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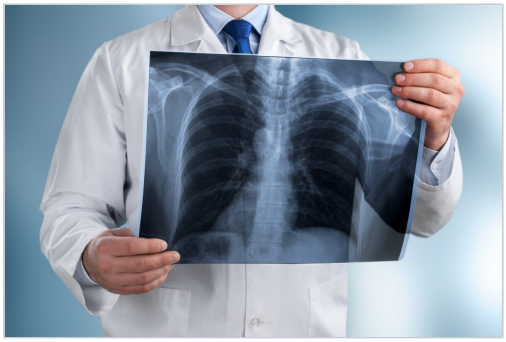
Application Note

Advantages of using ACQUITY PREMIER System and Columns with MaxPeak HPS Technology for Bioanalysis of Gefitinib – an EGFR Inhibitor

Nikunj Tanna, Robert S. Plumb

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Discovery bioanalysis laboratories are routinely tasked with developing rapid, sensitive, and robust methods for novel analytes. While the physico-chemical properties of these molecules may be known, their behavior within the analytical system is usually unknown and can negatively impact method development time, data quality, confidence in results, and decision making. In such cases, having an analytical system that minimizes sample loss and reduces analytical variability by minimizing analyte/surface interactions is desirable. Gefitinib, a small molecule EGFR inhibitor with no known metal sensitivity, was used as a candidate molecule. The ACQUITY PREMIER System and ACQUITY PREMIER Columns with MaxPeak High Performance Surface Technology improve chromatographic peak shape, sensitivity, and reduces analytical variability at the LLOQ for better low-level detection, reproducibility, and data quality.

Benefits

- Improved peak shape for better quality results
- Improved reproducibility for confidence in results
- Improved sensitivity for low-level detection
- Reduced loss of analyte

Introduction

An analytical system comprised of a chemically inert surface barrier technology can be valuable to scientists in discovery bioanalysis laboratories who are tasked with developing methods for novel analytes whose interactions within an analytical system may not be well understood.

ACQUITY PREMIER System and ACQUITY PREMIER Columns with MaxPeak High Performance Surface (HPS) Technology have been shown to significantly improve chromatographic performance for electron rich, metal sensitive compounds which have a propensity to adhere to metal surfaces. This improved performance can play a critical role in enhancing the role of LC-MS in the understanding of biology[1] of a disease as well as determining the metabolic fate of candidate molecules in discovery[2] and development.[3] Since these hybrid surfaces were engineered to minimize metal chelation, performance improvements for this class of analytes is not surprising.

In this application brief, we investigate the effect of these novel hybrid barrier surface technologies on metal insensitive compounds. Gefitinib (Iressa) was chosen as a candidate molecule for this study. Gefitinib is an epidermal growth factor receptor (EGFR) inhibitor and is indicated in non-small cell lung cancer (NSCLC) and certain breast cancer patients. There are no known reports of metal sensitivity exhibited by Gefitinib, however previous reported bioanalytical methods for this compound have all employed buffers in the mobile phase to improve chromatographic peak shape. Here, we show that using ACQUITY PREMIER and MaxPeak HPS Columns improves the robustness and reproducibility for bioanalysis of gefitinib.

Results and Discussion

Gefitinib (Iressa) dissolved in methanol was spiked into human plasma to create a calibration curve from 0.75–1000 ng/mL. Protein precipitation was performed on 100 µL of each sample in triplicate using 1:3 methanol. 250 µL of the supernatant was transferred to a LCMS vial and 10 µL of the sample was injected onto LC-MS/MS system. The LC-MS/MS methodology used for this separation is the same as described by Tanna ***et al***.[2] Briefly, separation was performed on an HSS T3 Column using a linear gradient employing water and ACN containing 0.1% formic acid as mobile phases A and B (5–75% B, 2.5 mins). For Gefitinib, MRM transition 448.22 → 128.03 was monitored for quantification. This same sample set was injected on both the ACQUITY PREMIER with MaxPeak HPS Column as well as conventional UPLC with conventional column hardware.

