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Reliability of computer-assisted method transfer between several column dimensions packed with 1.3–5 μm core–shell particles and between various instruments

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ABSTRACT

In this contribution, the possibility to automatically transfer RPLC methods between different column dimensions and instruments was evaluated using commercial modelling software. The method transfer reliability was tested with loratadine and its 7 related pharmacopeial impurities. In this study, state-of-the-art columns packed with superficially porous particles of 5, 2.6, 1.7 and 1.3 μm particles were exclusively employed. A fast baseline separation of loratadine and related impurities ($R_{s,min}$ = 2.49) was achieved under the best analytical conditions (i.e. column of 50 mm × 2.1 mm, 1.3 μm, 10–90% ACN in 5 min, T = 40 °C, pH = 3, F = 0.5 ml/min). This optimal method was successfully tested on columns packed with other particle sizes, namely 1.7 and 2.6 μm, to reduce pressure drop. The selectivities and retentions remained identical, while the peak widths were logically wider, leading to a reduction of peak capacity from 203 to 181 and 159 on the 1.3, 1.7 and 2.6 μm particles, respectively. On the minimum, the resolution was equal to 1.54 on the 50 mm × 2.1 mm, 2.6 μm stationary phase. Next to this, the method was transferred to columns of different lengths, inner diameters and particle sizes (100 mm × 3 mm, 2.6 μm or 150 mm × 4.6 mm, 5 μm). These columns were used on other LC instruments possessing larger dwell volumes. The modelling software employed for developing the original method was able to calculate the new gradient conditions to be used. The accuracy of prediction was excellent, as the average retention time errors between predicted and observed chromatograms were −0.11% and 0.45% when transferring the method to 100 mm × 3 mm and 150 mm × 4.6 mm columns, respectively. This work proves the usefulness and validity of HPLC modelling software for transferring methods between different instruments, column dimensions and/or flow rates.

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1. Introduction

The profiling of impurities and degradation products of pharmaceuticals is one of the most challenging tasks in liquid chromatography (HPLC), because of the requirements for both high-resolution and trace analysis, in addition to stringent regulatory and reporting guidelines [1,2]. Generally, reversed phase gradient methods are applied to increase peak capacity, enhance detection of highly retained impurities and shorten analysis time. In spite of these generic requirements, several isocratic conventional high performance liquid chromatographic (HPLC) methods can be found in pharmacopoeias. In most of the cases, they are time consuming (30–60 min long separations) and not sensitive enough. The current trend in pharmaceutical analytical research and development is to develop fast gradient ultrahigh-pressure liquid chromatographic (UHPLC) methods and then transfer them to any conventional HPLC or other UHPLC methods to different labs. The benefit of this approach is that the time spent on method development is drastically shorten thanks to the use of narrow bore short columns (typically 50 mm × 2.1 mm) instead of conventional standard bore long columns (e.g. 150 mm × 4.6 mm) [3]. On the other hand, the drawback of this methodology is the need for exactly the same stationary phase chemistry and column packing quality for the different column dimensions and some possible issues related to differences in systems dwell volume and extra-column variances [4]. Other effects, such as pressure and temperature gradients observed under UHPLC conditions, can also impact the selectivity and resolution when transferring methods between HPLC and UHPLC [5,6].

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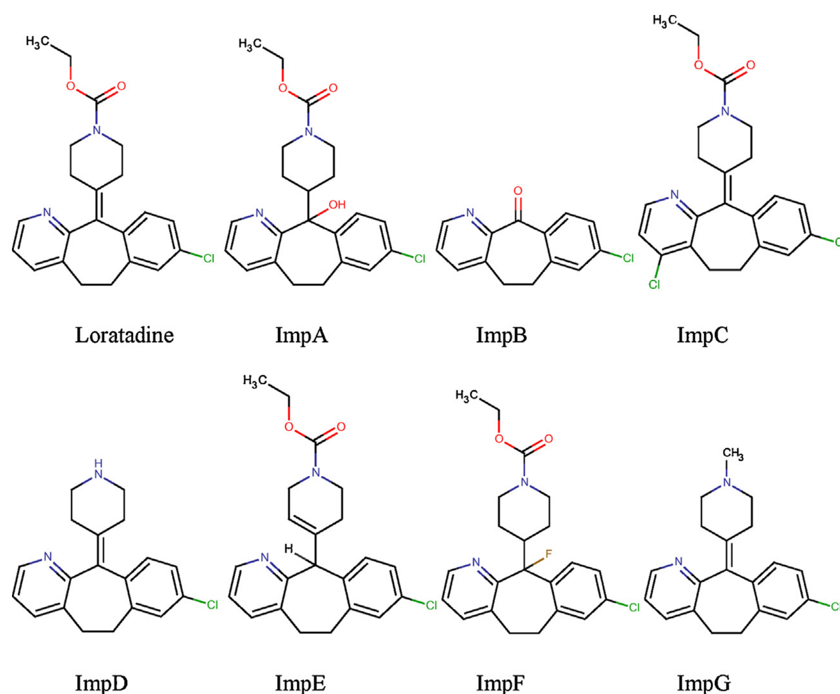

