SFC for fast and efficient impurity profiling in pharmaceutical quality control of carbamazepine – A Quality-by-Design based method development approach

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Abstract

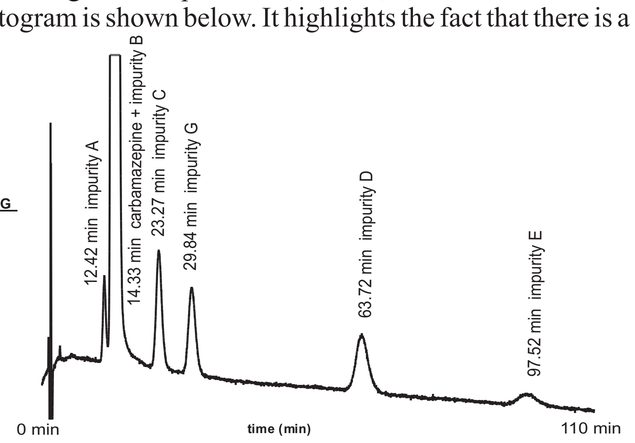
Detection of impurities in active pharmaceutical ingredients (API's) has always been a challenging task in pharmaceutical development. HPLC is a well-recognized analytical method for impurity profiling of various classes of drugs. However, some highly polar analytes interact only insufficiently on conventional RP columns and their analysis in still a challenge. Supercritical fluid chromatography (SFC) as orthogonal separation technique to HPLC may help to overcome these issue.

To check for the general potential of SFC separations as alternative, a state-of-the-art SFC method has been developed for impurity profiling of carbamazepine. Incorporating Quality by Design (QbD) principles to the method development approach by using the statistical software package Fusion QbD allows to study the relationship between chromatographic parameters (factors) and the resolution (response) between the peaks of interest.
In a screening phase the factors known to have major effect in column selectivity were studied. In the second phase the chromatographic parameters identified to affect the resolution were studied with additional instrument settings. In both phases statistical concepts with experimental design plans (Design-of-Experiments) are used as an efficient and fast tool to simultaneously gain knowledge about the influencing factors and interactions. An operating space within the design space is established and a verification study confirms the robustness of the final method.

Introduction

Carbamazine is widely used as an antiepileptic drug, which is described in the monograph of the European Pharmacopeia (E.P.). The purity testing of carbamazepine is accomplished in accordance to the monograph by using HPLC with UV-detection on a cyano column in isocratic mode with an eluent consisting of tetrahydrofuran, methanol, water (3:12:85, V/V/V). To 1000 mL of this solution 0.2 mL anhydrous formic acid and 0.5 mL triethylamine is added. The flow rate is 2.0 mL/min. On the basis of the synthetic route, the monograph recommends for testing of the impurities A, B, C, D, E, F and G.
A single run takes 110 min and a typical chromatogram is shown below. It highlights the fact that there is a need to reconsider and update the method.

Chromatogram of carbamazepine and its impurities A, B, C, D, E, F and G by using the E.P. method:



Design of Experiments

A Quality by Design (QbD) approach to define an operating space within the Design Space is often based on knowledge gained through Design-of-Experiments. In this case study, we used the statistic software package Fusion QbD to develop a state-of-the-art purity method for carbamazepine. An operating space within the design space is established and ensures a robust SFC method, which increases confidence in the ability to validate that method.

Selecting the experimental design is a critical step because not all statistical designs have the same ability to identify which factors are important and to quantify variable effects. Therefore, Fusion QbD software is using a two-step approach: In the first step a screening phase identifies the most influential operating parameters and in the second step an optimization phase are used to select the optimal values for method parameters.

