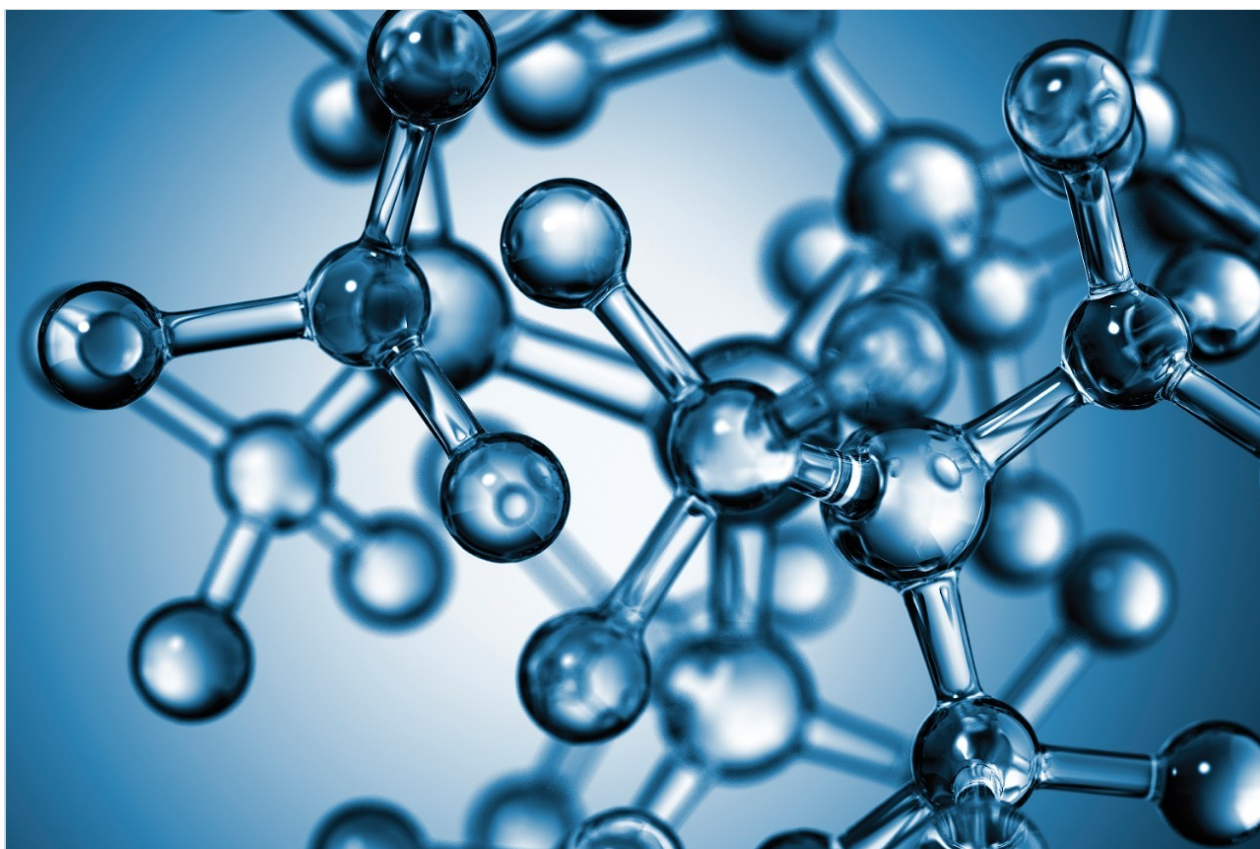


## Recovery of Metal-Sensitive Analytes on the Arc Premier Solution: System-to-System Reproducibility and a Multi-System Comparison to Conventional LC

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## Abstract

Metal-sensitive compounds can be challenging and time consuming to analyze on conventional LC technology, due to interactions with metal surfaces. Waters has a solution for the routine workflow with the MaxPeak High Performance Surfaces (HPS) Technology to effectively reduce non-specific adsorptive losses due to metal interactions, first introduced with the ACQUITY Premier Solution and now available on the Arc Premier Systems. The Arc Premier Solution combines ruggedness of the ACQUITY Arc with the MaxPeak Solution to improve recovery of metal-sensitive compounds. The results include reproducible HPLC performance from system-to-system without the need for time-consuming system preparation or for modifications to methods to reduce these interactions.

In this study, the Arc Premier Solution will be tested with phosphorylated compounds that can interact with metallic surfaces to evaluate system-to-system performance. The results demonstrate the superior system-to-system reproducibility of the Arc Premier Solution for a range of compounds, including metal-sensitive analytes. The performance of the Arc Premier Solution will also be compared to conventional LC technology, with all studies performed on multiple systems. Data analysis will demonstrate the statistical difference of the Arc Premier Solution to conventional LC technology for metal-sensitive compounds.

