

## Enhanced Performance of the Analysis for Veterinary Drugs with Metal Affinity Using ACQUITY™ Premier and Xevo™ TQ-S Micro

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

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### Abstract

The application of MaxPeak™ High Performance Surfaces (HPS) reduces interaction for metal sensitive compounds and so increases the response of these compounds in a liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Here we demonstrate the utility of the Waters™ ACQUITY™ Premier UPLC™ System-Xevo TQ-S micro for the analysis of a range of veterinary drugs representative of the major classes and show increased performance of MaxPeak High Performance Surfaces (HPS) for the known metal sensitive tetracyclines.

### Benefits

Sensitivity gains for metal sensitive veterinary drugs, extending detection limits of previous methods

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## Introduction

The monitoring of veterinary drugs in food materials is important for public health and trade. These drugs vary in chemical nature from very polar to very non-polar, but also some, notably the tetracyclines, have an affinity for metal ions which can create undesired interactions in chromatographic systems that use stainless steel flow paths. Here we show the application of a Waters ACQUITY Premier System, that prevents interaction with compounds that have a metal affinity by way of MaxPeak HPS, for the analysis of a broad range of veterinary drug compounds in milk that are representative of each of the classes, as well as an illustration of performance benefits when analyzing metal sensitive veterinary drugs compared to a typical stainless steel UPLC.

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## Experimental

Sample preparation was performed on samples of cow's milk spiked with a concentration range of 1–50 µg/kg for 18 veterinary drug standards, using the protocol described in reference (1). Samples were analyzed using a Waters ACQUITY Premier System or a Waters ACQUITY UPLC H-Class connected to a Waters Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer. Both UPLC systems had quaternary pumps and Flow Through Needle (FTN) autosamplers, being as similar as possible in flow path and gradient delivery. The ACQUITY Premier used an ACQUITY Premier BEH™ C<sub>18</sub> 1.7 µm 2.1 x 100 mm Column, the ACQUITY UPLC H-Class used an ACQUITY UPLC BEH C<sub>18</sub> 1.7 µm 2.1 x 100 mm Column. Both LC systems used solvents (A) 0.1% formic acid and (B) methanol, holding an initial mixture of 2% B for 2 minutes and then applying a gradient of 30%–80% B over 5 minutes, ramping quickly from 45% B to 70% B in the last 2 minutes. The MS/MS analysis used two transitions per compound. The multiple reaction monitoring (MRM) transitions can be found in reference (1) although source and collision cell parameters were optimized for this instrument.

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## Results and Discussion

When using the ACQUITY Premier-Xevo TQ-S micro System the selected 18 study compounds representing all

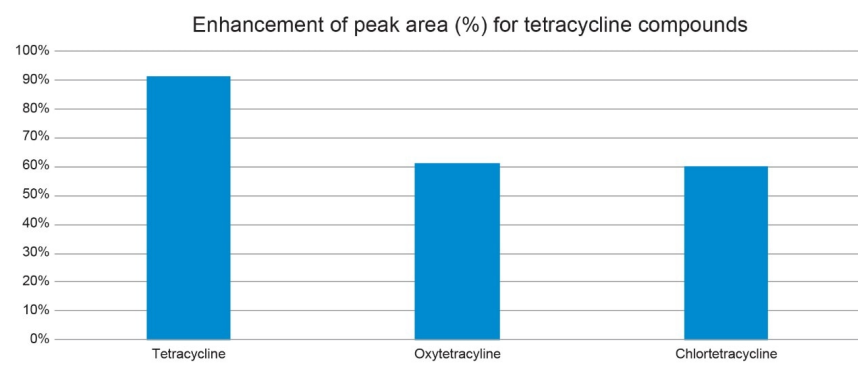
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major classes of vet drugs were successfully detected across the calibration range in matrix with good linearity. These results show that ACQUITY Premier is suitable for analysis of all major classes of veterinary drugs. Table 1 summarizes the linearity and  $R^2$  for the 18 vet drugs. The linear range for the compounds studied was found to be  $\leq 2$  to 50  $\mu\text{g/kg}$ ,  $R^2$  is  $>0.990$  with a residual of  $<15\%$ .

