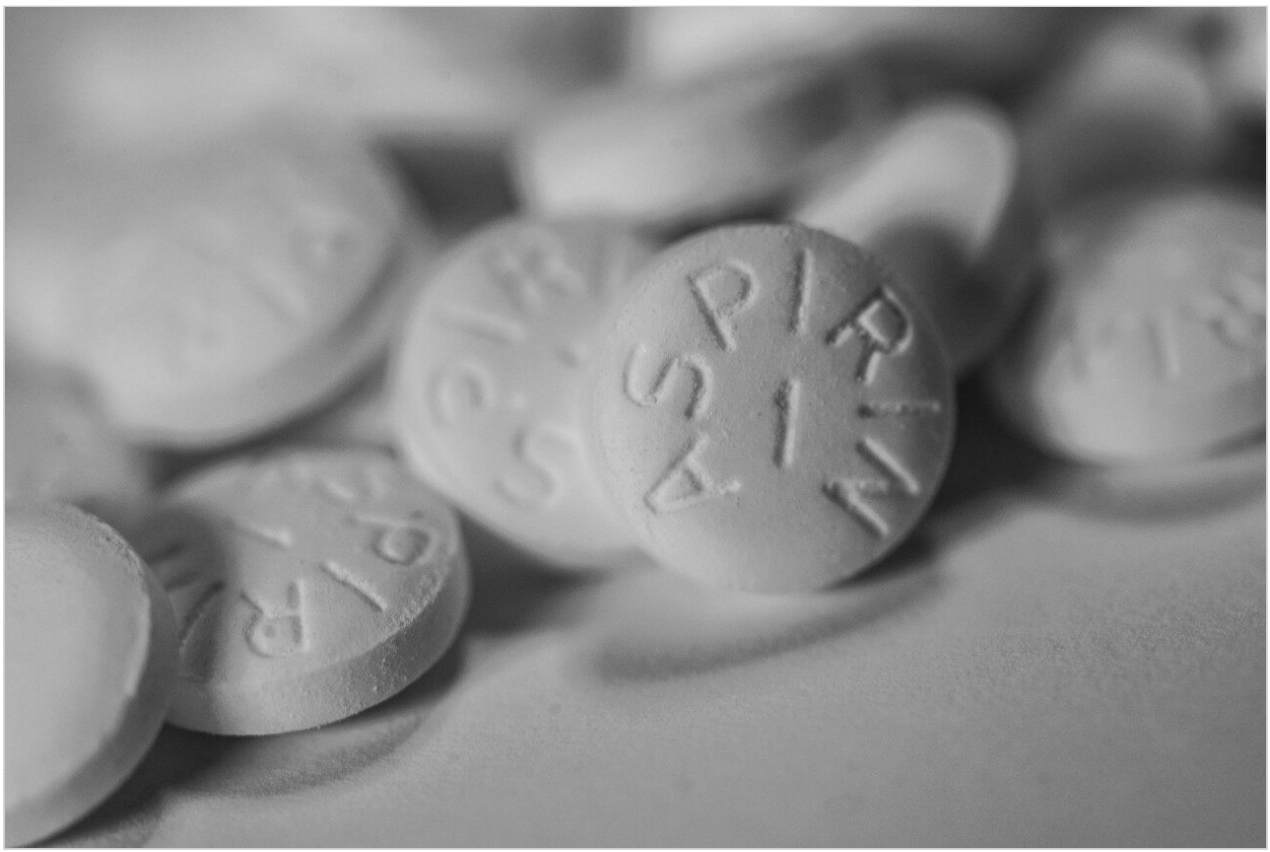
Waters™

Application Note

Development of a Robust Method for Analysis of Aspirin and Related Substances Using a Statistical Software and Quality-by-Design Approach

Margaret Maziarz, Paul D. Rainville, Tran Pham

Waters Corporation



Abstract

Aspirin is a class of medication available by prescription or over-the-counter (OTC) for the treatment of arthritis, fever, mild to moderate pain, and for prevention of cardiovascular events. In this study, a software-assisted development of an HPLC method for the analysis of aspirin active pharmaceutical ingredient (API) and its related substances is presented. The developed method separates all analytes of interests under MS compatible conditions, making it suitable for identification of known and unknown component by mass spectrometry (MS). The new method offers quick and reliable analysis of aspirin and related substances in tablet formulation.

Benefits

- Improve robustness of the developed methods using statistical software with experimental data modeling
- Increase efficiency of method development using the ACQUITY Arc System for columns and solvent screening in single experimental run
- Facilitate quick and accurate identification of sample components using mass spectral data from an ACQUITY QDa Detector in combination with UV data

Introduction

Analytical methods play a critical role during all stages of a drug product's life cycle. Since the data generated by the method is used to assess quality, efficacy, and safety of the drug products, it is essential that the analytical method is robust, precise, accurate, and reliable for its intended purpose.[1,2]

Development of methods is a complex process built around series steps to find conditions for robust and reproducible separation. The impact of different parameters (e.g. column chemistry, organic solvent, pH, gradient slope, flow rate, temperature) on the chromatographic separation is investigated during the process. Varying one-factor-at-the-time (OFAT) often results in a non-robust performance, while the quality-by-design (QbD) based approach enables fast and efficient development of robust methods. The concept of QbD was initially introduced for the pharmaceutical development of drug products but was further extended to the development of analytical procedures and is referred as Analytical Quality-by-Design (AQbD).[1-3] AQbD is a systematic based 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 a design space.[1] Applying AQbD-based approach with statistical tools for DoE and data modeling improves development of fit-for-purpose and highly robust methods. This increases the success of method validation and transfer.

