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Establishing column batch repeatability according to Quality by Design (QbD) principles using modeling software

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ABSTRACT

Column technology needs further improvement even today. To get information of batch-to-batch repeatability, intelligent modeling software was applied. Twelve columns from the same production process, but from different batches were compared in this work. In this paper, the retention parameters of these columns with real life sample solutes were studied. The following parameters were selected for measurements: gradient time, temperature and pH. Based on calculated results, batch-to-batch repeatability of BEH columns was evaluated. Two parallel measurements on two columns from the same batch were performed to obtain information about the quality of packing. Calculating the average of individual working points at the highest critical resolution ($R_{s,crit}$) it was found that the robustness, calculated with a newly released robustness module, had a success rate >98% among the predicted $3^6 = 729$ experiments for all 12 columns. With the help of retention modeling all substances could be separated independently from the batch and/or packing, using the same conditions, having high robustness of the experiments.

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1. Introduction

Column technology has been improved dramatically in the last thirty years, but batch-to-batch repeatability is still a critical issue, even today. There are several column tests to control the quality of columns. Most of them use small molecules and give some information about column chemistry, but outside of investigated conditions no further estimation of selectivity and retention behavior is given, although it is well known, that small differences in column chemistry and in eluent composition as well might result in dramatic effects on the separation.

Based on the experience with HPLC modeling [1] which was started under the leadership of Lloyd R. Snyder, new features were developed to be able to establish methods in accordance with Quality by Design (QbD) principles and also to continue the work of Dolan–Snyder [2]. A new and simple extension of 2-dimensional models into a third direction, the Cube (Fig. 1) could be established [3]. At the same time based on the high speed of modeling technology, ACN could be replaced by MeOH and the best methods could be found faster than before [4,5] and HPLC became more environmentally friendly. In this work we intended to use the same approach for column comparisons in more detail and try suggesting a new way of evaluation of batch-to-batch repeatability.

Producing columns by different vendors with the same separation characteristics is often problematic today. The importance of establishing well-working, robust methods in the pharmaceutical industry is also crucial. For this purpose – among other factors – one main factor is to have an efficient column, and a method which needs to be validated. Often is the production of a validated column discontinued by the manufacturer. Also starting materials are changed by selection of a different supplier of the chemicals which might cause retention alternation. Particle degradation or differences in the packing procedure can also occur, resulting in unacceptable chromatographic results. In these cases the method has to be revalidated in a tedious process called “post approval change” for regulatory acceptance. The aim of column selection is to find a column which is rugged enough to overcome the differences between batch-to-batch, and also helping to find a replacement column to meet the requirement of the authorities. Using rugged column, the next step is to find the best separation and shorten analysis time according to QbD principles [6] by allowing a control strategy and continual improvement.

There are different ways to find an acceptable column or a replacement one. For example with Column Match® we could help to find acceptable columns. The Snyder–Dolan column selectivity factor, Fs is critical for the replacement of columns in regulated

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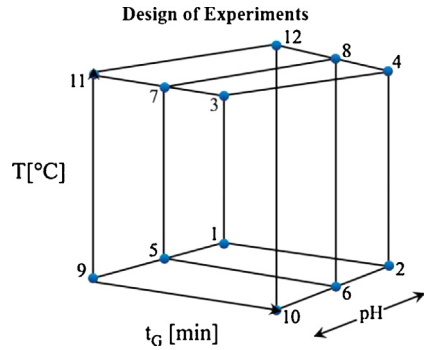


Fig. 1. Design of experiments, used to obtain the DryLab® 4 models. The experiments 1, 2, 5, 6, 9 and 10 were carried out at 20 °C, the experiments 3, 4, 7, 8, 11 and 12 were carried out at 50 °C. The experiments 1, 3, 5, 7, 9 and 11 were carried out with steep gradient t_{G1} = 1.5 min, the flat gradient experiments 2, 4, 6, 8, 10 and 12 with t_{G2} = 4.5 min. The pH of eluent A was 3.2 with exp. 1, 2, 3 and 4, it was 3.8 with exp. 5, 6, 7 and 8, and it was 4.4 with exp. 9, 10, 11 and 12.

processes [7], which are written down in validation protocols [8]. An additional approach is to use 3-dimensional resolution spaces for column selectivity and column performance comparisons [9–11] before validation. The advantage of this method is that no database was needed to characterize the selected columns. In this way information can be generated about the retention process [12,13] also. After creating a design space and validating the methods, one can work with acceptable resolution values at different working points and use the column without the need for a revalidation of the method [14–16]. Due to the much greater degree of flexibility, more and more companies are using modeling prior to validation and include the results into Knowledge Management documents [3].

2. Experimental

2.1. Chemicals

Acetonitrile (gradient grade), formic acid and sodium formate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Water was made freshly by ELGA Maxima (Lane End, UK).

2.2. Eluents

Eluent A was prepared by combining volumes of 10 mM formic acid (HCOOH) (=385 μL 98% HCOOH/1000 mL water) and 10 mM sodium formate (HCOONa) (=0.68 g HCOONa/1000 mL water). For the pH 3.2-solution 837 mL 10 mM HCOOH and 163 mL 10 mM HCOONa, for the 3.8-solution 467 mL 10 mM HCOOH and 533 mL 10 mM HCOONa, for the pH 4.4-solution 172 mL 10 mM HCOOH and 828 mL 10 mM HCOONa were mixed. Buffer compositions were calculated by Buffer Maker® buffer calculator software (BPP Marcin Borkowski, Marki, Poland) which reliability was tested at the method development part of amlodipine [9].

Eluent B was acetonitrile. For gradient elution two gradients with a factor 3 difference in the gradient time were selected: $t_{G,1}$ was 1.5 min, $t_{G,2}$ was 4.5 min, gradient range was 30 → 90%B. On our system at the flow rate of 0.8 mL/min the dwell volume means approximately 0.16 min delay in gradient (0.125 mL) and the t_0 was approximately 0.15 min. This gradient time is acceptable for achieving quick information about the column characteristics.

