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Analytical Quality by Design Based Method Development for the Analysis of Dexamethasone Phosphate and Related Compounds Using Arc Premier MaxPeak High Performance Surfaces (HPS) Technology

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Abstract

An Ultra High Performance Liquid Chromatography method was developed for the analysis of a mixture of metal chelating and non-chelating compounds using the Analytical Quality by Design (AQbD) approach. DryLab, Empower, and Waters systems were used to automate the method development process. The performance of the Arc Premier System in combination with MaxPeak Premier Columns was compared to standard stainless-steel hardware. Results showed that MaxPeak Premier Columns on an Arc Premier System provides great performance for the separation of metal chelating compounds compared to stainless-steel hardware. The final method uses a MaxPeak Premier BEH C_{18} Column (10 cm × 4.6 × 2.5 µm), 0.1% formic acid in acetonitrile as an organic solvent, and 10 mM ammonium formate in water aqueous mobile phase. The method showed excellent separations between the peaks, ideal peak shapes, high recoveries, and good reproducibility. For example, the USP tailing was ≤1.1 for all peaks including the phosphorylated compounds. These findings indicate that using the MaxPeak High Performance Surfaces (HPS) Technology for the analysis of metal chelating compounds is greatly beneficial in mitigating undesired interactions with metal surfaces and achieving excellent separations.

Benefits

- Show the superior advantages for using the Arc Premier System's MaxPeak HPS Technology for the analysis of phosphorylated compounds
- Show the increased efficiency of method development using Arc Premier System in combination with Empower 3 Chromatographic Data System (Empower CDS) and DryLab4 Software
- Shows the seamless integration between DryLab and Empower to fully automate the method development process

Introduction

Stainless-steel has been the most commonly used material to construct liquid chromatography instruments and columns due to its corrosion resistance,[1] manufacturability, and its inertness to a wide variety of chemical compounds. However, certain classes of analytes, such as metal chelating compounds/Lewis bases can interact with metal oxide films because of the electron deficient nature of these metal ions. For example, electron rich analytes such as phosphate and carboxylate groups can readily adsorb to the electron deficient surfaces of stainless-steel within the flow path of the chromatographic system. Such interactions can result in poor chromatographic peak shape, severe analyte losses, and quantitative inaccuracies.[2,3]

One approach that has previously been used to address this issue was adding metal chelators such as EDTA, citric acid, and acetone to the mobile phase.[4–7] Despite the potential advantages of these additives, the use of these chelators can have undesired impacts on chromatographic selectivity and MS detection sensitivity. Another alternative that has also previously been used to address the interactions between metal surfaces and metal-sensitive compounds was using metal free columns, such as Polyether ether ketone (PEEK). While the use of PEEK tubing in liquid chromatography columns has shown to be useful, it had major draw backs. For example, PEEK does not have the mechanical strength to withstand ultrahigh pressures (*i.e.*>5000 psi) that are normally required for UltraPerformance Liquid Chromatography (UPLC).[8] Additionally, PEEK tubing is not compatible with several organic solvents such as tetrahydrofuran (THF), dimethylsulfoxide (DMSO), and chlorinated hydrocarbons.[9,10]

To combat these issues, Waters has recently developed a family of technologies named MaxPeak High Performance Surfaces (HPS). The MaxPeak HPS LC surfaces are composed of a highly cross-linked layer related to that of ethylene bridged hybrid (BEH) chromatographic particles. These surfaces are designed to increase

analyte recovery, sensitivity, and reproducibility by mitigating undesired interactions with metal surfaces.

In this application note, a UHPLC method for the analysis of metal-sensitive pharmaceuticals/related compounds using MaxPeak HPS systems and columns was developed. These compounds are two phosphorylated active pharmaceutical ingredients (hydrocortisone phosphate triethylamine and dexamethasone sodium phosphate) and three dexamethasone phosphate related compounds. The chemical structures and the names of the five compounds are shown in Figure 1. The method development process was performed according to the Analytical Quality by Design (AQbD) principles. These principles are described in detail elsewhere.[11,12] Briefly, the AQbD is a systematic approach to method development that incorporates risk assessment and design of experiments (DoE) to investigate interaction effects on the method performance. The output of DoE identifies a region of robust operating conditions for the method, referred as Method Operable Design Region (MODR).