Aspirin is a common drug for relieving minor aches, pains, and fevers. It is also used for prevention of heart attacks and mini strokes.[5] Aspirin is available in tablets for oral administration, with 81–650 mg of aspirin per tablet. So far, only one method is found in literate for the simultaneous separation of aspirin and its six related substances, which operates under non-MS compatible conditions.[6] The United States Pharmacopeia specifies only one impurity in the USP monograph for aspirin tablets.[7]

In this application note, we develop a MS compatible method for aspirin API and its related substances using a Fusion QbD (by S-Matrix Corporation, Eureka, CA, USA) method development software. Fusion QbD method development software is applied to perform DoE studies, statistical data analysis, and mathematical modeling for an optimum and robust method. Experiments are run on an ACQUITY Arc System integrated with PDA and ACQUITY QDa detectors, controlled by Empower Data Chromatography Software (CDS). The final method separates all analytes of interests using MS compatible mobile phase and is suitable for the analysis of aspirin tablet formulations.

Experimental

Sample Description

Mixture with Aspirin and Related Substances

Separate stock solutions with related substances and aspirin API were prepared in diluent (60:40 water/acetonitrile with 0.1% formic acid) at 1.0 and 5.0 mg/mL, respectively. The API stock solution was diluted with diluent to 0.1 mg/mL and spiked with related substances at 10% level. Aspirin and its related substances specified by the European Pharmacopeia[6] used in this study are listed in Table 1.

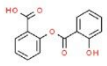
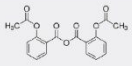
Compound	Name	Molecular formula	Monoisotopic mass (Da)	Structure
Aspirin API	2-Acetoxybenzoic acid, O-Acetylsalicylic acid	$C_9H_8O_4$	180.04	
Impurity A	p-Salicylic acid, 4-hydroxybenzoic acid	$C_7H_6O_3$	138.03	
Impurity B	4-Hydroxy-1,3-benzenedicarboxylic acid, 4-Hydroxyisophthalic acid	$C_8H_6O_5$	182.02	
Impurity C	Salicylic acid, 2-Hydroxybenzoic acid, o-Hydroxybenzoic Acid	$C_7H_6O_3$	138.03	
Impurity D	Acetylsalicylsalicylic acid, 2-(Acetyloxy)benzoic acid	$C_{16}H_{12}O_6$	300.06	
Impurity E	2-((2-hydroxybenzoyl)oxy) benzoic acid, salsalate	$C_{14}H_{10}O_5$	258.05	
Impurity F	2-Acetoxybenzoic anhydride, O-acetylsalicylic anhydride,	$C_{18}H_{14}O_7$	342.07	

Table 1. List of compounds for method development.

Aspirin Drug Tablets

Crushed tablets were dissolved in diluent (60:40 water/acetonitrile with 0.1% formic acid) at 1.6 mg/mL of aspirin by sonication for 10 minutes. After extraction, sample test solutions were centrifuged for 10 minutes at 3000 rpm and diluted to 0.1 mg/mL with diluent. Solutions were filtered through 0.2 µm Nylon syringe (Waters P/N WAT200524 <https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/wat200524-acrodisc-syringe-filter-nylon-13-mm-02--m-aqueous-100-pk.html>) filter prior analysis.

Conditions

LC system:	ACQUITY Arc System, column heater/cooler with passive pre-heater
Detection:	PDA and ACQUITY QDa
Vials:	LCMS Maximum Recovery 2 mL volume, P/N 600000670CV

Method Development Conditions

Column(s):	All columns 4.6 x 100 mm, 2.5 µm XSelect HSS T3 XSelect BEH C_{18} XSelect CSH C_{18} XSelect HSS PFP
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	10–25 µL
Flow rate:	1–1.5 mL/min
Mobile phase:	A: acetonitrile B: methanol C: 100 mM formic acid in water D: 100 mM ammonium hydroxide in water
Gradient:	5–95% organic solvent
Wash solvents:	Purge/Sample wash: 60:40 water/acetonitrile Seal wash: 90:10 water/acetonitrile
Detector settings:	PDA: 210–400 nm (derived at 237 nm)

Final Method Conditions

Column(s):	XSelect HSS T3, 4.6 x 100 mm, 2.5 µm
Column temp.:	40 °C

Sample temp.:	10 °C
Injection volume:	10 µL
Mobile phase:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Wash solvents:	Purge/sample wash: 60:40 water/acetonitrile Seal wash: 90:10 water/acetonitrile
Detector settings:	PDA: 210–400 nm (derived at 237 nm)

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	1.3	95.0	5.0	6
0.10	1.3	95.0	5.0	6
7.00	1.3	5.0	95.0	6
8.50	1.3	5.0	95.0	6
8.60	1.3	95.0	5.0	6
12.00	1.3	95.0	5.0	6

MS Conditions

MS detector:	ACQUITY QDa (extended performance)
Ionization mode:	ESI-
Acquisition range:	50–450 *m/z*
Capillary voltage:	-0.6 kV

Cone voltage:	2 V
Data:	Centroid

Software

Chromatography data system (CDS):	Empower 3 FR4 SR2
Method development software:	Fusion QbD Software from S-Matrix Corporation, Version 9.9.0.650 SR2b

Results and Discussion

The method development approach used in this study begins with defining the method performance goals and conducting risk assessment of critical method parameters, followed by the screening and optimization DoE studies (Figure 1).

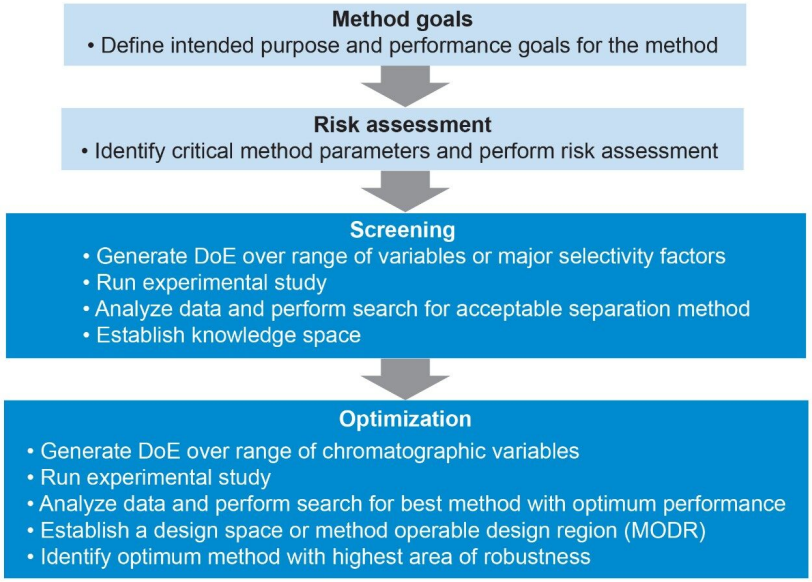


Figure 1. Method development approach for the study.

