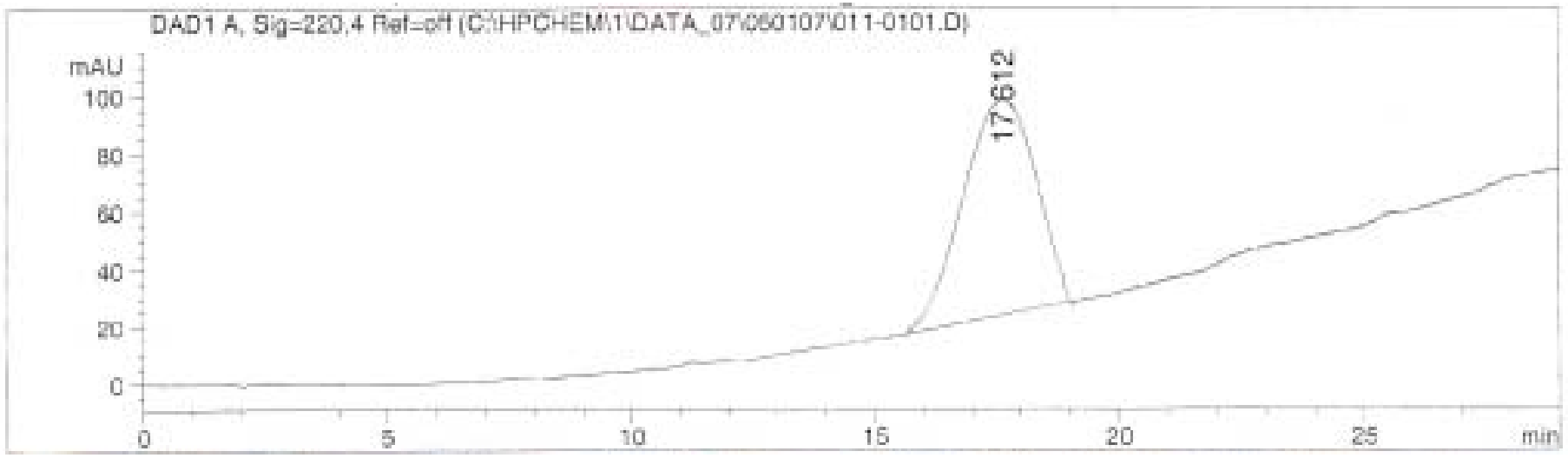
- **We mimicked the analytical conditions and standard preparations ranging from 1.0 to 100 ppm.**
- **Method 535 specifies a column temperature of 65 °C, but does not discuss the reason for this.**

Observations

- **We experimented with different column temperatures.**
- **While running the initial samples at room temperature we found that many of the analytes, especially the Acetochlor OA, resulted in two peaks.**

Acetochlor OA at room temperature shows two distinct peaks, at 65 °C they merge into a single peak





