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Nota de aplicación

Analytical Quality by Design Based Method Development for the Analysis of Valsartan and Nitrosamines Impurities Using UPLC-MS

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Abstract

An UltraPerformance Liquid Chromatography method is developed for the analysis of Valsartan and a mixture of six genotoxic impurities using the Analytical Quality by Design (AQbD) approach. DryLab, Empower, and Waters systems were used to automate the method development process. The final method used an HSS T3 Column (10 cm × 2.1 × 1.7 µm), Methanol as an organic solvent, and 0.1% formic acid in water aqueous mobile phase. The developed method showed excellent robustness and reproducibility. For example, the retention time percentage relative standard deviation (%RSD) of peaks from a standard mixture was 0.9% over a three day analysis. These findings indicate that using the AQbD approach and automated software helps gaining a deep knowledge about the method and as a result developing a highly robust and reproducible method.

Benefits

- Show the straightforward method development capabilities of the ACQUITY UPLC H-Class 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
- Develop a high performance and robust method for the analysis of Valsartan and a mixture of six genotoxic impurities

Introduction

Genotoxic impurities are defined by the ICH S2 (R1) guideline as substances that have been demonstrated to cause deleterious changes to the genetic material regardless of the mechanism.[1] The primary concern related to such impurities that they have the potential to interact with human cells to cause mutations and cancer, even at the lowest levels. As such, genotoxic impurities should be avoided all at once and if not possible, reduced below a defined level. The advent of these impurities in various angiotensin receptor blocker drugs (ARB) such as valsartan, losartan, and irbesartan has caused broad recalls of these drugs from the markets and made this issue a focus for regulatory agencies including the FDA and the European Medicines Agency (EMA). In order to comply with regulations, it is critical that analytical methods that are used for the analysis of these compounds are accurate, robust, and sensitive. One way to develop robust and

accurate methods is employing the Analytical Quality by Design principles (AQbD) in the method development process. The AQbD is a systematic approach for method development that starts with predefined objectives and provides rational understanding of the effects of chromatographic factors on the method performance.[2] In this approach multiple variables are screened to provide a broad knowledge about the impact of the studied factors on the method performance. This knowledge is used to establish the method operable design region (MODR) which corresponds to the multi-dimensional combination of factors that have been verified to meet the method performance criteria. The outcome of this approach is a fit for purpose, well designed, understood, and robust method that delivers the expected performance throughout its lifecycle.[3,4]

In this application note, a software assisted AQbD approach was implemented to develop a method for the analysis of valsartan standard and six genotoxic impurities (NDMA, NMBA, NDEA, NEIPA, NDIPA, and NDBA).

The experiments were performed using an ACQUITY UPLC H-Class 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 as an AQbD software in this study. Details about the use of the AQbD principles for method development on an ACQUITY UPLC H-Class PLUS System has previously been described.[5]

Experimental

Materials and Standard Preparations

Valsartan and six nitrosamines (genotoxic impurities) were all purchased from Toronto Research Chemicals (North York, Ontario, Canada). Stock solutions of these compounds were prepared by accurately weighing the desired amounts of each standard and dissolving them in methanol as a solvent. The stock solutions were then used to make a test mixture that contains valsartan and all the impurities. This mixture was prepared by diluting the stock solutions of each one of the standards in 80/20 (v/v) water/methanol as sample solvent. The final concentration of each analyte in the test mixture were approximately: 0.1 mg mL^{-1} valsartan and 0.07 mg mL^{-1} for each impurity. The specifics of these compounds such as names and molecular masses are shown in Table 1 below.

Analyte	Common name	Monoisotopic mass (Da)
NDMA	N-nitrosodiethylamine	75.05
NMBA	N-nitro-N-methyl-4-aminobutyric acid	146.07
NDEA	N-nitrosodiethylamine	102.08
NEIPA	N-nitrosoethylisopropylamine	116.09
NDIPA	N-nitrosodiisoropylamine	130.11
NDBA	N-nitrosodibutylamine	158.14
API	Valsartan	435.22

Table 1. Genotoxic impurities and the active pharmaceutical ingredient (API) used in this study.

LC Conditions

LC system:	ACQUITY UPLC H-Class System with Quaternary Solvent Manager (QSM), Sample Manager (FTN), Column Manager, two CM Aux, PDA Detector, QDa Mass Detector
Detection:	PDA and QDa
Column(s):	HSS T3 Columns, 2.1 × 100 mm, 1.8 µm pH range: 1–8
Column temp.:	30–60 °C
Sample temp.:	10 °C
Injection volume:	3 µL
Flow rate:	0.4
Mobile phase A:	0.1% (v/v) Formic acid in water
Mobile phase B:	Acetonitrile and methanol

Gradient:	2 to 98% 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:	245 nm

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 DryLab4
MS software:	Empower 3

Results and Discussion

The analytical quality by design as stated earlier is a systematic approach to method development that starts with predefined objectives and is based on sound science. Employing the AQbD principles in analytical

method development 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”. [6] 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 1. Details about each of these steps are described next.