Method Goals

Method goals are set of analytical objectives that describes the intended purpose of the method, what will be measured, and the performance criteria for the measurements. For the aspirin and related substances, the method performance goals included:

- Method should separate aspirin active pharmaceutical ingredient (API) and the related substances with a USP resolution ≥2.5 between all the analytes.
- Method must operate under MS-compatible conditions for identification by mass spectrometry.
- Method must meet the system suitability criteria:
  - USP peak tailing ≤1.2
  - % RSD of peak areas ≤2.0
  - % RSD of retention times ≤1.0
- Method should meet the assay acceptance criteria of 90.0–110.0% specified in the USP monograph for Aspirin Tablets[7] and precision of less than 5% RSD for replicate preparations..

Risk Assessment

In the risk assessment, critical method parameters (CMP) are identified and assessed for the highest impact on the quality of the data generated by the method.[1] The high-risk parameters that may affect the method's ability to meet the goals are assessed based on the sound chromatographic science, previous knowledge, and experience.

For aspirin and related substances, selection of method parameters for risk assessment was based on the analyte's information found in literature and scientific experience. In this case, column chemistry, mobile phase pH, buffer, and solvent type were identified as critical method parameters to have the greatest impact on the selectivity, retentivity, and peak shape (Figure 2). Therefore, they were studied in the screening phase of the method development. Other chromatographic parameters including flow rate, gradient time, slope and injection volume can affect the resolution between analytes. These parameters can be easily controlled, therefore were not considered critical. For the sample preparation, choice of solvent and extraction procedure for the drug substance and product were considered to have significant impact on the accuracy and precision of the measurements generated by the analytical procedure and stability of the sample solutions.

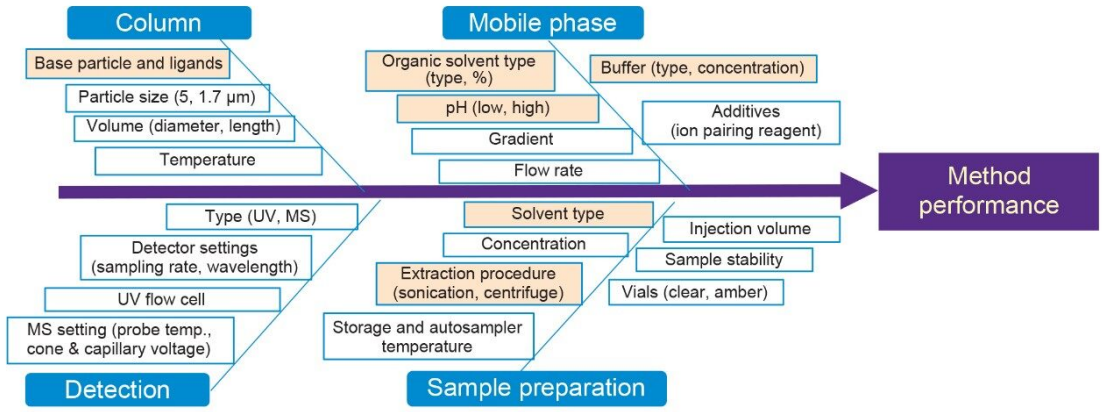


Figure 2. Fishbone diagram for risk assessment of method parameters. Critical parameters are indicated in yellow.

Design of Experiment (DoE)

Using Fusion QbD Software, separate DoE studies were created for screening and optimization phases. Fusion QbD method development software used partial factorial to uniformly select random points from the entire experimental space to create the most efficient experimental design with fewer runs and less time compared to full factorial.[8-10] The DoE at each phase was exported to the Empower Software, which automatically generated a sample set method (injection sequence) with instrument methods, equilibration and column conditioning steps for the entire DoE run. After completing the runs, the processed data was imported to Fusion QbD for statistical analysis and numerical search for best conditions.

Screening DoE

The goal of screening was to identify the best conditions for an acceptable separation of aspirin API and its associated related substances. The parameters with greatest impact on the selectivity were screened including column chemistry, organic solvent, pH, and gradient time. Columns with different base particles and ligands were selected to reflect wide selectivity range. Using a column manager, all columns were screened with acetonitrile and methanol in one chromatographic run, without the need to manually switch columns. Due to the acidic nature of the analytes, low pH mobile phases were selected by blending 0.1% formic acid and with 0.1% ammonium hydroxide. Variables and their ranges for DoE screening study with Fusion QbD Software included:

- Columns: CSH C_{18}, BEH C_{18}, HSS T3, HSS PFP
- Solvents: acetonitrile and methanol

- Mobile phase pH: 2.74, 3.40, 4.97
- Gradient time: 7–13 minutes with 5–95% organic

Method parameters that were kept constant included flow rate at 1 mL/min, injection volume at 10 µL and column temperature at 40°C. Empower results and chromatographic trace data were imported to Fusion QbD Software for trend responses generation and numerical search for the best method based on the user defined separation goals.

The response goal settings for the method entered in the Fusion QbD Software included:

- Number of peaks with USP resolution ≥2.5: maximize between 5 and 6
- Number of peaks with USP tailing ≤1.2: maximize between 6 and 7
- First peak - retentivity (k*): maximize between 2.0 and 3.0
- Last peak - retention time: minimize between 6 and 8

The numerical search determined that the best separation can be achieved with acetonitrile, 8.3 minutes gradient 0.1% formic acid in water (pH 2.74), and HSS T3 Column. The chromatographic separation run under these conditions in Empower resulted in a baseline separation between all the peaks (Figure 3).

