

Nota applicativa

## Challenge Accepted: Arc Premier System Increases Sensitivity and Reproducibility for Hard-to-See Compounds

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### Abstract

In this application note, we employ three applications to investigate the performance benefits achieved by the Waters Arc Premier System when paired with a MaxPeak Premier Column. The first application uses adenosine nucleotides as a probe to study the effects of the inert MaxPeak High Performance Surfaces (HPS) Technology on critical performance parameters, such as detection sensitivity and reproducibility. The study shows that adenosine phosphorylated derivatives were readily detected on the Arc Premier System starting at a concentration 0.5 µg/mL, with appreciable peak areas and significantly less tailing. The peaks remain undetected on the other two LC systems, the standard stainless-steel system and standard biocompatible metal-alloy system. The second application, steroid phosphate drug analysis, demonstrates improved peak shape, sensitivity, reproducibility, and quantification accuracy for metal-sensitive compounds on the Arc Premier System. Finally, the third application, RP analysis of a mix of non-metal-sensitive compounds, shows that an analytical method can be successfully transferred among a standard stainless-steel system, a standard biocompatible metal-alloy system, and an Arc Premier System, regardless of stainless-steel columns or MaxPeak Premier Columns for non-metal-sensitive analysis.

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## Benefits

- MaxPeak High Performance Surfaces (HPS) Technology minimize uncertainty in detection and quantitation for problematic metal-sensitive analytes
- Improved peak shape, sensitivity, reproducibility, and quantitation accuracy
- Seamless method transfers across the Arc Premier System and standard stainless-steel and biocompatible metal-alloy systems for non-metal-sensitive analytes

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## Introduction

Historically, analytes containing electron-rich moieties impose challenges on metal-based LC systems and columns, such as stainless-steel (SS), or biocompatible titanium and nickel-cobalt alloys (MP35N). Those electron-rich moieties, including phosphate groups, uncharged amines, and deprotonated carboxylate groups, are susceptible to chelate with metal surfaces across the LC systems and columns.<sup>1</sup> As a result, poor peak shape, reduced sensitivity, poor reproducibility, and robustness were measured on those LC systems with standard stainless-steel columns for metal-sensitive compounds. These metal-analyte interactions adversely affect the development of a successful analytical method. Workaround solutions, such as employing chelating reagents, PEEK tubing, and system passivation, are either not permanent, incompatible with certain solvents, or reduce ionization efficiency with MS detection.<sup>2</sup>

To address the challenges of analyzing metal-sensitive compounds, Waters developed the ACQUITY Premier System, Arc Premier System, and MaxPeak Premier Columns. While the ACQUITY Premier System offers performance advantages to scientists performing research and method development with sub-2- $\mu\text{m}$  column chemistry, the Arc Premier System is designed to support routine LC analysis and method development with  $\geq 2.5$   $\mu\text{m}$  column chemistry. The ACQUITY and Arc Premier Systems and MaxPeak Premier Columns are based on MaxPeak HPS Technology, where HPS are comprised of a highly crosslinked layers similar to bridged-ethyl hybrid silica.<sup>3</sup> MaxPeak HPS Technology provides a highly effective barrier that minimizes undesired interactions with metal surfaces.

In this study, we applied adenosine and its phosphorylated derivatives as a probe to show how chromatographic surfaces can significantly influence detection of metal-sensitive analytes. Then, we highlight and emphasize the

benefits of the Arc Premier Solution using steroid phosphate drug analysis as an example. Finally, chromatographic performance equivalency is demonstrated using a mix of non-metal-sensitive compounds on a standard system, a standard bio system, and an Arc Premier System.

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## Experimental

### LC Systems

LC systems	ACQUITY Arc	ACQUITY Arc Bio	Arc Premier
Wetted flow path surface materials	Stainless steel	MP35N/Titanium	MaxPeak HPS
UV detector	PDA2998	TUV2489	PDA2998
Pump type	QSM (Quaternary Solvent Manager)		
Column compartment	CHA (Column Heater with Active Preheating)		

### Data Management

Chromatography software:

Empower 3 FR5

### ATP/ADP/AMP/Adenosine Analysis

#### Sample Description

Disodium adenosine triphosphate (ATP) and sodium adenosine diphosphate (ADP) were purchased from Sigma and adenosine monophosphate (AMP) and adenosine were purchased from Acros. All samples were dissolved in sample diluent comprised of 95% mobile phase A and 5% of mobile phase B to concentrations in the range of 0.5 µg/mL to 200 µg/mL.

## Method Conditions

LC systems	ACQUITY Arc and ACQUITY Arc Bio	Arc Premier
Column(s):	XSelect HSS T3 XP, 100 Å, 2.5 µm, 4.6 mm × 50 mm (p/n: 186006157)	XSelect Premier HSS T3, 100 Å, 2.5 µm, 4.6 × 50 mm (p/n: 186009858)
Column temp.:	35 °C	
Sample temp.:	10 °C	
Injection volume:	15 µL	
Flow rate:	1.5 mL/min	
Mobile phase A:	10 mM ammonium acetate, pH 6.8 in 99.8/0.2 water/acetonitrile	
Mobile phase B:	8 mM ammonium acetate, pH 6.8 in 79.8/20.2 water/acetonitrile	
UV wavelength:	260 nm	