Method 535 refers to these stereoisomers

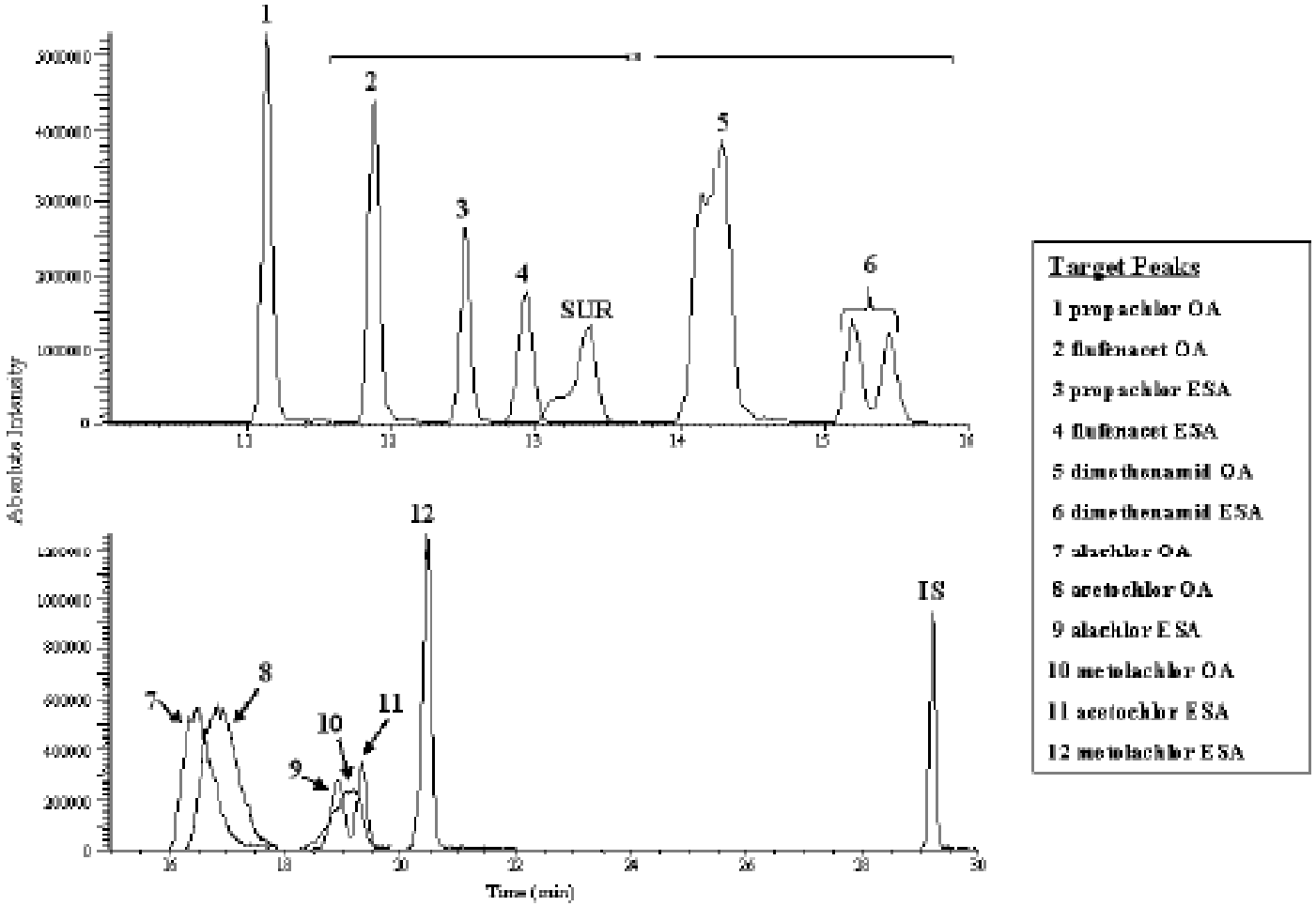
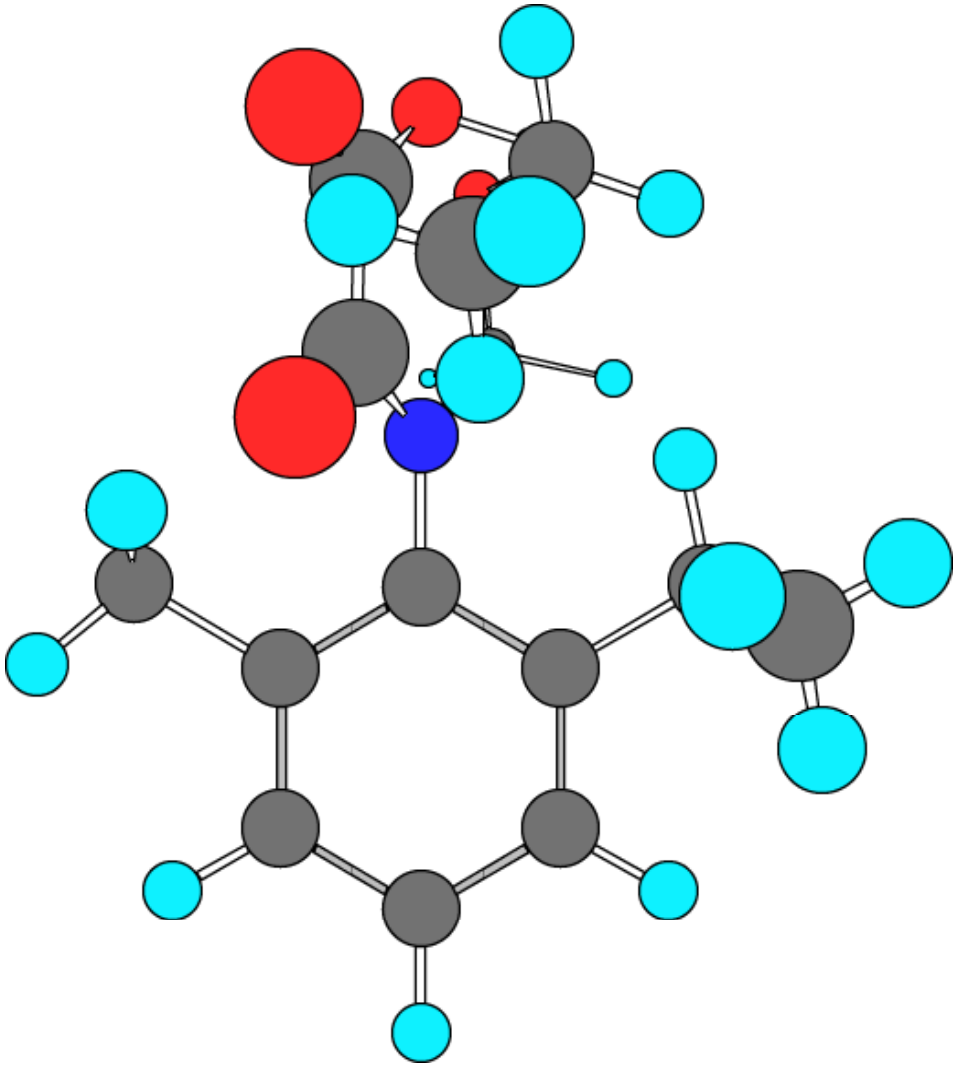


Figure 1. Ion trap LC/MS/MS chromatogram of a standard containing the target analytes at the concentrations listed in Table 10. Note there some of the analytes (SUR, 5, and 6) have two partially resolved peaks due to steroisomers.