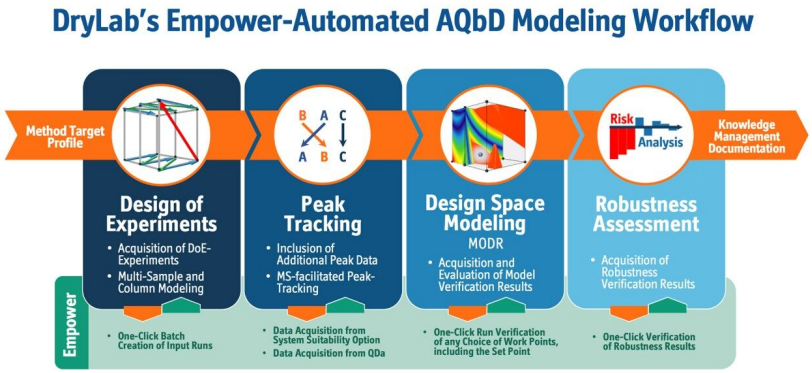

Figure 1. A workflow shows the multiple steps that are involved in DryLab - Empower AQbD method development process.

Design of Experiment

Design of Experiment (DOE) is defined by the ICH as “a structured, organized method for determining the relationship between factors affecting a process and the output of that process”. DryLab uses the DOE approach to develop robust LC methods in compliance with AQbD principles. In this part of the AQbD method development process a linear gradient working method is selected and the variables that need to be optimized are defined. Several experimental designs are available within the 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. In this DOE gradient time, ternary modifier composition (methanol), and temperature were all selected as variables to be studied. The total number of experiments included in this study were 12 experiments detailed in Figure 2. When the DOE was selected, it was 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. This was automatically and

seamlessly done by a single click within DryLab software. This automation feature is only possible with Empower CDS and Waters systems. This feature is very beneficial as it significantly shortens the method development time by eliminating the time needed to manually generate methods and method sets for these experiments. It also eliminates any transcription error.

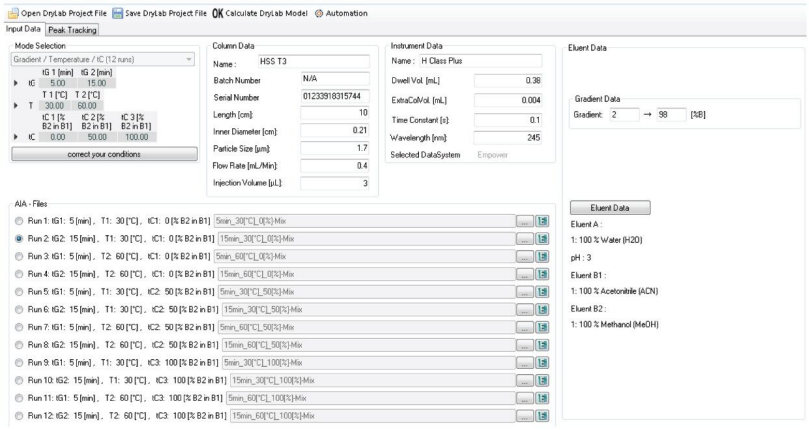

Figure 2. A screenshot from DryLab software shows the 3D DOE and all the variables that were studied in this work.

Peak Tracking

After all the experiments were run, 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, tailing and symmetry for these peaks. The peaks were then automatically tracked over the 12 different chromatograms. The peak tracking in DryLab software is mostly based on the area of the peaks. 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 making the molecular masses of the analytes available. The software also automatically imports the Apex *m/z* (ACQUITY QDa Mass Detector) values for all the peaks which helps confirming that the peaks are accurately tracked.

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. It should be noted here that in addition to the seven peaks correspond to the analytes present in the mixture, it was observed that there are additional three peaks of unknown impurities. As such, two resolution maps were created, one map for the ten peaks and the other for the only the seven peaks. Figure 3 shows the resolution maps that were obtained based on the 12-run experiment. As can be seen in Figure 3A, using the original linear gradient profile of 2–98 %B (100% methanol) and a t_G=25 min offers good separation of all peaks (critical pair resolution, $R_{s,crit,}$=1.6). However, if only the seven peaks of interest were taken into consideration, a very wide range of experimental conditions can be found to achieve a baseline resolution for all the analytes as can be seen in Figure 3B. Moreover, a minimum resolution of the critical pair of 17.6 was predicted to be achieved when only the seven analyte peaks are considered for modeling under the same conditions. Since the separation of valsartan and the six GTIs was the goal of this work, it was decided that further experiments will only focus on separation of these analytes.