## Gradient Table

Time (min)	%A	%B
0	99	1
0.2	99	1
7.2	5	95
8.5	5	95
8.6	99	1
10.0	99	1

## HCP/BMP/DMP and Related Compounds Analysis

### Sample Description

Hydrocortisone phosphate (HCP) triethylamine, betamethasone sodium phosphate (BMP), dexamethasone sodium phosphate (DMP), dexamethasone, and dexamethasone acetate were purchased from Sigma Aldrich. All samples were dissolved in sample diluent comprised of 90% water and 10% acetonitrile to concentrations in the range of 1 µg/mL to 200 µg/mL

## Method Conditions

LC systems	ACQUITY Arc and ACQUITY Arc Bio	Arc Premier
Column(s):	XBridge BEH C <sub>18</sub> XP, 130 Å, 2.5 µm, 4.6 mm × 50 mm (p/n: 186006037)	XBridge Premier BEH C <sub>18</sub> , 130 Å, 2.5 µm, 4.6 × 50 mm (p/n: 186009847)
Column temp.:	40 °C	
Sample temp.:	10 °C	
Injection volume:	30 µL	
Flow rate:	1.5 mL/min	
Mobile phase A:	10 mM ammonium formate, pH 3.0	
Mobile phase B:	Acetonitrile	
UV wavelength:	260 nm	

## Gradient Table

Time (min)	%A	%B
0	82	18
3.0	82	18
7.0	50	50
8.0	50	50
8.1	82	18
10.0	82	18

## Non-metal-sensitive Compounds Analysis

### Sample Description

Amitriptyline hydrochloride was purchased from Sigma Aldrich and leucine enkephalin was obtained from Waters (p/n: 186006013 <<https://www.waters.com/nextgen/us/en/shop/standards--reagents/186006013-leucine-enkephalin.html>> ). Both were dissolved in 50:50 water:acetonitrile and then mixed with Waters ACQUITY UPLC MS-Start-Up Solution 2 (p/n: 700002741 <<https://www.waters.com/nextgen/us/en/shop/standards--reagents/700002741-acquity---quattro-micro-or-quattro-premier-ms-start-up-solution-.html>> ).

## Method Conditions

LC systems	ACQUITY Arc and ACQUITY Arc Bio	Arc Premier
Column(s):	XBridge BEH C <sub>18</sub> XP, 130 Å, 2.5 µm, 4.6 mm × 50 mm (p/n: 186006037)	XBridge Premier BEH C <sub>18</sub> , 130 Å, 2.5 µm, 4.6 × 50 mm (p/n: 186009847)
Column temp.:	40 °C	
Sample temp.:	10 °C	
Injection volume:	15 µL	
Flow rate:	1.8 mL/min	
Mobile phase A:	0.01% formic acid in water	
Mobile phase B:	0.01% formic acid in acetonitrile	
UV wavelength:	205 nm	

## Gradient Table

Time (min)	%A	%B
0	95	5
1.0	95	5
7.0	30	70
7.1	10	90
8.0	10	90
8.1	95	5
10.0	95	5

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## Results and Discussion

### ATP/ADP/AMP/Adenosine Analysis: Assessing Inert Surfaces

Due to their electron-rich nature, phosphate groups in a compound can interact with exposed metal sites, leading to adsorption on LC systems. As a result, in the absence of any ion pairing reagents, these compounds are ideal probes for assessing exposed metal surfaces. Adenosine nucleotide and its phosphorylated derivatives, ATP, ADP, and AMP were analyzed using a RP LC method for this purpose. Since the number of phosphate

groups in phosphorylated adenosine derivatives designates the degree the compound will bind, we chose the ATP and ADP region to demonstrate how the metal surfaces on LC system and column impact the detection of metal sensitive compounds (Figure 1). On both standard stainless steel (SS) system and standard Bio MP35N systems with a SS column, both ATP and ADP are undetected at lower concentrations and detected when the concentration increased to 25 µg/mL but with strong tailing (USP tailing >2.0). When the same sample was injected onto an Arc Premier System paired with a MaxPeak Premier Column, both ATP and ADP were readily detected starting at a concentration as low as 0.5 µg/mL, with appreciable peak areas and gaussian peak shape (USP tailing < 1.5), This demonstrates significant improvement in detection sensitivity for metal sensitive compounds at low concentrations compared with standard SS and standard Bio MP35N systems.

