Application Note

Method Robustness Testing Using Empower™ Method Validation Manager Software Aided by the Empower Sample Set Generator to Automate Method Creation

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Abstract

Robustness testing is a measure of a method's ability to remain unaffected by the minor changes of chromatographic parameters. In this work, robustness of a method for the analysis of naphazoline hydrochloride, pheniramine and associated related substances was investigated using Empower Method Validation Manager (MVM) Software. The robustness multivariable design of experiments (DoE) was created to study the effect of

individual parameters and their interaction on the chromatographic resolution between the peaks. The Empower Sample Set Generator (SSG) was used to automatically create a sample set method or injection sequence, instrument methods, and method sets required to perform the entire study in one run. The effect plots generated by the Empower MVM Software clearly showed which parameters have the greatest effect on the method performance and provided knowledge for establishing a set of controls to assure method meets the expected criteria during routine use.

Benefits

- Enhance method understanding and identify acceptable operating conditions through method robustness testing using a multivariable DoE with Empower Method Validation Manager (MVM) Software
- Automate creation of chromatographic methods for robustness testing with Empower Sample Set Generator (SSG) tool
- Identify the effect of method parameters and their interaction on the performance using effects plots generated by the Empower MVM Software

Introduction

Robustness testing is a critical task to demonstrate that the method remains unaffected by the minor changes in chromatographic parameters, providing an indication of its reliability during routine use.[1,2] Performing robustness during method development stage should be considered so that the critical parameters affecting method performance can be identified and optimized in the process. The parameters and appropriate associated ranges to be tested experimentally should be selected based on prior knowledge and risk assessment.[2] Implementing risk assessment into robustness helps to identify the risk associated with how each parameter impacts the data generated by the method. Based on the outcome of robustness, a control strategy can be established to define method's acceptable operating ranges, minimize and control source of variability.

Method robustness is typically determined by using either one factor at a time (OFAT) or multivariable approach with a design of experiments (DoE).[3] With an OFAT approach, only one factor (or chromatographic parameter) is investigated while others remain unchanged. This is a time-consuming process and often important interactions between variables such as temperature changes with flow rate are not identified. The multivariate DoE approach

shows the effect of each independent parameter and multiple parameters simultaneously.

In this work, robustness of a method for the analysis of naphazoline hydrochloride (HCl), pheniramine maleate, and associated related substances was assessed using Empower MVM Software, aided by the Empower SSG to automate method creation. The robustness DoE was created to study the multivariable effect and the effect plots were used to determine which parameters had the most impact on method performance.

Experimental

Compounds (Table 1) were purchased from Sigma-Aldrich and Toronto Research Chemicals (TRC). Mass spectrometry grade reagents and solvents were obtained from Honeywell.

Sample Description

Standard solutions

Individual stock solutions were prepared in methanol at 4.0 mg/mL. Stock solutions were diluted with 80:20 water/methanol diluent to make a mixture standard solution for robustness testing containing naphazoline HCl and pheniramine maleate active ingredients at 0.1 mg/mL and related substances at 10 µg/mL. List of the compounds used in this study is shown in Table 1.

Compound	Name	Formula
Pheniramine maleate	PHE API	$C_{20}H_{24}N_2O_4$
2-benzylpyridine	PHE Imp. A	$C_{12}H_{11}N$
4-benzylpyridine	PHE Imp. B	$C_{12}H_{11}N$
Naphazoline HCl	NAPH API	$C_{14}H_{15}ClN_2$
N-(2-Aminoethyl)-2-(naphthalen-1-yl) acetamide	NAPH Imp. A	$C_{14}H_{16}N_2O$
1-naphthylacetic acid	NAPH Imp. B	$C_{12}H_{10}O_2$
1-Naphthylacetonitrile	NAPH Imp. C	$C_{10}H_7CH_2CN$
β-naphazoline	NAPH Imp. D	$C_{14}H_{14}N_2$

Table 1. List of compounds used in this study

Final method

LC system:	Arc™ Premier System, column manager with active pre-heating, PDA Detector
Vials:	LCMS Maximum Recovery 2 mL volume, p/n: 600000670CV
Detection:	UV at 260 nm
Column:	XSelect™ Premier CSH C_{18}, 4.6 x 150 mm, 2.5 µm (p/n: 186009874)
Column temp.:	44°C
Sample temp.:	10°C
Injection volume:	5.0 µL

