

Application Note

## ACQUITY PREMIER Solution Improves the UPLC-MS Analysis of Deferoxamine – an Iron Chelating Drug

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Cheryl Boissel, Thomas H. Walter, Stephen J. Shiner

Waters Corporation



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## Abstract

HPLC separations of metal-sensitive analytes are known to be impacted by interactions of the analytes with the metal surfaces in HPLC instruments and columns. This causes effects ranging from peak broadening and tailing to loss of peak area and high injection-to-injection variability. The ACQUITY PREMIER Solution mitigates these effects by employing the novel surface technology MaxPeak High Performance Surfaces. Here we compare UPLC separations for the iron chelating drug deferoxamine obtained using a standard UPLC system and column vs the ACQUITY PREMIER Solution. The results show that higher peak areas and improved injection-to-injection reproducibility are achieved using the ACQUITY PREMIER Solution.

### Benefits

- The ACQUITY PREMIER Solution showed higher and more consistent peak areas for deferoxamine compared to a standard UPLC system and column
- No conditioning or unusual mobile phase additives were required to achieve reproducible separations using the ACQUITY PREMIER Solution

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## Introduction

Iron chelating drugs are used for the treatment of iron overload,<sup>1</sup> and also have immunomodulatory effects which make them potentially useful for treating microbial and viral infections, including COVID-19.<sup>2,3</sup> One of these drugs is deferoxamine (sold under the brand name Desferal). It has been reported that when analyzing deferoxamine using HPLC, complexation with iron in the chromatographic system causes the formation of unexpected peaks and variation in retention time.<sup>4</sup> These effects were mitigated by purging the system and column with a solution of deferoxamine and adding EDTA to the mobile phase. While effective, these steps add time and complexity to the separation method, and the use of EDTA in the mobile phase makes the method incompatible with mass spectrometry detection. Here, we describe an evaluation of the ACQUITY PREMIER System and a PREMIER column (together referred to as the ACQUITY PREMIER Solution) for the analysis of deferoxamine. The ACQUITY PREMIER Solution employs novel surface technology (MaxPeak High Performance Surfaces) to mitigate the interactions between metal-sensitive compounds and the metal surfaces in UPLC systems and columns.<sup>5</sup> The results are compared to those obtained using a conventional UPLC system and column.

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# Experimental

## Sample Preparation

Deferoxamine mesylate salt and thiourea were purchased from Millipore Sigma. Stock solutions were prepared at 1 mg/mL in 10% acetonitrile/90% water and 2 mg/mL in 90% acetonitrile/10% water, respectively. For the injection reproducibility study, the test mixture contained 300 µg/mL thiourea, and 5 µg/mL deferoxamine mesylate salt in 5% acetonitrile/95% 10 mM ammonium formate pH 3.00 (aq). Thiourea was used as the void volume ( $V_0$ ) marker. Three consecutive 2 µL injections of this mixture were made on fresh columns. For the calibration curve investigations, deferoxamine mesylate salt was prepared at concentrations of 1, 2.5, 5, 10, 20, and 30 µg/mL in 5% acetonitrile/95% 10 mM ammonium formate pH 3.00 (aq).

## Method Conditions

### LC Conditions

|                   |                                                                                                                 |
|-------------------|-----------------------------------------------------------------------------------------------------------------|
| Systems:          | ACQUITY UPLC H-Class QSM, FTN Sample Manager with CM<br>ACQUITY PREMIER H-Class QSM, FTN Sample Manager with CM |
| Detection:        | ACQUITY QDa Mass Detector                                                                                       |
| Columns:          | ACQUITY PREMIER HSS T3, 1.8 µm, 2.1 x 50 mm;<br>ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 50 mm                        |
| Column temp.:     | 30 °C                                                                                                           |
| Sample temp.:     | 15 °C                                                                                                           |
| Injection volume: | 2 µL                                                                                                            |
| Flow rate:        | 0.5 mL/min                                                                                                      |