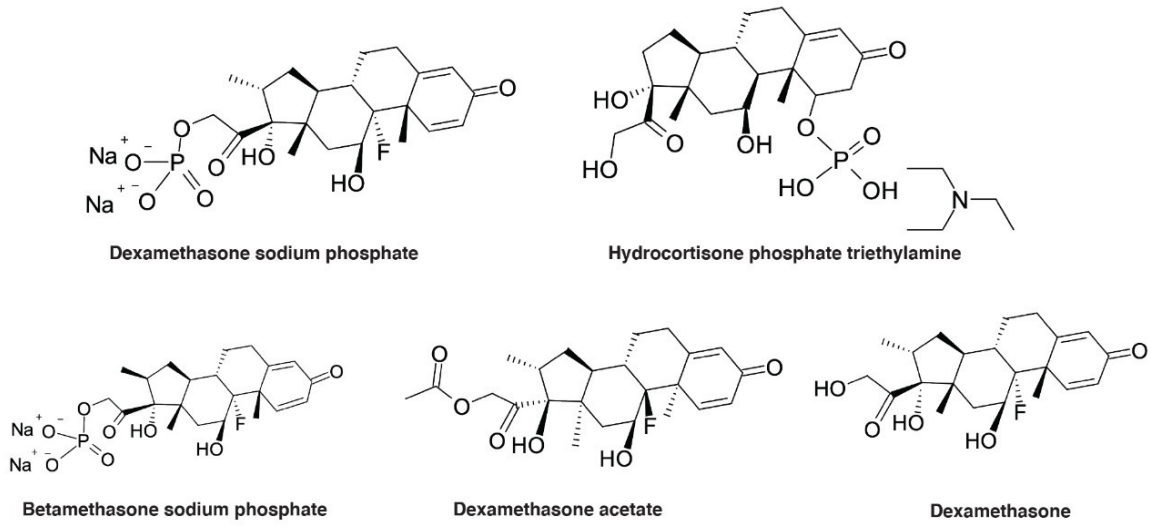


Figure 1. Chemical structures of dexamethasone sodium phosphate, hydrocortisone phosphate triethylamine, and the dexamethasone phosphate related compounds.

The experiments were performed using an Arc Premier System that is equipped with a column manager and solvent select valve to allow for automated exploration of a wide range of conditions. DryLab4 method development software was used in this study to automate the method development process according to AQbD principles.

Experimental

Materials and Standard Preparations

Hydrocortisone phosphate triethylamine, dexamethasone sodium phosphate, betamethasone sodium phosphate, dexamethasone, and dexamethasone acetate were all purchased from the United States Pharmacopeia (USP) (Rockville, MD, USA). Stock solutions of these compounds were prepared by accurately weighing the desired amounts of each standard and dissolving them in 50/50 (v/v) water/acetonitrile solvent. The stock solutions were then used to make a test mixture that contains the two APIs and three dexamethasone phosphate related compounds. This mixture was prepared by diluting the stock solutions of each one of the standards in 90/10 (v/v) water/acetonitrile as sample solvent. The final concentration of each analyte in the test mixture were approximately: 0.1 mg mL^{-1} Hydrocortisone phosphate triethylamine, dexamethasone sodium phosphate and 0.07 mg mL^{-1} for each related compound.

LC Conditions

LC system:	Arc Premier System with Quaternary Solvent Manager (rQSM), Sample Manager (rFTN), Column Manager, and a CM-Aux, PDA Detector, ACQUITY QDa Mass Detector
Detection:	PDA
Column(s):	1. HSS T3 Column, 2.5 µm, 4.6 × 100 mm, pH range: 1–8 2. BEH C_{18} Column, 2.5 µm, 4.6 × 100 mm, pH range: 1–8
Column temp.:	30–60 °C
Sample temp.:	10 °C
Injection volume:	3 µL
Flow rate:	0.5

Mobile phase A:	0.1 % (v/v) Formic acid in water
Mobile phase B:	Acetonitrile and methanol (0.1 % Formic acid)
Gradient:	10 to 90% B/5 or 15 min*. Gradient starts at t=0 and a final hold of 2 minutes was applied before returning to initial conditions.
UV detection:	254 nm

* Two different gradients of 5 and 15 minutes were explored in this experiment.