Flow rate:	1.2 mL/min
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in methanol

Gradient Table

Time (min)	%A	%B	Curve
Initial	93.0	7.0	6
1.00	93.0	7.0	6
15.00	15.0	85.0	6
16.00	15.0	85.0	6
16.10	93.0	7.0	6
21.00	93.0	7.0	6

Data Management

Chromatography software:	Empower 3 Feature Release 5 Service Release 5 (FR5 SR5)

Results and Discussion

The robustness of a method for the analysis of naphazoline HCl, pheniramine maleate, and associated related substances was conducted by performing a multivariable DoE study, utilizing Empower MVM Software. The chromatographic separation was performed using a XSelect Premier CSH C_{18} based on previously described method[4], with some modifications of the chromatographic conditions. The column temperature, flow rate, composition of the organic solvent at the beginning and end of the gradient were optimized to achieve a robust separation. A representative chromatograph acquired using the final method conditions is shown in Figure 1.

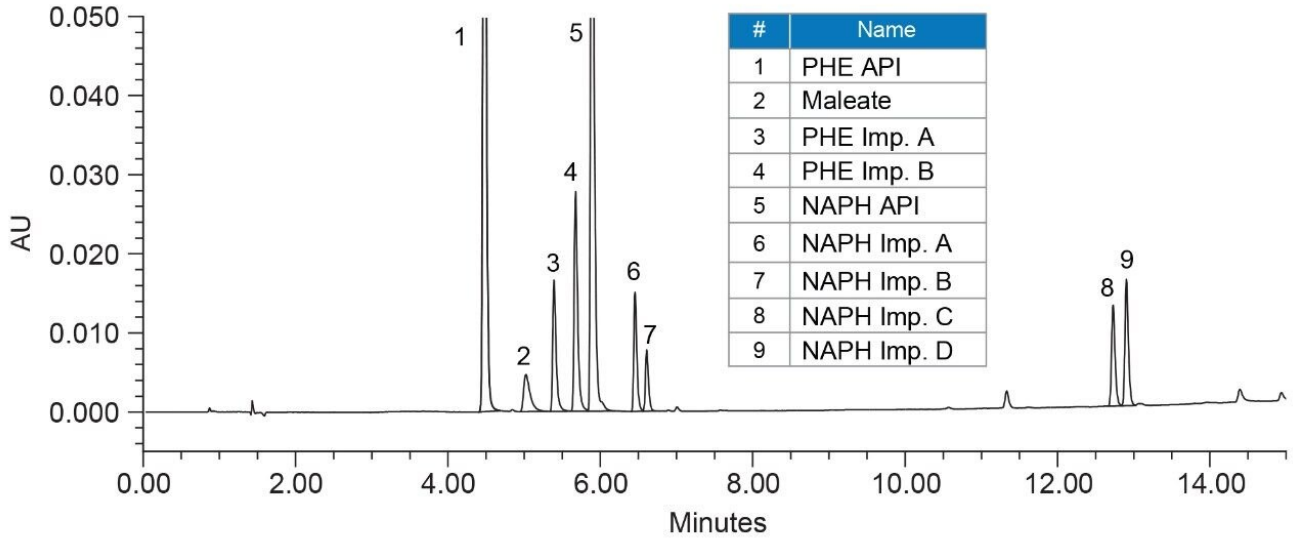


Figure 1. Separation of naphazoline HCl , pheniramine and associated related substances using final method.

About Empower MVM

The Empower Method Validation Manager (MVM) is a compliant-ready software that automates, streamlines, and simplifies methods validation workflows.[4,5] The entire method validation process is performed within a single software application, from creating a validation protocol method to acquiring, reviewing, analyzing, approving, and reporting validation data, including a full complement of statistical results. The validation tests and data are checked during the study for adherence to the validation requirements and acceptance criteria, with secure data storage and audit trails.

About Empower SSG

The Empower Sample Set Generator (SSG) tool automates the creation of instrument methods, method sets, and sample set methods, while varying the chromatographic parameters.[5] The Empower method sets and instruments methods are automatically created and structured in the sample set method according to the experiment design as a ready-to-run injection sequence. Automating creation of chromatographic methods minimizes transcription errors that may arise during the manual process and time spent generating methods, providing confidence that all chromatographic runs are completed with correctly created methods.