2.3. Sample

The test samples contained 10 μg/mL amlodipine and its European Pharmacopeia (Ph. Eur.) impurities (A, B, D, E, F, G, H) in the same concentration. Amlodipine and its impurities were purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM, Strasbourg, France). Sample solvent was (acetonitrile:water) (30:70 v/v). The structures of the compounds are shown in Fig. 2.

2.4. Columns

12 different columns of Acquity BEH C18 50 mm × 2.1 mm, 1.7 μm (see Table 1) were purchased from Waters (Milford, USA).

2.5. Equipment and software

UHPLC separations were made with Waters Acquity UHPLC with Binary Solvent Manager, Sample Manager, Column Manager which enabled to work with 4 columns and PDA detector. The UV detection was performed at 237 nm. The system has 0.125 mL dwell volume and 0.0025 mL extracolumn volume.

The chromatographic data were obtained by Empower 3 chromatography data software (CDS) (Waters, Milford, MA, USA). After integration, the chromatograms were exported into the AIA/ANDI-format (*.cdf) (including retention times and peak areas) and were transferred to the modeling tool for further processing of the peak tracking. Separation modeling was carried out with DryLab® 4, version no. 4.2.0.7. (Molnár-Institute, Berlin, Germany), which also included the Snyder–Dolan hydrophobicity subtraction database (Column Match).

2.6. Design of experiments (DoE)

The conditions for carrying out the 12 experiments prior to calculation of the Cube (12 experiments) are described in the legend of Fig. 1.

2.7. Processing of the experiments: time requirement for 4 columns

The lower temperature (20 °C) was selected for the first six experiments (see Fig. 1) because column-heating can be obtained faster than column cooling. To get reliable information of temperature we selected lower temperature than ambient one. The most modern instruments are allowing to use the temperature about 5 °C. Temperature equilibration time (t_{TE}) was 30 min to achieve stable conditions for temperature. In this time the columns were started to wash with (water:acetonitrile)-mixtures for a period of 6–7 min/column.

Measurement with pH_1 buffer: 3 injections with $t_{G,1}$ gradient time carried out on each column were carried out (see run no. 1 in Fig. 3). After the 2nd repetition we had a fairly stable pH-equilibrium. Following this, no. 2 ($t_{G,2}$) experiment was started (see Fig. 3). Injection time (t_{inj}) took 1 min. In this period the return from 90%B to 30%B eluent composition reached eluent equilibration on the 50 × 2.1 column. At a flow rate of 0.8 mL/min, this means 0.8 mL of 30%B-eluent was passing through the column.

One column has a time requirement (t_{req}) with pH_1 buffer according to the equation:

$$t_{req} = 3 \cdot (t_{inj} + tG_1) + (t_{inj} + tG_2) = 7.5 + 5.5 = 13\,\text{min} \quad (1)$$

The required time for 4 cubes for 4 columns:

$$t_{cube} = 4_{no.col.} \times 3_{no.buffers} \times 2_{temp.layers} \times t_{req} = 4 \times 3 \times 2 \times 13 = 312\,\text{min} \quad (2)$$

In practice the flow rate have to be increased slowly and also we have to wait to have a stable flow ($t_{st.flow}$), therefore 15 min

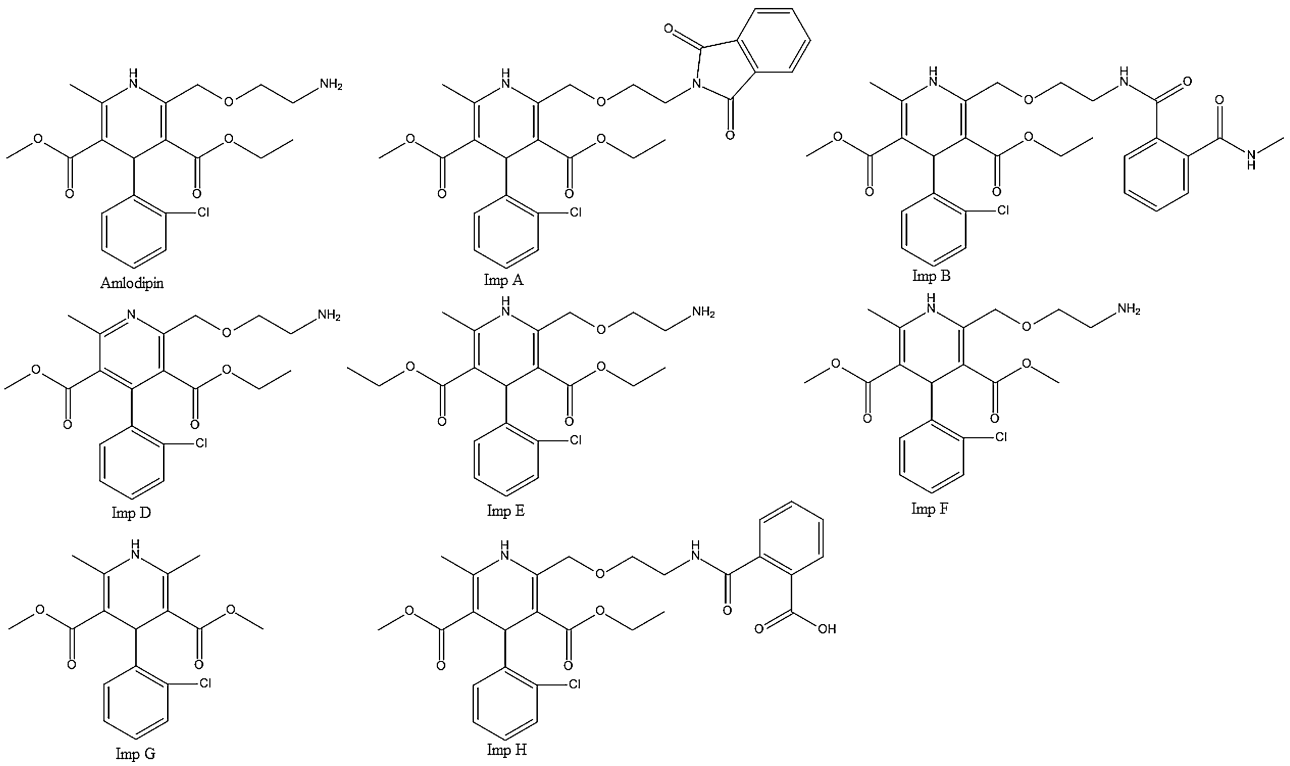


Fig. 2. The molecular structures of the compounds used in this work; amlodipine and impurities A, B, D, E, F, G, H.