MS Conditions

MS system:	ACQUITY QDa Mass Detector
Ionization mode:	ESI+
Acquisition range:	100–500 Da
Capillary voltage:	0.8 kV
Source temp.:	600 °C
Cone voltage:	15 V

Data Management

Chromatography software:	Empower 3 Chromatographic Data System and DryLab 4

Results and Discussion

To properly evaluate the benefits of using High Performance Surfaces Technology for the analysis of metal chelating compounds it was interesting and necessary to compare a High Performance Surface LC system to a standard stainless-steel system. To do this, the two systems were used in parallel to develop a method for the separation of a mixture of these metal chelating compounds. Comparisons between the two systems at the different stages of the method development process will be demonstrated when applicable.

The method development process was performed in compliance with the AQbD principles as stated earlier. Employing these principles allows for a better understanding of the various chromatographic effects on the performance of the method. Further, it facilitates defining a robust design space where all the method performance goals are met. This design space offers flexibility with regards to regulations as any alteration within this space is not considered to be a change and does not require "a regulatory post approval process".[13] DryLab is an AQbD software that is commercially available. It is used combination with Empower to develop methods in compliance with the AQbD principles and it automates the whole method development process by creating all the needed methods within the CDS (Empower). The workflow for DryLab-Empower for automated method development process involves multiple steps can be seen in Figure 2. Details about each of these steps are described next.

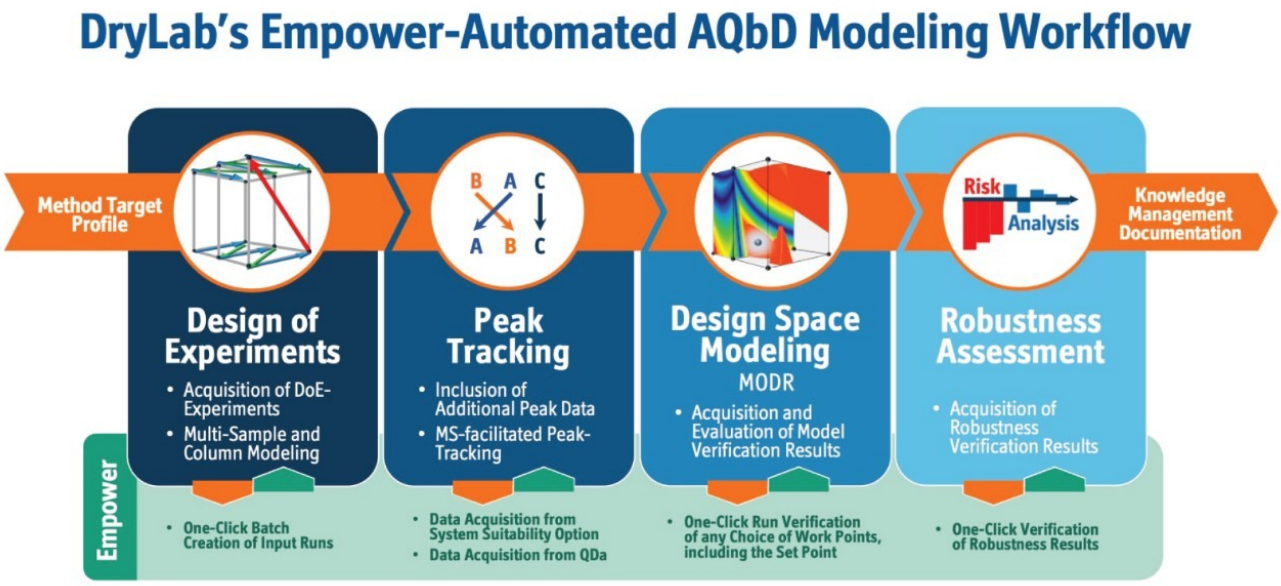


Figure 2. A workflow shows the multiple steps that are involved in DryLab-Empower AQbD method development process.