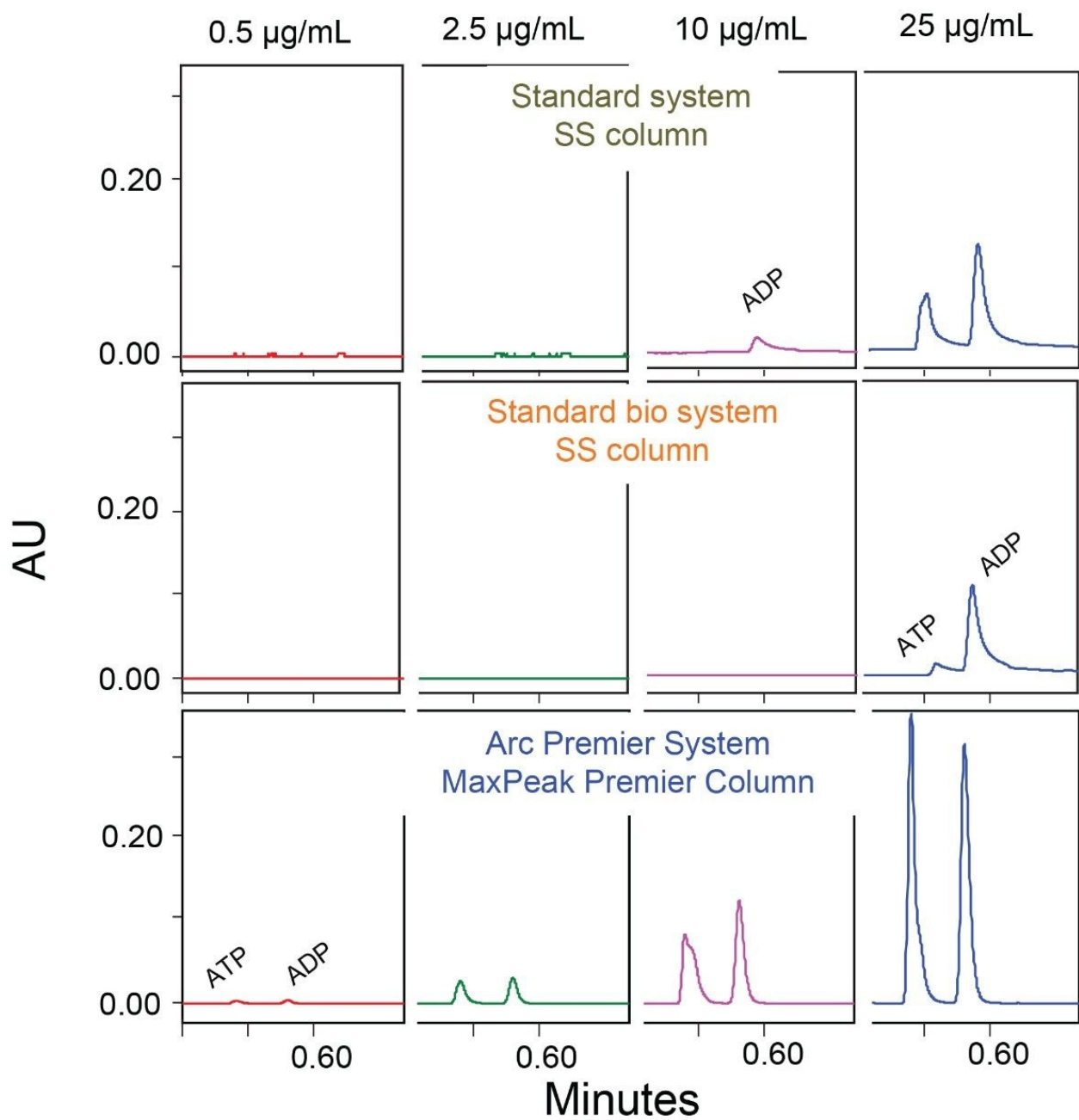


Figure 1. A comparison of detection of ATP and ADP with concentration increasing from 0.5 µg/mL to 25 µg/mL on the standard SS and the standard biocompatible LC systems paired with SS columns, and the Arc Premier System paired with a MaxPeak Premier Column.

For standard SS and standard MP35N bio LC systems, it is not uncommon to see poor repeatability across injections for metal-sensitive compounds. The extent of the variability depends on the specific compounds, the system history and exposure, the mobile phases, etc.<sup>4</sup> Figure 2 shows the comparison of the first injection versus the 100th injection of the ATP mix on these three LC systems. During repeated injections, ATP evolves from being undetectable to detectable on both the standard SS and standard bio systems with SS columns, and the same for ADP on the standard bio system. Additionally, the peak area of AMP increases with the number of injections on both standard SS and standard bio systems. On the Arc Premier System with a MaxPeak Premier Column, no dramatic peak area differences were observed when comparing the first injection with the 100th injection. The improved peak area repeatability and peak shape observed on the Arc Premier System is attributed to the effective suppression of metal-analyte interactions by the proprietary MaxPeak HPS Technology.