Table 1
The parameters of the Waters Acquity BEH C18 50 mm × 2.1 mm, 1.7 μm columns which were used in the experiments.

Number of the column	Batch number	90%/10% diameter ratio	Pore volume (cm^3/g)	Pore size (Å)	Surface area (m^2/g)	Metal content			Carbon load (%)	^{18}C surface coverage (μmol/m^2)	Production year
						Na (ppm)	Al (ppm)	Fe (ppm)			
Waters Acquity BEH C18 50 × 2.1 mm, 1.7 μm (hybrid)											
1	154	1.51	0.75	140	188	1	<1	3	17.58	3.16	2007 November
2	158	1.49	0.75	146	189	2	<1	3	17.69	2.91	2008 February
3	159/1	1.49	0.75	149	184	<1	<1	<1	17.37	3.05	2008 March
4	159/2	1.49	0.75	149	184	<1	<1	<1	17.37	3.05	2008 March
5	169	1.49	0.75	145	186	3	2	4	17.82	3.22	2008 September
6	170	1.52	0.75	144	185	2	<1	3	17.74	3.08	2008 September
7	179	1.51	0.75	139	183	<1	1	2	17.37	3.05	2008 December
8	182	1.51	0.68	135	180	<1	1	2	17.30	3.16	2009 January
9	205/1	1.49	0.68	138	179	<1	<1	<1	17.02	3.04	2011 October
10	205/2	1.49	0.68	138	179	<1	<1	<1	17.02	3.04	2011 October
11	209	1.44	0.71	140	183	1	<1	1	17.19	3.08	2011 December
12	221	1.50	0.70	138	183	1	<1	1	17.40	3.07	2012 November

per column should be calculated with. This means, on 4 columns 3 buffer solutions 2 times are needed (because of the temperature layers), i.e.

$$t_{\text{flow}} = t_{\text{st.flow}} \times 4_{\text{no.col.}} \times (2_{\text{buffer change at lower } T} + 2_{\text{buffer change at higher } T}) = 15 \times 4 \times 4 = 240\,\text{min} \quad (3)$$

Changing eluent composition takes ca. 5 min, that has to be done four times on each column, takes additional 80 min (t_{eluent}).

Summarizing the total time (t_{total}), we get

$$t_{\text{total}} = t_{\text{TE}} + t_{\text{cube}} + t_{\text{flow}} + t_{\text{eluent}} = 60 + 312 + 240 + 80 = 692\,\text{min} \approx 11.5\,\text{h} \quad (4)$$

So far the total time required for 4 columns is somewhat more than 11.5 h, i.e. 3 h/column.

3. Results and discussion

3.1. Peak tracking and peak movements

An HPLC method of amlodipine was described in the European Pharmacopoeia with an analysis time of ca. 60 min. Especially

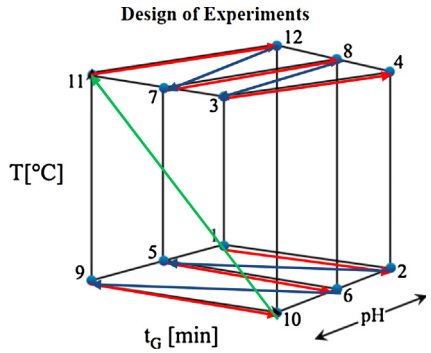


Fig. 3. The process of measuring the design space. First the lower temperature (T_1) experiments were selected on each column then the higher temperature (T_2) experiments on each column. The red arrows are representing the change of the gradient time (t_{cube}) (s. Eq. (2)), the blue arrows are representing the change of the buffers (t_{eluent}) and the green arrow is representing the change of the temperature (t_{TE}). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the separation of 3 impurities B, G and H was problematic due to unknown effects, which were first assumed to be pH dependent problems of the different columns selected [9]. As it can be seen in Fig. 2, the measured compounds are chemically either neutral (impurities A, B, G), or acidic (impurity H) or some are basic (amlodipine, impurities D, E, F). All these next considerations were elaborated by modeling DryLab®4-Cubes, which are based on the fundamentals described in the theory of solvophobic interactions [12,13]. If the elution is performed at an acidic pH (pH is less than basic compound's pK_a-2), the basic amino groups are completely protonated, highly hydrated and less retained. As they become less polar (i.e. the ratio of the protonated vs. neutral molecules will be lower) they will dissolve less in water which has a decisive influence on retention. This can be visualized by DryLab®4 if we are measuring near pK_a values of the compounds. Impurity H is the only component of the mixture, which has an acidic character because of the carboxylic group, thus it is protonated (neutral) at lower pH (below pK_a-2). A transition can be observed between the ratio of the protonated and deprotonated structures by increasing the pH (near pK_a). Thus the retention time of impurity H could be predicted. The temperature causes impurity G and B to elute with different speeds, due to their large difference between their molecular weight (MW_G = 335.8, MW_B = 570.0) and accessible molecular surface area. G elutes faster with the temperature change and the result is, that the peaks are changing their positions.

Finally it has to be emphasized, that this sample or similar samples are useful for column characterization, moreover this approach is practically much more representative than generic column tests. This point is an important milestone in column testing and it is a major message of this paper.

3.2. Behavior of columns

Multifactorial column testing was started by Snyder and Dolan in 2000 already [2], however they turned in 2004 to a different type of column characterization approach, to the so called "Hydrophobicity Subtraction Database", which is a part now of the DryLab software [7]. Based on the requirement of many column users, the Molnar group continued with the multifactorial evaluation idea presented in the Dolan–Snyder work [2]. Two other widely used column characterization descriptions are the Tanaka approach [17–19] and the so-called SRM 870 (Standard Reference Material) approach [18–20]. The Tanaka approach determines 6 chromatographic properties with 8 test compounds in order to achieve empirical column characteristic parameters such as hydrophobicity, hydrophobic selectivity shape selectivity, hydrogen bonding capacity, total ion-exchange capacity and acidic ion-exchange capacity [19,21]. The SRM 870 approach uses 5 test compounds in order to model the character of column. This also uses empirical equations for finding replaceable columns such as describing the hydrophobicity and silanol activity of the column [19,20]. These approaches based on empirical assumptions in order to describe the behavior of the reversed phase. Most approach uses Principal Component Analysis (PCA) [17–19,21–24] in order to separate different groups which are similar. Another option is to use else statistical or chemometrical tools [20,25–27].

