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Using an innovative Quality-by-Design approach for development of a stability indicating UHPLC method for ebastine in the API and pharmaceutical formulations

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ABSTRACT

A stability-indicating ultra high performance liquid chromatographic (UHPLC) method has been developed for purity testing of ebastine and its pharmaceutical formulations.

Successful chromatographic separation of the API from impurities was achieved on a Waters Acquity UPLC BEH C18, 50 mm × 2.1 mm, 1.7 μm particle size column with gradient elution of 10 mM acetate buffer pH 6.2 and a mixture of acetonitrile/2-propanol (1:1) as the mobile phase. Incorporating Quality by Design (QbD) principles to the method development approach by using the chromatography modeling software DryLab®4 allows the visualization of a "Design Space", a region in which changes to method parameters will not significantly affect the results as defined in the ICH guideline Q8 (R2). A verification study demonstrated that the established model for Design Space is accurate with a relative error of prediction of only 0.6%.

The method was fully validated for specificity, linearity, accuracy and precision, and robustness in compliance to the ICH guideline Q2 (R1). The method was found to be linear in the concentration range from the quantification limit (LOQ) to 125% of the specification limit for ebastine and each of the impurities with correlation coefficients of not less than 0.999. The recovery rate was between 98.15 and 100.30% for each impurity. The repeatability and intermediate precision (RSD) were less than 3.2% for ebastine and each of the impurities.

The robustness of the developed method was studied by varying the six parameters: gradient time, temperature, ternary composition of the eluent, flow rate and start and end concentration of the gradient at 3 levels (+1, 0, −1). The resulting 729 experiments were performed *in silico* from the previously constructed model for Design Space and showed that the required resolution of 2.0 can be reached in all experiments. To prove the stability-indicating performance of the method, forced degradation (acid and base hydrolysis, oxidation, photolytic and thermal stress conditions) of ebastine was carried out. Baseline separation could be achieved for all peaks of the impurities, the degradation products and the API. Total run time was only 4 min, which is an impressive 40-fold increase in productivity in comparison to the method published in the Ph. Eur. monograph and allowed purity testing of more than 360 samples per day.

1. Introduction

Ebastine (1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(diphenylmethoxy)piperidin-1-yl]butan-1-one) is a second generation, non-sedating antihistamine mainly used for allergic rhinitis [1] and available in different pharmaceutical formulations (tablets, oro-dispersible tablets and syrup). It is official in the European Pharmacopoeia [2] where its purity testing is accomplished by using high performance liquid chromatography (HPLC) with UV-detection on a Spherisorb cyano column (250 mm × 4.6 mm, 5 μm particle size) in isocratic mode with an eluent consisting of 35 vol% acetonitrile and 65 vol% phosphoric acid pH = 5.0 and a flow rate of 1 ml/min. On the basis of the synthetic route, the monograph recommends for testing of the impurities A, C, D, and E, while impurities B, F & G are potential degradation products (see Fig. 1).

The resolution between the corresponding peak of impurity C and D is required of not less than 2.0. This method is not state-of-the-art because the retention factor *k* is between 0.25 and 70 and therefore far away from the recommended *k*-value between

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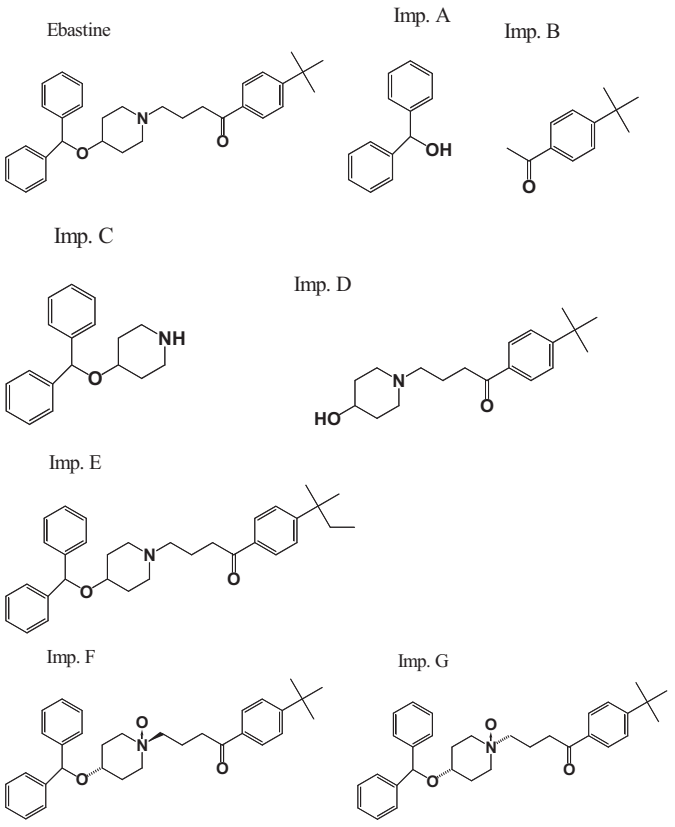

Fig. 1. Chemical structures of ebastine and its synthesis and degradation impurities.

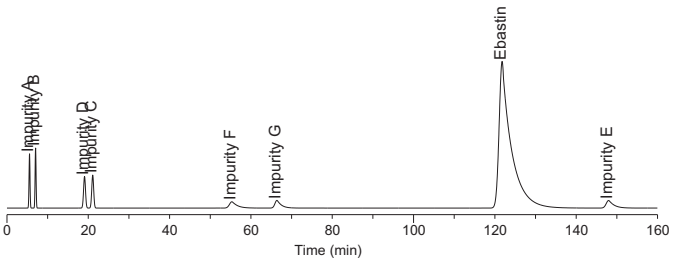

Fig. 2. Typical chromatogram of a selectivity standard solution containing ebastine and its impurities A–G by using the purity method published in the European Pharmacopoeia (conditions: Spherisorb cyano column, 250 mm × 4.6 mm and 5 μm particle size in an isocratic mode with an eluent consisting of 35 vol% acetonitrile and 65 vol% phosphoric acid pH = 5.0 and a flow rate of 1 ml/min).