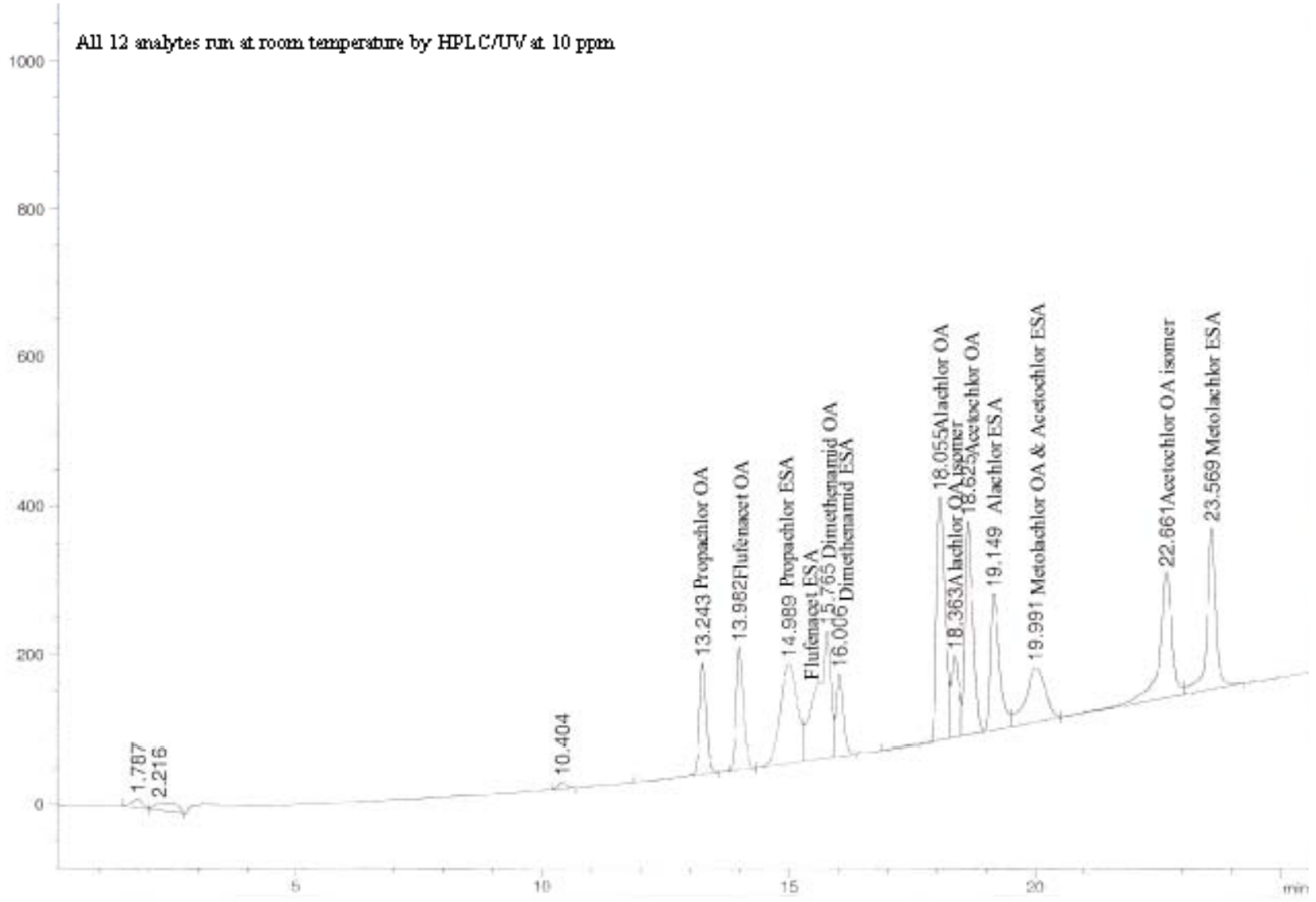
Confirmation of the Identity of the Isomers

- **Wondering about the identity of these individual peaks we repeated the experiment with the LC/MS/MS.**
- **The two peaks observed each had identical mass spectra.**
- **Our hypothesis is that these are rotamers around a single bond, and when heated the separation was lost.**

Postulation on the rotation of the molecule



With HPLC/UV we were able to separate & quantify the analytes



Conclusion

- **All compounds in Method 535 can be quantified by LC/UV with a detection limit of about 100 ppb.**
- **The LC/MS/MS performs extremely well for clean samples at levels below 100 ppb.**
- **LC/UV can be a cost-effective way of screening out high level samples and provide information for dilution prior to final quantification by LC/MS/MS.**

Questions