Amlodipine and its Ph. Eur. impurities were perfect molecules to characterize the behavior of a column, because all the 3 eluent parameters have some effect to at least one compound. There was the mentioned movement of the peaks (H, G, B) in all the different cubes, as the blue lines reflected it, along which peaks overlapped. The red zones are the MODRs (Method Operating Design Regions) in which the critical resolution is higher than 1.5 ("baseline resolution") (see Fig. 4)

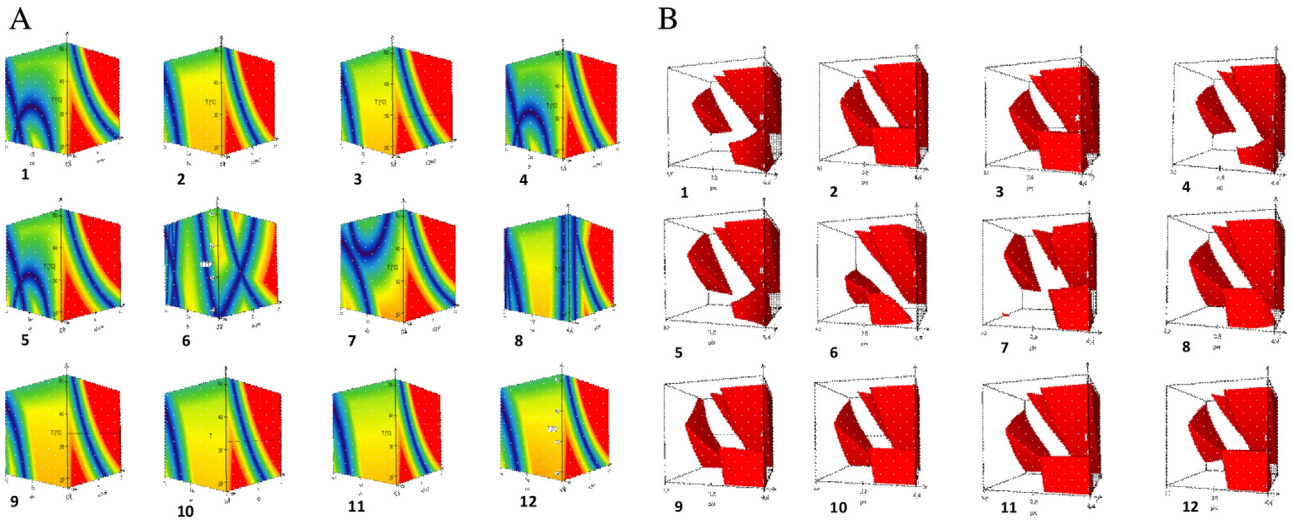


Fig. 4. The DryLab®4 models (A) and the MODRs (Method Operating Desing Regions) (B) of the examined 12 BEH C18 columns. 3–4 and 9–10 are from the same production batch.