Method Target Profile

Method targets are the analytical objectives that describe the intended performance goals of the method and the performance criteria for the measurements. For the dexamethasone phosphate/hydrocortisone phosphate and related substances, the method performance goals included:

- The critical pair USP resolution should be ≥1.5. The critical resolution represents the resolution between the two components of the chromatogram with the lowest calculated resolution between them.
- Method must operate under MS-compatible conditions for identification by mass spectrometry.
- Method must meet the system suitability criteria:
    - USP peak tailing ≤1.5
    - %RSD of peak areas ≤2.0
    - %RSD of retention times ≤1.0

Risk Assesment

In this part of the AQbD method develpmet processs, the critical method parameters (CMP) are identified and assessed for their impact on the quality of the data generated by the method.[14] The parameters that pose the highest risks on the ability of the method to achieve the desired method performance goals are evaluated based on the sound chromatgraphic science and previous experience.

For dexamethasone phosphate/hydrocortisone phosphate and related substances, method parameters were evaluated based on the analytes information found in literature[15] and scientific experience. The parameters that were identified to have the greatest impact on the selectivity, resolution, retentivity, and peak shape were column chemistry, interaction with metal surfaces, temperature, gradient time, and the organic solvent composition. As such, these variables were studied in the scouting phase of the method development. Other chromatographic parameters including flow rate, injection volume can affect the resolution between analytes. These parameters can be easily controlled, therefore were not considered critical.

Design of Experiment

The DOE approach was implemented in this part of the study to develop a method in compliance with AQbD principles. The CMP that has been identified from the previous step were defined to be included in the DOE. Several experimental designs are available within the DryLab Software to be used for method development depending on the type/number of variables that are desired to be studied. In this study a three variable (3D) experimental design was selected. Gradient time, ternary modifier composition(methanol), and temperature were

all selected as variables to be scouted. The total number of experiments included in this DOE was 12 and this was performed for two stationary phases (HSS T3 and BEH C_{18}) on each chromatographic system. When the DOE was selected, it was automatically exported to Empower creating all the methods and method sets that are needed for these runs. It also created and exported all the necessary conditioning/equilibration methods and method sets. All method parameters that were studied in this part are listed in Table 1.

	Settings		
Input parameter		Standard ACQUITY ARC	ARC PREMIER
Column type	Variable	▪ Standard BEH C_{18} 2.5 µm × 4.6 × 100 mm (pH range: 1–12) ▪ Standard HSS T3 2.5 µm × 4.6 × 100 mm (pH range: 2–8)	▪ Max Peak™ HPS BEH C_{18} 2.5 µm × 4.6 × 100 mm (pH range: 1–12) ▪ Max Peak™ HPS HSS T3 2.5 µm × 4.6 × 100 mm (pH range: 2–8)
Aqueous mobile phase and the pH	Constant	10 mM ammonium formate (no pH adjustment)	10 mM ammonium formate (no pH adjustment)
Column temperature	Variable	30–60 °C	30–60 °C
Gradient time	Variable	5–15 minutes	5–15 minutes
Flow rate	Constant	0.5 mL min^{-1}	0.5 mL min^{-1}
Injection volume	Constant	3 µL	3 µL

Table 1. Summary of range of chromatographic conditions that were studied in the scouting experiments.

Peak Tracking

After all experiments were run on the two systems, data were processed in Empower before they were imported to DryLab Software. Processing in Empower involved integrating only all the peaks of interest in the resulting chromatograms and calculating some relevant chromatographic parameters such as resolution, USP tailing and symmetry for these peaks. For each DOE, the peaks were then automatically tracked over the 12 different chromatograms. This was performed four times for the two systems as detailed in Table 1. DryLab Software tracks peaks across the different chromatograms based on their areas. While this feature is reasonably accurate in tracking the peaks, sometimes manual intervention for assigning the peaks is necessary. For example, in the case of coelution the software allows for manual "splitting" of coeluting peaks and reordering/turning peak positions which makes peak tracking more accurate. Another important feature in the software that enables even more accurate tracking by automatically importing the Apex *m/z* (ACQUITY QDa Mass Detector) values for all the peaks which helps confirming that the peaks are accurately tracked.

Results of this step clearly indicates that the Arc Premier System in combination with the HPS MaxPeak Columns shows superior performance compared to the standard ACQUITY Arc System/stainless-steel columns. For example, as can be seen in Figure 3, the initial scouting conditions show extreme performance differences for peak shapes and areas with MaxPeak HPS versus stainless-steel. It should be noted here that HSS T3 MaxPeak Premier Column also showed superior performance on the Arc Premier System compared to the stainless-steel HSS T3 Column on the standard ACQUITY Arc System. As such, the decision was made that further steps of the method development process will be performed only on the Arc Premier System using the MaxPeak Premier Columns.