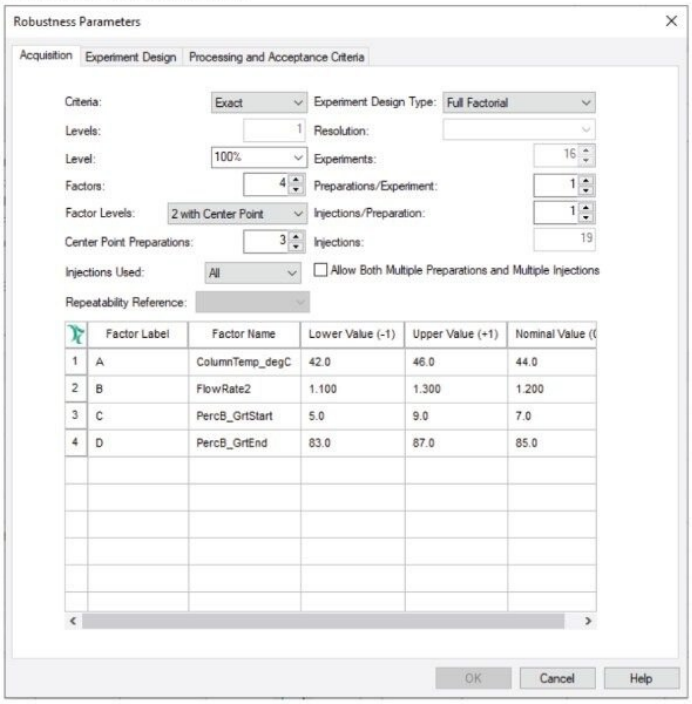
Robustness study

First, in risk assessment, parameters that may have the most impact on method performance were identified based on previous knowledge. The four parameters (factors) were investigated in this work and included column temperature, flow rate, composition (%) of organic solvent at the start and end of the gradient (Table 2). The effect of these parameters and their ranges was investigated experimentally using a robustness test in the Empower MVM Software. A DoE study was created to assess the multivariable effect on the chromatographic resolution between peaks, with acceptance criteria set to a USP Resolution ≥ 1.5. Using a full factorial for four factors with 2 levels (low and high), sixteen design points or experiments with a combination of different instrument conditions (Figure 2) were created for the robustness study.

Factor	Parameter	Center point (CP)	Low level	High level
A	Column temperature (°C)	44.0	42.0 °C	46.0 °C
B	Flow rate (mL/min)	1.200	1.100	1.300
C	% B at the gradient start	7.0	5.0	9.0
D	% B at the gradient end	85.0	83.0	7.0

Table 2. Factors (chromatographic parameters) for robustness study with Empower MVM Software.

A. Factors for acquisition



B. Experiment design

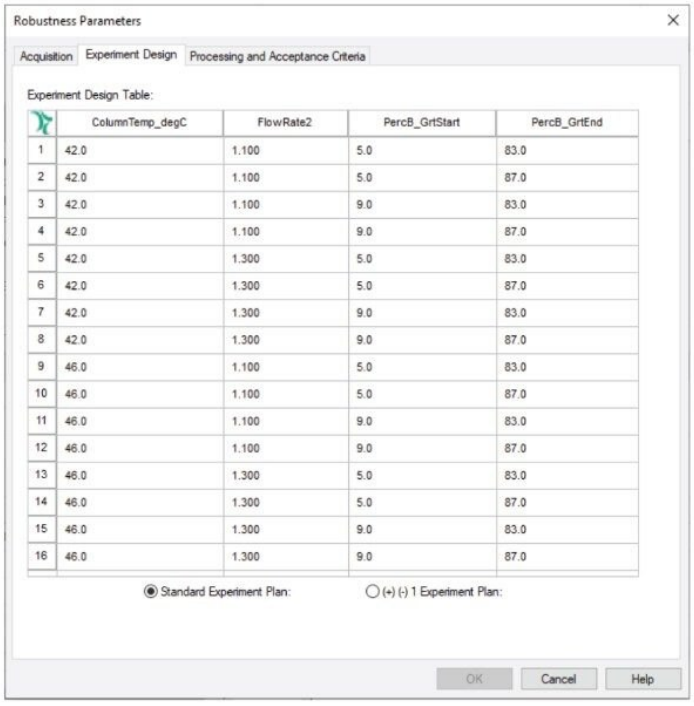


Figure 2. Setting up Empower MVM Software for robustness study. User defines the selection of study factors for

acquisition (A) and Empower automatically creates the experiment design (B).