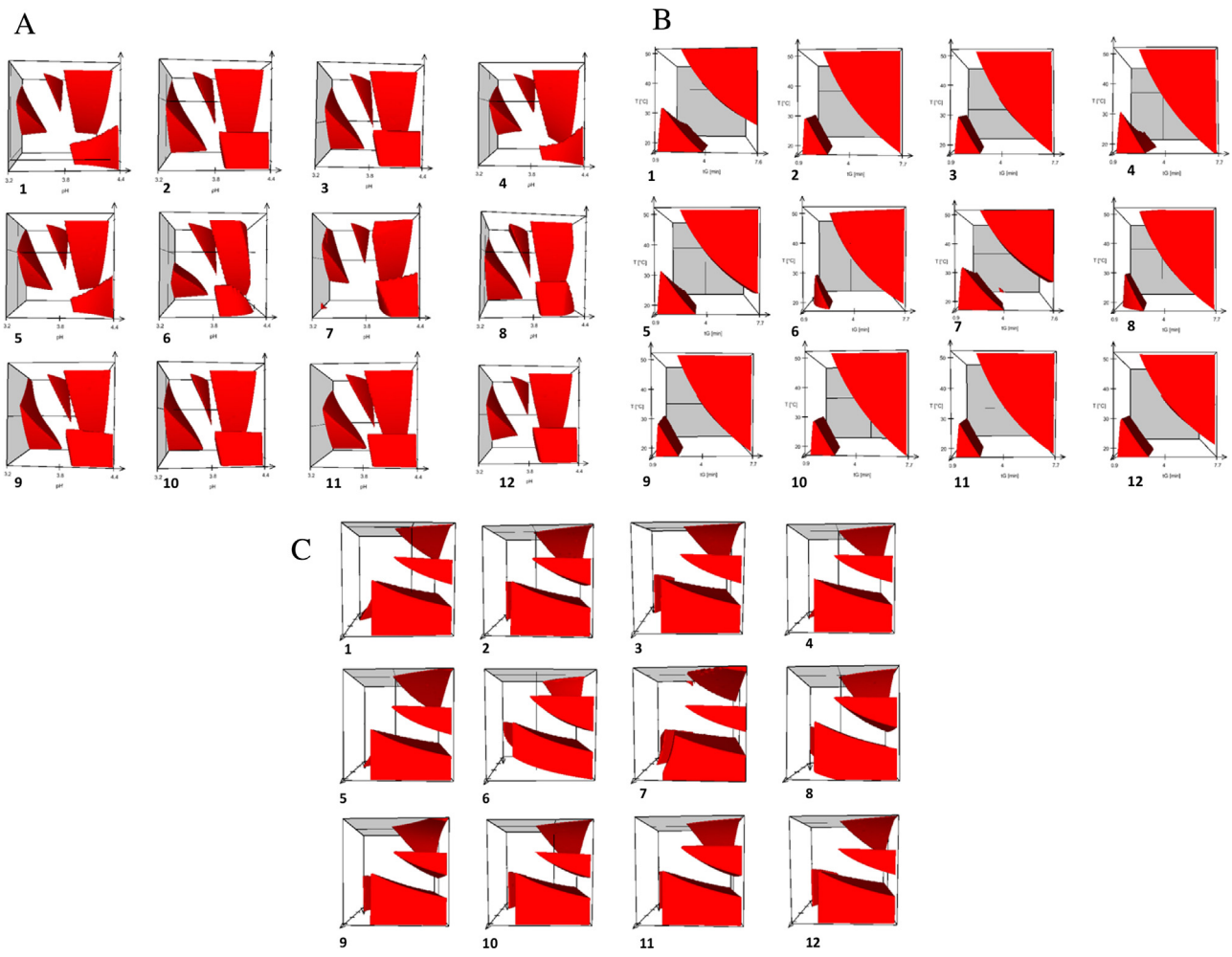


Fig. 5. Different aspects of the MODRs from different views. (A) Side view from the pH axis, (B) front view from the gradient time axis, (C) view from the top of the cube.

Different aspects to select "equivalent" columns were used. Three different groups could be isolated: I. 1-4-5, II. 2-3-9-10-11-12 and III. the "others" (6-7-8) (see Fig. 5). The selection of columns into this 3 groups is based on the graphical similarity of their cubes, i.e. they were selected as "Equivalent Columns" as their MODR's (the irregular red bodies = design spaces, inside their cubes) were almost identical. This is the reason to group them, to find alternative possibilities to replace a column, which might be not available anymore.

Temperature and pH could cause impurities B, G, H to overlap. The gradient-time-decrease strongly reduces the critical resolution between impurity D and F. At the column 6 and 8, two more critical peak-pair could be identified (impurity B and G with impurity E). The basic compounds were measured with longer retention, thus impurity E can overlap with either H (at higher pH) or G (at higher temperature).

The divergence between the measured cubes shown in chromatograms was acceptable. (see Fig. 6), the MODRs are in the same place in the cube, and only the conditions (shape) differs. The highest critical resolution at the working points each proves the assumption, that the columns has similar processing methods in their production (Table 2) as the deviation is not high. Although columns no. 6 and 8 have conspicuous differences in the retention of the basic compounds (see the red boxes of Fig. 6). The column characteristic values were provided by the manufacturer (see Table 1). These reflect the initial stage of columns but do not give any information about the change of the characteristics during using the column. These eluted later, presumably the longer use of the column in practical work in the routine lab. The usage affects directly the accessible silanol-group concentration and activity. However even with these column adjustments could be done, so that the method would remain valid, as far as the working point remains inside the MODR. This is a great advantage to gain more flexibility in the Quality Control (QC) department as far as prerequisites for it, namely to use the concept as described here, with well-designed experiments and combined with the modeling software was fulfilled.

Table 2
Conditions of the working points of each column at the highest critical resolution ($R_{s,crit}$). These are almost the same conditions: $t_{G,\ average}$ = 7.58 min (s = 0.00 min, 0% RSD), $T_{average}$ = 33.77 °C (s = 2.38 °C, 7.0% RSD), $pH_{average}$ = 4.30 (s = 0.08, 1.9% RSD). Critical peak resolution average is 3.32 (s = 0.07, 2.1% RSD).

No.	t_G (min)	T (°C)	pH	($R_{s,crit}$)	Robustness (% of successful experiments with $R_{s,crit}$ > 1.5)
1	7.58	38.30	4.21	3.31	94.51%
2	7.58	32.42	4.40	3.40	99.18%
3	7.58	31.69	4.26	3.33	95.88%
4	7.58	34.63	4.21	3.42	93.83%
5	7.58	36.09	4.26	3.35	95.61%
6	7.58	32.42	4.33	3.30	93.69%
7	7.58	36.83	4.21	3.16	92.87%
8	7.58	30.95	4.23	3.29	91.22%
9	7.58	33.89	4.40	3.37	98.77%
10	7.58	32.42	4.23	3.29	90.81%
11	7.58	30.95	4.40	3.28	99.59%
12	7.58	34.63	4.40	3.28	100.00%