## Benefits

- Arc Premier Solution provides superior system-to-system reproducibility for metal-sensitive compounds
- MaxPeak HPS Technology provides improved recoveries for metal-sensitive analytes at low concentration
- Arc Premier Solution demonstrates improved performance for metal-sensitive compounds compared to conventional, stainless-steel LC technology

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## Introduction

Metal-sensitive compounds, such as phosphorylated compounds, have posed unique challenges on conventional LC, including poor recovery, irreproducible results, and poor precision. Historically strategies to address these analytical challenges include passivation or using of ion-pairing reagent in the mobile phase. The former is time consuming and requires multiple flushing steps to prepare the system for analysis, while the latter may require redevelopment of the method and is not compatible with many detection techniques. In both instances, system-to-system reproducibility can be impacted by the system or mobile phase preparation.

To address these challenges, Waters has introduced the Arc Premier Solution. This solution combines the high throughput of HPLC technology optimized for use with 2.5  $\mu\text{m}$  particle chemistry, first introduced by Waters, with the ACQUITY Arc with MaxPeak HPS Technology to provide a highly reproducible system compatible with metal-sensitive compounds. In this study, system-to-system studies will be performed to evaluate reproducibility for a wide range of analytes on both the Arc Premier Solution and conventional LC technology. Data analysis will be performed using t-test to demonstrate the statistical significance of the studies.

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## Experimental

### Method 1: Adenosine, Adenosine Diphosphate, Adenosine Triphosphate

#### Sample Description

Stock solutions of adenosine (Acros), adenosine diphosphate (ADP) (Sigma/Aldrich), adenosine triphosphate (ATP) (Acros) were prepared individually at 2 mg/mL in 95/5 mobile phase A: mobile phase B. Calibrants and samples were prepared through serial dilutions of a mixture of the raw materials. Samples and standards were prepped from 0.2 to 200  $\mu\text{g/mL}$ .

#### Method Conditions

|             |   |
|-------------|---|
| LC systems: | Arc Premier QSM-R, Arc Premier FTN-R,<br>MaxPeak Column Heater-Active, and<br>Conventional LC system  |
| Detection:  | Arc Premier 2489 UV/Vis or Arc Premier<br>2998 PDA  |
| Wavelength: | 260 nm  |
| Column(s):  | Arc Premier Solution: XSelect Premier HSS<br>T3 XP 2.5 $\mu\text{m}$ , 4.6 x 50 mm Column (p/n:<br>186009858)<br><br>Conventional LC: XSelect HSS T3 XP 2.5 $\mu\text{m}$ ,<br>4.6 x 50 mm, Column (p/n: 186006157) |

|                   |   |
|-------------------|---|
| Column temp.:     | 40 °C   |
| Sample temp.:     | 10 °C   |
| Injection volume: | 15 µL   |
| Flow rate:        | 1.5 mL/min  |
| Mobile phase A:   | 8 mM ammonium acetate pH 6.8 in 99.8%<br>water and 0.2% acetonitrile    |
| Mobile phase B:   | 6.4 mM ammonium acetate pH 6.8 in 79.8%<br>water and 20.2% acetonitrile |
| Gradient:         | 0.2 minute hold at 1% B followed by a 7<br>minute gradient to 95% B.    |

## Method 2: Hydrocortisone Phosphate, Dexamethasone, Dexamethasone Phosphate, Dexamethasone Acetate

### Sample Description

Standards were obtained for hydrocortisone phosphate triethylamine, dexamethasone sodium phosphate, dexamethasone, and dexamethasone acetate were purchased from Sigma/Aldrich. A mixed stock solution was prepared at 2 mg/mL of hydrocortisone phosphate and dexamethasone sodium phosphate, and 0.6 mg/mL dexamethasone, and dexamethasone acetate in 50/50 water:acetonitrile. Calibrants and samples were prepared through serial dilutions of a mixture of the raw materials. Samples shown are at 25 µg/mL for hydrocortisone phosphate and dexamethasone sodium phosphate, and 7.5 µg/mL for dexamethasone and dexamethasone acetate.

|            |   |
|------------|---|
| LC system: | Arc Premier QSM-R, Arc Premier FTN-R,<br>MaxPeak Column Heater-Active, and Absorbance<br>Detector (2489 or 2998). Conventional system |
| Detection: | Arc Premier 2489 UV/Vis or Arc Premier 2998<br>PDA  |

|                   |   |
|-------------------|---|
| Wavelength:       | 260 nm  |
| Column(s):        | Arc Premier Solution: XBridge Premier BEH C <sub>18</sub><br>Premier 2.5 $\mu$ m 4.6 x 50 mm (p/n: 186009847)<br>Conventional LC: XBridge BEH C <sub>18</sub> 2.5 $\mu$ m 4.6 x 50<br>mm (p/n: 186006037) |
| Column temp.:     | 40 °C   |
| Sample temp.:     | 10 °C   |
| Injection volume: | 30 $\mu$ L  |
| Flow rate:        | 1.5 mL/min  |
| Mobile phase A:   | 10 mM ammonium formate pH 3.0 in water  |
| Mobile phase B:   | Acetonitrile  |
| Gradient:         | 3 minute hold at 18% B followed by a 4 minute<br>gradient to 50% B.   |

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## Results and Discussion

Many compounds are known to interact with metallic surfaces. To address these challenges, historically several approaches have been taken, including system passivation or conditioning, or the use of mobile phases containing ion pairing reagents. The Arc Premier Solution takes advantage of MaxPeak Coatings to reduce these interactions and provide improved recovery without the need for either of these approaches.