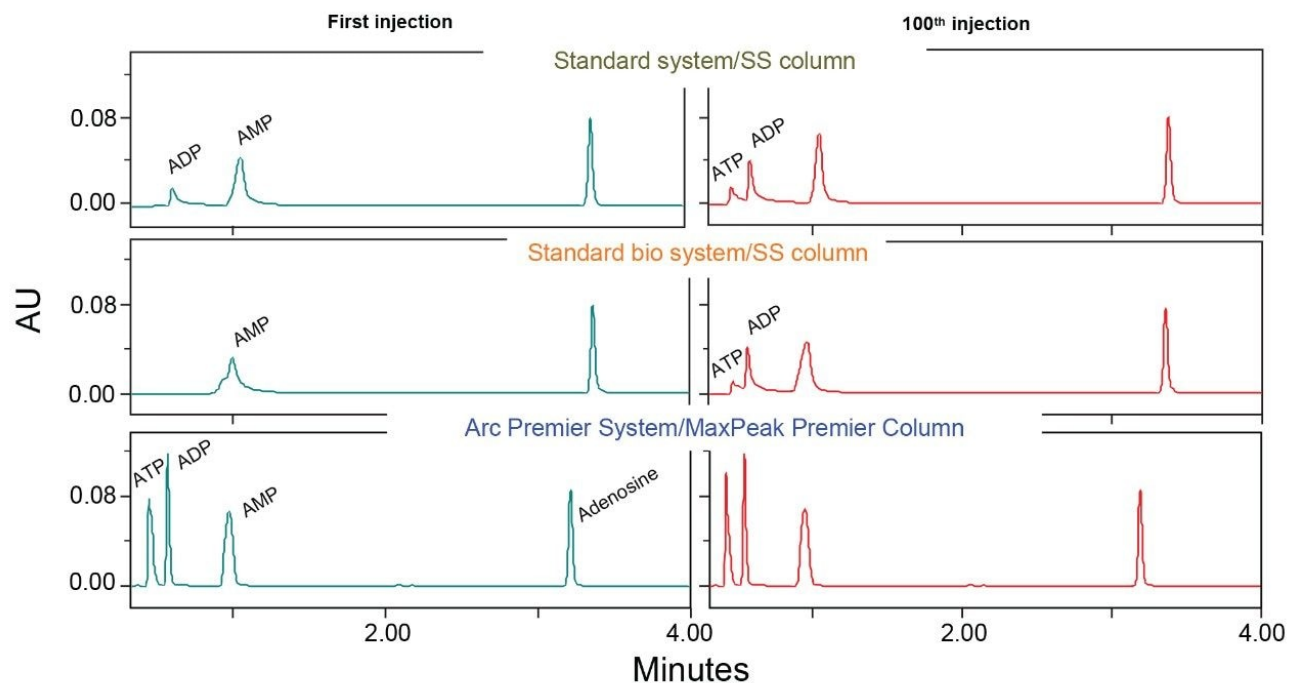


Figure 2. A comparison of first injection versus 100th injection with concentration of ATP, ADP, and AMP at 10  $\mu\text{g}/\text{mL}$  and adenosine at 5  $\mu\text{g}/\text{mL}$  on the standard SS system, the standard bio system, and the Arc Premier System.

The employed RP separation may not be sufficiently robust to deal with complex sample matrices since it does not provide retention for all the nucleotides, where ATP is seen to elute near the void. However, the ATP mix

serves as a useful probe to study the effectiveness of inert chromatographic surfaces.

## HCP/BMP/DMP and Related Compounds Analysis: Improving Sensitivity, Reproducibility, and Quantitation Accuracy

HCP and DMP, corticosteroids, relieve inflammation and are used to treat endocrine disorders and certain forms of immune and allergic conditions.<sup>5</sup> Similar to ATP, the electron-rich phosphate groups in HCP and DMP are susceptible to adsorption on metal surfaces. Figure 3 compares the HCP/DMP and related compounds analysis on the standard SS system, standard bio system, and the Arc Premier System at concentration 1 µg/mL and 25 µg/mL respectively. Three compounds, including HCP, BMP, and DMP, are severely affected by the metal surfaces in the standard SS and standard bio systems with SS columns. At a concentration as low as 1 µg/mL, these three compounds are barely detectable on the standard system; and presented low peak intensities and strong tailing on the standard bio system, which caused difficulty in integrating the peaks consistently. Conversely, on the Arc Premier System combined with a MaxPeak Premier Column, sharp and symmetrical peaks are observed for these three compounds. When the concentration increased to 25 µg/mL, the peaks of HCP, BMP, and DMP gained intensities on the standard SS and standard bio systems, however, they presented much broader and with greater tailing than those on the Arc Premier System.