2 and 10. Moreover, a single run takes 160 min and is therefore not acceptable for routine analysis. A typical chromatogram of a selectivity standard solution containing ebastine and its impurities A–G is given in Fig. 2.

Other HPLC methods, which have been reported in the literature for determination of ebastine in pharmaceutical preparations [3–6] as well as HPLC with tandem MS detection for simultaneous determination of ebastine and its metabolites in physiological samples [7,8] are not suitable for the determination of impurities in ebastine or require derivatization steps. Of these methods only one [6] is claimed to be stability indicating without a derivatization step. A forced degradation study was performed to provide an indication of the stability indicating property of the method but it fails to give information's of the potential impurities.

Hence, there is a need for the development and validation of a simple, fast and reliable stability indicating UHPLC method for the determination of ebastine and its impurities and degradation products in the API and in pharmaceutical formulations.

In the past, the common practice to develop an analytical method in liquid chromatography was performed by a trial-and-error approach, for example by varying one-factor-at-a-time (OFAT) and examine the resolution of peaks until the best method was found. This approach was time-consuming and required a large amount of manual data interpretation. It often resulted in a non-robust performance when transferred into another lab because interactions between factors were not considered. The OFAT approach should only be used if the user wants to understand selectivity changes, i.e. if everything is fixed and only one factor is varied and if the factor of interest is known not to have an interactive effect with any other factor studied [9].

A more systematic concept uses experimental design plans as an efficient and fast tool for method development. In a full or fractional factorial design a couple of experiments are carried out in which one or more factors are changed at the same time. Using statistic tools the effect of each factor on the separation can be calculated and the data be used to find the optimum separation. Typical examples are the widespread use of the Plackett–Burman design or the Monte-Carlo simulation in a multivariate data analysis software package (e.g. Fusion AE) [10–14].

A very smart and computer-assisted way of developing a chromatographic method is by using software modeling packages (e.g. DryLab, ChromSword, ACD/LC simulator). Based on a small number of experiments these software applications can predict the movement of peaks in reversed-phase liquid chromatography separations when changing the mobile phase composition or pH, temperature, flow rate and the column dimensions and particle size [15–22]. Other widespread strategies in HPLC method development uses the molecular structure, or physicochemical properties such as logP, logD and pKa of the sample components to estimate their retention and optimal separation conditions [23].

Since the US Food and Drug Administration announced in 2002 its "Pharmaceutical Current Good Manufacturing Practices (cGMPs) for the 21st Century" initiative [24], a Quality-by-Design approach in pharmaceutical development is requested.

Quality-by-Design (QbD), as defined by the ICH guideline Q8 (R2) [25], is "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". Although the ICH guideline Q8 (R2) doesn't explicitly discuss analytical method development, the QbD concept can be extended and results in a systematic approach that includes defining methods goal, risk assessment, developing of a Design Space, implementing a control strategy and continual improvement to increases method robustness and knowledge. The novelty and opportunity in this approach is that working within the Design Space of a specific method can be seen as an adjustment and not a (post approval) change.

In our laboratory we have been using the chromatography modeling software DryLab® for many years now in HPLC method development and it results in a better understanding of our methods (for example the movement of peaks and how a method should be communicated) and the degree of robustness [26,27]. By using the QbD approach the fundamental of a systematic method development has not changed. The introduction of an early risk-assessment helps to identify critical analytical parameters and to concentrate on them in method development [28].

The aim of our study was to apply Quality-by-Design principles to build in a more scientific and risk-based multi-factorial approach to the development and validation of a new stability indicating UHPLC method for ebastine and its impurity and degradation products in the API and pharmaceutical formulations.

2. Experimental

2.1. Chemicals and eluents

Methanol, acetonitrile and 2-propanol were HPLC-gradient grade, all other chemicals were at least analytical grade, and purchased from Merck (Darmstadt, Germany). Water used was purified by a Milli-Q academic water purification system (Millipore, Eschborn, Germany).

2.2. UHPLC equipment and chromatographic conditions

An Acquity UPLC® H-class system consists of a Quaternary Solvent Manager with Solvent Selection Valve, Sample Manager, Column Manager, and Photo-Diode Array detector, controlled by Empower® 2 C/S-software (Waters, Eschborn, Germany) was used for all experiments. The dwell volume of the system is 0.12 ml. Four different Acquity UPLC® columns BEH C18, HSS T3, BEH Phenyl, HSS C18 SB, with the dimension 50 mm × 2.1 mm and 1.7 μm particle size were used in the study (Waters, Eschborn, Germany). All chromatographic experiments were performed in the gradient mode. Eluent A was 10 mM acetate buffer at pH 6.2 and eluent B was prepared by mixing different volumes of the organic solvents acetonitrile and 2-propanol. Eluent C was methanol (for screening experiments only). The flow-rate was set to 0.5 ml/min and the injection volume was 5 μl.

The temperature in the screening experiments were at 35 °C, and 35 °C and 70 °C in the optimization experiments. The UV detection was carried out at 210 nm and the UV spectra were taken in the range of 200–400 nm.

2.3. Software

DryLab®4 chromatography modeling software package (Molnar-Institute, Berlin, Germany) was used for screening and optimization of gradient time, temperature, solvent composition and pH to separate a mixture of ebastine and seven impurities. The software package includes the PeakMatch and 3-D-Robustness modules.

