Systematic Protocol Utilizing High Performance Surface Technology for the Improved Separation and Quantification of Synthetic Peptides and Associated Impurities

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Introduction

Antibiotics have saved millions of lives since they were discovered, but over prescription of these compounds has led to resistant strains of bacteria becoming more prevalent. In these cases, specialized cyclic peptide antibiotics have been shown to be successful in combatting specific and resistant strains.[1] Due to this, macrocyclic antibiotics usage has increased in recent years. The availability of streamlined workflows and good prior knowledge on peptides may allow for better risk assessment, potentially decreasing method development time for similar compounds of interest.

Analytical quality by design (AQbD) has become a topic of interest for regulatory agencies over recent years, and the principles of AQbD are now being adopted into regulatory guidances. Within this study, a risk-based approach, created with AQbD principles in mind, was used during method development to determine high risk variables. The risk assessment was conducted based on prior knowledge, literature review, and screening studies to ensure confidence in the quality and consistency of the final method.

Risk Assessment

Research on the highest risk variables has been carried out previously.[2] These high-risk variables are illustrated in Figure 1. Column chemistry is usually one of the highest risks. Recent work at waters has identified two columns to be the best starting points for peptide analysis. The recommended columns are the XSelect™ Premier Peptide CSH™ C18 and the XBridge™ Premier Peptide BEH™ C18 Columns.[2] Another risk noted in this work is the surface analyte interactions which were found to be reduced with the MaxPeak™ Premier technology.

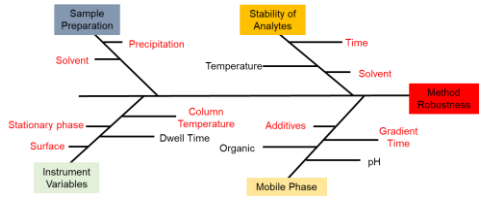

Figure 1: Ishikawa diagram to show some method development parameters that were evaluated. The high-risk variables found are in red.

Method

LC Systems:	Arc™ Premier QSM-r System
Column:	XSelect Premier Peptide BEH C_{18} 130Å, 2.5 µm, 4.6 mm X 150 mm Column
Detectors:	Waters Arc Premier 2998 PDA, 214nm
	ACQUITY™ QDa™ Mass Detector
Column Temp:	60°C
Injection Volume:	10µL
Mobile Phase A:	0.1% Formic Acid in Water
Mobile Phase B:	0.1% Formic Acid in Acetonitrile

Sample Preparation:
The solvents used to dissolve the peptide was observed as a high-risk variable. Preparation of the peptides in 30:70 Water:DMSO led to an 80% degradation of some of the compounds in 3 days. The use of 100% DMSO as a solvent resolved these stability issues.

All peptides were prepared in 100% DMSO at 0.1 mg/mL concentration for the initial panel. The focus gradient for Dalbavancin impurities was at 0.1mg/ml for the Dalbavancin and 0.25 mg/ml for the A40926.

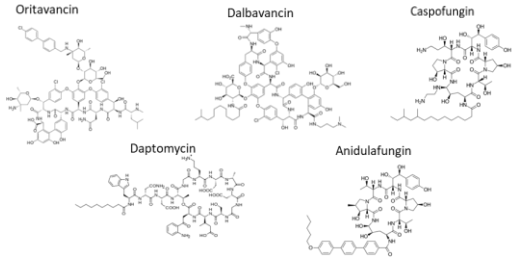

Figure 2: Structures of the compounds included in the macrocyclic peptide panel.

Results and Discussion

Basing our starting point on previous knowledge and good risk assessment, an initial gradient of 5-55% acetonitrile was tested. This gradient was run on two columns: the XSelect CSH and XBridge BEH with 0.1% formic acid or trifluoracetic acid in both the water and acetonitrile mobile phase. Of these 4 combinations the XSelect CSH column was chosen for its ability to best meet our predefined analytical target profile (ATP) needs. From this point a slightly changed gradient going from 10-70% acetonitrile in 20 minutes was created that provided acceptable peak shape, resolution, and reproducibility for all compounds. Final separation can be seen in Figure 3B.

The same workflow was used to develop a method for separating dalbavancin, and its impurities shown in Figure 5. This separation proved to be more difficult so other variables were investigated such as gradient time and temperature. The final method that was able to accomplish our ATP used the same column and additives, but with a gradient of 23-40% acetonitrile in 20 minutes and an increased column temperature of 80 ° C.

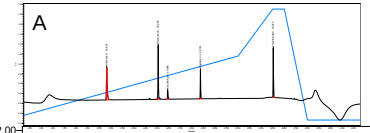

Figure 3A: Gradient 0.5-55% acetonitrile over 20 minutes. Anidulafungin elutes during the wash step of the gradient.