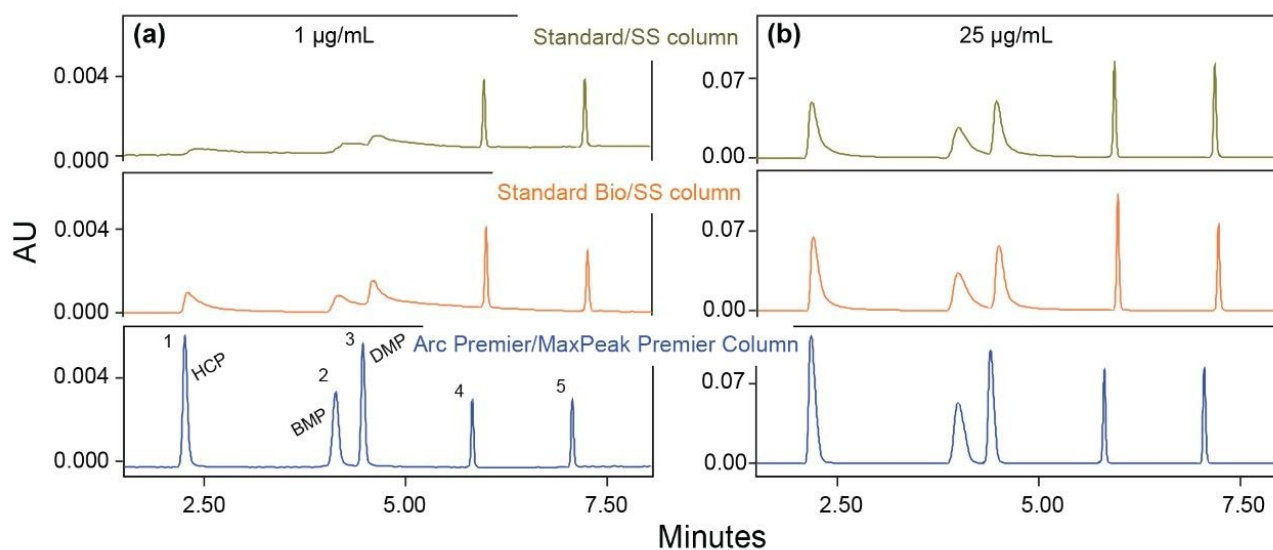


Figure 3. A comparison of HCP/DMP and related compounds on the standard system (SS column), standard bio system (SS column) and the Arc Premier System (MaxPeak Premier Column) at concentrations (a) 1 µg/mL for 1, 2, and 3, 0.3 µg/mL for 4 and 5; and (b) 25 µg/mL for 1, 2, and 3, 7.5 µg/mL for 4 and 5. (Peak labels: 1 - HCP, 2 - BMP, 3 - DMP, 4 - dexamethasone, 5 - dexamethasone acetate.)

Broad and tailing peaks would impact the data reproducibility and quantitation accuracy. The peak area %RSD of six sets of precision samples using HCP as a representative compound are compared among the standard system, standard bio system and the Arc Premier System (Figure 4). Out of six sets of precision samples, the Arc Premier System gave the lowest peak area %RSD, in the range of 0.05%–0.3%; the standard system gave the largest peak area %RSD, in the range of 0.8%–1.4%; while the standard bio system was intermediate, 0.15%–0.4%. Even though the  $R^2$  of three HCP calibration curves from these three systems appeared in the same order (Figure 5), we observed that both the standard SS and standard bio systems deviated from linearity at low concentrations using log scale of peak area vs. concentration (Figure 6a), and they also deviated from consistent response factor (peak area/concentration) at low concentrations (Figure 6b). The direct impact is the % deviation of quantitation of HCP at lower concentrations. At concentration 5 µg/mL, analysis of the replicate injections shows larger variabilities among six sets of calibration curves for the standard SS and standard bio systems (the highest % deviation was 22% for the standard SS system; 15% for the standard Bio system) (Figure 7). For the Arc Premier System, the average % deviation is 0.4% with the highest at 2.6% among six sets of calibration curves of

HCP. Employing the Arc Premier System with a MaxPeak Premier Column, great improvements are achieved in data reproducibility, sensitivity, linear dynamic range, and quantitation accuracy for steroid phosphate drug analysis.

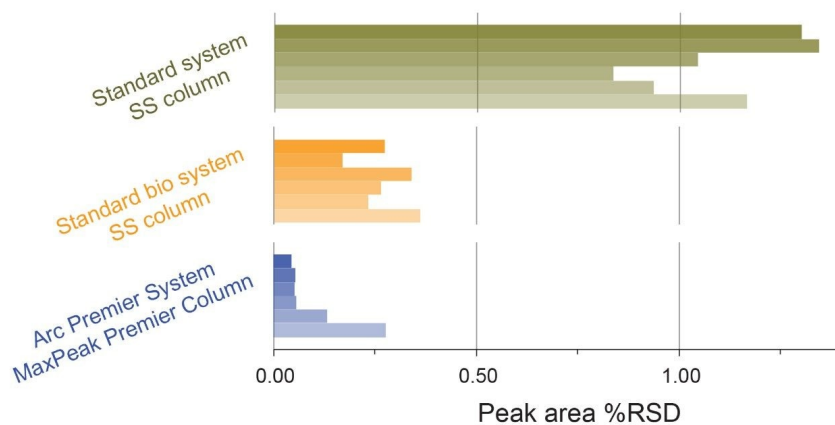


Figure 4. A comparison of peak area %RSD for six sets of precision samples (each bar representing peak area %RSD for six replicates in each set) of HCP peak at concentration 25  $\mu\text{g}/\text{mL}$  on the standard system, standard bio system, and Arc Premier System.