In the following study, we will demonstrate the system-to-system reproducibility for these challenging analytes, including statistical analysis. For the following study, three Arc Premier QSM-R Systems were tested with a wide range of compounds (Figure 1), including those that contain moieties that might interact with metallic surfaces. All the tests were performed with mobile phases that would not impact that interaction, *i.e.* without the use of ion-pairing reagents. For each system, each test involved over 100 injections on each system. Analysis for each system

is represented by 36 sequential injections for ATP, ADP, and 36 injections over 3 days for all other compounds. Data showing calibration curves represents the aggregate of 6 replicates per concentration on three systems. Comparison testing of multiple (n=3) systems was performed for both the Arc Premier Systems and conventional LC systems.

For the studies described, the metal-sensitive compounds analyzed each contained a phosphate group, or positively charged analyte which is known to interact with the charged species on metal surfaces. To probe the impact of the chemical structure on the interaction, chemical analytes with increasing number of phosphate groups were tested together, including ADP and ATP. Furthermore, the non-phosphorylated analogs (adenosine and dexamethasone) were used as controls.

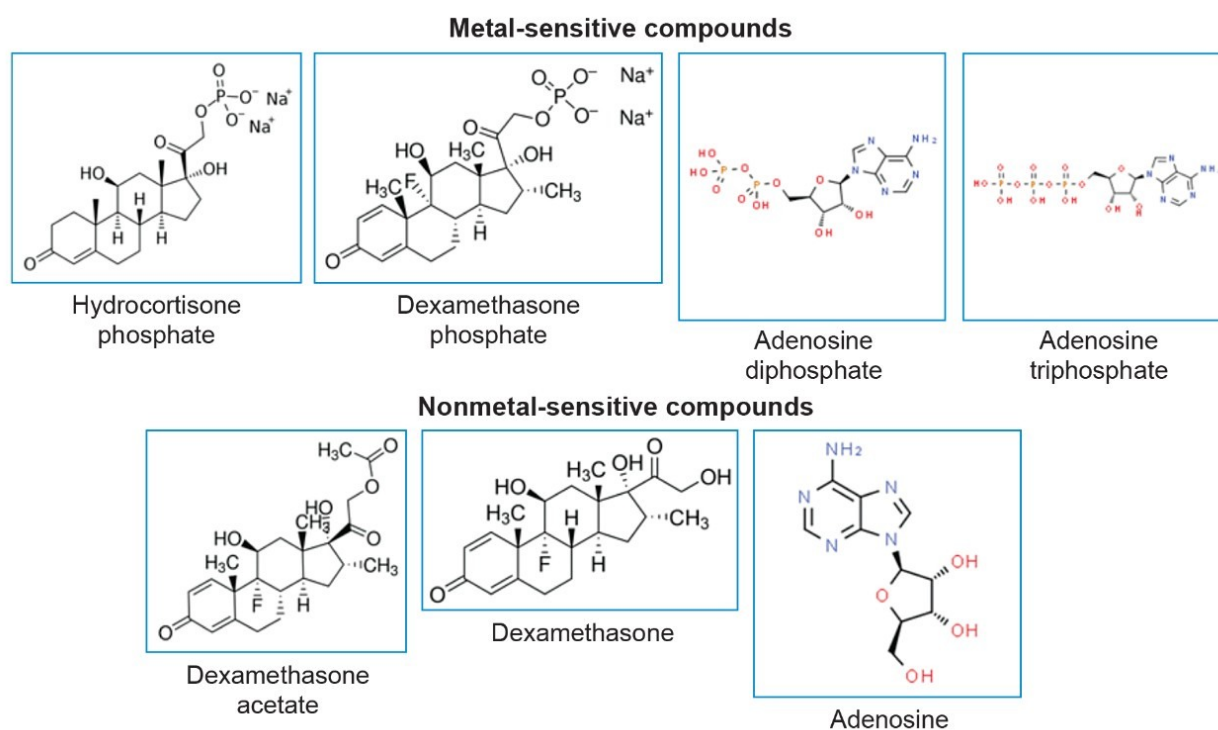


Figure 1. Chemical structures of compounds tested on ACQUITY Arc Premier Solution and Conventional LC technology.