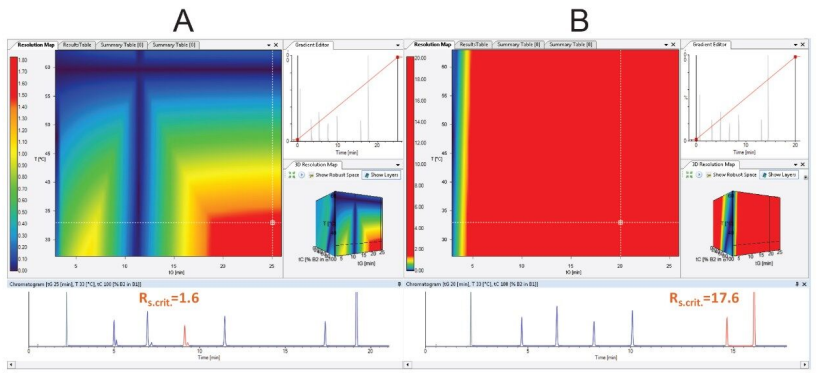

Figure 3. DryLab screenshots of resolution maps. A represents the MODR for the separation of ten peaks including three unknown impurities and B represents the MODR for only the seven peaks of interest. The red color represents the design space where the minimum resolution between all the peaks is achieved (1.5 in this case). The crosshair pointer location on the corresponding graphs represent the Working Point conditions.

Robustness Assessment

In this part of the method development process the robustness of the final method that was obtained from the models in the previous experiment (Figure 3B) was assessed. This assessment takes into account the instrument tolerance limits at the selected Working Point and the failures that can occur because of

fluctuations in method variables and parameters. Figure 4A shows the robustness assessment for the final method and the instrument tolerances for the different chromatographic parameters. As can be seen, the assessment shows that 100% of the times the method would provide a minimum resolution of 16 between all of the seven peaks.

Further, the robustness assessment also shows 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 work it was found, as can be seen in Figure 4B, that the two most critical parameters that affect the resolution are the flow rate and the gradient time. This contributes to a more efficient method control strategy by identifying the critical separation parameters.

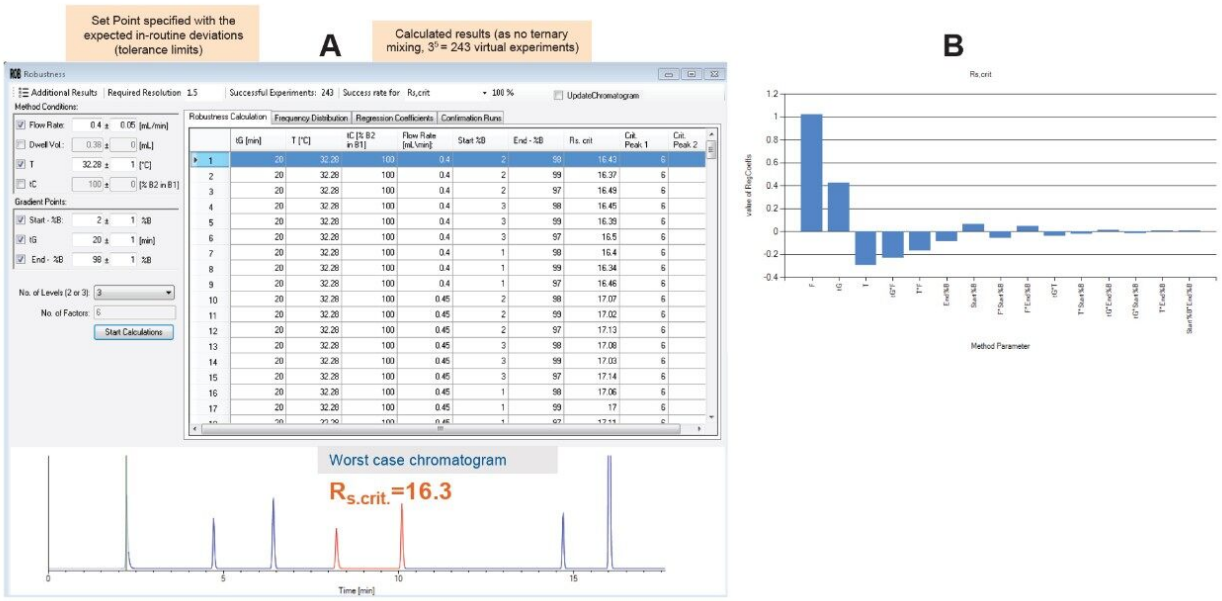

Figure 4. Screenshots of Drylab Robustness assessment module. A represents the calculated results of 243 virtual experiments with the expected deviations based on the instrument tolerances and B represents Regression Coefficients ranked by their impact on critical resolution.