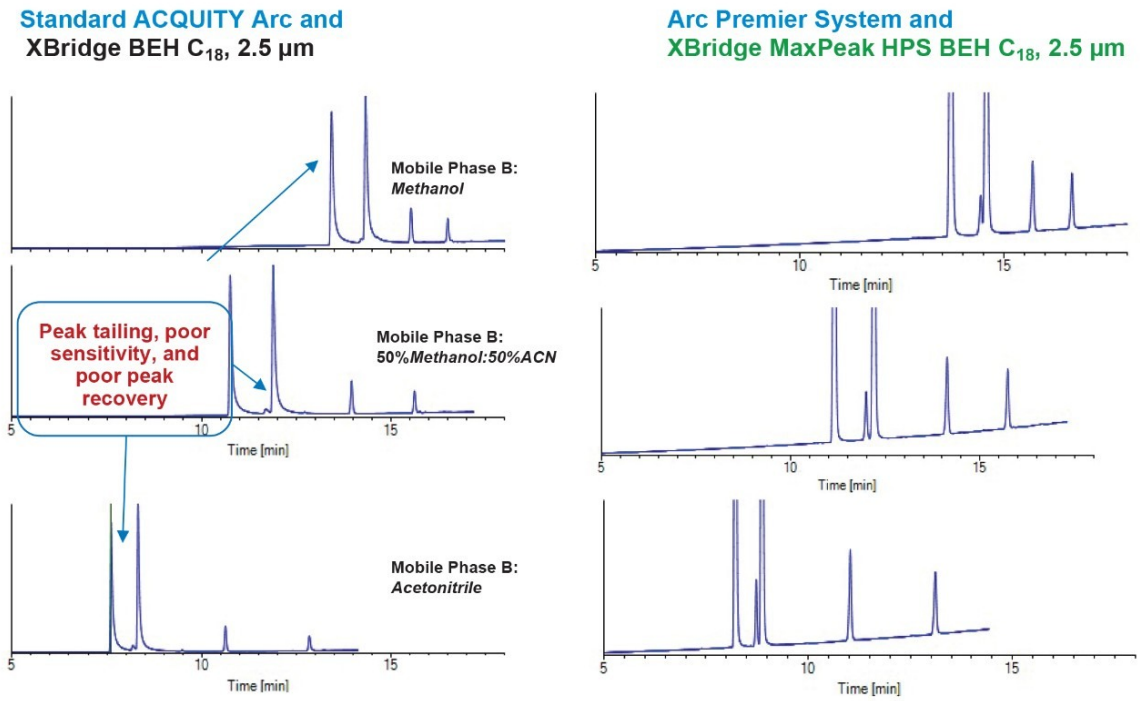


Figure 3. Representative chromatograms from the 12 DOE runs. A: represents three experiments that were performed on a Standard ACQUITY Arc System under different scouting conditions and B represents three experiments that were performed on the Arc Premier System under the same conditions. Conditions in common between all chromatograms are: Mobile phase A: 10 mM Ammonium formate in water, flow rate 0.50 mL/min, temperature 30 °C, 0.0–15 min, and 10–90% B linear gradient. Variations in conditions are detailed in the figure.

Design Space Modelling

Next, when the data was processed and all the peaks were correctly tracked, the software automatically builds the models to create a resolution map that shows the combinations of conditions where the desired resolution between the peaks is achieved. Since the initial scouting conditions showed that good separations can be

achieved on both MaxPeak HPS Columns (the HSS T3 and the BEH C_{18}), two resolution maps were created and compared for these columns on the Arc Premier System. Figure 4 shows a comparison between the resolution maps that were obtained based on the 12-run experiment for these two columns. As can be seen in Figure 4, using the original linear gradient profile of 10→90 %B (0.1% formic acid in acetonitrile) and a tG=15 min offers good separation of all peaks on both columns (critical pair resolution, $R_{s,crit,.}$=2.1). It also shows a very wide range of experimental conditions (red area around the current method conditions) were a minimum baseline resolution for the critical pair can be achieved. Although both MaxPeak Columns showed very comparable results, the MaxPeak Premier BEH C_{18} Column showed slightly better peak shapes and critical pair resolution. For example, the USP tailing for all the analytes on the MaxPeak HPS BEH C_{18} Column was less than 1.2 compared to less than 1.4 on the HSS T3 MaxPeak Column.