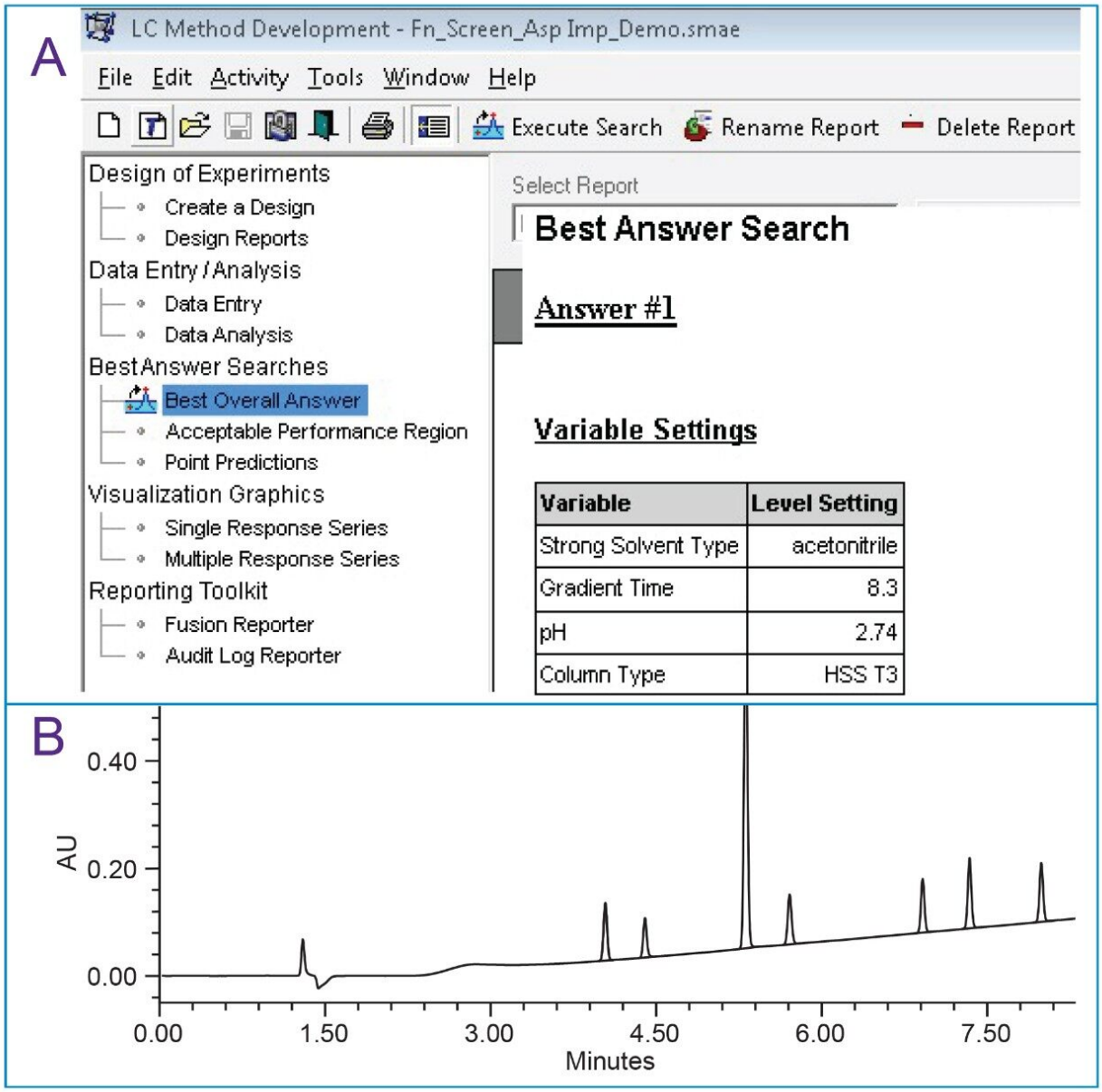


Figure 3. Result from the screening. Numerical search for best method conditions from the Fusion QbD Software (A) and chromatographic separation in Empower at 237 nm (B).

Optimization DoE

While the method from the screening step provided acceptable chromatographic separation, the conditions were further optimized to reduce run time and increase the injection volume to obtain higher sensitivity for impurities, while meeting the separation goals settings. In optimization, the impact of gradient time, injection volume, flow rate, and column temperature were investigated. Ranges for DoE optimization with Fusion QbD

Software included:

- Gradient time: 6–8 minutes
- Injection volume: 10–25 µL
- Flow rate: 1.0–1.5 mL/min
- Column temperature: 40–44 °C

After importing results from Empower to Fusion, the optimum method was identified to be with a flow rate of 1.3 mL/min, 7 minutes, injection volume of 10 µL, and column temperature at 40 °C. These conditions represented the center point (T) inside the box of the acceptable performance region (Figure 4). The prediction points (A–D) around the rectangle represented the confidence limits at which method met the goals for a USP resolution, peak tailing, k*, and retention time of last peak. The area within the rectangle, referred as design a space or method operable design region (MORD), represented a region of acceptable and robust method performance. Overall, the unshaded area indicated conditions under which the method met all the separation goal settings, while shaded area represented an unacceptable performance region.

The conditions of the prediction points (A–D) from Figure 4 were exported from Fusion to Empower to experimentally verify that the method remains unaffected by the changes of the flow rate and gradient time. The chromatographic data showed acceptable separation under the set of tested conditions (Figure 5). Furthermore, the response surface plots were evaluated visually to examine the combined effects of the method variables on the responses such as USP resolution and peak tailing. For example, the impact of gradient time and flow rate demonstrated that the best resolution was achieved with gradient time of 7 minutes (Figure 6A) and least peak tailing at about 1.3 mL/min flow rate (Figure 6B). These were indicated by the red color and shallow slope.