## Arc Premier Solution: System-to-System Reproducibility

For system-to-system reproducibility of the Arc Premier Solution, the peak area of each analyte was compared. As described above, each set of data represents 36 injections per system acquired for ATP and APD sequentially and over 3 days for all other compounds. Comparison of the peak areas of the metal-sensitive compounds on each of the three systems (Figure 2) shows high precision and comparable results on each system. Specifically, the %RSD for

each system was not more than 0.28% for any one analyte, confirming the superior precision. For each of the phosphorylated compounds, average peak areas were comparable and within 0.9–2.2% for all metal-sensitive analytes. Given the number of injections (36) and the length of the study, these results demonstrate the superior precision of the Arc Premier Solution, and consistent performance from system-to-system.

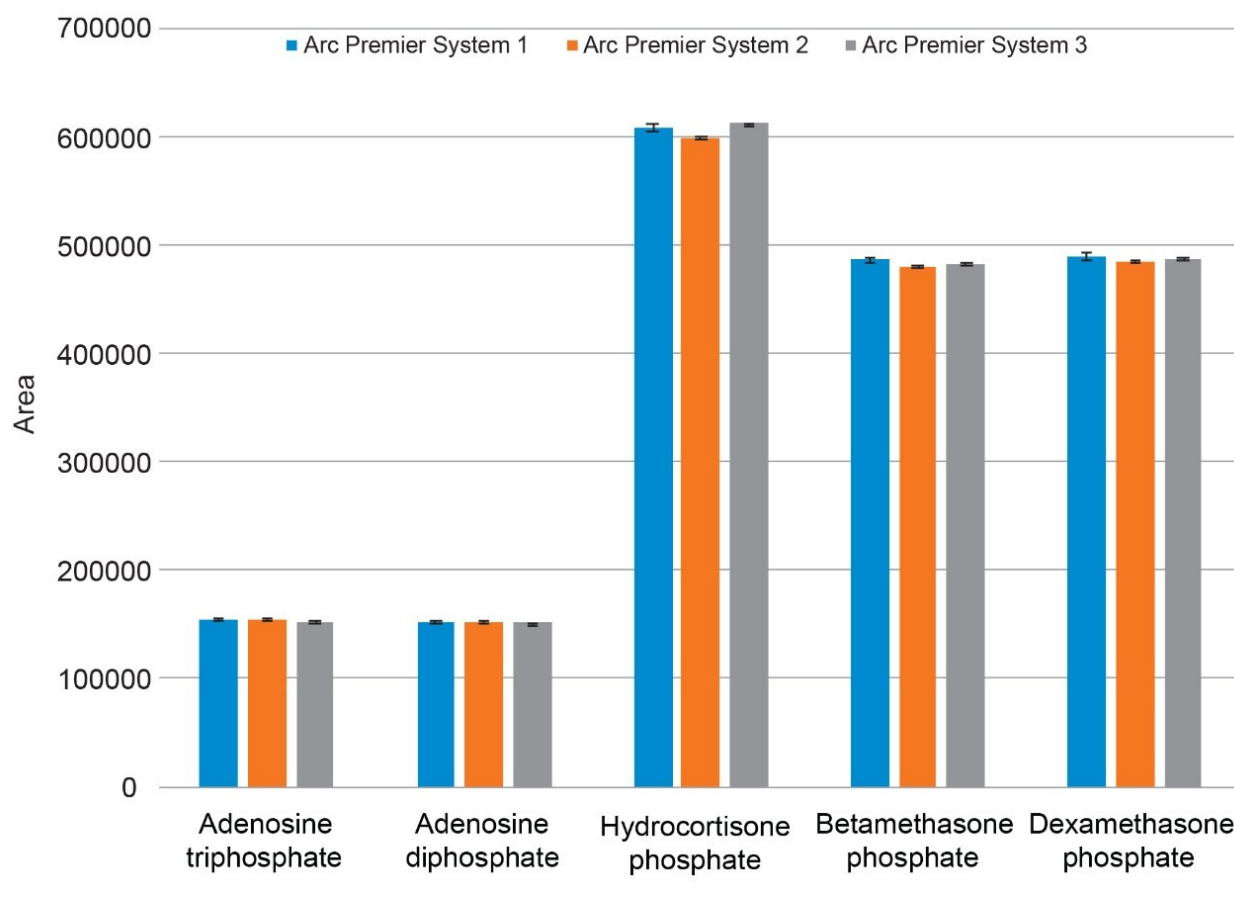


Figure 2. The peak area comparison of metal-sensitive compounds across 3 Arc Premier QSM-R Systems. Analysis for each system is represented by 36 injections. Standard error bar represents +/- 3 standard deviation ( $\sigma$ ) on each system.