The LLOQ achieved on both systems was 0.75 ng/mL and the calibration curve was linear from 0.75–1000 ng/mL on both systems. However, as shown in Figure 1, the ACQUITY PREMIER System and MaxPeak HPS Column showed a superior peak shape and higher area counts for gefitinib at the LLOQ level. On the conventional system and column, although the LLOQ peak at 0.75 ng/mL is quantifiable there is slight tailing observed which could cause higher variability in integration resulting in higher %CV. This phenomenon is illustrated in Figure 2 which compares the %CV at the lower concentration levels on the novel system and column combination vs the conventional system and column. For the two lowest points on the calibration curve, the %CV observed for on the ACQUITY PREMIER System and MaxPeak HPS Column is <5%. The %CV for the LLOQ (0.75 ng/mL) and 1 ng/mL levels on the standard system/column combination is 17.5% and 11.5% respectively. This improved performance is most significant at the lower concentrations. Since there are fewer analyte molecules present at the low concentration levels, any loss of these molecules on the analytical system due to metal interactions is exacerbated, resulting in higher variability in results at the lowest concentrations. The ACQUITY PREMIER System/Column minimizes these unwanted interactions, thereby resulting in more consistent area counts and peak shape, even at the lowest concentration levels, thereby reducing the variability in measurement as demonstrated by the lower %CV observed in Figure 2. Although the %CV values observed on the conventional system/column are acceptable based on the industry accepted bioanalytical method validation guidelines, the higher variability can be a cause of concern, especially when dealing with late stage clinical samples where the matrix variation is higher than safety assessment FTIH studies.

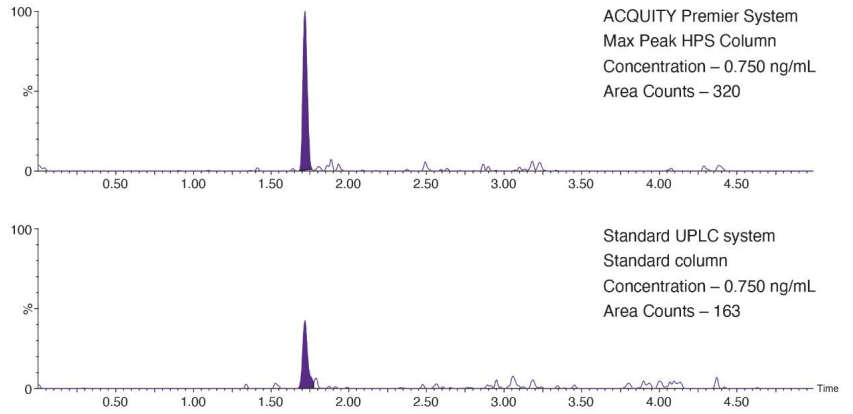


Figure 1. Gefitinib – LLOQ comparison between ACQUITY PREMIER System with MaxPeak HPS Column vs standard ACQUITY LC system with standard column.

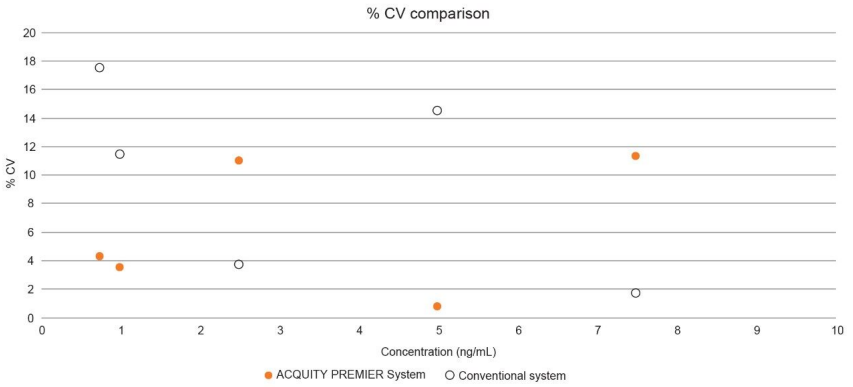


Figure 2. %CV comparison between ACQUITY PREMIER System with MaxPeak HPS Column vs standard ACQUITY LC system with standard column.

Conclusion

The inert nature of the ACQUITY PREMIER System and MaxPeak HPS Column can be valuable tools for the scientists when trying to rapidly and robustly develop methods for compounds whose chromatographic performance and metal sensitivity may not be well characterized. It may also provide additional confidence when quantifying analytes at extremely low concentration levels by potentially improving the reproducibility of integration, thereby reducing variability of the assay as highlighted by the lower %CV observed for gefitinib in this study.

References

1. Kerri Smith and Paul Rainville. Utilization of MaxPeak High Performance Surfaces for Improved Separation and Recovery of Analytes Associated with the Tricarboxylic Acid Cycle. Waters Application Note, 720006727EN, 2019.

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3. Fadi L Alkhateeb, Ian Wilson, Margaret Maziarz, and Paul Rainville. Ultra High-Performance Liquid Chromatography Method Development for Separation of Formoterol, Budesonide, and Related Substances using an Analytical Quality by Design Approach. *Journal of Pharmaceutical and Biomedical Analysis*, Vol 193, 2021.

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