Statistical analysis was calculated using the MVA statistical software (Novia, Frankfurt, Germany).

2.4. Standard and sample preparation

A selectivity standard stock solution containing ebastine (Arevipharma, Radebeul, Germany) and all seven impurities (in-house standard substances) was prepared with acetonitrile as solvent and protected from light by use of amber glass ware. Final dilution was made in a mixer of 10 mM ammonium acetate pH 6.2 and acetonitrile (50:50, v/v). This selectivity standard solution was used in all screening and optimization experiments.

A sample solution of ebastine oro-dispersible tablets (Aristo Pharma, Berlin, Germany) was prepared in a mixer of 10 mM ammonium acetate pH 6.2 and acetonitrile (50:50, v/v). The sample solution was filtered through a 1 μm-glassfiber filter and the resulting clear solution was used for the UHPLC determination.

3. Results and discussion

3.1. Development strategy

Our innovative development strategy follows Quality-by-Design (QbD) principles and can be divided into the five steps (1) definition of method goals, (2) risk assessment, (3) design of experiments with screening and optimization steps, (4) Design Space that includes model building, working point selection and verification, just as method validation and (5) method control strategy based on the knowledge gained about the developed method.

3.2. Definition of method goals

The primary goal of developing an UHPLC stability indicating method is generally to separate the API from impurities (resolution $R_s > 2.0$) that may impact the quality of the pharmaceutical formulation. From the general equation $R_s = 0.25^* N^{1/2}[(\alpha - 1/\alpha)](k/1+k)$ it is obvious that the selectivity parameter α has the greatest impact on resolution. Selectivity can be changed by modification of the mobile phase composition, column chemistry and temperature [23]. Other factors, such as the need for short analysis times (<10 min) are also considered. Crucial for the Quality-by-Design approach is to create a visual "Design Space", in which the method is robust.

3.3. Risk assessment

In an early risk assessment the critical parameters should be identified. That could be method factors which may affect extraction of the compounds of interest in sample preparation (e.g. extraction method, extraction time, extraction solvent) [9] as well as settings in the instrumental analysis. For example the UV spectra of ebastine and its impurities were evaluated to select the detection wavelength, where is maximum response for the detection of all impurities. Further on the critically influential separation parameters stationary phase, gradient time tG, temperature T, ternary composition tC of the eluent B and pH of the eluent A were identified [29].

3.4. Design of experiments

3.4.1. Definition of critical parameters

As the result of the risk assessment, the four parameters gradient time tG, temperature T, ternary composition of the eluent B and pH of the eluent A were optimized – after choosing the best stationary phase – due to their strong known influential effect on selectivity.

3.5. Screening experiments for selection of stationary and mobile phase

The development of an UHPLC method for the analysis of impurities is a critical task to ensure quality of the API and the drug product. Therefore, a column and mobile phase screening was performed to achieve orthogonality in separation and to make sure that all impurities were well separated from the API peak. The screening strategy applies an automated column selection system and a number of UHPLC columns, varying in hydrophobicity and silanol activity. The columns were evaluated using a reversed phase column selectivity chart (free available on www.waters.com), which compares RP-columns of different vendors. The following four stationary phases with different selectivity's were selected:

- Acquity UPLC® BEH C18 (ethylene bridged hybrid technology particle)
- Acquity UPLC® HSS T3 (high strength silica based C18 particle with endcapping)
- Acquity UPLC® BEH Phenyl (ethylene bridged hybrid technology particle with a alternative selectivity because of the phenyl ligand)
- Acquity UPLC® HSS C18 SB (high strength silica particle without any endcapping and therefore a different selectivity than the "HSS T3" column)

Table 1
Results of the screening experiments: in the screening experiment the highest critical resolution for the separation of ebastine and its impurities could be found on the Acquity UPLC® BEH C18 column with acetonitrile and 2-propanol gradients, respectively.

Stationary phase	Eluent B	Retention time of last peak [min]	Critical resolution $R_{s,crit}$
Acquity UPLC® BEH C18	Methanol	3.27	1.64
	Acetonitrile	2.80	1.94
	2-Propanol	1.77	1.88
Acquity UPLC® HSS T3	Methanol	3.51	<1.5
	Acetonitrile	3.31	<1.5
	2-Propanol	2.08	1.57
Acquity UPLC® BEH Phenyl	Methanol	3.33	<1.5
	Acetonitrile	2.69	<1.5
	2-Propanol	1.93	<1.5
Acquity UPLC® HSS C18 SB	Methanol	3.41	<1.5
	Acetonitrile	2.22	<1.5
	2-Propanol	2.08	<1.5

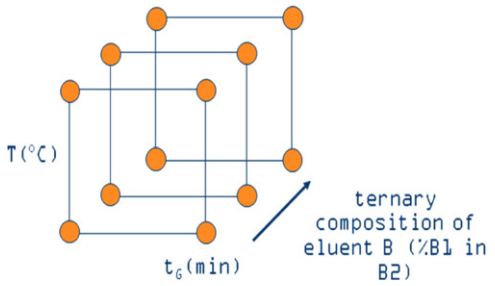

Fig. 3. Experimental design for a three dimensional UHPLC method optimization.

In the initial screening experiment these 4 different stationary phases were tested against linear gradients of 10–90% methanol, acetonitrile and 2-propanol, respectively, in 10 mM acetate buffer at pH 6.2. The pH of the buffer was selected based on our prior knowledge and will be confirmed in a later section of this paper.