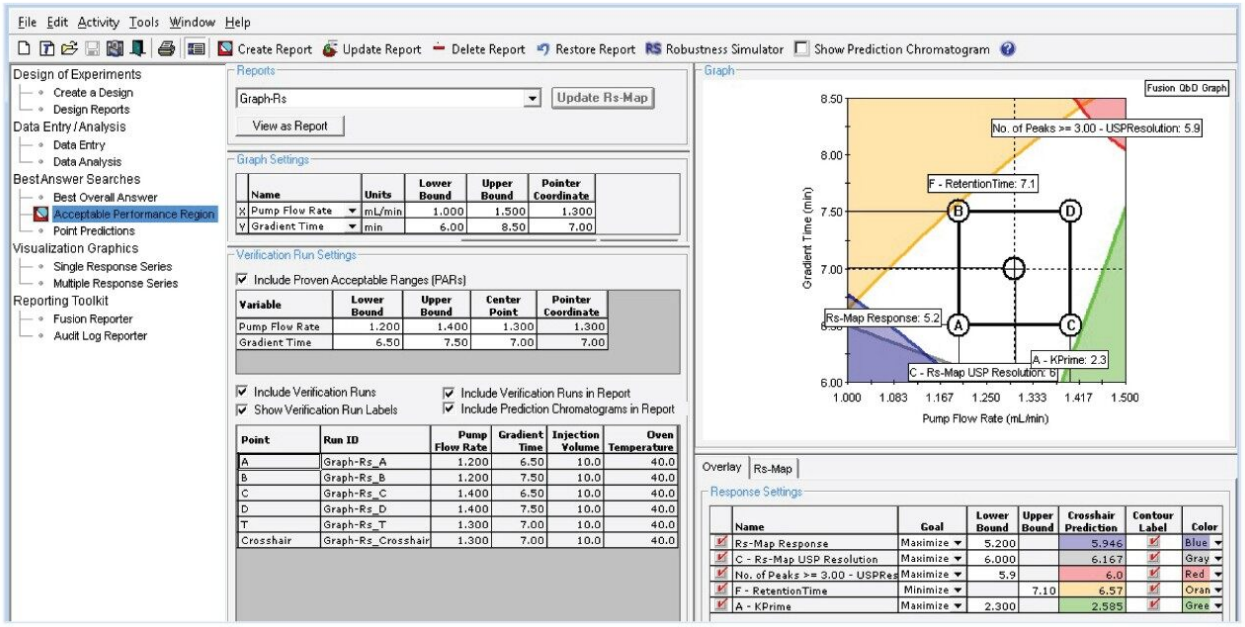


Figure 4. Acceptable performance region of the method determined by the Fusion QbD Software. The proven acceptable range (PAR) rectangle, or design space, with a robust area of acceptable method performance.

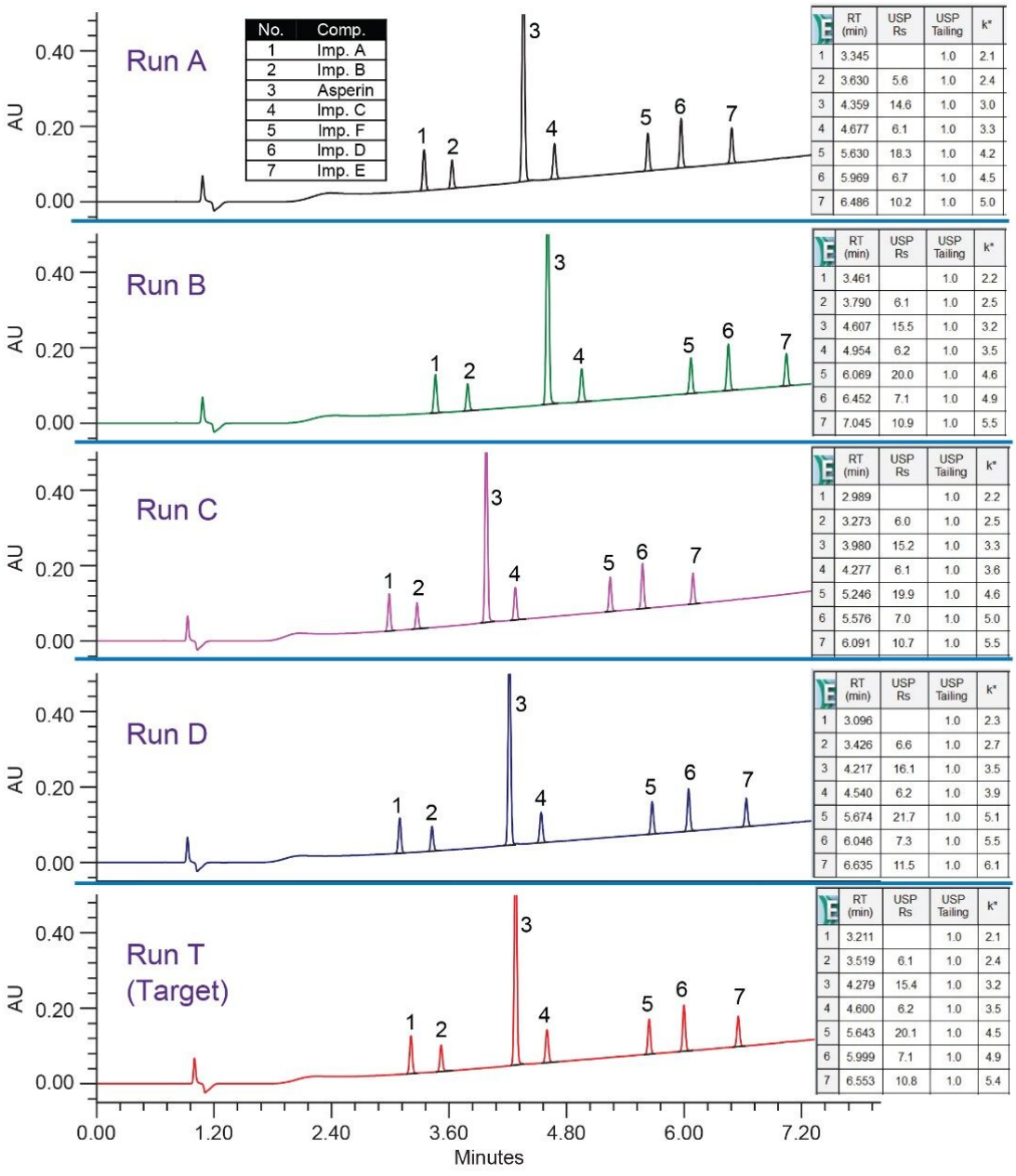


Figure 5. Verification of the prediction points (A–D) around the design space and final method (run T) in the center.