The chromatographic methods required to run the entire study were automatically created using Empower SSG Software. The Empower SSG automatically created a sample set method according to the experiment design as a ready-to-run injection sequence (Figure 3). The sample set method included the experiment name, and method sets with instruments methods for each experiment. Injections of blank and equilibration steps throughout the run were included as instructed by the user.

Apply Table Preferences: Robustness

	SampleName	Sample Preparation	Experiment Name	Function	Method Set / Report or Export Method	Robustness	ColumnTemp degC	Flow Rate2	PercB GrtStart	PercB GrtEnd
1				Equilibrate	Robust_Study5_CP					
2	Blank			Inject Samples	Robust_Study5_CP	☐				
3	APIs w/10ug/mL RS _CP1	Preparation 1	Center Point	Inject Samples	Robust_Study5_CP	☑	44.0	1.200	7.0	85.0
4	APIs/RS 10ug/mL _CP2	Preparation 2	Center Point	Inject Samples	Robust_Study5_CP	☑	44.0	1.200	7.0	85.0
5	APIs 100ug/mL _CP3	Preparation 3	Center Point	Inject Samples	Robust_Study5_CP	☑	44.0	1.200	7.0	85.0
6				Equilibrate	Robust_Study5_1					
7	APIs w/10ug RS _1	Preparation 1	Experiment 1	Inject Samples	Robust_Study5_1	☑	42.0	1.100	5.0	83.0
8	APIs w/10ug RS _2	Preparation 1	Experiment 2	Inject Samples	Robust_Study5_2	☑	42.0	1.100	5.0	87.0
9				Equilibrate	Robust_Study5_3					
10	APIs w/10ug RS _3	Preparation 1	Experiment 3	Inject Samples	Robust_Study5_3	☑	42.0	1.100	9.0	83.0
11	APIs w/10ug RS _4	Preparation 1	Experiment 4	Inject Samples	Robust_Study5_4	☑	42.0	1.100	9.0	87.0
12				Equilibrate	Robust_Study5_5					
13	APIs w/10ug RS _5	Preparation 1	Experiment 5	Inject Samples	Robust_Study5_5	☑	42.0	1.300	5.0	83.0
14	APIs w/10ug RS _6	Preparation 1	Experiment 6	Inject Samples	Robust_Study5_6	☑	42.0	1.300	5.0	87.0
15				Equilibrate	Robust_Study5_7					
16	APIs w/10ug RS _7	Preparation 1	Experiment 7	Inject Samples	Robust_Study5_7	☑	42.0	1.300	9.0	83.0
17	APIs w/10ug RS _8	Preparation 1	Experiment 8	Inject Samples	Robust_Study5_8	☑	42.0	1.300	9.0	87.0
18				Equilibrate	Robust_Study5_9					
19	APIs w/10ug RS _9	Preparation 1	Experiment 9	Inject Samples	Robust_Study5_9	☑	46.0	1.100	5.0	83.0
20	APIs w/10ug RS _10	Preparation 1	Experiment 10	Inject Samples	Robust_Study5_10	☑	46.0	1.100	5.0	87.0
21				Equilibrate	Robust_Study5_11					
22	APIs w/10ug RS _11	Preparation 1	Experiment 11	Inject Samples	Robust_Study5_11	☑	46.0	1.100	9.0	83.0
23	APIs w/10ug RS _12	Preparation 1	Experiment 12	Inject Samples	Robust_Study5_12	☑	46.0	1.100	9.0	87.0
24				Equilibrate	Robust_Study5_13					
25	APIs w/10ug RS _13	Preparation 1	Experiment 13	Inject Samples	Robust_Study5_13	☑	46.0	1.300	5.0	83.0
26	APIs w/10ug RS _14	Preparation 1	Experiment 14	Inject Samples	Robust_Study5_14	☑	46.0	1.300	5.0	87.0
27				Equilibrate	Robust_Study5_15					
28	APIs w/10ug RS _15	Preparation 1	Experiment 15	Inject Samples	Robust_Study5_15	☑	46.0	1.300	9.0	83.0
29	APIs w/10ug RS _16	Preparation 1	Experiment 16	Inject Samples	Robust_Study5_16	☑	46.0	1.300	9.0	87.0

Figure 3. Sample set method for robustness test generated using Empower SSG Software.