Veterinary drugs	Linear range (R <sup>2</sup> )
Sulfanilamide	2–50 µg/kg (0.9947)
Salbutamol	1–50 µg/kg (0.9988)
Carbadox	1–50 µg/kg (0.9894)
Sulfamerazine	1–50 µg/kg (0.9942)
Sulfamethazine	1–50 µg/kg (0.9943)
Ractopamine	2–50 µg/kg (0.9913)
Phenylbutazone	2–20 µg/kg (0.9902)
Ciprofloxacin	2–50 µg/kg (0.9939)
Penicillin G	2–20 µg/kg (0.9908)
Enrofloxacin	2–50 µg/kg (0.9930)
Dexamethasone	1–50 µg/kg (0.9995)
Oxacillin	1–50 µg/kg (0.9911)
Lincomycin	1–50 µg/kg (0.9968)
Tetracycline	2–50 µg/kg (0.9912)
Oxytetracycline	2–50 µg/kg (0.9940)
Chlortetracycline	1–50 µg/kg (0.9950)
Erythromycin	2–50 µg/kg (0.9960)
Chloramphenicol	2–50 µg/kg (0.9960)

Table 1. Linear range of in sample concentration in µg/kg and R<sup>2</sup> of the 18 veterinary drugs spiked in milk matrix.

Comparing the data generated by the two systems it was clear that there were sensitivity gains for the metal sensitive tetracycline compounds when using a system that utilizes HPS. Figure 1 shows the difference in peak area (%) across the calibration points between the MaxPeak HPS and a standard UPLC System without these surfaces. Response for these compounds were increased by 60 to 90% when using MaxPeak HPS, as these compounds are no longer interacting with the metal surfaces inside the UPLC system and column and resulting in an increased response in terms of area counts. Figure 2 shows the MRM chromatograms for the three tetracyclines between these two systems.



*Figure 1. Difference in peak area (%) between MaxPeak HPS and an untreated stainless steel UPLC system for tetracycline compounds in a milk matrix.*

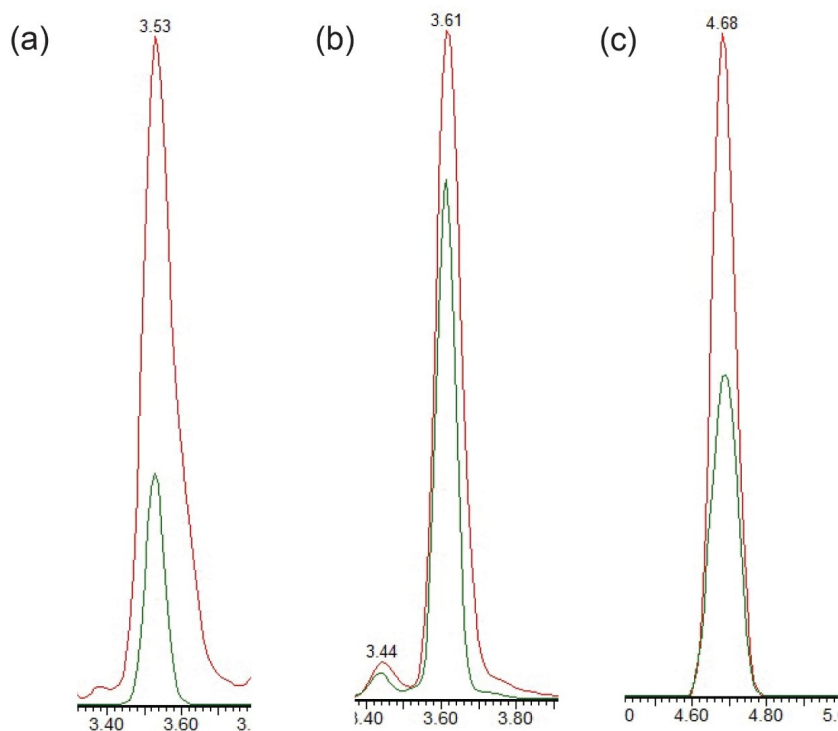


Figure 2. Overlaid chromatograms showing the effect of HPS on known metal sensitive compounds. HPS – (red) and stainless steel (green) for (a) Tetracycline (ESI+, 445.45 > 410.00), (b) Chlortetracycline (ESI+, 479.30 > 444.18), and (c) Oxytetracycline (ESI+, 461.26 > 426.13).

## Conclusion

ACQUITY Premier is suitable for the analysis of veterinary drugs in food matrices. Further to this, reduced metal interactions of the MaxPeak HPS of this system enhances the sensitivity of metal sensitive compounds, in this example the tetracyclines when analyzing veterinary drugs in a milk matrix.

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## References

1. Michael S. Young, Kim Van Tran. Optimized Extraction and Clean-up Protocols for LC-MS/MS Multi-Residue Determination of Veterinary Drugs in Milk. Waters Application Note, [720004089EN](#), August 2011.

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## Featured Products

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ACQUITY UPLC H-Class PLUS System <<https://www.waters.com/10138533>>

Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <<https://www.waters.com/134798856>>

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