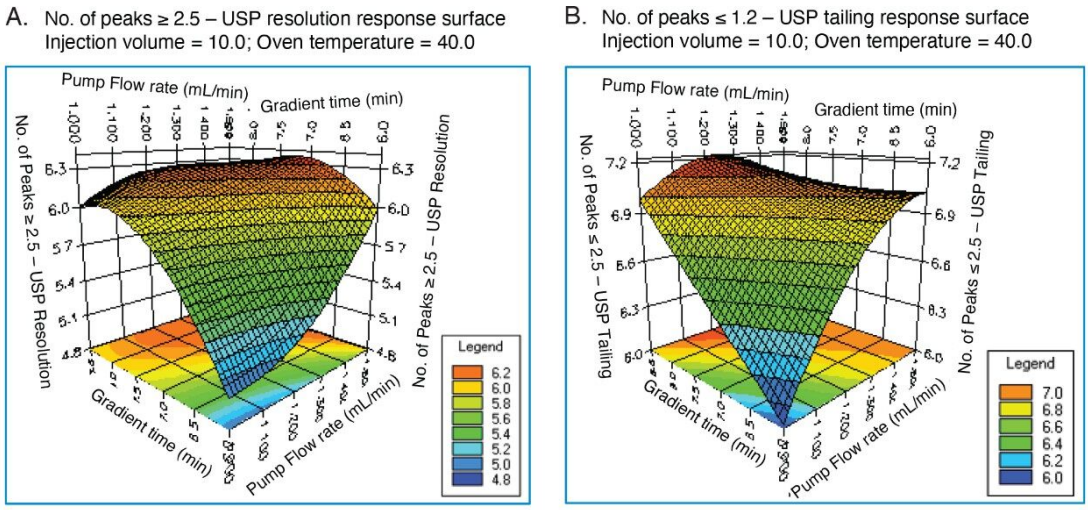
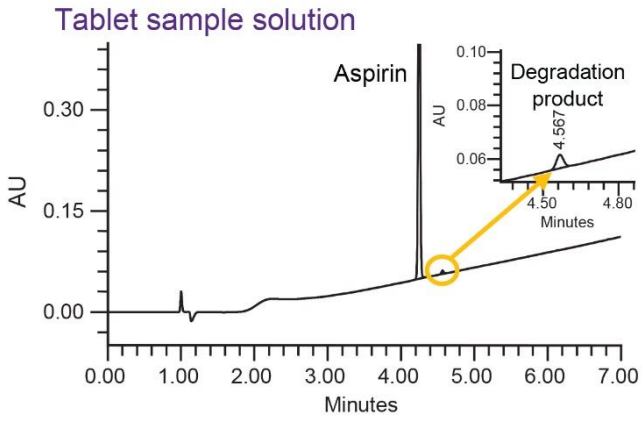


Figure 6. Response surface graphs from Fusion QbD Software showing the interaction of variables on USP resolution (A) and USP peak tailing.

Optimization of the Sample Diluent

Suitability of various diluents was evaluated during the method development to ensure aspirin solubility and stability in the solution. It was observed that aspirin was degrading in the diluent containing 80:20 water/methanol. According to the previously published studies, aspirin was found to undergo hydrolysis under aqueous conditions to salicyclic acid (impurity C).[11] In this case, a study was conducted to investigate the degradation of aspirin in diluents containing different ratios of water to organic solvents. For the study, aspirin tablets were dissolved in 80:20, 60:40, and 50:50 water to acetonitrile and methanol solvents containing 0.1% formic acid. Data showed that diluents with water/methanol resulted in greatest aspirin degradation compared to water/acetonitrile (Figure 7). Therefore, diluent with 60:40 water/acetonitrile with 0.1% formic acid was used to prepare standards and tablet sample solutions. In addition, all solutions were stored at -20 °C.