To ensure that nonmetal-sensitive compounds were not affected, system-to-system reproducibility was also analyzed for the non-phosphorylated analogs. As demonstrated in Figure 3, the Arc Premier Solution shows superior repeatability for these compounds as well. For all analytes and individual systems, the %RSD were less than 0.26% for 36 injections over multiple days. In addition, average peak areas were comparable and within 1% on all three systems. These results indicate, regardless of the analyte, the Arc Premier Solution delivers consistent system-to-system results.

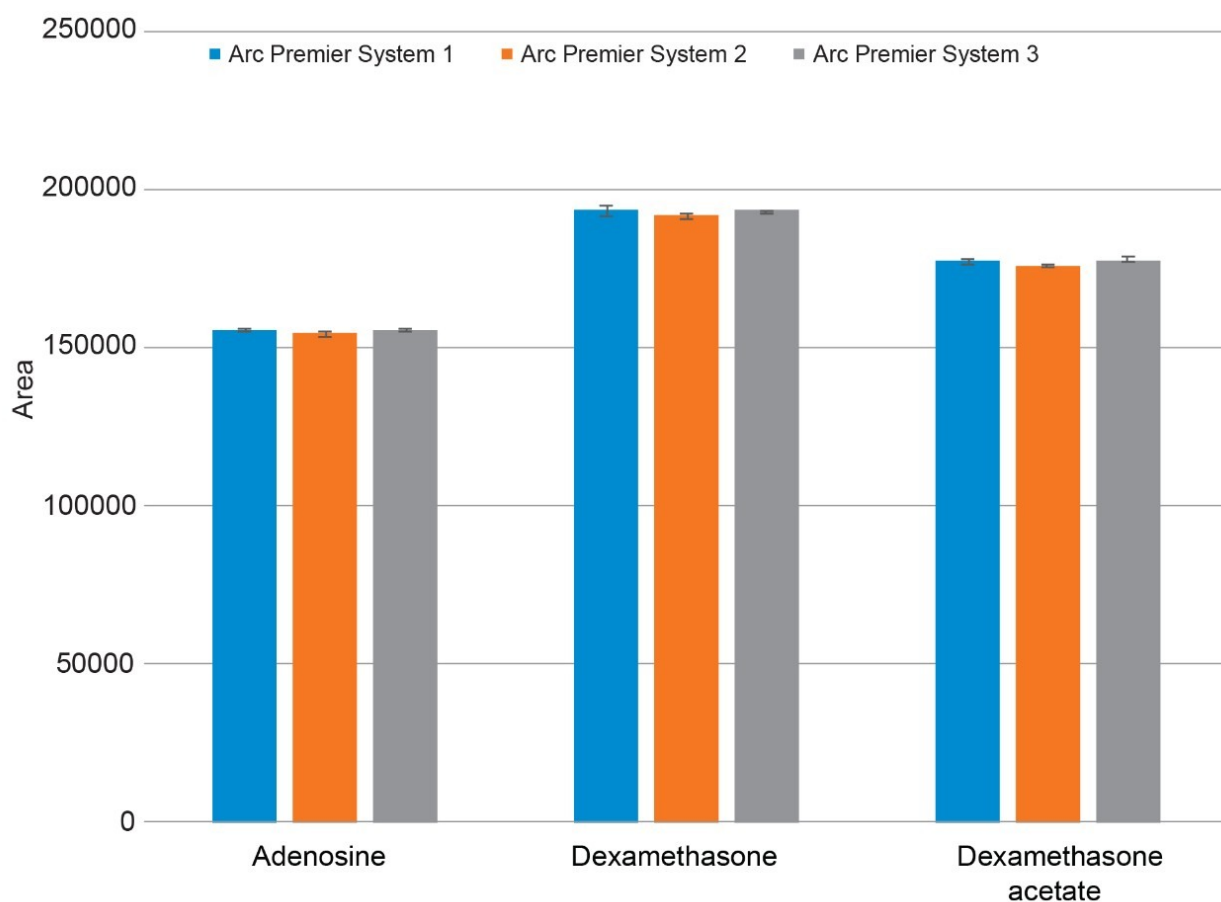


Figure 3. The peak area comparison of compounds that are not metal-sensitive across 3 Arc Premier QSM-R Systems. Standard error bar represents +/- 3 standard deviation ( $\sigma$ ) on each system.

## Multi-System Comparison of Arc Premier Solution to Conventional LC

In addition to demonstrating consistent system-to-system performance, the Arc Premier Solution has shown to provide improved performance for metal-sensitive compounds compared to conventional LC systems.<sup>1</sup> To further evaluate the differences across the two systems, data was collected on multiple Arc Premier Systems as well as conventional LC systems. All data was collected as previously described.