## LC Conditions

|                         |                                                        |
|-------------------------|--------------------------------------------------------|
| Mobile phase A:         | 10 mM Aqueous ammonium formate pH 3                    |
| Mobile phase B:         | 95/5 Acetonitrile/200 mM aqueous ammonium formate pH 3 |
| Gradient:               | 5 to 50% B in 2.65 min (linear)                        |
| Run time per injection: | 5 min                                                  |

## MS Conditions

|                                 |                 |
|---------------------------------|-----------------|
| System:                         | ACQUITY QDa     |
| Mass scan range:                | 50–800 Da       |
| Ionization mode:                | Positive scan   |
| Cone voltage:                   | 15 V            |
| Sample rate target:             | 8 points/sec    |
| Capillary voltage:              | Positive 1.5 kV |
| SIR polarity (+):               | Positive        |
| SIR mass (DA) for thiourea:     | 77              |
| SIR mass (DA) for deferoximine: | 561             |

## Gradient Table

| Time (min) | Flow (mL/min) | %A | %B | Curve   |
|------------|---------------|----|----|---------|
| 0.00       | 0.5           | 95 | 5  | Initial |
| 2.65       | 0.5           | 50 | 50 | 6       |
| 3.15       | 0.5           | 50 | 50 | 6       |
| 3.2        | 0.5           | 95 | 5  | 6       |

## Data Management

Chromatography software:

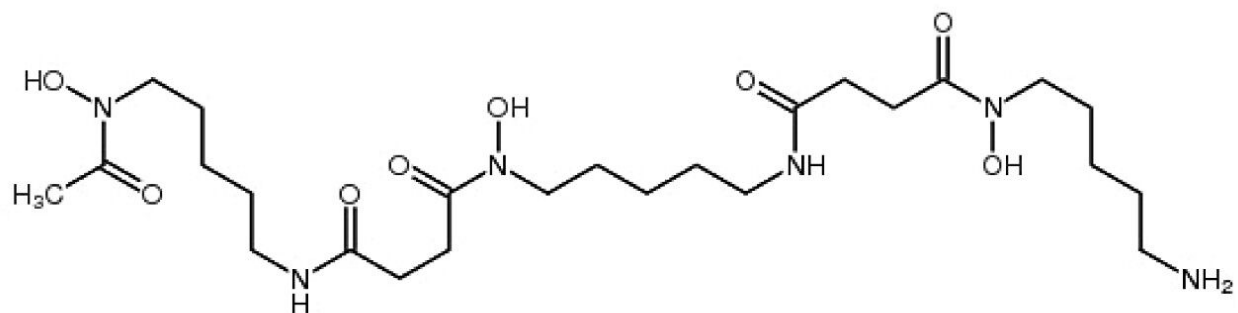
Empower 3 FR 4

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## Results and Discussion

As shown in Figure 1, deferoxamine is a highly polar strong base, with a logP of -2.2. Consequently, HSS T3 columns were chosen in order to take advantage of their ability to retain polar bases. Because of the weak UV absorbance of deferoxamine, we used an ACQUITY QDa Mass Detector. Good peak shape and sensitivity were achieved using an acetonitrile gradient with an aqueous mobile phase containing ammonium formate (pH 3). As shown in Figure 2B, when using a standard UPLC system and column, the peak area variability for deferoxamine was found to be relatively high, with a 16.8% relative standard deviation (RSD) over ten injections. As shown in Figure 3, the peak area gradually increased over the ten injections, as observed for other metal-sensitive analytes.<sup>6</sup> In contrast, when using the ACQUITY PREMIER Solution, the deferoxamine peak area RSD was only 2.1% over ten injections. In addition, the average peak area was 62% higher relative to that obtained using a standard system and column. This difference was found to be statistically significant at >95% confidence using a t-test. Since it has been shown that the reduction in peak area caused by interactions with metal surfaces is most severe at low mass loads,<sup>5</sup> we determined the peak areas for deferoxamine at several different loads, ranging from 2–60 ng, using both the ACQUITY PREMIER Solution

and a standard UPLC system and column. As shown in Figure 4, the results indicate that the greatest increases in peak area obtained using the ACQUITY PREMIER Solution were observed at the lowest loads. At 2–5 ng loads, the peak areas were approximately 60% higher compared to the standard system and column, while at the 40–60 ng loads the peak areas were about 32% higher.