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 well with the observed performance. For example, the prediction that the Working Point conditions would result in a critical pair resolution of 17.6 was verified in practice as shown in

Table 2. This experiment was repeated for 15 times over three days to test the reproducibility of the method. It was found the developed method was very reproducible with %RSD values for retention times, peak areas, and resolution of less than 1% for all the runs. Summary of all the these results over the three days are summarized in table 2. Figure 5 shows five replicate injections from day one of this experiment.

Analyte	Average tR	%RSD	Average area	%RSD	Rs	%RSD	Average tR	%RSD	Average area	%RSD	Rs	%RSD	Average tR	%RSD	Average area	%RSD	Rs	%RSD
NDMA	1.82	0.23	186390	0.87	NA	NA	1.83	0.22	181919	0.50	NA	NA	1.81	0.37	183171	0.62	NA	NA
NDBA	3.52	0.09	88159	0.08	17.8	0.1	3.56	0.82	88344	0.26	18.2	0.8	3.48	0.32	89054	0.31	19.0	0.1
NDEA	5.45	0.05	171360	0.14	26.4	0.1	5.49	0.73	170085	0.20	26.3	0.4	5.41	0.20	168783	0.22	26.2	0.2
NEIPA	7.53	0.07	106297	0.18	25.1	0.1	7.57	0.62	104908	0.20	24.9	0.5	7.50	0.13	103670	0.43	24.7	0.1
NDIPA	9.60	0.06	155434	0.29	23.3	0.1	9.63	0.49	153998	0.20	23.2	0.2	9.56	0.10	152567	0.16	23.1	0.2
NDBA	14.52	0.04	121057	0.82	57.0	0.0	14.60	0.24	119285	0.25	56.7	0.3	14.50	0.08	117344	0.26	56.3	0.2
Valspatan	15.95	0.02	961622	0.08	17.7	0.0	16.06	0.12	966832	0.04	17.8	0.8	15.94	0.13	974682	0.06	17.6	0.7

Table 2. Summary of peak areas, retention times, and resolution (Rs) for the seven analytes in the mixture when analyzed under the Working Point conditions detailed in Figure 5.

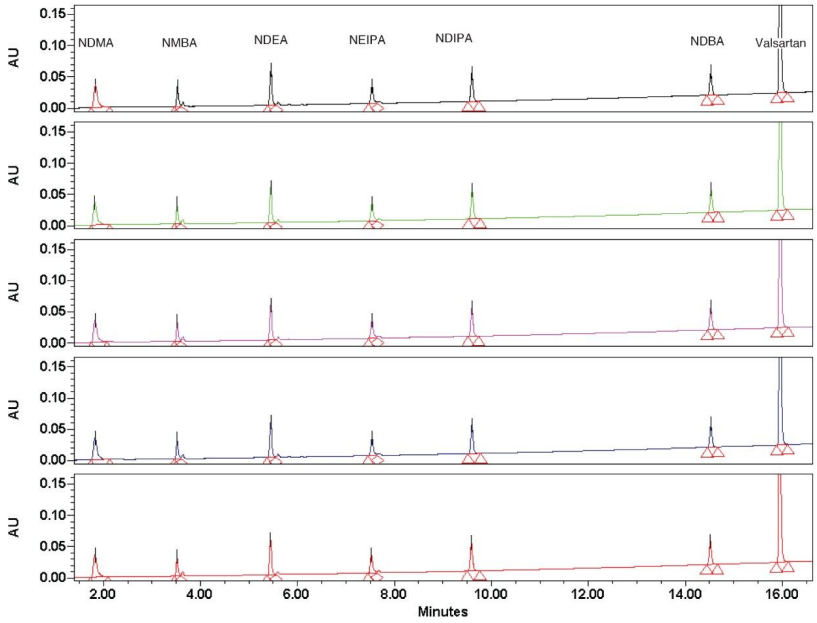

Figure 5. Five replicate injections of valsartan and the six GTIs under the Working Point conditions. These conditions are: Temperature = 33 °C, and Flow rate = 0.40 mL min^{-1}. The gradient profile was a linear gradient of Methanol from 2–98% over 25 minutes. Column; 100 mm × 2.1 mm HSS T3, 1.8 µm.

Conclusion

- Systematic AQbD Method Development have shown, that the separation between valsartan and NDBA is depending strongly on the gradient time
- The use of DryLab in conjunction with Empower and Waters systems is very beneficial for automating the whole method development process
- Employing the AQbD principles in analytical method development helps obtaining robust and reproducible method
- The use of the ACQUITY QDa Mass Detector is essential in method development as is facilitates tracking peaks across the different chromatograms

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720007033, December 2020