The robustness data showed that the method met the limit for a USP Resolution ≥1.5 for most compounds, except for naphazoline peak (Figure 4). The resolution for naphazoline peak in experiments 9, 10, and 12 was found to be below 1.5 and was flagged by the software. The effect plots were used to determine which factor had most the impact on the resolution by visually examining the magnitude of the effect. For NAPH API peak, factor A (column temperature) had a significant impact on the resolution with negative effect, indicating loss in resolution with increase of column temperature (Figure 5). Furthermore, factor B (flow rate) had a positive impact effect on NAPH API, improving resolution when flow rate increased. For related substances, individual factors showed

positive and negative impact effects and interactions of multi-variables had minimum effects on the resolution (Figure 6).

Summary_USP Rs_Components

Sample Set ID: 1108 Result Set ID: 1816

Processed Channel Descr.: 2998 PDA 260.0 nm (2998 (210-400)nm)

USP Resolution Summarized by Name
Channel: 2998

	SampleName	Maleate	PHE-Imp A	PHE-Imp B	NAPH	NAPH-Imp D	NAPH-Imp A	NAPH-Imp B	NAPH-Imp C
1	APIs w/10ug RS_1	6.2	2.1	3.4	2.7	7.2	2.3	16.0	1.8
2	APIs w/10ug RS_2	6.2	2.0	3.4	2.6	7.1	2.3	15.6	1.8
3	APIs w/10ug RS_3	7.3	1.7	3.4	3.0	7.3	2.3	16.6	1.9
4	APIs w/10ug RS_4	7.2	1.6	3.4	2.8	7.2	2.4	16.0	1.8
5	APIs w/10ug RS_5	5.4	3.0	3.4	3.1	7.4	2.0	16.9	2.0
6	APIs w/10ug RS_6	5.4	2.8	3.4	3.0	7.3	2.0	16.4	2.0
7	APIs w/10ug RS_7	6.8	2.3	3.4	3.3	7.5	2.1	17.4	2.1
8	APIs w/10ug RS_8	6.7	2.2	3.4	3.2	7.4	2.1	17.0	2.0
9	APIs w/10ug RS_9	3.9	3.8	4.0	***1.4***	7.1	2.2	15.5	2.1
10	APIs w/10ug RS_10	3.9	3.7	4.0	***1.2***	7.0	2.2	15.1	2.1
11	APIs w/10ug RS_11	5.0	3.2	4.1	1.6	7.2	2.2	16.0	2.2
12	APIs w/10ug RS_12	5.0	3.2	4.1	***1.4***	7.1	2.3	15.7	2.2
13	APIs w/10ug RS_13	3.0	4.7	4.0	1.8	7.3	1.9	16.5	2.4
14	APIs w/10ug RS_14	3.1	4.5	4.0	1.6	7.2	1.9	16.0	2.3
15	APIs w/10ug RS_15	4.6	3.8	4.1	1.9	7.4	2.0	16.9	2.4
16	APIs w/10ug RS_16	4.5	3.8	4.1	1.8	7.3	2.0	16.5	2.4

Figure 4. Component summary report in Empower Software showing USP Resolution between peaks over the robustness experiments. No resolution is assigned to the first peak. The limits for resolution are specified in the processing method and the out-of-specification (OOS) results are flagged.

A. Validation data for naphazoline peak

	Component	Experiment	Factor A	Factor B	Factor C	Factor D	Data Faults	Assessed Field	Assessed Field Value
1	NAPH	Experiment 1	42.0	1.100	5.0	83.0	☐	USP Resolution	2.7
2	NAPH	Experiment 2	42.0	1.100	5.0	87.0	☐	USP Resolution	2.6
3	NAPH	Experiment 3	42.0	1.100	9.0	83.0	☐	USP Resolution	3.0
4	NAPH	Experiment 4	42.0	1.100	9.0	87.0	☐	USP Resolution	2.8
5	NAPH	Experiment 5	42.0	1.300	5.0	83.0	☐	USP Resolution	3.1
6	NAPH	Experiment 6	42.0	1.300	5.0	87.0	☐	USP Resolution	3.0
7	NAPH	Experiment 7	42.0	1.300	9.0	83.0	☐	USP Resolution	3.3
8	NAPH	Experiment 8	42.0	1.300	9.0	87.0	☐	USP Resolution	3.2
9	***NAPH***	Experiment 9	46.0	1.100	5.0	83.0	☑	USP Resolution	***1.4***
10	***NAPH***	Experiment 10	46.0	1.100	5.0	87.0	☑	USP Resolution	***1.2***
11	NAPH	Experiment 11	46.0	1.100	9.0	83.0	☐	USP Resolution	1.6
12	***NAPH***	Experiment 12	46.0	1.100	9.0	87.0	☑	USP Resolution	***1.4***
13	NAPH	Experiment 13	46.0	1.300	5.0	83.0	☐	USP Resolution	1.8
14	NAPH	Experiment 14	46.0	1.300	5.0	87.0	☐	USP Resolution	1.6
15	NAPH	Experiment 15	46.0	1.300	9.0	83.0	☐	USP Resolution	1.9
16	NAPH	Experiment 16	46.0	1.300	9.0	87.0	☐	USP Resolution	1.8