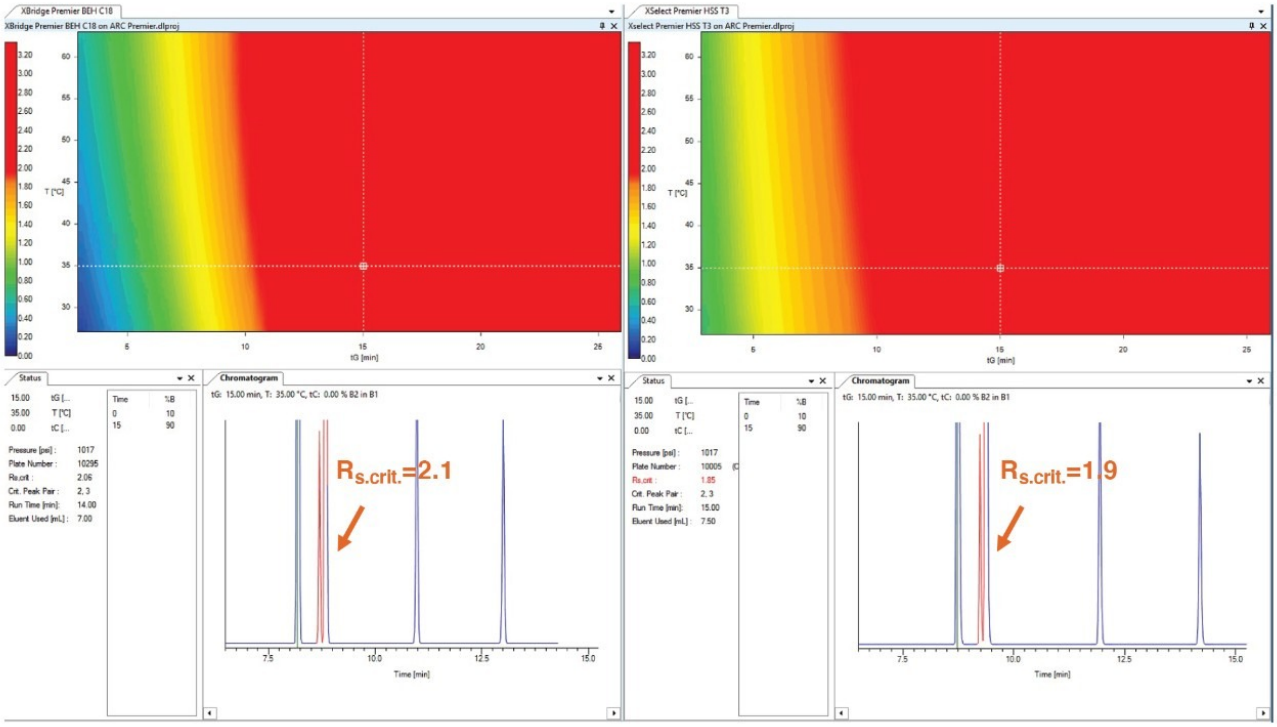


Figure 4. DryLab screen images represent separation models for the two Premier Columns on the Arc Premier System. On the axis of the colored resolution maps the experimental parameters are plotted (x-axis: Temperature (T) and y axis is the Gradient Time (tG). The color code describes the critical resolution of the chromatogram obtained at a certain set of tG and T conditions. The cursor in the resolution maps points to the condition of the "Working Point".

Robustness Assessment and Control Strategy

In this part of the study the robustness of the final method that was obtained from the models in the previous

experiment (Figure 5) was assessed. This assessment considers the instrument tolerance limits at the selected Working Point and the failures that can occur because of fluctuations in method variables and parameters. Figure 5A shows the robustness assessment for the final method on the Premier BEH C_{18} Column. As can be seen, the assessment shows that 100% of the times the method would provide a minimum resolution of 2.1 between all the five peaks.