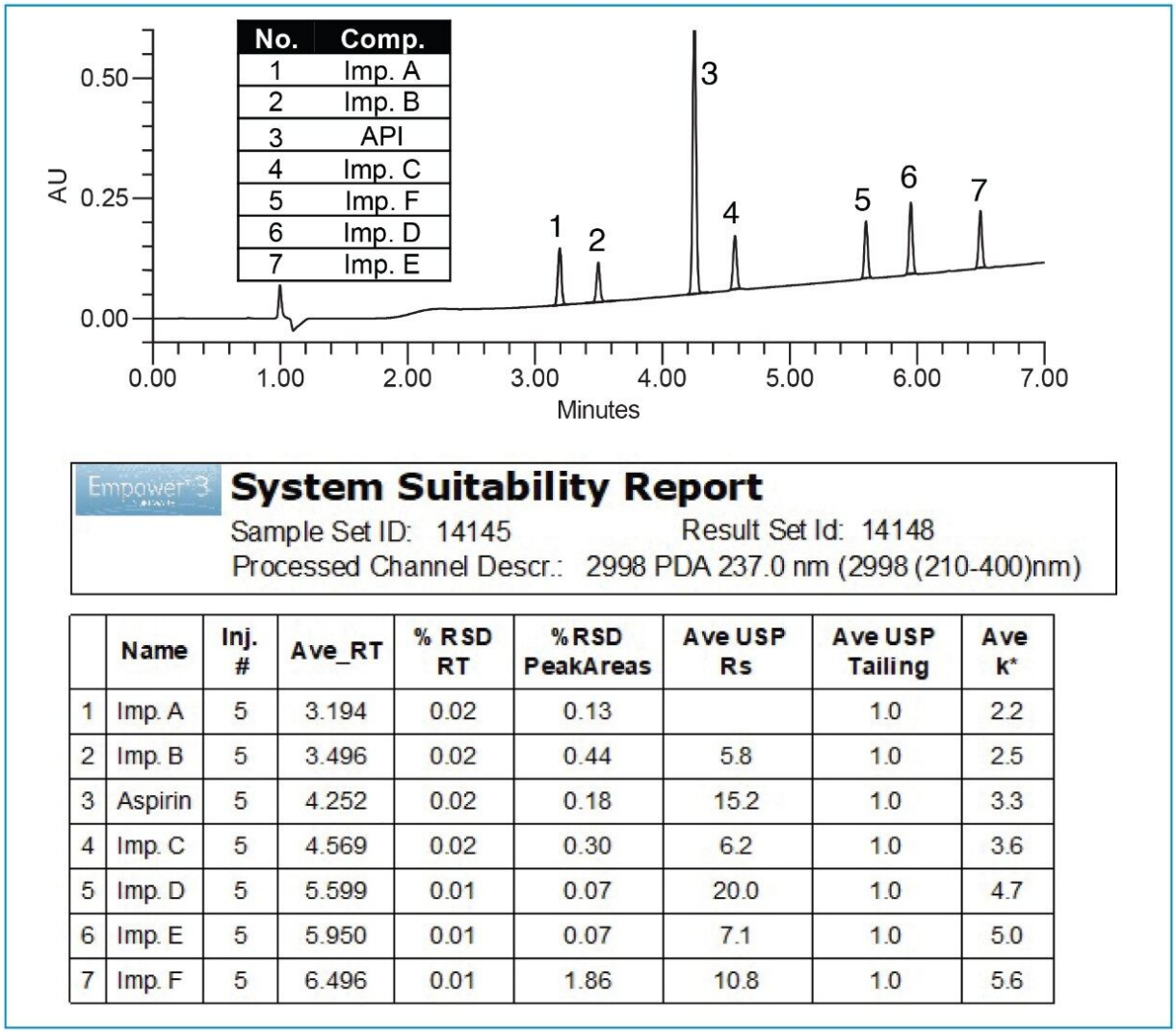
Diluent Water/methanol	% degradant
80:20	2.57
60:40	2.46

Diluent Water/acetonitrile	% degradant
80:20	0.53
70:30	0.43
60:40	0.36
50:50	0.36

Figure 7. Diluent study for optimization of sample preparation. All diluents with 0.1% formic acid.

Final Method

The success of the method development was evaluated by comparing results generated by the method against the performance requirements listed under the method goals. Operating under MS compatible conditions as described in the experimental section, final method separated all analytes with a USP resolution well above the requirement of ≥2.5 and met all system suitability criteria (Figure 8). The accuracy was determined by calculating % recovery of aspirin from the tablet sample solutions (Figure 9). The % recovery ranged from 94.2 to 97.0% which met the assay criteria of 90–110% listed in the USP monograph for aspirin tablets.[7] Furthermore, precision of six replicate preparations met the specification less than 5% RSD.



Empower 3

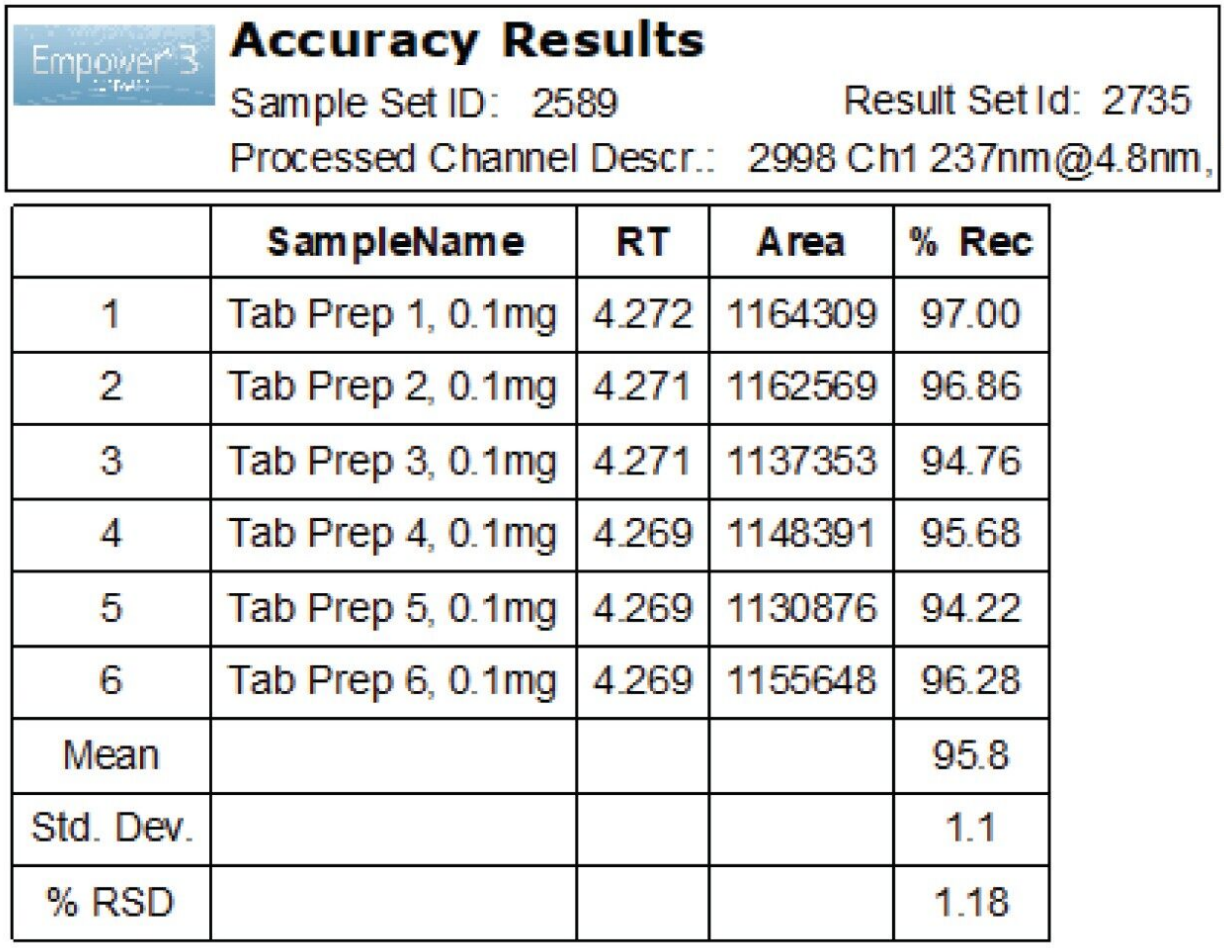
System Suitability Report

Sample Set ID: 14145 Result Set Id: 14148

Processed Channel Descr.: 2998 PDA 237.0 nm (2998 (210-400)nm)