B. Factors & interactions effect plots

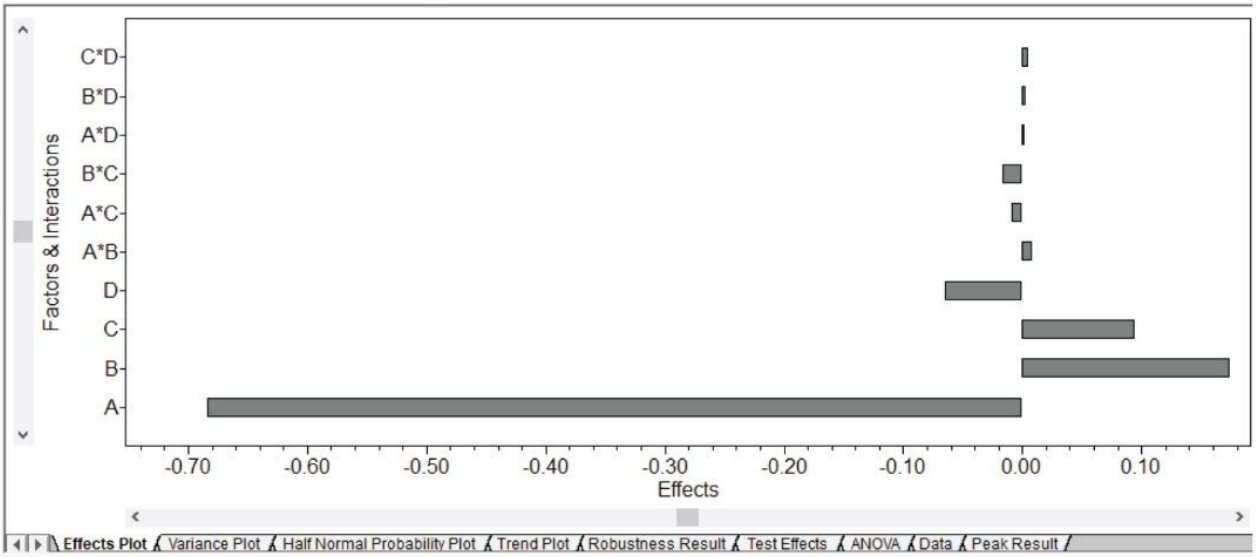


Figure 5. Validation results for naphazoline peak generated using Empower MVM Software. USP Resolution over

the robustness experiments with out-of-specification (OOS) results flagged (A) and effect plot of factors and their interactions on the USP Resolution (B).