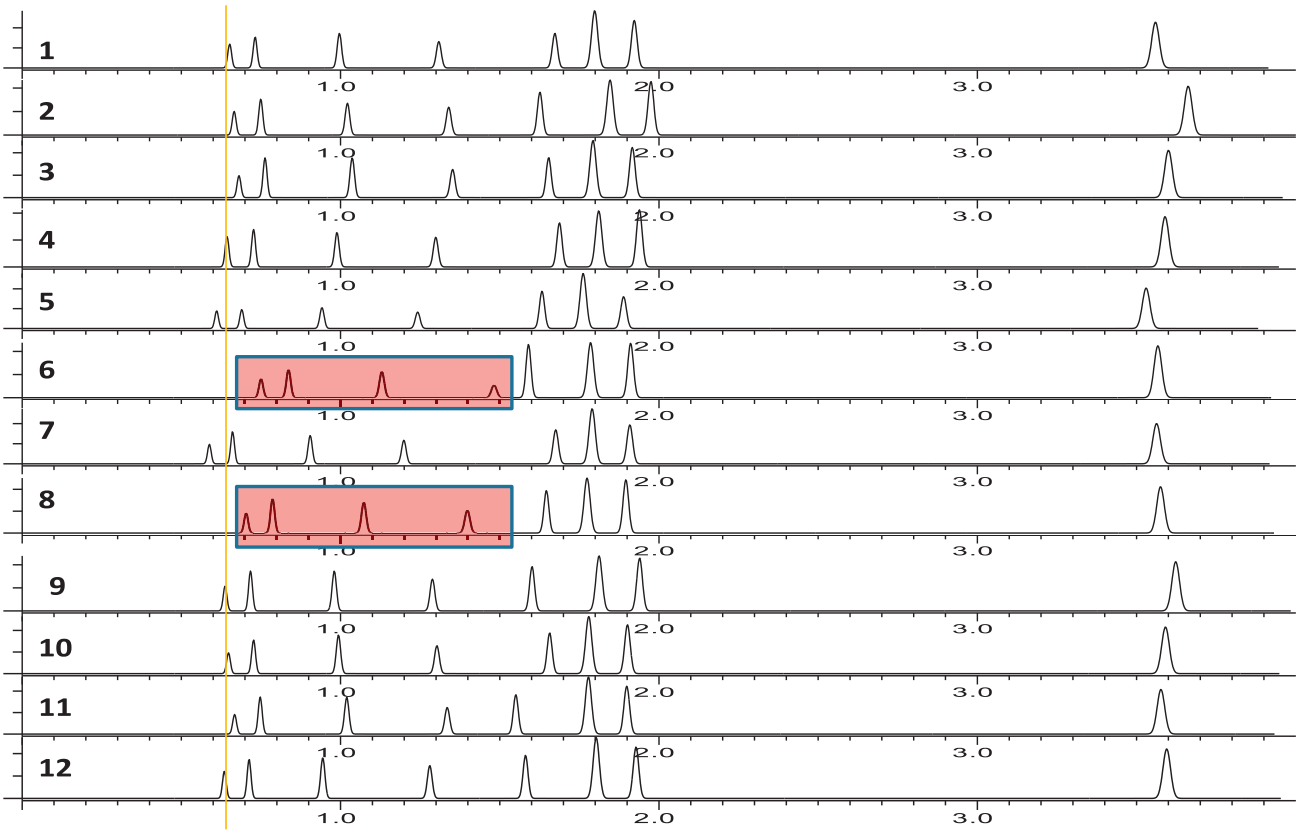


Fig. 6. Chromatograms of the highest resolution of each column. The retention order is impurities D, F, amlodipine, impurities E, H, G, B, A. Basic compounds (red boxes) are retarded more in the columns no. 6 and 8. The critical resolution however is sufficient with all columns. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

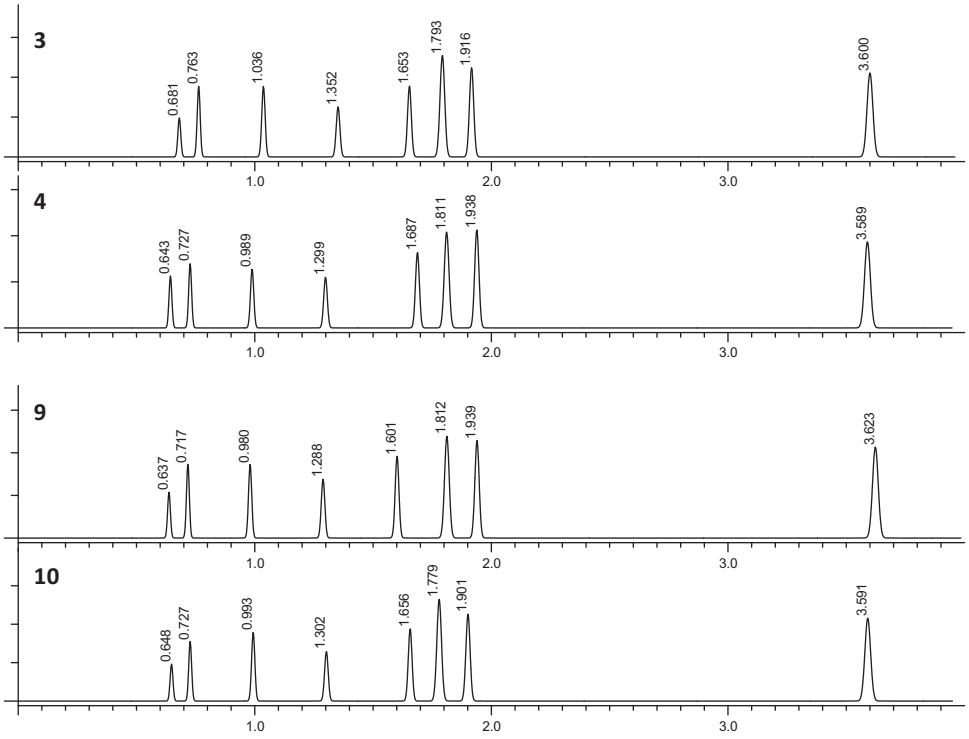


Fig. 7. The chromatograms of the identical batches (3–4 and 9–10) at the working point of the highest critical resolution.

Table 3
The retention times (t_R), its averages and standard deviations (SD) of the same batches in minutes. The average standard deviations of all peaks were 0.02 min (0.018 min at 3–4 and 0.023 min at 9–10) (ca. 1.6%).

No.	Impurity D			Impurity F			Amlodipine			Impurity E		
	t_R	Average	SD	t_R	Average	SD	t_R	Average	SD	t_R	Average	SD
3	0.681	0.662	0.027 (4.1%)	0.763	0.745	0.025 (3.4%)	1.036	1.013	0.033 (3.3%)	1.352	1.326	0.037 (2.8%)
4	0.643			0.727			0.989			1.299		
9	0.637	0.643	0.008 (1.2%)	0.717	0.722	0.007 (1.0%)	0.980	0.987	0.009 (0.9%)	1.288	1.295	0.010 (0.8%)
10	0.648			0.727			0.993			1.302		

No.	Impurity H			Impurity G			Impurity B			Impurity A		
	t_R	Average	SD	t_R	Average	SD	t_R	Average	SD	t_R	Average	SD
3	1.653	1.670	0.024 (1.4%)	1.793	1.802	0.013 (0.7%)	1.916	1.927	0.016 (0.8%)	3.600	3.595	0.008 (0.2%)
4	1.687			1.811			1.938			3.589		
9	1.601	1.629	0.039 (2.4%)	1.812	1.796	0.023 (1.3%)	1.939	1.920	0.027 (1.4%)	3.623	3.607	0.023 (0.6%)
10	1.656			1.779			1.901			3.591		

It should be mentioned, that method robustness is dependent on the position of the working point as well as on the size of the instrument-dependent tolerance limits, which can be modeled within the Robustness Module of DryLab®4.