Experiment Constants

Constant Name	Constant Value	Units
Pump Flow Rate	1.500	mL/min
Injection Volume	1.0	uL
Wavelength	230	nm
Initial % Strong Solvent	1.0	%
Final % Strong Solvent	25.0	%

Study Variable Settings

Name	Units	Range/Level(s)
Strong Solvent Type		Methanol, Iprop, ACN
Gradient Time	(min)	8.00 - 16.00
Oven Temperature	(°C)	30.0 - 55.0
Column Type		BEH, BEH 2-EP, Diol, 1-AA

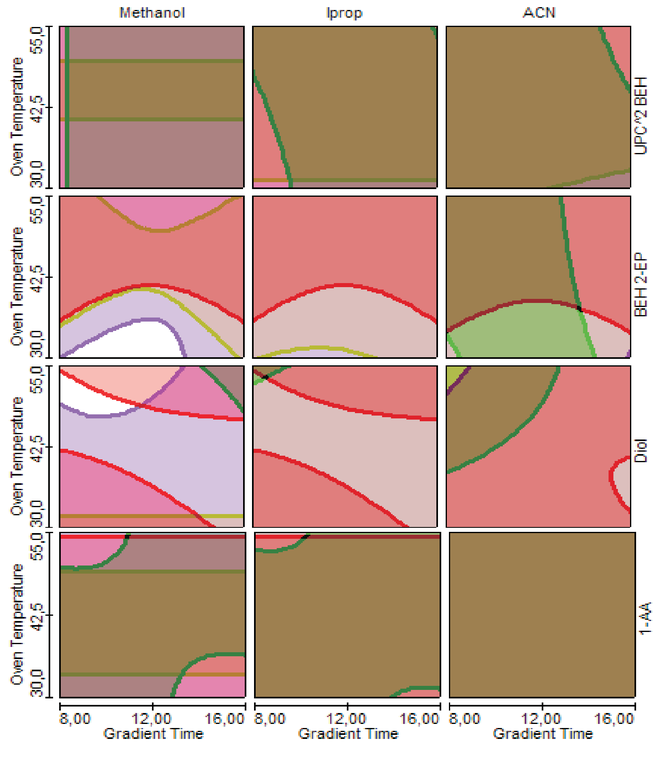
Once the experiments were run and the chromatograms integrated, the method performance goals (multiple peak responses) of the screening phase were calculated.

Response Variable Goals

Name	Goal	Lower Bound	Upper Bound	Color
No. of Peaks	Maximize	8.0	---	Red
No. of Peaks >= 2,00 - USPResolution	Maximize	5.0	---	Green
No. of Peaks <= 1,50 - SymmetryFactor	Maximize	7.0	---	Purple
Carbamazepin - SymmetryFactor	Minimize	---	1.50	Lime

This so-called trend-response approach eliminates the requirement for time-consuming and error-prone peak tracking. However, it has to be taken into account that - without peak tracking - any knowledge about the chromatographic behaviour (peak movements) of the analytes cannot be collected.

For each modeled response a contour plot (response surface plot) can be generated, and these plots can be overlaid to visualize the knowledge space. The following figure shows the overlay graph for the four columns with methanol, iso-propanol and acetonitrile as the organic eluent. The white unshaded area highlights the experimental region where all performance goals are met.



Optimization

The best results (conditions) of the screening phase were used for the optimization phase in addition to other chromatographic parameters determine the optimum SFC method.

Experiment Constants

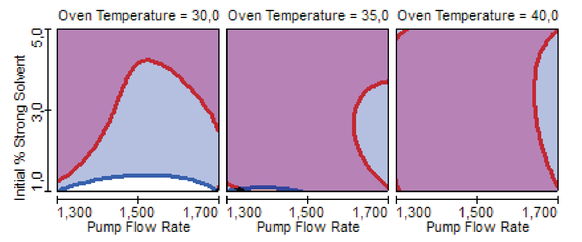
Constant Name	Constant Value	Units
Column Type	2-EP	
Eluent	Methanol	
Injection Volume	1.0	uL
Wavelength	230	nm
Gradient Time	5.00	min
Final % Strong Solvent	15.0	%

Experiment Variables

Name	Units	Range/Level(s)
Pump Flow Rate	(mL/min)	1.300 - 1.700
Initial % Strong Solvent	(%)	1.0 - 5.0
Oven Temperature	(°C)	30.0 - 40.0