LogP: -2.2  
MW: 560.7 g/mol

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Figure 1. Chemical structure and key properties of deferoxamine (DFO).

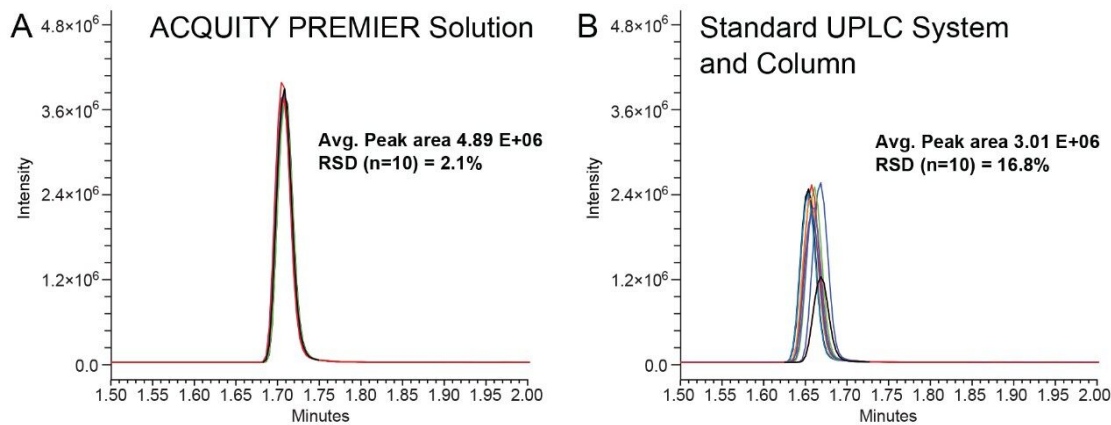


Figure 2. (A) Overlay depicting 10 replicate injections of deferoxamine mesylate obtained using the ACQUITY PREMIER Solution. (B) Overlay depicting 10 replicate injections of the same analyte using a standard UPLC system and a standard column. ACQUITY UPLC HSS T3, 100 Å, 1.8 μm, 2.1 x 50 mm Columns were used in this study. The mass load of deferoxamine mesylate was 10 ng. Acetonitrile gradient separations were carried out with a 10 mM ammonium formate (pH 3.0) aqueous mobile phase, a flow rate of 0.5 mL/min, and a temperature of 30 °C. The deferoxamine peak was detected using an ACQUITY QDa Detector with SIR positive  $m/z$  561.0.