Columns with the same batch were also measured as mentioned above. Because the particles were from the same batch, the quality of the column packing-procedure could be discussed. Some user would like to consider the "true" peak widths for the model that is based on such fast gradients. However DryLab is not a peak-shape, but a movement modeling tool. In DryLab the "true" peak width is replaced by a virtual plate number, which is based on the comparison of the separation quality of critical peak pairs.

The average retention time variations in the modeled chromatograms (Fig. 7) had 0.02 min standard deviation (ca. 1.6%) (Table 3). The consequence is that the two-two columns are equal. The difference between the retention times of these columns can be explained by the measurement error. This means that the repeatability is only dependent of the duration in the usage of the columns.

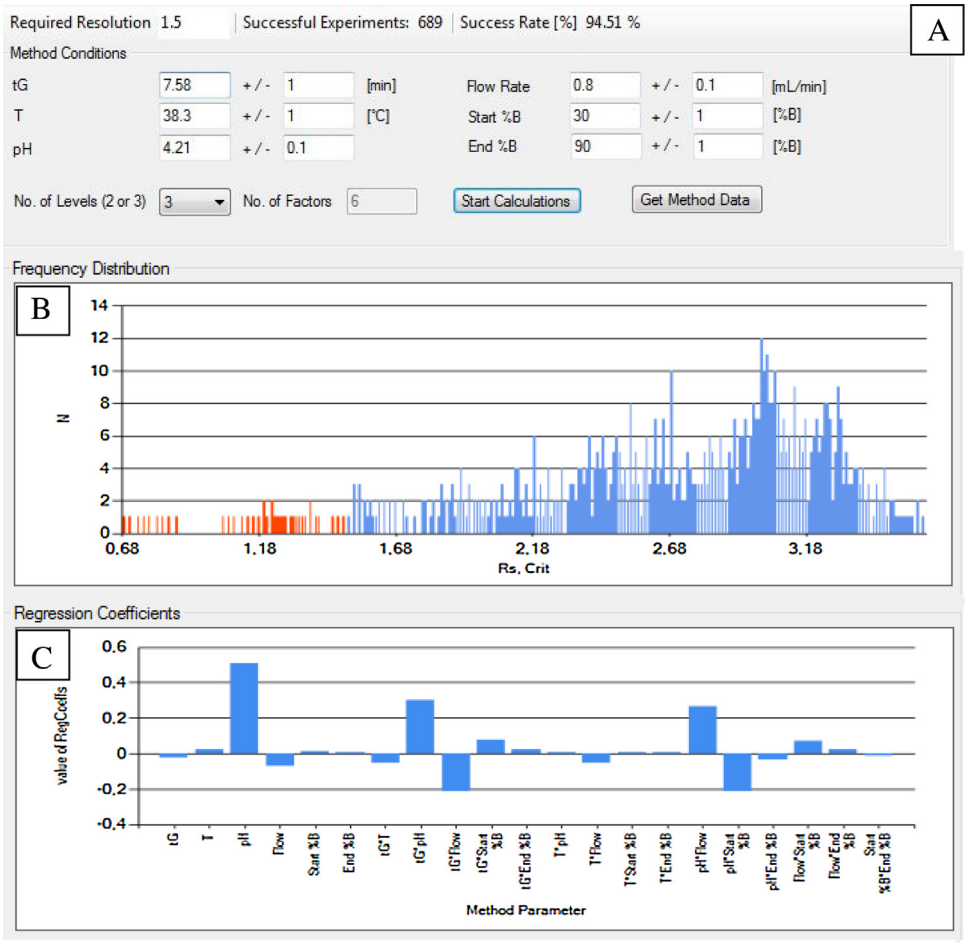


Fig. 8. The examined parameters and their levels of column no. 1. (A) (Batch no. 154) in the DryLab®4 robustness module. Picture B (frequency distribution of the critical resolution) shows the success rate (blue) (94.51%) in $3^6 = 729$ virtual experiments. Picture C shows the influence of several parameters to the robustness (regression coefficients) of the 729 of runs. The parameters are t_G, T, pH, $\%B_{start}$, $\%B_{end}$ and their combined effects. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