Table 1 shows the retention time of the last eluting peak (in all cases impurity E) and the critical resolution. It can be seen that the highest critical resolution was found with acetonitrile and 2-propanol gradients on the Acquity UPLC® BEH C18 column and therefore these combinations were chosen for further method optimization.

3.6. Optimization phase

The screening experiments are followed by optimization of chromatographic performance using the DryLab® modeling software, where the selectivity changes as movement of peaks can be studied without the need for extensive laboratory experiments.

Therefore the experimental data from 12 experiments are entered into the software, which then simulates and predicts separations for a very large number of variations in chromatographic conditions based on the limited data set obtained in the 12 experiments.

Initial input data were acquired under the following conditions: gradient times of 2 min and 6 min (10–90%B), temperatures of 35 °C and 70 °C and the ternary composition of the organic eluent B were of (a) 100% acetonitrile, (b) acetonitrile/2-propanol (70:30, v/v), and (c) acetonitrile/2-propanol (40:60, v/v). Higher 2-propanol concentrations were not possible because of the high backpressure, which 2-propanol causes. The aqueous eluent A was 10 mM acetate buffer pH = 6.2. Twelve ($2 \times 2 \times 3$) experimental runs were performed according to the design of experiments shown in Fig. 3 [28].

In this approach the chromatograms obtained by two gradient time (tG), two column temperature (T), and three ternary eluent compositions were necessary in order to build 2D-models and further on a 3D-model of the critical resolution by using DryLab software. The ranges between these factors were large enough to induce peak movements to discover hidden peaks [22].

3.7. Design Space

After processing and checking the accuracy of data, the retention times of 8 peaks of interest (ebastine and impurities A–G) were matched in each of the chromatograms by using the PeakMatch module of the DryLab® software. The peak tracking process was based on peak area and confirmed by the UV-spectra of the compounds. All data were transferred automatically into the modeling software DryLab®4 but small adjustments for the peak width had to be made to meet real column performance in the simulated chromatograms. For each of the three experimental sets 2-dimensional resolution maps of gradient time tG against column temperature T were generated and the start gradient optimized for a short runtime (see Fig. 4a–c).

The color code in these resolution maps represents the value of the critical resolution, with warm "red" colors show large resolution values ($R_s > 2.0$) and cold "blue" colors show low resolution values ($R_s < 0.5$) [22].

The three 2-D resolution maps were used to create a 3-D-resolution cube (see Fig. 5), in which the combined influence of the optimized parameters is visualized.

Each point in this 3-D resolution cube stands for a highly accurate chromatogram. A comparison of predicted and experimental retention times for seven verification points within the 3D resolution cube was found to be excellent (for details see Section 3.8).

By resetting the resolution option shown in the 3D resolution map, the robust region can be isolated and studied. The visual inspection of the cube shows a large red region ($R_s > 2.0$), where the method is very robust and the resolution of all peaks in the chromatogram are well baseline separated from each other. The three pictures in Fig. 6 visualize the Design Space from different angles.

In an additional set of experiments the influence of the pH was studied. It is critical to select a pH in which the method is robust, to ensure that minor changes in the buffer preparation will not have a negative influence on the resolution of peaks. Based on prior experiments, in which we used a mass spectrometry suitable eluent, we know that with an acetate buffer at pH of 6.2 the method is robust. So we investigated the variation of pH of the aqueous eluent between pH 6.0 and pH 6.85 and created the 2-D resolution map of R_s vs. pH and gradient time, which is shown in Fig. 7.

It can be seen from the resolution map that the method is robust in the pH range between 6.0 and 6.4 and a gradient time between 3 and 5 min and provides the highest peak resolution ($R_s > 2.0$).

3.8. Working point selection and verification

From the previously constructed Design Space the working point was selected by visual examination looking for the highest critical resolution ($R_{s,crit}$) and best robustness of the method. At this point small changes of the critical parameters gradient time, temperature and ternary composition of the eluent B as well as flow rate, gradient slope and shape, column dimensions and dwell volume have no negative influence on the separation of all peaks. This working point was found in the cube at tG = 3.0 min, T = 60 °C and tC = acetonitrile/2-propanol (50:50, v/v) and a predicted chromatogram is shown in Fig. 8.

A set of seven verification runs was carried out to ensure that the predicted (*in silico*) results can be confirmed experimentally and the accuracy was found to be excellent with a relative error