Response Variable Goals

Name	Goal	Lower Bound	Upper Bound	Color
No. of Peaks	Maximize	8.0	---	Red
No. of Peaks >= 1,50 - USPResolution	Maximize	7.0	---	Blue



Further optimization and final method

Experiment Constants

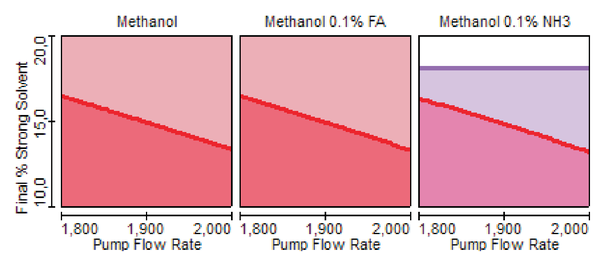
Constant Name	Constant Value	Units
Column Type	2-EP	
Injection Volume	1.0	uL
Oven Temperature	30.0	°C
Wavelength	230	nm
Gradient Time	4.00	min
Initial % Strong Solvent	1.0	%

Experiment Variables

Name	Units	Range/Level(s)
Strong Solvent Type		Methanol, Methanol 0.1% FA, Methanol 0.1% NH3
Pump Flow Rate	(mL/min)	1.800 - 2.000
Final % Strong Solvent	(%)	10.0 - 20.0

Response Variable Goals

Name	Goal	Lower Bound	Upper Bound	Color
Last Peak - RetentionTime	Minimize	---	2.60	Red
No. of Peaks >= 2,00 - USPResolution	Maximize	7.0	---	Blue
No. of Peaks <= 1,50 - SymmetryFactor	Maximize	7.0	---	Orange
Carbamazepin - SymmetryFactor	Minimize	---	1.50	Purple

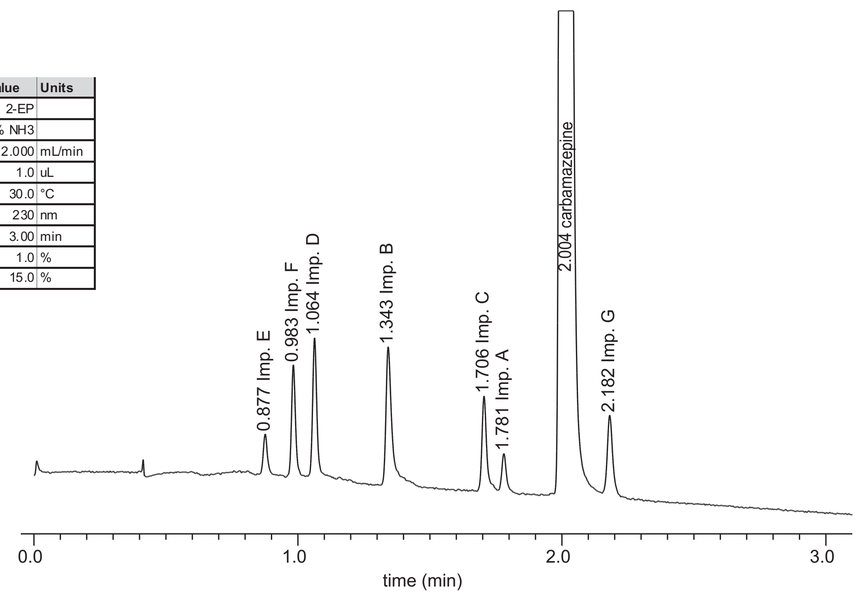


The method development approach leads to a final SFC method with an analysis time of only 3 min, which is an impressive 37-fold increase in productivity in comparison to the HPLC method published in the E.P.
All impurities are baseline separated.

Chromatogram of the final method

Experiment Constants

Constant Name	Constant Value	Units
Column Type	2-EP	
Strong Solvent	MeOH 0.1% NH3	
Flow Rate	2.000	mL/min
Injection Volume	1.0	uL
Oven Temperature	30.0	°C
Wavelength	230	nm
Gradient Time	3.00	min
Initial % Strong Solvent	1.0	%
Final % Strong Solvent	15.0	%



The method develoment approach will be published soon

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