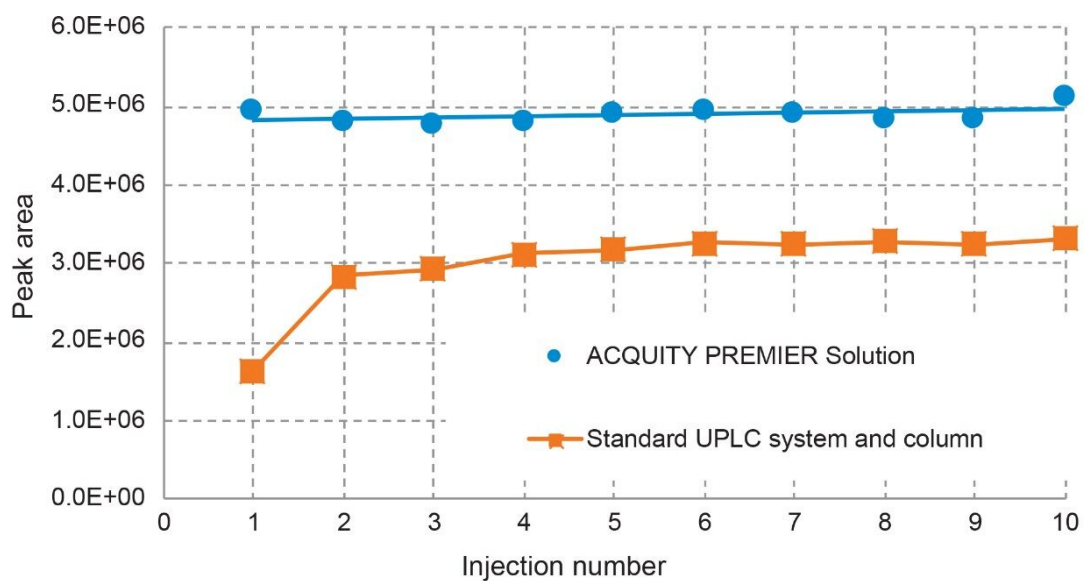


Figure 3. Plot of deferoxamine peak area obtained using the ACQUITY PREMIER Solution (blue circles) compared to the peak area obtained using a standard UPLC system and column (orange squares) for 10 sequential injections.



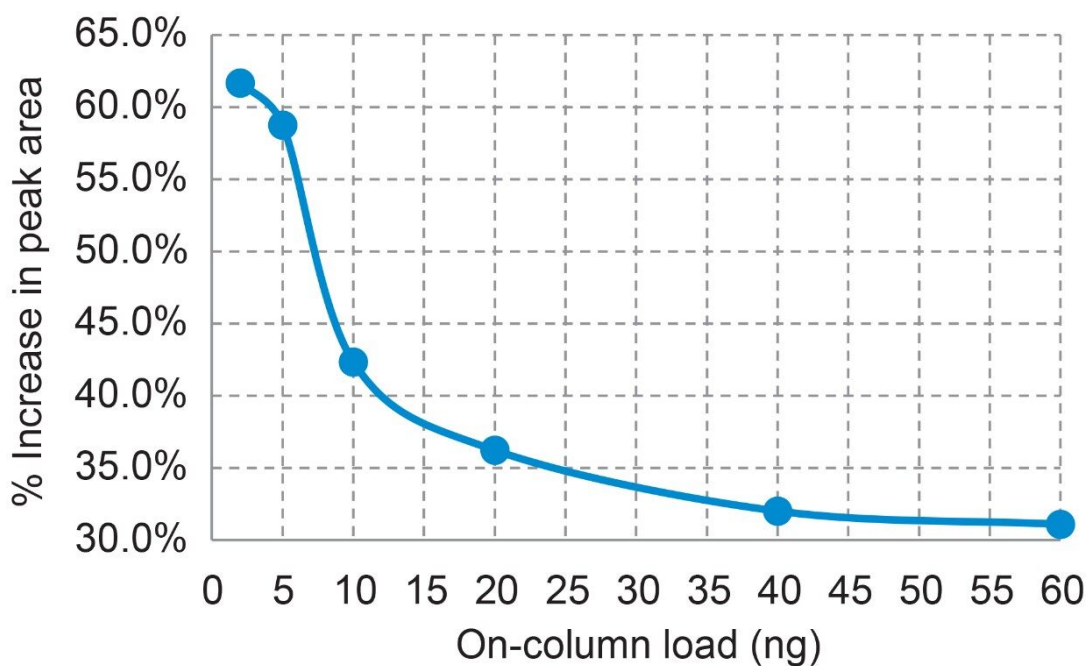


Figure 4. Plot of the relative increase in peak area of deferoxamine obtained using the ACQUITY PREMIER Solution compared to the peak area obtained using a standard UPLC system and column vs. the on-column mass load of deferoxamine mesylate. The separation conditions were the same as for Figure 2.

## Conclusion

These results demonstrate that, relative to a standard UPLC system and column, the ACQUITY PREMIER Solution results in higher peak areas and better peak area reproducibility for deferoxamine. The relative difference in peak area for the ACQUITY PREMIER Solution vs. a standard UPLC system and column was also shown to increase with decreasing mass load. These results are consistent with those previously reported for other metal-sensitive analytes when using the ACQUITY PREMIER Solution, and further demonstrate the value of this solution when analyzing such analytes. It's notable that unlike most of the other analytes that show adsorption to metal surfaces, deferoxamine is basic and lacks phosphate and carboxylate groups. This suggests that other iron chelators that lack acidic groups may also benefit from the use of the ACQUITY PREMIER Solution.

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## References

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