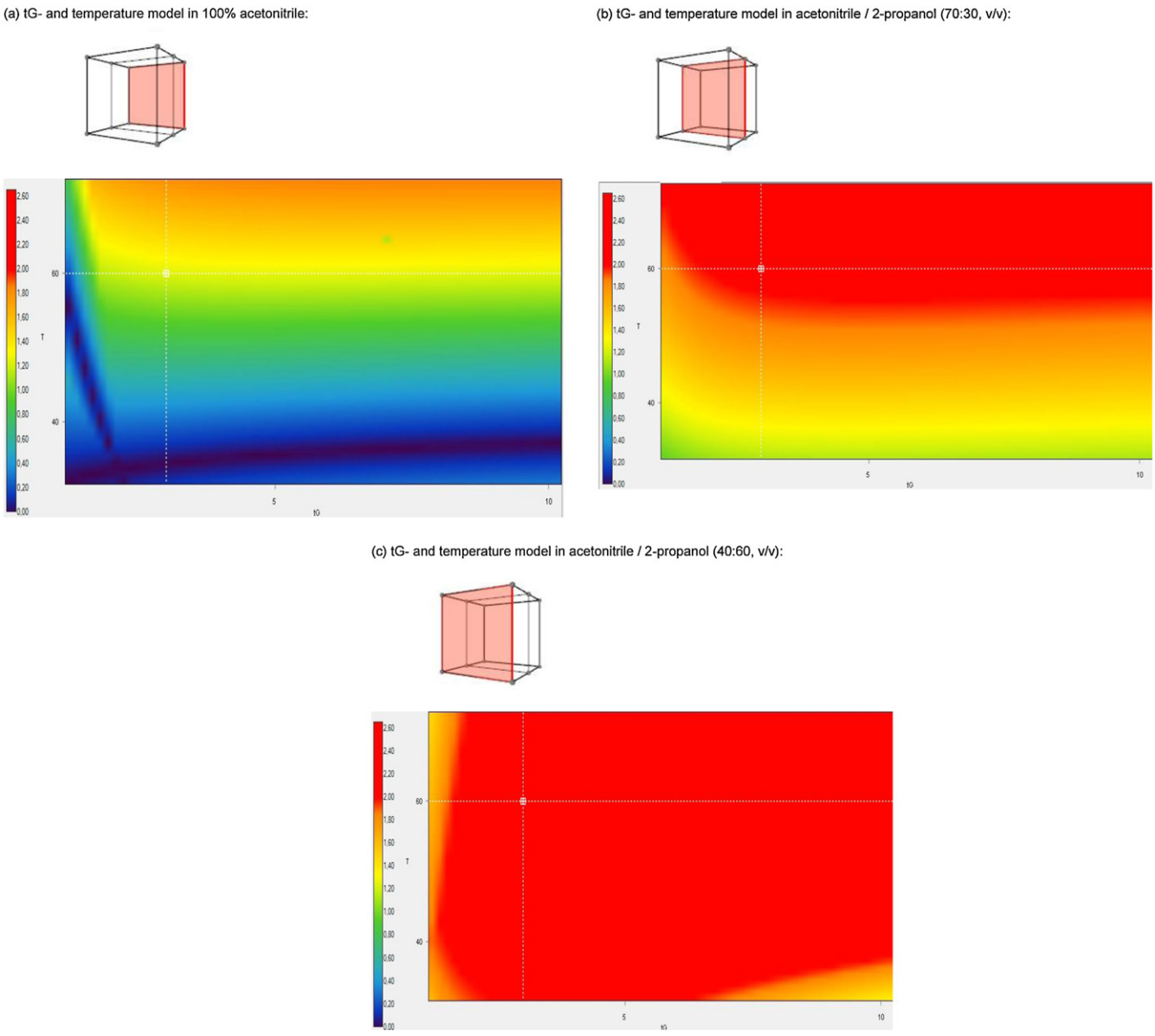

Fig. 4. (a) tG- and temperature model in 100% acetonitrile: the warm "red" color shows a region, in which the resolution of the critical peak pair is higher than 2.0 ($R_{s,crit} > 2.0$). It can be seen that with pure acetonitrile, a resolution is only possible at elevated temperature and longer gradient time. (b) tG- and temperature model in acetonitrile/2-propanol (70:30, v/v): With a ternary eluent composition of 30% 2-propanol in acetonitrile the warm "red" region is much broader - indicating the resolution of the critical peak pair is higher than 2.0 ($R_{s,crit} > 2.0$). At a column temperature of 60 °C and a gradient time of 3 min baseline separation of all peaks are possible. (c) tG- and temperature model in acetonitrile/2-propanol (40:60, v/v): The broad warm "red" region shows that the method is very robust, when an ternary eluent composition of 60% 2-propanol in acetonitrile is used: At a column temperature between 40 and 70 °C and gradient time between 2 and 10 min there will be always a resolution of the critical peak pair of higher than 2.0 ($R_{s,crit} > 2.0$) – indicating baseline separation of all peaks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of prediction of only 0.6%, as shown in Table 2. This is also in compliance to previous reported data [30,31].

3.9. Method validation

A validation study in compliance to the ICH guideline Q2 (R1) [32] was performed. The validation data can be shown in Table 3.

An important part of the validation study is the robustness of the developed method. The ICH guideline Q2 (R1) [33] define the robustness as ". . ..the reliability of an analysis with respect to deliberate variations in method parameters. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage".

The robustness of the developed method was studied with the aid of the Robustness module of the DryLab®4 software without the need for further experiments. This module used the constructed 3D resolution cube for multi-factorial robustness calculations [22]. The six parameters tG (3 min ± 0.3 min), T (60 °C ± 6 °C), tC (50% ± 5% 2-propanol in acetonitrile), flow rate (0.5 ml/min ± 0.1 ml/min) and the %start (30% ± 2%) and %end (90% ± 2%) of the gradient were varied at 3 levels (+1, 0, −1). These tolerances are much larger than the instruments specification for precision and allow an evaluation of the robustness of the method, especially when transferred into other labs and performed on different equipment. From the results

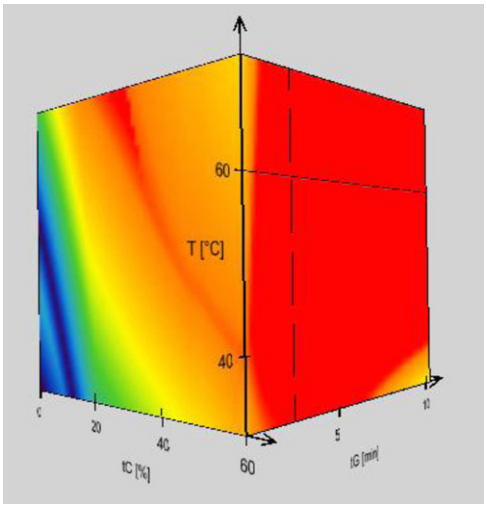

Fig. 5. The three 2-dimensional resolution maps were used to create a 3-D-resolution cube, in which the combined influences of the optimized parameters are visualized: Each point in these 3-D resolution cube stands for a highly accurate chromatogram. A comparison of predicted and experimental retention times for five validation points within the 3D resolution cube was found to be excellent.

of the 729 experiments it can be seen that the required resolution of 2.0 can be reached in all experiments (see Table 4. A figure of the frequency of the distribution of the resolution values $R_{s,crit}$ for all 729 experiments of the robustness study can be found in the Supplementary Figure S-1). Therefore, the developed method is robust against small changes of chromatographic parameters.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2013.01.032.