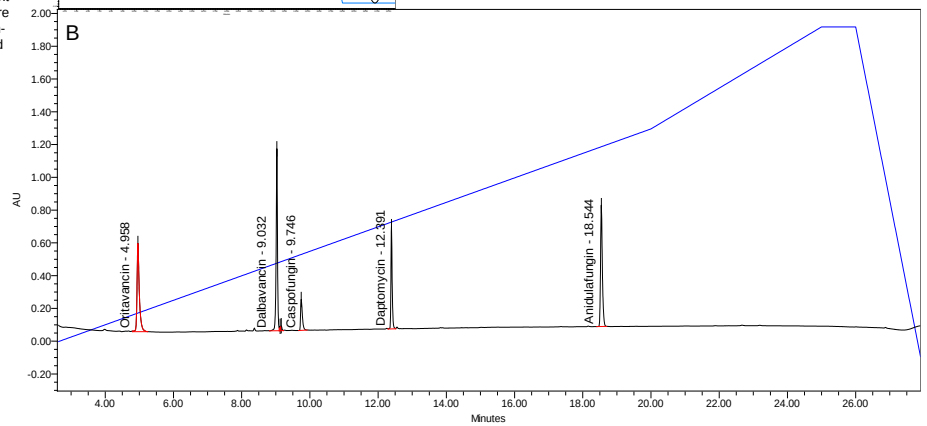

Figure 3B: An adjusted gradient to 10-70 % acetonitrile over 20 minutes allows all 5 compounds to elute in the gradient.

	MaxPeak Premier RSD	Stainless Steel RSD
Height	0.50%	4.00%
Area	0.50%	4.30%
Retention time	0.04%	0.09%

Figure 4A: Comparison of the relative standard deviation (RSD) of the height, area, and retention time on MaxPeak Premier versus stainless steel systems and columns. A lower RSD on the MaxPeak Premier system and column suggests a more reproducible method.

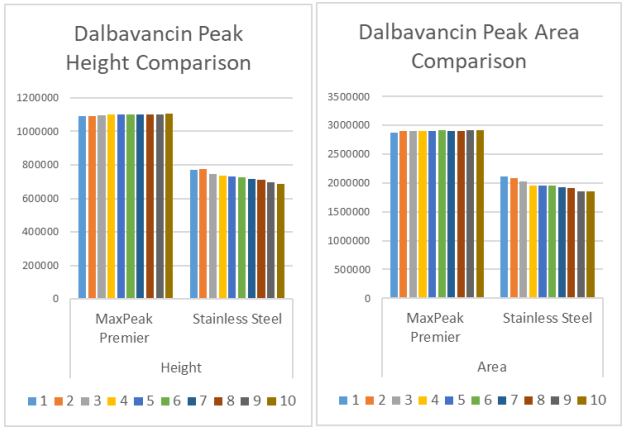

Figure 4B: Comparison of the analysis of dalbavancin on MaxPeak Premier versus stainless steel systems and columns. Peak height and area improved on MaxPeak Premier system and column when compared to stainless steel in both count and consistency.

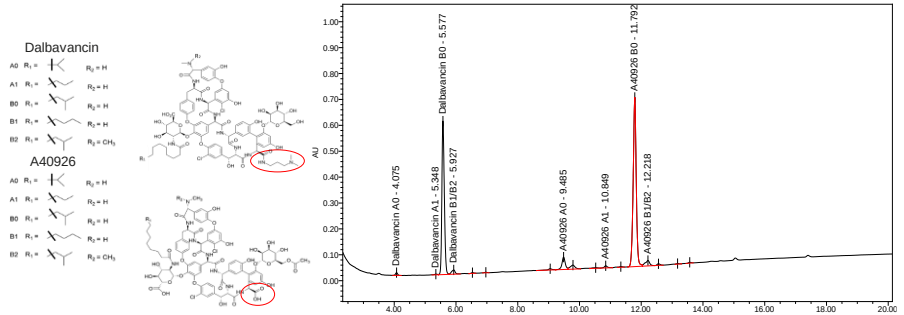

Figure 5: Dalbavancin and its known impurity's structure and labeled chromatogram. Mass spectral data provided by the QDa Mass Detector was used to suggest peak identification of the impurities.[3]

CONCLUSION

- Method development using a systematic protocol can assist in the rapid method development and the creation of a quality method.
- The use of risk assessment can ensure that all critical method parameters are evaluated.
- MaxPeak™ Premier systems and columns can provide improved chromatographic area and height compared to stainless steel systems.

References:
1. Luther, A.; Bisang, C.; Obrecht, D. Advances in macrocyclic peptide-based antibiotics, Bioorganic & Medicinal Chemistry (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.08.006
2. Yang, H.; Koza, S.;Shiner, S. Reversed-Phase Column Performance for Peptide and Peptide Mapping Separations, Waters.com 2023, 720008035.
3. Zhu, D.; Ping, L.; Hong, Y.; Shen, J.; Weng, Q.; He, Q.; Simultaneous Quantification and Pharmacokinetic Study of Five Homologs of Dalbavancin in Rat Plasma Using UHPLC-MS/MS. Molecules. 2020 Sep 8;25(18):4100. doi: 10.3390/molecules25184100. PMID: 32911715; PMCID: PMC7570859.