Analysis of the average peak area of the three systems, as well as the standard deviation of peak area revealed clear differences across from the Arc Premier Solution to the conventional LC system (Figure 4). Both sets of systems show comparable recovery of the adenosine, an analyte that does not typically interact with metallic surfaces when comparing the average peak area over three systems, acquired over a set of 100 injections. In contrast, ATP and ADP, both phosphorylated compounds that can interact with metallic surfaces, show dramatic improvement in peak areas without the use of passivation of the system or ion pairing reagents in the mobile phase over the same time



period.

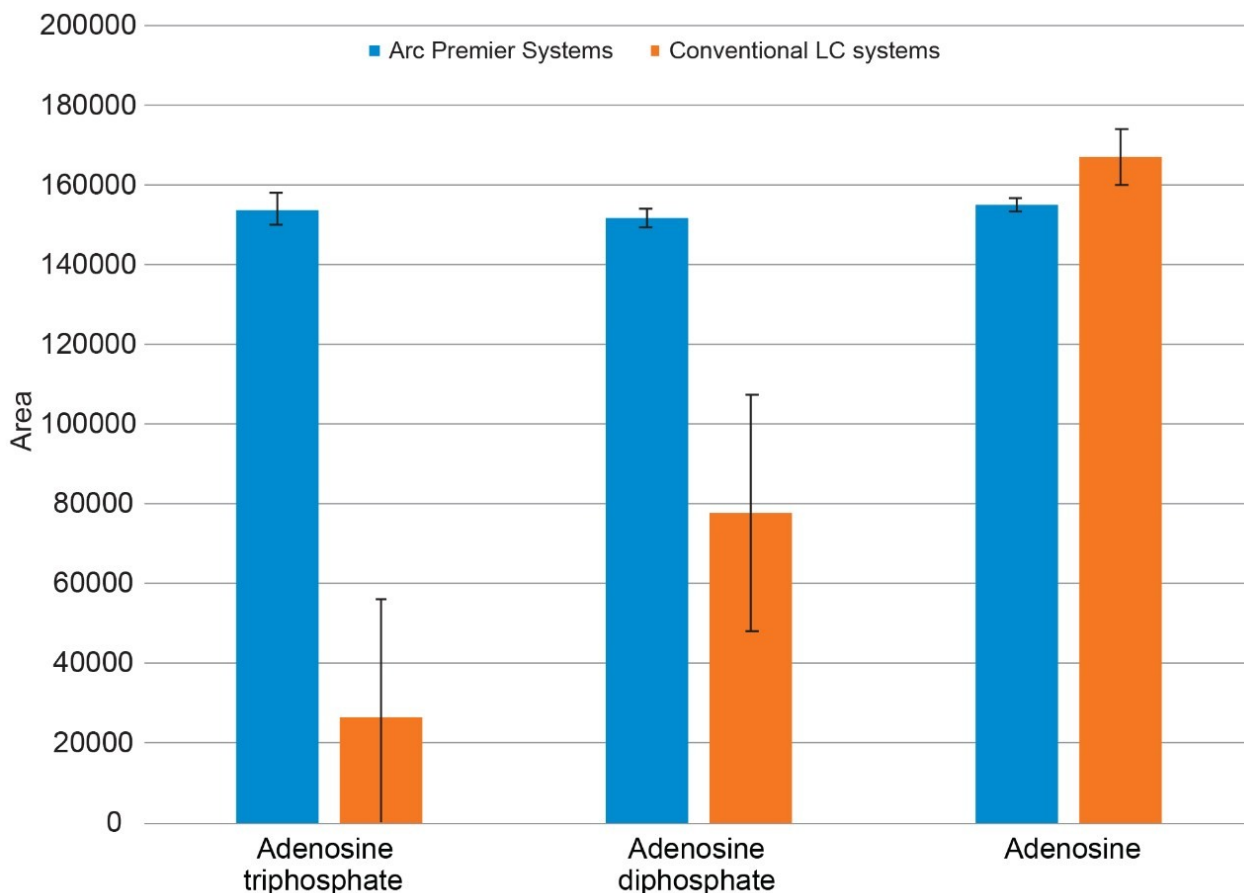


Figure 4. Average area comparison of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine for Arc Premier Systems (blue) and conventional LC systems (orange). Standard error bar represents  $\pm 3$  standard deviation ( $3\sigma$ ) on each system.

In contrast, comparing the response factors or peak area/concentration for metal-interacting compounds, the difference between the Arc Premier Solution and conventional LC technology is apparent (Figure 5 and 6). Most notably the lower points of the calibration curve for both ATP and ADP on the conventional LC did not produce any integrable peaks, with the LOQ being much higher on the conventional LC vs Arc Premier Systems. The Arc Premier solution gave a relatively flat change for both ATP and ADP from 1–200  $\mu\text{g/mL}$ , without the need to passivate or modify the system or use complex ion-pairing mobile phases. Overall, the data suggests there is loss of the phosphorylated compounds on the conventional LC system, likely due to absorption onto metal surface. In contrast, the Arc Premier System has minimal changes in the response factors over the concentration range.