The robustness study was completed by confirming the separation on five different batch numbers of the Acquity UPLC® BEH C18 column.

The ebastine standard solution was subject to acid and base hydrolysis, oxidation, photolytic and thermal stress conditions. The baseline separation of all peaks of the impurities and degradation products and the API indicate the stability-indicating performance of the method. Peak purity was confirmed by the UV- and MS-data.

3.10. Method control

In this innovative Quality by Design approach a control strategy was implemented to ensure that the developed UHPLC method is performing as intended. Based on the validation data and the robustness of the method, the risk assessment indicates that there is extensive knowledge gained about the performance of the method. Therefore, the only one control element which is needed in our method control strategy is a system suitability test [33]. The resolution of the critical peak pair ($R_{s,crit}$) impurity C and D, which has the lowest resolution of all impurity peaks, was chosen as a system suitability test parameter and should not be less than $R_{s,crit} > 2.0$.

A summary of the chromatographic parameters and tolerances of the final method are given in Table 5. The method was applied

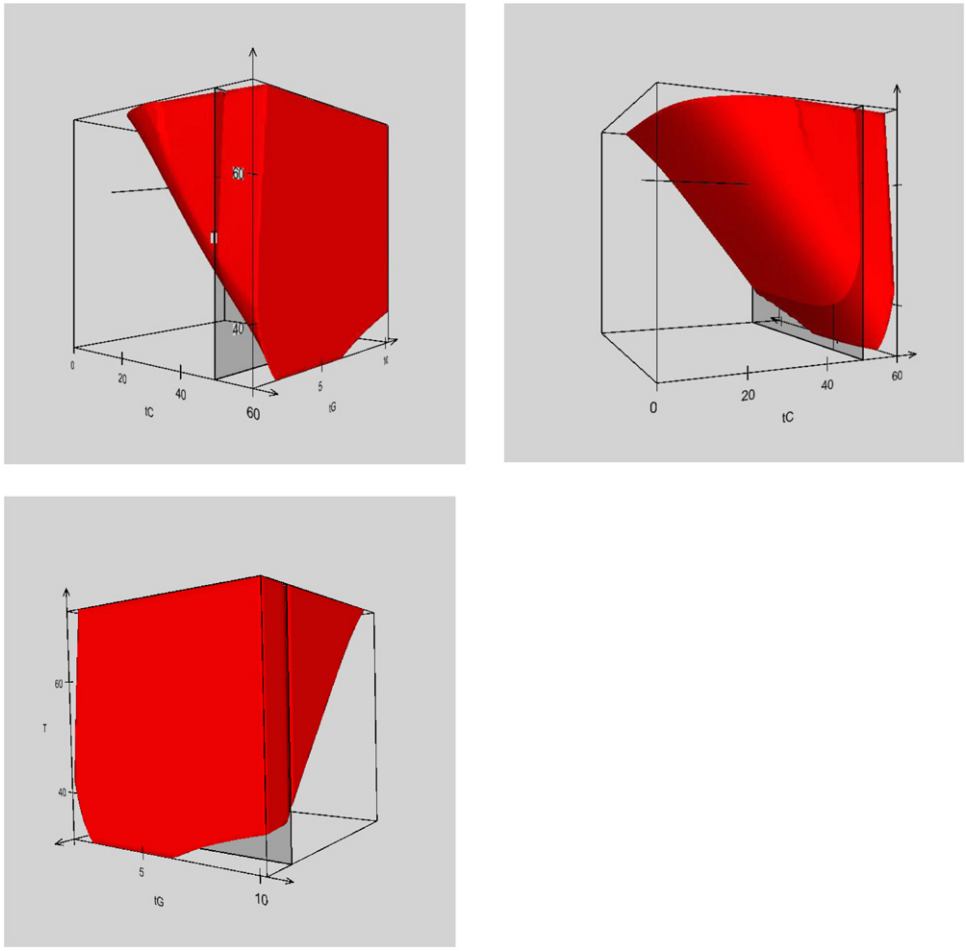

Fig. 6. By resetting the resolution option shown in the 3D resolution map, the robust region can be isolated and studied (the robust region is where the resolution of all peaks in the chromatogram are well baseline separated from each other; $R_s > 2.0$). The following three pictures visualize the Design Space from different angles.

Table 2
Verification study: a comparison of predicted and experimental retention times of all components are shown below and found to be excellent (relative error of prediction is 0.6%).