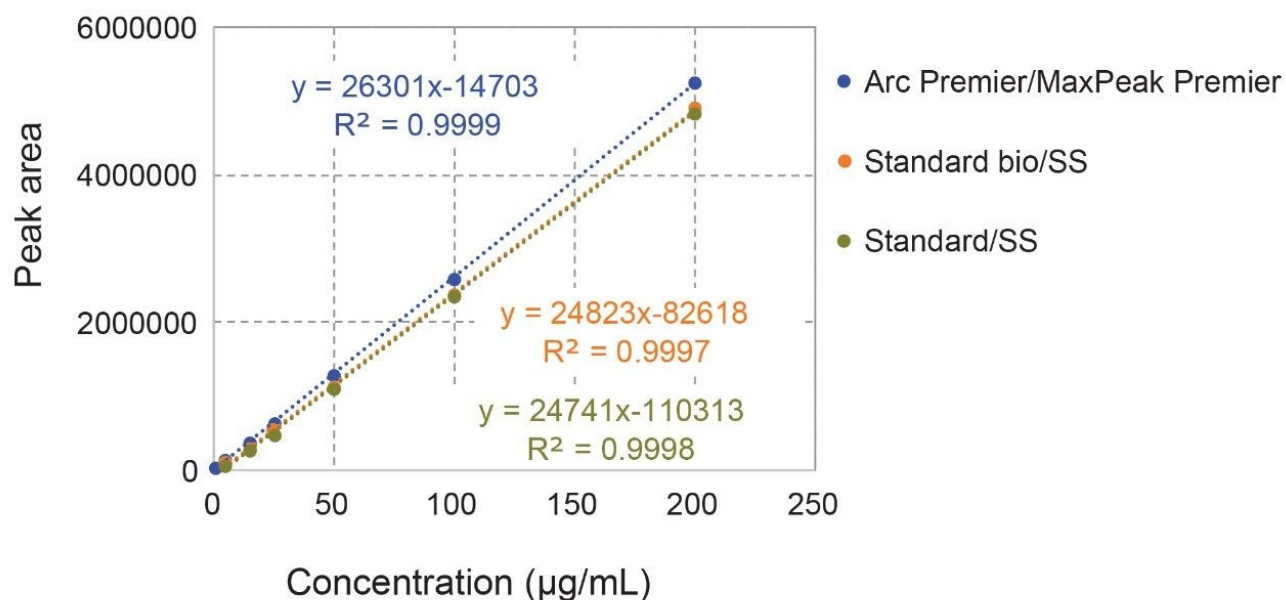


Figure 5. The calibration curves for HCP of three LC systems. All calibration curves were determined with 1/x weighting. Calibration range for standard system (SS column) and standard bio system (SS column) are 5 µg/mL–200 µg/mL and for the Arc Premier System (MaxPeak Premier Column) is 1 µg/mL–200 µg/mL.

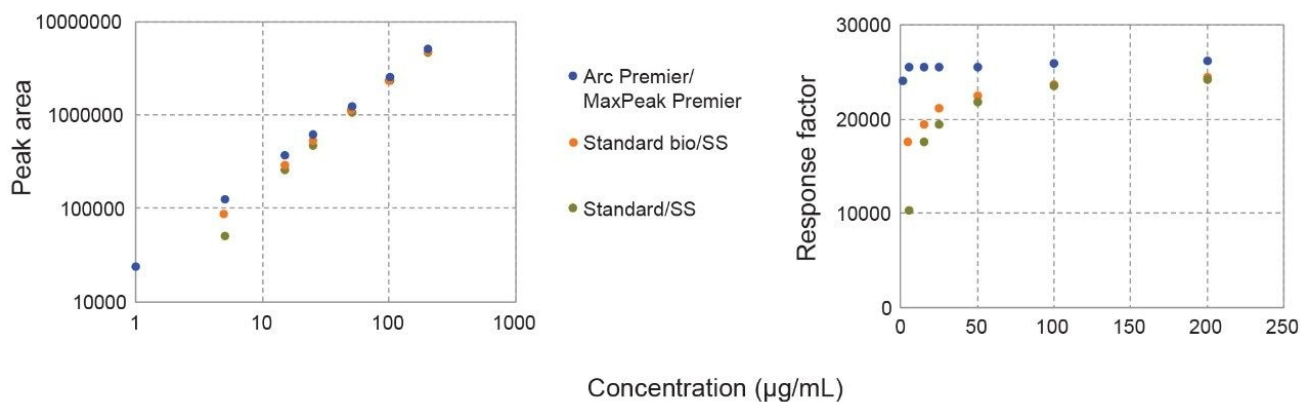


Figure 6. Two alternative plots for HCP standards. (a) Log plot for peak area vs. concentration; (b) response factor (peak area/concentration) vs. concentration. Calibration range 5 µg/mL–200 µg/mL for standard SS and standard bio systems and 1 µg/mL–200 µg/mL for the Arc Premier System.