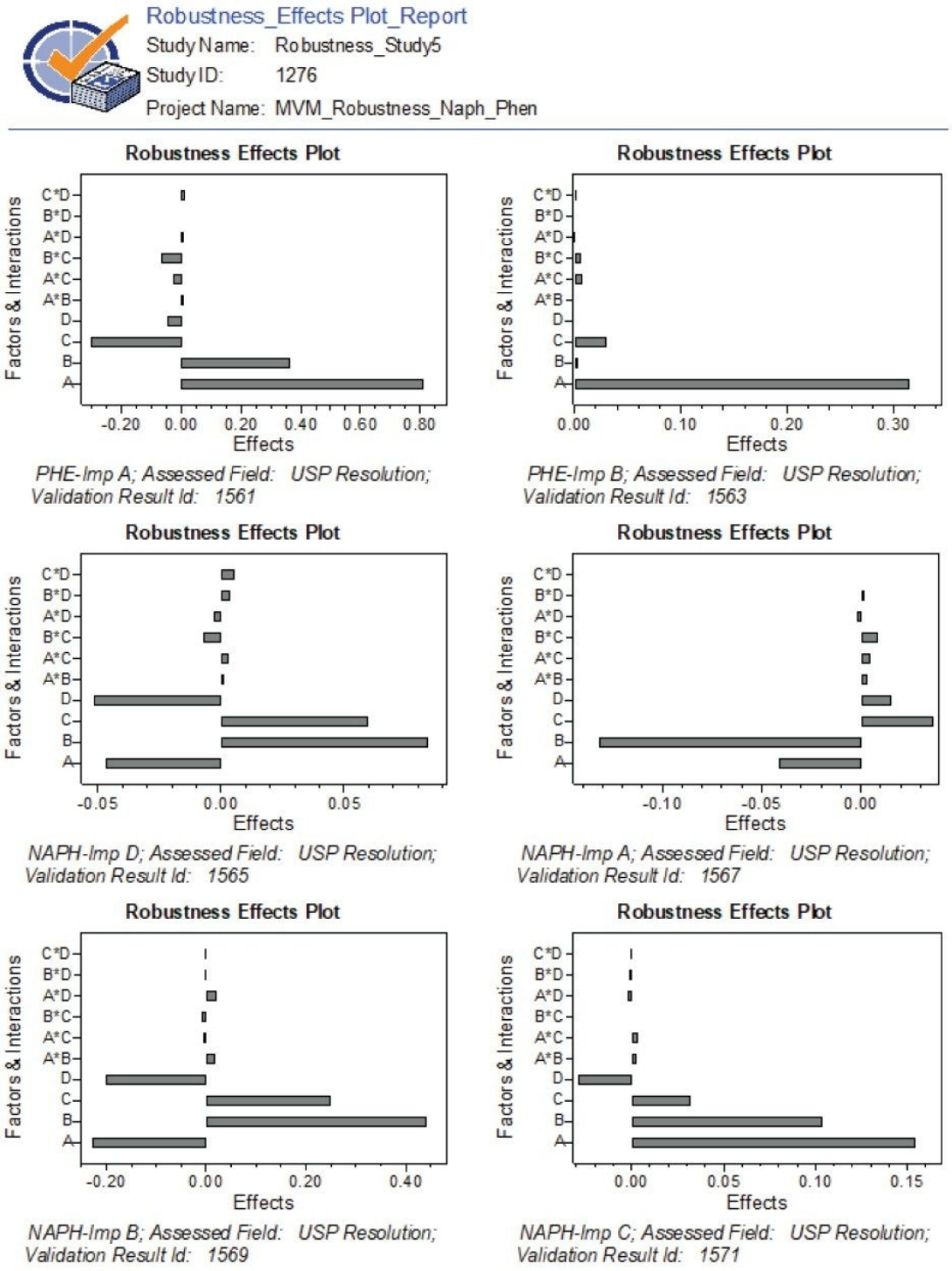


Figure 6. Robustness results for related substances generated using Empower MVM Software. Effect plots of

factors and their interactions on the resolution of each individual component. Factors include A: column temperature, B: flow rate, C: %B at gradient start, D: %B at the gradient end.

Control strategy

A control strategy consists of a set of controls to ensure that the analytical method performs as expected during routine use throughout its lifecycle and is based on the enhanced understanding of the method performance characteristics derived from risk assessment and robustness evaluation.[2] Understanding the effect on the method performance facilitates identification of parameters that should be controlled and appropriately defining method's operating range over which performance of the method is unaffected.

In this work, the DoE robustness study demonstrated sensitivity of the method to column temperature, specifically impact on the resolution between the most critical pair of peaks, PHE-Imp A and NAPH API. Restricting column temperature setting of 44.0±1.0 °C was recommended to achieve the required resolution of ≥1.5. Other parameters including flow rate of 1.2±0.1 mL/min, composition of organic at gradient start of 7.0±2.0% and gradient end of 85.0±2.0% provided acceptable operating range over which resolution criteria was met.

Conclusion

Robustness of a method for the analysis of naphazoline hydrochloride, pheniramine and associated related substances was assessed using a multivariable DoE study with Empower MVM Software. The Empower SSG Software automated generation of chromatographic methods to run the entire study. The effect plots clearly indicated parameters with the most impact on the method performance and helped to define method's operating conditions to meet the performance criteria.

Evaluating method robustness using a multivariable DoE helps to identify the effect of method parameters and robust operating conditions. It facilitates development of a control strategy to reduce and control source of variability during routine use.

References

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