	Working point		Verification point 1		Verification point 2		Verification point 3		Verification point 4		Verification point 5		Verification point 6	
	tG [min]	3.0	tG [min]	2.7	tG [min]	3.3	tG [min]	3.3	tG [min]	2.7	tG [min]	3.0	tG [min]	3.0
	T [°C]	60	T [°C]	66	T [°C]	66	T [°C]	54	T [°C]	54	T [°C]	60	T [°C]	60
	tC [%ACN]	50	tC [%ACN]	50	tC [%ACN]	50	tC [%ACN]	50	tC [%ACN]	50	tC [%ACN]	55	tC [%ACN]	45
Peak name	DryLab [min]	Exp. [min]	DryLab [min]	Exp. [min]	DryLab [min]	Exp. [min]	DryLab [min]	Exp. [min]	DryLab [min]	Exp. [min]	DryLab [min]	Exp. [min]	DryLab [min]	Exp. [min]
Imp. C	0.65	0.65	0.62	0.62	0.63	0.63	0.68	0.68	0.67	0.67	0.67	0.66	0.62	0.63
Imp. D	0.75	0.76	0.72	0.73	0.73	0.74	0.78	0.79	0.76	0.77	0.77	0.77	0.72	0.74
Imp. A	0.90	0.90	0.85	0.85	0.87	0.88	0.95	0.95	0.91	0.91	0.92	0.91	0.87	0.88
Imp. B	1.39	1.40	1.30	1.30	1.40	1.40	1.49	1.50	1.38	1.38	1.43	1.41	1.35	1.37
Imp. F	1.68	1.69	1.56	1.56	1.72	1.73	1.81	1.82	1.63	1.63	1.73	1.71	1.64	1.65
Imp. G	1.87	1.88	1.73	1.73	1.93	1.94	2.02	2.04	1.80	1.81	1.92	1.90	1.83	1.84
Ebastine	2.14	2.17	2.00	2.02	2.24	2.27	2.28	2.32	2.03	2.06	2.19	2.20	2.08	2.12
Imp. E	2.30	2.34	2.14	2.16	2.41	2.45	2.46	2.51	2.17	2.21	2.35	2.36	2.24	2.28

Table 3
Validation study: validation data of the final analytical procedure: the results given prove that the acceptance criteria are met and therefore the UHPLC purity method for ebastine are fully validated in compliance to the ICH guideline Q2 (R1).

Test	Test details	Acceptance criteria	Results							
			Imp. A	Imp. B	Imp. C	Imp. D	Imp. E	Imp. F	Imp. G	Ebastine
Specificity			There are no peaks present in the chromatogram of the sample solvent and eluent at the retention time window of ebastine (RT = 2.2 min) and its known impurities and degradation products (RT = 0.7–2.5 min)							
Linearity	5 concentration points in the range 0.1–0.5 μg/ml	Coefficient of correlation > 0.999	0.99943	0.99978	0.99986	0.99907	0.99981	0.99975	0.99978	0.99931
Limit of detection	Calculated from the 95% estimation interval of the calibration line		0.058 μg/ml	0.035 μg/ml	0.028 μg/ml	0.070 μg/ml	0.032 μg/ml	0.037 μg/ml	0.031 μg/ml	0.061 μg/ml
Limit of quantification	Calculated from the 95% estimation interval of the calibration line		0.085 μg/ml	0.053 μg/ml	0.042 μg/ml	0.102 μg/ml	0.048 μg/ml	0.055 μg/ml	0.046 μg/ml	0.090 μg/ml
Accuracy and precision (repeatability)	$n = 9$	RSD < 5.0%	1.55%	3.18%	2.67%	2.62%	0.55%	0.58%	0.65%	2.34%
(Operator A)		Recovery rate between 98.0 and 102.0%	99.33%	98.15%	98.78%	98.15%	99.91%	100.3%	99.87%	98.51%
Intermediate precision	$n = 9$	RSD < 5.0%	0.95%	1.74%	1.73%	2.77%	0.89%	0.77%	0.53%	2.43%
(Operator B)		Recovery rate between 98.0 and 102.0%	100.27%	99.80%	98.65%	98.17%	99.68%	100.24%	99.90%	98.35%
		Mean-*t*-test must comply	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
System precision	$n = 10$	RSD < 2.0%	0.27%	1.31%	0.57%	1.40%	0.50%	1.00%	1.19%	1.86%

Table 4
Robustness study: the robustness of the developed method was studied with the aid of the 3-D Robustness Module of the DryLab®4 software. The six parameters tG (3 min ± 0.3 min), T (60 °C ± 6 °C), tC (50% ± 5% 2-propanol in acetonitrile), flow rate (0.5 ml/min ± 0.1 ml/min) and the %start (30% ± 2%) and %end (90% ± 2%) of the gradient were varied at 3 levels (+1, 0, −1). Only the first and last fifteen runs of the 729 experiments are shown here. All runs are within the required resolution of $R_{s,crit} > 2.0$.

Run No.	tG [min]	T [°C]	tC [%2-PrOH in ACN]	Flow rate [ml/min]	Start [%B]	End [%B]	R_s (crit.)
1	3	60	50	0.5	30	90	2.44
2	3	60	50	0.5	30	92	2.43
3	3	60	50	0.5	30	88	2.44
4	3	60	50	0.5	32	90	2.76
5	3	60	50	0.5	32	92	2.73
6	3	60	50	0.5	32	88	2.79
7	3	60	50	0.5	28	90	2.17
8	3	60	50	0.5	28	92	2.18
9	3	60	50	0.5	28	88	2.17
10	3	60	50	0.6	30	90	2.46
11	3	60	50	0.6	30	92	2.45
12	3	60	50	0.6	30	88	2.46
13	3	60	50	0.6	32	90	2.86
14	3	60	50	0.6	32	92	2.85
15	3	60	50	0.6	32	88	2.87
...							
715	2.7	54	45	0.6	32	90	2.52
716	2.7	54	45	0.6	32	92	2.51
717	2.7	54	45	0.6	32	88	2.53
718	2.7	54	45	0.6	28	90	2.07
719	2.7	54	45	0.6	28	92	2.07
720	2.7	54	45	0.6	28	88	2.07
721	2.7	54	45	0.4	30	90	2.2
722	2.7	54	45	0.4	30	92	2.19
723	2.7	54	45	0.4	30	88	2.2
724	2.7	54	45	0.4	32	90	2.41
725	2.7	54	45	0.4	32	92	2.39
726	2.7	54	45	0.4	32	88	2.42
727	2.7	54	45	0.4	28	90	2.06
728	2.7	54	45	0.4	28	92	2.06
729	2.7	54	45	0.4	28	88	2.06