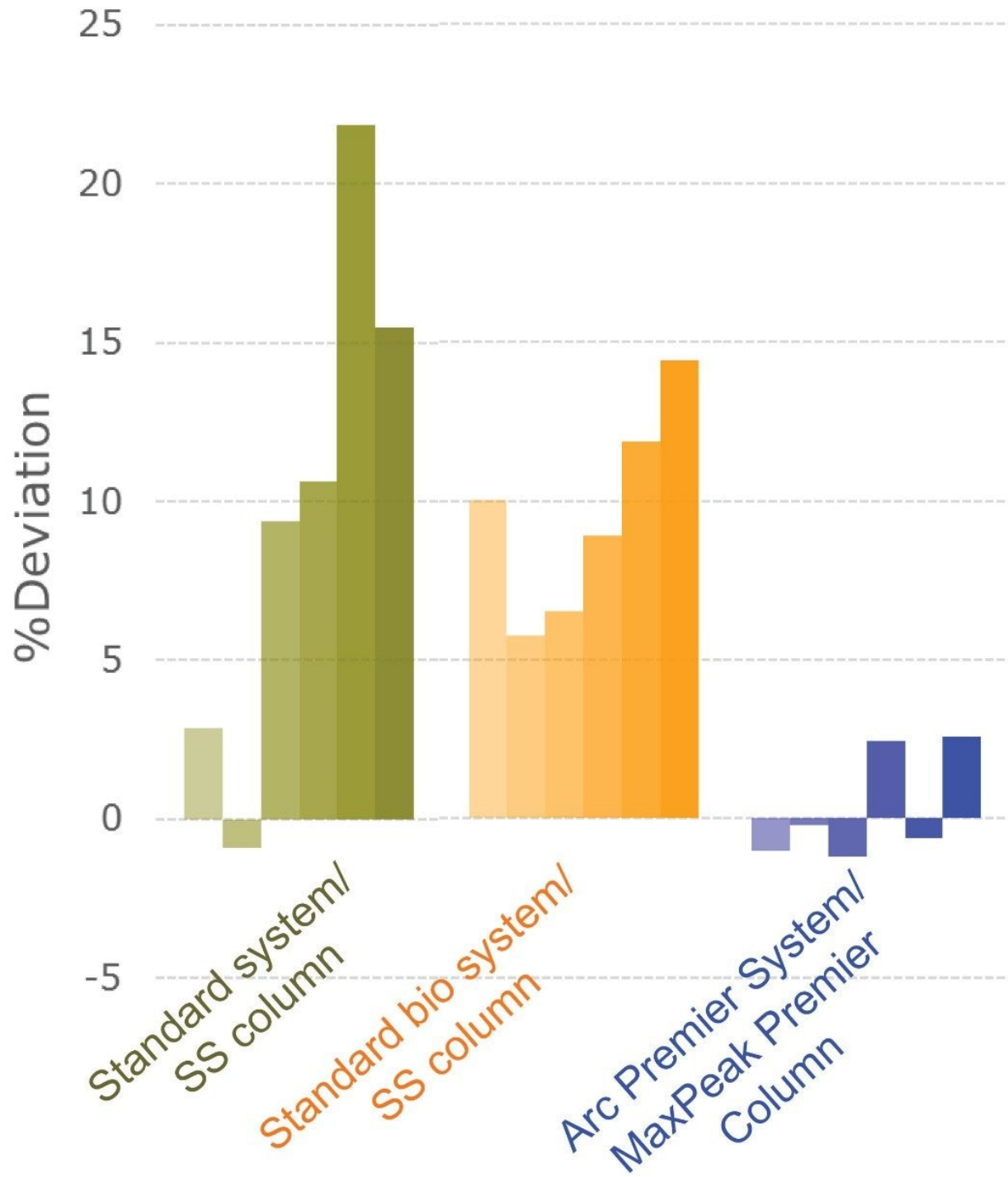


Figure 7. A comparison of % deviation of quantification accuracy at 5 µg/mL for six sets of calibration curves of

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HCP.

## Non-metal-sensitive Compounds Analysis: Method Transfer

In the above ATP/ADP/AMP/adenosine and HCP/BMP/DMP and related compounds analysis cases, the benefits of the Arc Premier System combined with a MaxPeak Premier Column are manifested in improved sensitivity, reproducibility, dynamic linear range, and quantitation accuracy for metal-sensitive compounds. For non-metal-sensitive compounds in these two cases, such as adenosine, dexamethasone, and dexamethasone acetate, the peak shape, detection sensitivity, and data reproducibility are comparable among the three LC systems (data not shown). It indicates that an LC method for non-metal-sensitive compounds can be transferred among a standard SS system, standard bio system, and Arc Premier System regardless the types of columns used (stainless-steel or MaxPeak Premier Column).

To illustrate the LC method transferability among the standard SS system, the standard bio system, and the Arc Premier System, a mix of seven non-metal-sensitive compounds with different chemical and physical properties were selected as examples. Both the standard SS and standard bio systems used columns with SS hardware, and the Arc Premier System used a MaxPeak Premier Column with MaxPeak HPS hardware. Comparable chromatographic profiles with similar retention times and peak shapes were observed among these three systems (Figure 8). Table 1 showed the comparison of the retention time standard deviation (RT SD) and peak area %RSD for six replicate injections for these seven compounds at a concentration of 10 µg/mL each. The retention time standard deviation is within 0.05–0.22 seconds, and the peak area %RSD is within 0.04%–1% for all the seven compounds on the standard SS system, standard bio system, and Arc Premier System. The transferability of non-metal-sensitive compounds analysis among these three LC systems is demonstrated by the comparable chromatographic performance.