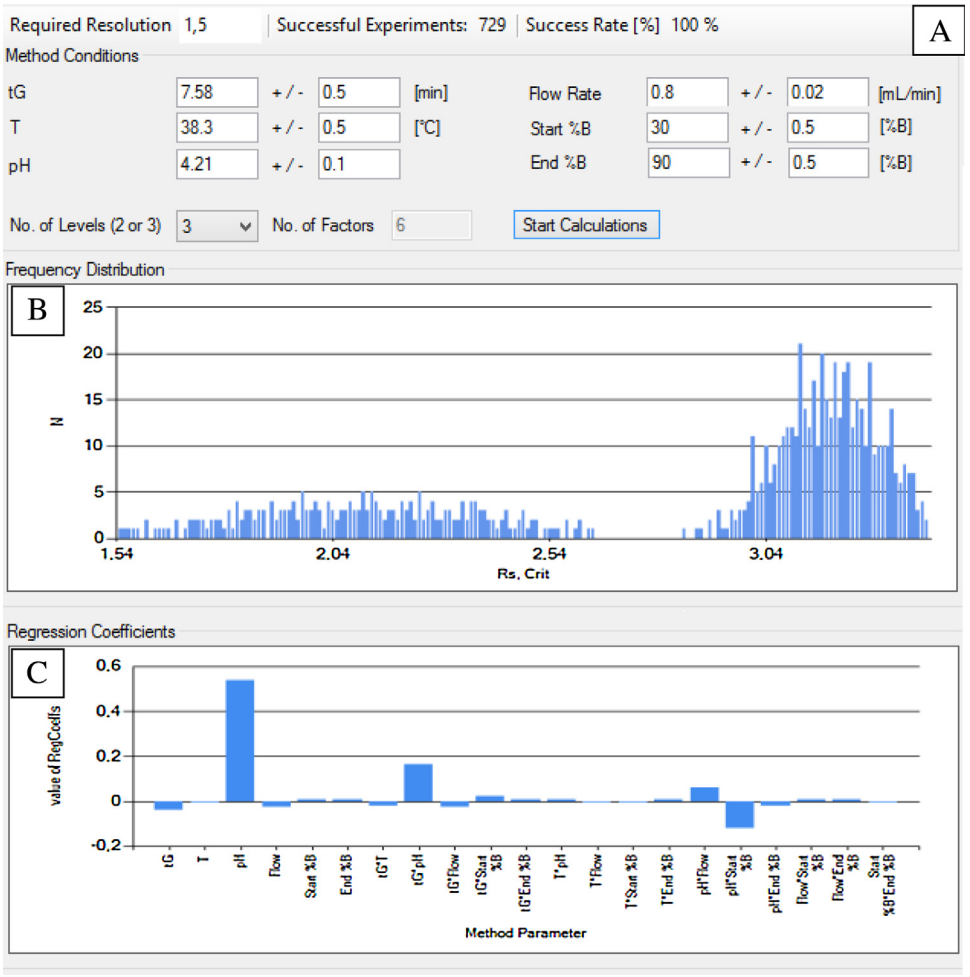


Fig. 9. The tolerance limits were changed to the performance values of the instrument (C). Regression coefficients (A) and the frequency distribution of the critical resolution (B) in the 729 virtual experiments of column no. 1 (Batch 154) in the DryLab®4 Robustness Module.

3.3. Robustness calculation

One of the most important goals in method development is the determination of conditions where the method is robust and small variations will not reduce the Analytical Target Profile (ATP), which is the critical resolution [28,29]. In our study the main focus in this respect was the batch-to-batch repeatability in a given type of column. The prediction without knowledge of the peak movement is not possible. At the working points of each column, successful model experiments were calculated by the Robustness Module of DryLab®4. The following 6 parameters and their interactions were investigated at 3 levels: the three variables t_G, T, pH, the flow rate F, and the initial and final compositions of the mobile phase $\%B_{start}$ and $\%B_{end}$ (Fig. 8). As it can be seen in Fig. 9, the influence of the parameters is different on separation. If the conditions such as the pH, are decisive (have a great influence to the success of the separation), and are kept under control, one will achieve more successful experiments. In our study exact amounts of chemicals (calculated

Table 4
The % rate of successful experiments at the working point (WP) of each column (Table 1 and at the average working point ($t_{G,average}$ = 7.58 min, $T_{average}$ =33.77 °C, $pH_{average}$ = 4.30). The criteria of the critical resolution were 1.5. The comparing tolerance limits were the values seen on Fig. 8 and the system performance tolerance limits were the values seen on Fig. 9.

No.	WP of each column (% success rate)		Average WP (% success rate)	
	Comparing tolerance limits	System performance tolerance limits	Comparing tolerance limits	System performance tolerance limits
1	94.51	100.00	99.45	100.00
2	99.18	100.00	99.18	100.00
3	95.88	100.00	99.59	100.00
4	93.83	100.00	100.00	100.00
5	95.61	100.00	99.86	100.00
6	93.69	100.00	93.28	100.00
7	92.87	99.18	99.31	100.00
8	91.22	96.84	100.00	100.00
9	98.77	100.00	98.77	100.00
10	90.81	94.92	99.18	100.00
11	99.59	100.00	99.59	100.00
12	100.00	100.00	100.00	100.00

with Buffer Maker®) to adjust the pH were used, instead of titration; hence less errors could be obtained. The pH adjustment is one of the most critical parameter in liquid chromatography. According to IUPAC recommendation [30], making reproducible buffer is based on mass measurement. Also significant improvement can be observed through changing the individual best working points of each column to the average value (Table 4) (except for column 6, where the basic compounds eluted later). Our results showed that columns from different batches are equivalent and without any difficulties one can use a new column when an old one is out of order. Based on these experiments one can suggest to use a modeling software to evaluate of equivalence of new column or to evaluate of equivalence of replacement one. Any restriction to a method might be a reason to be out of compliance, which is causing a great deal of post approval changes, is expensive and is often unnecessary. A DoE based peak-movement understanding is the solution. This knowledge can be elaborated by method modeling.

4. Summary

As in the old European Pharmacopoeia method there were several problems with the repeatability of the separation, one first believed, that the pH or the column chemistry caused the rather low quality of the method. Kormány et al. [9] proved however that only the temperature was a critical factor and they could reduce the time of the analysis from 60 min below 6 min. The goal of this study was to investigate a tool for evaluating the column replacement from the same vendor and estimate equivalency from column-to-column. In the pharmaceutical industry Quality by Design (QbD) is a basic principle and LC separations must follow this. Without using modeling software it is very hard to find conditions where the method is robust (QbD). Even in reversed phase chromatography small variations in surface chemistry result in differently deteriorated separations. First the movements of peaks had to be clarified. As test compounds, neutral, basic and acidic molecules were used. Their influence was easily learned with the help of the DryLab®-Cubes. All three model parameters (t_G, T, pH) did influence the separation selectivity, causing different degrees of peak overlaps. Thus the sample was perfect for carefully searching robust areas. Working points were set at the highest critical resolution values inside of MODR. Comparing these points the difference between the 12 columns is negligible because of the low SD values. To prove this thesis, the robustness was calculated at each working point. The success rate of the experiments was ca. 90%, which means 1 measurement will fail from 10. This is not rugged enough in the pharmaceutical industries; hence this rate had to be increased. It can be done by reducing the tolerance limits of the parameters to the best capabilities of our LC-instruments or change the working points, and could set a new working space. With the comparison of 12 columns, we averaged the conditions of the working points, and could set a new working space. Measuring their individual robustness, we got significant improvements among the successful experiments. The new working point is more robust for the columns, so we intend to work here. Hence we can confidently declare that these columns are fully interchangeable, a revalidation process is not necessary.

Finally we can state that using resolution cubes helps evaluating column equivalency and show the best and most robust working conditions for a given set of solutes. In pharmaceutical analysis the QbD approach is a basic need using HPLC. The quality of stationary phase is one of the most important parameters and using an intelligent program gives firm bases to evaluate it.

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