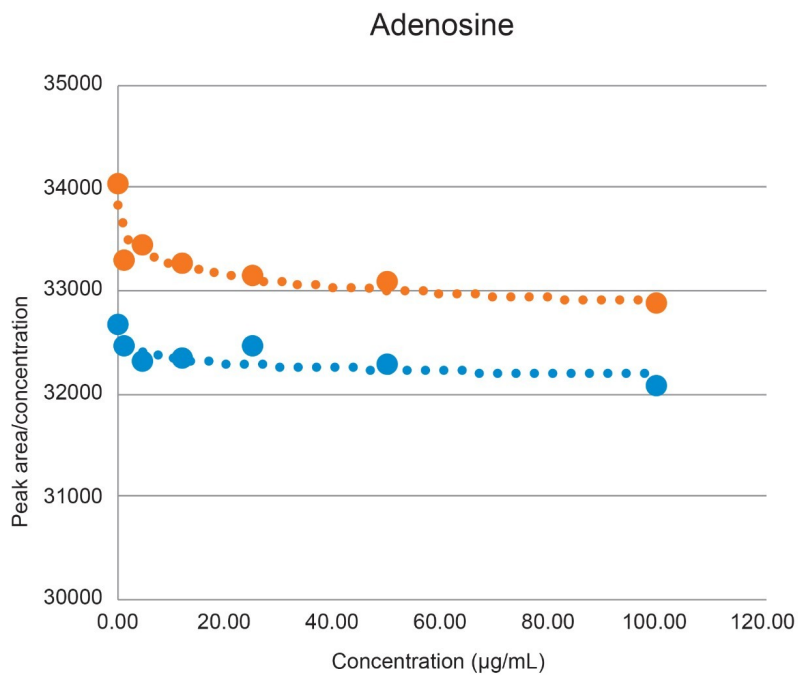


Figure 5. Average Response factor of adenosine for 3 Arc Premier Systems (blue) and 3 Conventional LC systems (orange) across the calibration range of 1–100 µg/mL.

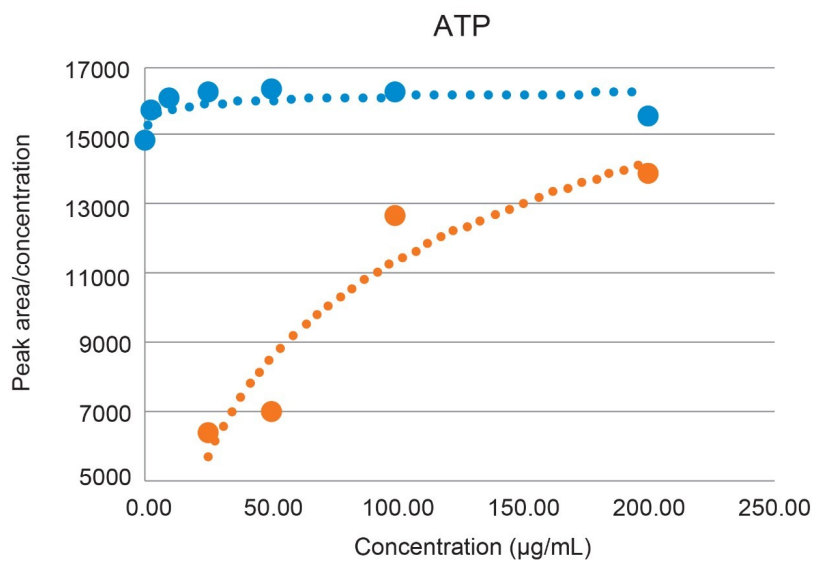


Figure 6. Average Response factor of ATP for 3 Arc Premier Systems (blue) and 3 Conventional LC systems (orange) across the calibration range of 1–200 µg/mL.