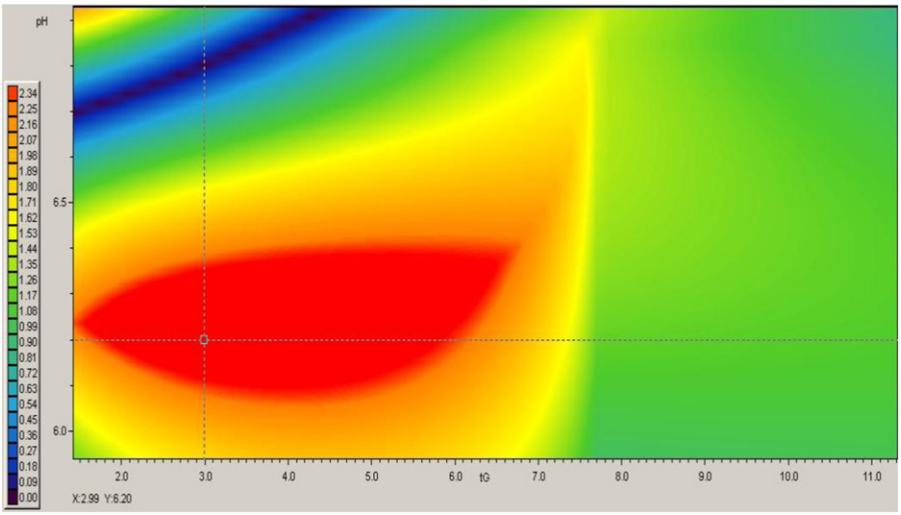

Fig. 7. In an additional set of experiments the influence of the pH was studied. The pH of aqueous eluent A was varied between pH 6.0 and pH 6.85 and the figure show the 2-D resolution map of R_s vs. pH and gradient time: It can be seen from the resolution map that the method is robust in the pH range between 6.0 and 6.4 and a gradient time between 3 and 5 min and provides the highest peak resolution ($R_s > 2.0$).

Table 5
Description of the final analytical procedure incl. the tolerances.

Column	Acquity UPLC® BEH C18; 2.1 mm × 50 mm, 1.7 μm (Waters)
Eluent A	10 mM acetate buffer pH6.2 (±0.1 pH units)
Eluent B	50% (±5%) 2-propanol in acetonitrile
Gradient	Linear increase from 30% (±2%) to 90% (±2%) of eluent B in 3.0 min (±0.3 min), followed by re-equilibration
Stop time	4 min
Flow rate	0.50 ml/min (±0.10 ml/min)
Column temp.	60 °C (±6 °C)
Injection volume	5 μl
Detection	UV 210 nm

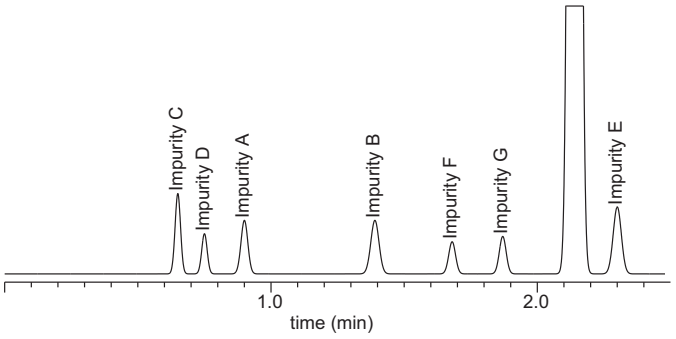

Fig. 8. Predicted chromatogram for conditions at the working point (for details see text).

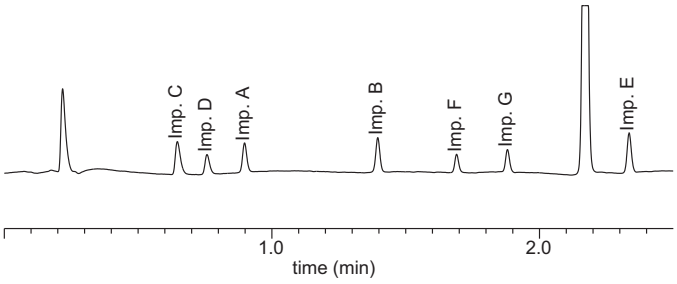

Fig. 9. Experimental chromatogram of a real sample spiked with impurities A–G for conditions at the working point (for details see text).

for purity testing of impurities and degradation products in ebastine API and different pharmaceutical formulations and can also be used for assay and dissolution testing of the drug product. A typical chromatogram of selectivity standard solution (ebastine spiked with all impurities A–G) is shown in Fig. 9.

The method history report stated that the new method has been used for the last two years without any issues.

4. Summary

An innovative Quality by Design approach for development of a stability indicating fast and reliable UHPLC method has been presented in this article. All previously defined method goals were met: Baseline separation of ebastine and its impurities were achieved with a critical resolution $R_{s,crit} > 2.0$.

A Design Space – a volume in which the method is robust – is defined and visualized. Also, the predicted retention times and resolution values are in excellent agreement with experimental values. The method was fully validated in compliance with ICH guidelines and a robustness study was performed by varying six chromatographic parameters. The analysis time is less than 4 min, which is a impressive 40-fold increase in productivity in comparison to the method published in the E.P. monograph and allows purity testing of more than 360 samples per day.

All experiments (screening and optimization study as well as the verification and validation study) were made within one week. Together with planning and reporting the whole method development process was finished within three weeks.

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