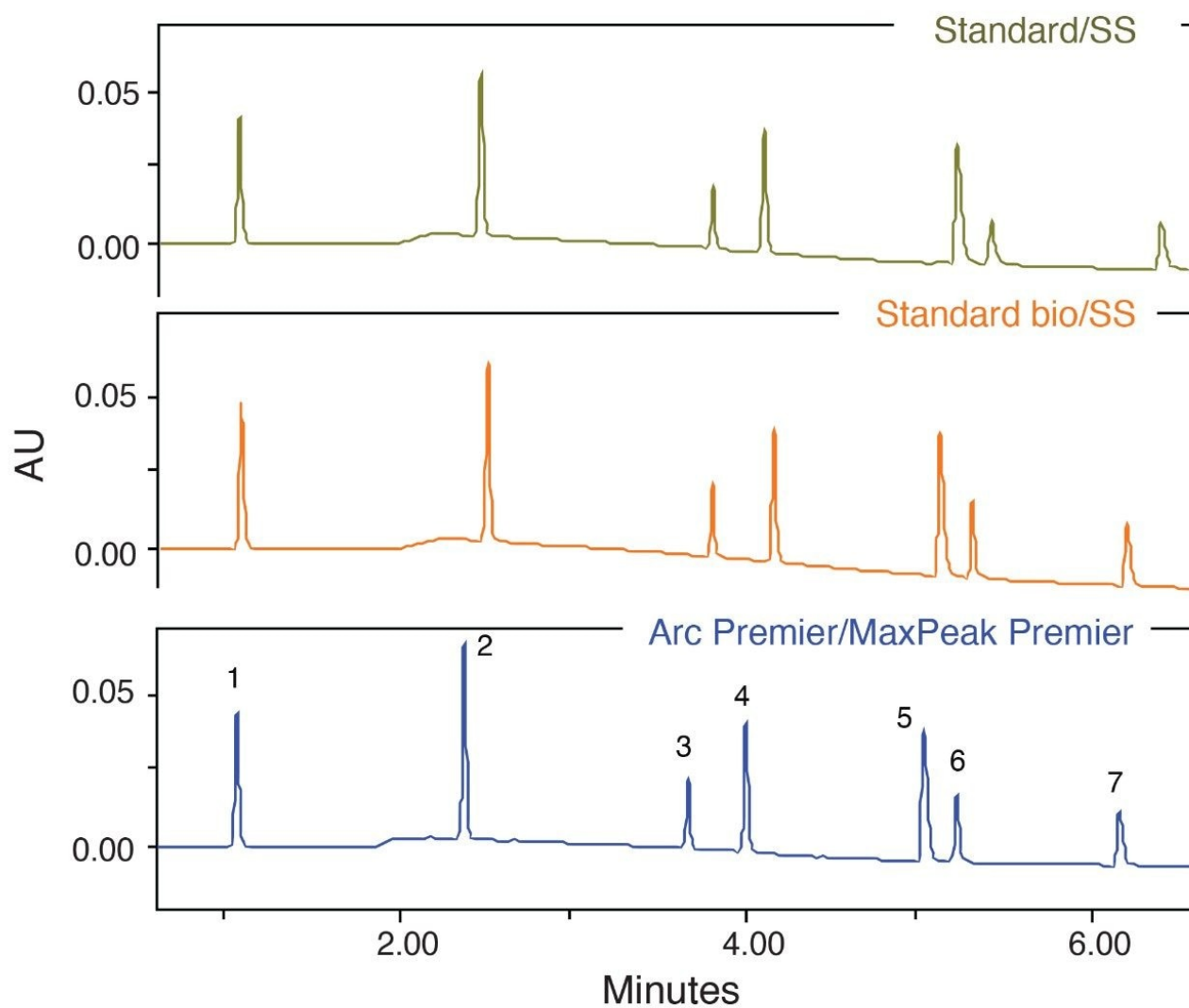


Figure 8. A comparison of a RP application of non-metal-sensitive compounds on three systems (peak labels: 1 - acetaminophen, 2 - caffeine, 3 - leucine enkephalin, 4 - sulfadimethoxine, 5 - amitriptyline, 6 - reserpine, 7 - terfenadine). Concentration is at 10  $\mu\text{g}/\text{mL}$  for each compound.

Peaks	Attributes	Standard SS	Standard bio/SS	Arc Premier/ MaxPeak Premier
Acetaminophen	RT SD (sec)	0.05	0.05	0.05
	Area %RSD	0.07	0.05	0.10
Caffeine	RT SD (sec)	0.06	0.07	0.06
	Area %RSD	0.06	0.04	0.08
Leucine enkephalin	RT SD (sec)	0.09	0.09	0.09
	Area %RSD	0.10	0.07	0.09
Sulfadimethoxine	RT SD (sec)	0.10	0.05	0.06
	Area %RSD	0.04	0.05	0.07
Amitriptyline	RT SD (sec)	0.16	0.07	0.12
	Area %RSD	0.14	0.18	0.10
Reserpine	RT SD (sec)	0.15	0.08	0.12
	Area %RSD	0.13	0.29	0.18
Terfenadine	RT SD (sec)	0.22	0.06	0.17
	Area %RSD	0.21	0.91	0.14

Table 1. Comparison of repeatability (n=6) of retention time standard deviation (RT SD) and peak area %RSD of non-metal-sensitive compounds (each at concentration 10 µg/mL) analysis among the standard SS system (SS column), standard bio system (SS column), and Arc Premier System (MaxPeak Premier Column).

## Conclusion

The Arc Premier System with MaxPeak Premier Columns, built with Waters' proprietary MaxPeak High Performance Surfaces Technology, provides a solution to historically challenging analytes, which have the tendency to adsorb to metal surfaces found in traditional stainless-steel LC systems and biocompatible metal-alloy LC systems. Applying ATP/ADP/AMP/adenosine mix as a probe, detection sensitivity and peak shape were significantly improved on the Arc Premier System compared to the other two metal-based LC systems. In steroid phosphate compounds analysis, improvements in data precision, sensitivity, and % deviation of quantitation are achieved by employing the Arc Premier System with MaxPeak Premier Column. Finally, for non-metal-sensitive compounds analysis, the Arc Premier System with MaxPeak Premier Columns offer comparable chromatographic performance compared to the traditional SS and biocompatible metal-alloy LC systems.

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## Featured Products

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[ACQUITY Arc Bio System <https://www.waters.com/134966135>](https://www.waters.com/134966135)

[Arc Premier System <https://www.waters.com/waters/nav.htm?cid=135083359>](https://www.waters.com/waters/nav.htm?cid=135083359)

[2998 Photodiode Array \(PDA\) Detector <https://www.waters.com/1001362>](https://www.waters.com/1001362)

[2489 UV/Visible \(UV/Vis\) Detector <https://www.waters.com/515198>](https://www.waters.com/515198)

[Empower Chromatography Data System <https://www.waters.com/10190669>](https://www.waters.com/10190669)

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