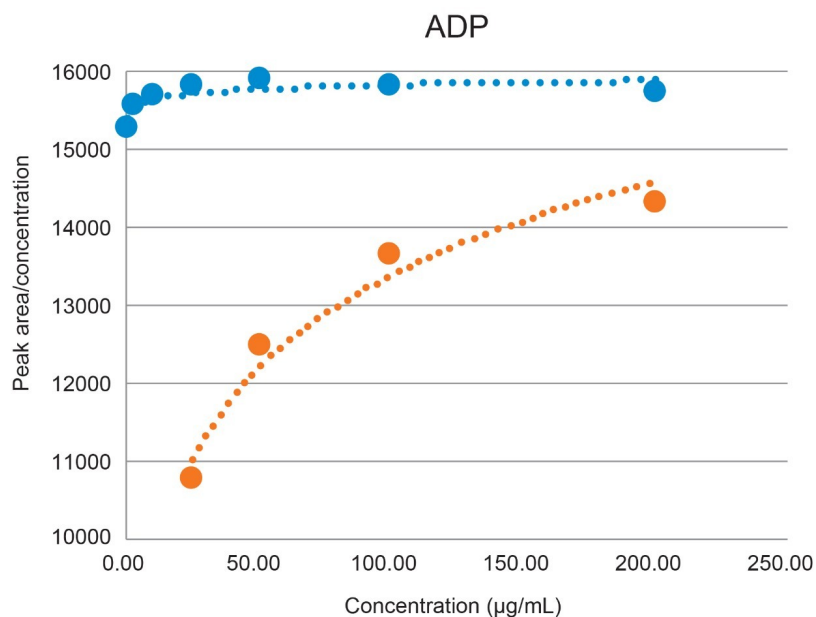


Figure 7. Average Response factor of ADP for 3 Arc Premier Systems (blue) and 3 Conventional LC conventional system (orange) across the calibration range of 1–200 µg/mL.

## Comparison and Statistical Analysis of Arc Premier and Conventional LC

Analysis of the aggregate system data for both Arc Premier Solution and conventional LC shows marked differences in system-to-system reproducibility for metal-sensitive compounds. As described above, the data represents the average values across the three systems tested by type. The results (Table 1) demonstrate the superior precision of peak area for nonmetal interacting analytes (adenosine) and superior performance for metal-sensitive phosphorylated compounds on the Arc Premier Solution. When compared to conventional LC, the differences are apparent for the metal-sensitive compounds. For example, precision for ATP shows a 63x improvement over conventional LC technology and ADP shows a 41x improvement over conventional LC technology.

| Analyte   | Arc Premier Solution | Conventional LC |
|-----------|----------------------|-----------------|
| Adenosine | 0.09%                | 0.07%           |
| ATP       | 0.25%                | 15.77%          |
| ADP       | 0.15%                | 6.07%           |

Table 1. %RSD for phosphorylated compounds on Arc Premier Systems and conventional LC systems. Values are average of 3 systems for each type.

A t-test was performed to evaluate the data from the Arc Premier Systems and conventional LC Systems. Analysis showed statistically different results for the precision values of ATP and ADP with the Arc Premier Solution having statistically lower variability relative to the conventional systems. P values for the t-test are presented on Table 2.

| Parameter             | Adenosine         | ATP                | ADP                |
|-----------------------|-------------------|--------------------|--------------------|
| Area                  | 0.800             | $2.330 * 10^{-22}$ | $8.295 * 10^{-21}$ |
| Area %RSD (precision) | $6.609 * 10^{-3}$ | $1.058 * 10^{-5}$  | $1.999 * 10^{-4}$  |

Table 2. P-values for t-test comparing three Arc Premier Solution to three conventional LC systems.

When comparing system-to-system reproducibility of the Arc Premier Systems and conventional LC systems, similar trends, as described above, are observed. For metal-sensitive (ATP and ADP), the Arc Premier Solution produced superior precision values of less than 5% between the three systems while the conventional LC technology had %RSD's of 12–38% when comparing multiple systems (Table 3). For metal-sensitive compounds Arc Premier Solution showed about an 8.6x improvement for ATP and a 3.5x improvement for ADP over conventional LC. These results indicate the superior performance for the Arc Premier Solution for these challenging metal-sensitive compounds.

| Analyte | Arc Premier Solution | Conventional LC |
|---------|----------------------|-----------------|
| ATP     | 4.39%                | 37.92%          |
| ADP     | 3.67%                | 12.73%          |

Table 3: Total %RSD for all peaks across 3 Premier Systems and 3 conventional systems at 25 µg/mL. Values are the aggregate of 150 injections on each of the systems.

## Conclusion

The Arc Premier Solution demonstrates superior system-to-system reproducibility, while also giving improved precision and sensitivity relative to conventional LC systems for phosphorylated compounds known to be metal sensitive. As described previously, greater precision and peak area were observed across multiple Arc Premier Systems relative to the conventional LC system for metal-sensitive compounds. The peak area differences were most prominent at low concentrations of the analytes, where adsorption onto conventional LC technology significantly impacted recovery of the analyte. The data indicates the ability of the Arc Premier Solution to consistently produce repeatable results for a wide range of analytes over many injections, while also providing confidence that system-to-system reproducibility will ensure that each system will give the same result.

## References

1. Zhimin Li, Z.; Shah, D.; Dovell, A.; Martin, A., Grzonka, Chris; Dyke, J. Challenge Accepted: Arc Premier System Increases Sensitivity and Reproducibility for Hard-to-See Compounds, Waters Application Note, 720007267EN <<https://www.waters.com/nextgen/us/en/library/application-notes/2021/challenge-accepted-arc-premier-system-increases-sensitivity-and-reproducibility-for-hard-to-see-compounds.html>> , June 2021.

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720007330, August 2021

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