A control strategy was also proposed based on the outcomes of the robustness and risk assessments to determine controls that need to be put in place to obtain consistent performance. The robustness assessment showed the range of resolution values that can be expected during routine use and all the method parameters that have the highest influence on separations. For example, in this study it was found that the two most critical parameters that affect the resolution are the flow rate and the gradient time (Figure 5B). This contributes to a more efficient method control strategy by identifying the critical separation parameters. These parameters can be setup and easily controlled using the instrument method. Another critical factor that significantly affects the performance of the method is the interaction of metal chelating compounds with stainless-steel surfaces. As such, using surfaces that eliminates or reduces such interactions will greatly minimize this risk.

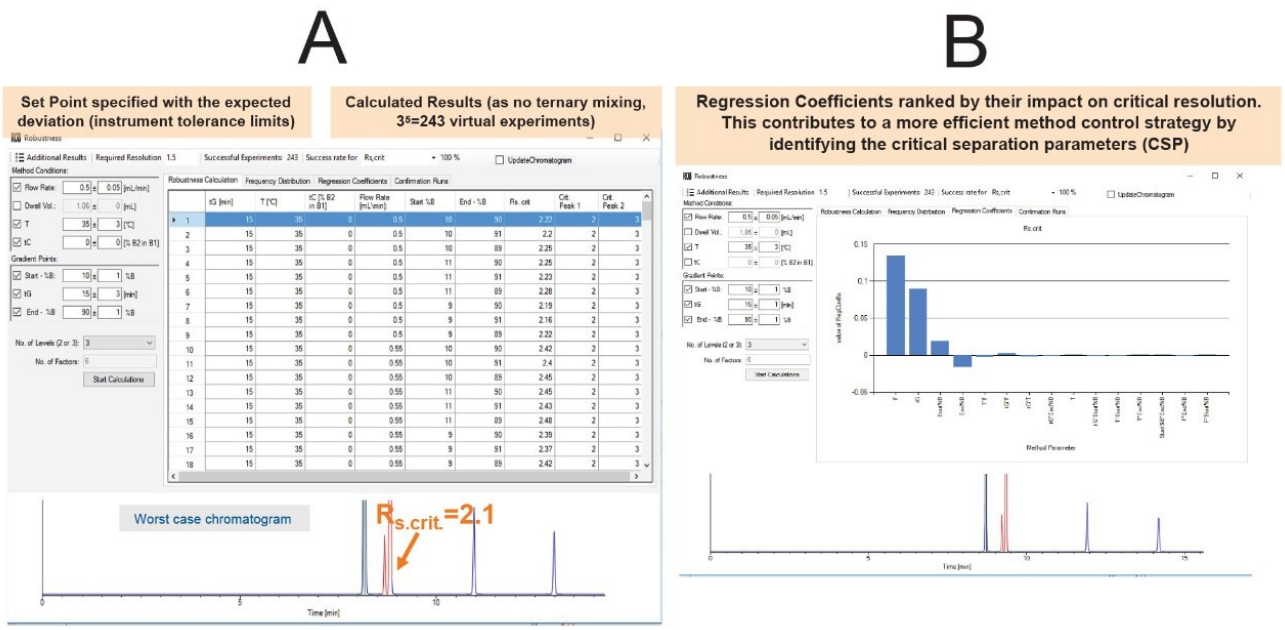


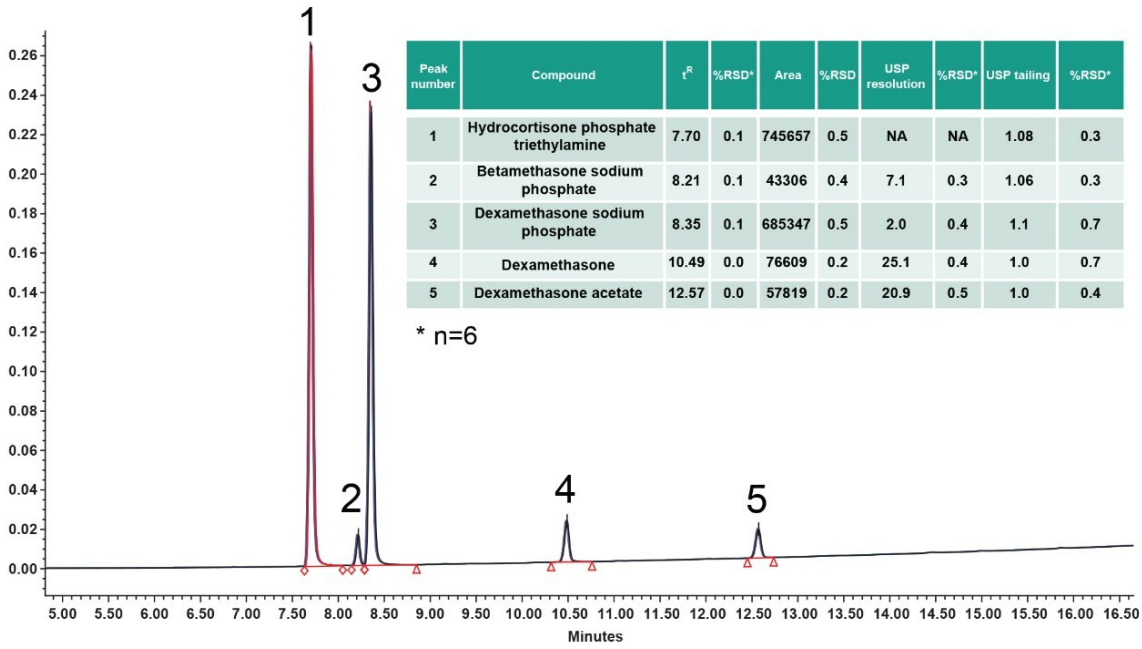
Figure 5. Screen images of Drylab robustness assessment module. A represents the calculated results of 243 virtual experiments with the expected deviations based on the instrument tolerances and B represents Regression Coefficients ranked by their impact on critical resolution.

Verification of DryLab Modeling

In order to verify the results as predicted based on the Robustness Assessment, it was important to compare

these results with actual runs of the analytes. To do this, multiple verification experiments were run under the final conditions that were obtained from the Robustness Assessment. These experiments showed that the predicted performance agreed reasonably well with the observed performance. For example, the prediction that the Working Point conditions would result in a critical pair resolution of 2.1 