	Name	Inj. #	Ave_RT	% RSD RT	%RSD PeakAreas	Ave USP Rs	Ave USP Tailing	Ave k*
1	Imp. A	5	3.194	0.02	0.13		1.0	2.2
2	Imp. B	5	3.496	0.02	0.44	5.8	1.0	2.5
3	Aspirin	5	4.252	0.02	0.18	15.2	1.0	3.3
4	Imp. C	5	4.569	0.02	0.30	6.2	1.0	3.6
5	Imp. D	5	5.599	0.01	0.07	20.0	1.0	4.7
6	Imp. E	5	5.950	0.01	0.07	7.1	1.0	5.0
7	Imp. F	5	6.496	0.01	1.86	10.8	1.0	5.6

Figure 8. System suitability results for 5 replicate injections of the standard mixture with aspirin API and related substances. Final method at 237 nm.



Accuracy Results

Sample Set ID: 2589 Result Set Id: 2735

Processed Channel Descr.: 2998 Ch1 237nm@4.8nm,

	SampleName	RT	Area	% Rec
1	Tab Prep 1, 0.1mg	4.272	1164309	97.00
2	Tab Prep 2, 0.1mg	4.271	1162569	96.86
3	Tab Prep 3, 0.1mg	4.271	1137353	94.76
4	Tab Prep 4, 0.1mg	4.269	1148391	95.68
5	Tab Prep 5, 0.1mg	4.269	1130876	94.22
6	Tab Prep 6, 0.1mg	4.269	1155648	96.28
Mean				95.8
Std. Dev.				1.1
% RSD				1.18

Figure 9. Recovery of aspirin API from the tablet's formulation.

Control Strategy

A control strategy was proposed based on the outcome of the risk assessment and experimental studies to determine controls that need to be put in place in order to obtain consistent performance and output in data quality that meets the criteria.

The study showed that flow rate and gradient time have the most effect on the resolution between analytes. These parameters can be setup and easily controlled using the instrument method. As for sample preparation, aspirin API and tablet formulations should be dissolved in diluent containing 40–50% acetonitrile with 0.1% formic acid. Standard and sample solutions should be stored -20 °C to minimize degradation.

Conclusion

A robust method for reliable and quick analysis of aspirin and related substances was successfully developed using a Fusion QbD method development software. The final method met all method performance goals and criteria. It operated under MS compatible conditions, which allowed the use of an ACQUITY QDa Mass Detector for peak identity confirmation. Utilizing Fusion QbD Software in conjunction with the Empower CDS streamlined the entire method development process, from DoE generation to design space establishment. Seamless interface enabled quick export of Fusion DoE to Empower for data acquisition and import of results to Fusion for statistical modeling. Response surface graphs generated by Fusion helped to identify variables that had the most impact on the method responses such as resolution or peak tailing. Finally, a control strategy was established to identify parameters that should be controlled to ensure method will meet the performance goals on routine basis.

Overall, applying DoE based approach increases robustness of the developed methods and assures data quality generated by the method. Using Fusion QbD method development software, ACQUITY Arc System and Empower CDS enables analytical laboratories to quickly develop reliable and reproducible analytical methods. As a result, methods can be successfully validated and transferred across laboratories and to contract partners.

References

1. Tome, T. Zigart, N. Casar, Z. Obreza, A. Development and Optimization of Liquid Chromatography Analytical Methods by Using AQbD Principles: Overview and Recent Advances. *Organic Process Research and Development.* 2019, 23, 1784–1802.
2. Guadin, K. Ferey, L. Quality by Design: A tool for Separation Method Development in Pharmaceutical Laboratories. *LCGC.* 2016, 29 (10), 16025.
3. Zigart, N. Casar, Z. Development of a Stability-Indicating Analytical Method for Determination of Venetoclax Using AQbD Principles. *ACS Omega*. 2020, 5, 17726–17742.
4. Proposed New USP General Chapter: The Analytical Procedure Lifecycle <1220>, The United States Pharmacopeia Convention. *Pharmacopeia Forum,* 43(1), Jan-Feb 2017.

5. The American Society of Health-System Pharmacists®
https://medlineplus.gov/druginfo/meds/a682878.html <
https://medlineplus.gov/druginfo/meds/a682878.html> , January 2021.

6. *Ph. Eur. Monograph.* Acetylsalicyclic Acid. The European Pharmacopeia 10.0. 01/2017:0309.

7. USP monograph for Aspirin Tablets. *United States Pharmacopeia* USP 43-NF 38, official May 1, 2020.

8. Fusion QbD Software, LC Method Development User's Guide. S-Matrix Corporation.

9. Fusion QbD Software, LC Method Development Tutorial. S-Matrix Corporation.

10. Fusion QbD Software, Data Exchange User's Guide – Waters Empower 2 and 3. S-Matrix Corporation.

11. Bakar, S.K. Niazi, S. Stability of Aspirin in Different Media. Research Articles. 1983, 72:9, 1024–1026.

Featured Products

ACQUITY Arc System <https://www.waters.com/134844390>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

Empower Chromatography Data System <https://www.waters.com/10190669>

Fusion Method Development Software <https://www.waters.com/waters/Fusion-Method-Development-Software/nav.htm?cid=10137325>

720007177, March 2021