was verified in practice as shown in Figure 6. Additionally, it was found the developed method was very reproducible with %RSD values for retention times, peak areas, and resolution of ≤0.5% for six replicate injections. It was also observed that the USP tailing for all the peaks was ≤1.1 indicating that the Arc Premier System in combination with the MaxPeak Premier BEH C_{18} Column provides great peak shapes for phosphorylated compounds.

Final Method Developed on XBridge Premier BEH C18, 2.5 µm with Arc Premier



Peak number	Compound	t^R	%RSD*	Area	%RSD	USP resolution	%RSD*	USP tailing	%RSD*
1	Hydrocortisone phosphate triethylamine	7.70	0.1	745657	0.5	NA	NA	1.08	0.3
2	Betamethasone sodium phosphate	8.21	0.1	43306	0.4	7.1	0.3	1.06	0.3
3	Dexamethasone sodium phosphate	8.35	0.1	685347	0.5	2.0	0.4	1.1	0.7
4	Dexamethasone	10.49	0.0	76609	0.2	25.1	0.4	1.0	0.7
5	Dexamethasone acetate	12.57	0.0	57819	0.2	20.9	0.5	1.0	0.4

* n=6

Figure 6. Six replicate injections of the final method under the "Working Point" conditions of 35 °C and 0.50 ml min^{-1} flow rate. The gradient profile was a linear gradient of Acetonitrile from 10–90% over 15 minutes. Solvent is mobile phase initial conditions. Column: 10 cm x 4.6 mm XSelect Premier BEH C_{18}, 2.5 µm on an Arc Premier System.

Conclusion

- The analysis of phosphorylated compounds using an Arc Premier System in combination with MaxPeak

Premier Columns provides superior chromatographic performance when compared to stainless-steel hardware.

- The use of DryLab in conjunction with Empower and Waters systems is very beneficial for automating the whole method development process.
- Efficient method development process that needed only 12 experimental runs.
- Employing the AQbD principles in analytical method development helps obtaining robust and reproducible method.

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