Fig. 1. Structure of loratadine and its related impurities.

Computer modelling softwares can be applied to improve the throughput as well as maximize information about method selectivity during the method development process. The most recent version of some modelling programmes has the ability to optimize the design space mainly by measuring and visualizing the effects of mobile phase composition: gradient time and shape, pH, ionic strength, ternary eluent composition, additive concentrations and temperature [7–9]. Different experimental designs (DoE) are suggested by these softwares such as the one including twelve experiments for the simultaneous optimization of three variables (i.e. gradient programme, mobile phase temperature and pH) [10]. Moreover, these computer programmes allow calculating the impact of not only the DoE variables but also some other parameters such as column length, internal diameter, particle size, flow-rate and system dwell volume [11]. Taking into account these possibilities, a direct geometrical method transfer can be performed between different column geometries and instruments without any additional experimental work. However, this feature of the software is not well described and its reliability not yet been reported.

The aim of this study was to evaluate the reliability of calculated geometrical method transfer between several state-of-the-art column dimensions and core–shell particle sizes. In addition, the accuracy of method transfer was also evaluated between systems, possessing different gradient dwell volumes. For this purpose, loratadine was selected as test probe, since it is a well known H_1 histamine antagonist and several pharmaceutical companies have this active substance in their portfolio. Loratadine is used to treat different allergies and is structurally close to tricyclic antidepressants (MW = 383 g/mol). Several methods are reported on the determination of loratadine related impurities from pharmaceuticals by conventional HPLC methods [12,13]. A few UHPLC methods are also reported for the measurement of loratadine but only in environmental samples [14]. To the best of our knowledge, there is no UHPLC method dealing with the separation of loratadine and its related impurities (impurity profiling). Another goal of this work was to show the congeniality of Kinetex C18 stationary phase chemistry family, independently from particle size and column geometry, and the effects of frictional heating and pressure (between 200 and 950 bar) on the selectivity in gradient elution mode.

2. Experimental

2.1. Chemicals, columns

The mobile phase used in this work was a mixture of acetonitrile and 30 mM phosphate buffer. Acetonitrile (gradient grade), phosphoric acid, sodium dihydrogen phosphate and standard reference buffers (pH 2.00, 4.01 and 7.00) were purchased from Merck (Darmstadt, Germany). For the measurements, water was prepared freshly using ELGA Purelab UHQ water (ELGA, Lane End, UK). The buffer was filtered before use on regenerated cellulose filter membrane, 0.2 μm pore size (Sartorius, Goettingen, Germany).

The test samples contained 10 μg/ml Loratadine and its European Pharmacopoeia (Ph.Eur.) impurities (A, B, C, D, E, F, G). Fig. 1 shows the structure of the test solutes. Real-life samples were prepared from loratadine API (1 mg/ml) and spiked with all the impurities at 0.1% level. Loratadine and its impurities were purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM, Strasbourg, France). Sample solvent was acetonitrile:water 10:90 (v/v).

The Kinetex C18 columns (50 mm × 2.1 mm, 1.3 μm; 50 mm × 2.1 mm, 1.7 μm; 50 mm × 2.1 mm, 2.6 μm; 100 mm × 3 mm, 2.6 μm and 150 mm × 4.6 mm, 5 μm) were purchased from Phenomenex (Torrance, USA).

2.2. Equipment and softwares

Acquity I-Class UPLC system (dwell volume, V_D = 0.1 ml, extra-column volume, V_{EC} = 7.5 μl), Acquity H-Class UPLC system (V_D = 0.4 ml, V_{EC} = 12 μl) and Alliance e2695 HPLC system (V_D = 1 ml, V_{EC} = 29 μl), all from Waters (Milford, USA) were employed in this study.

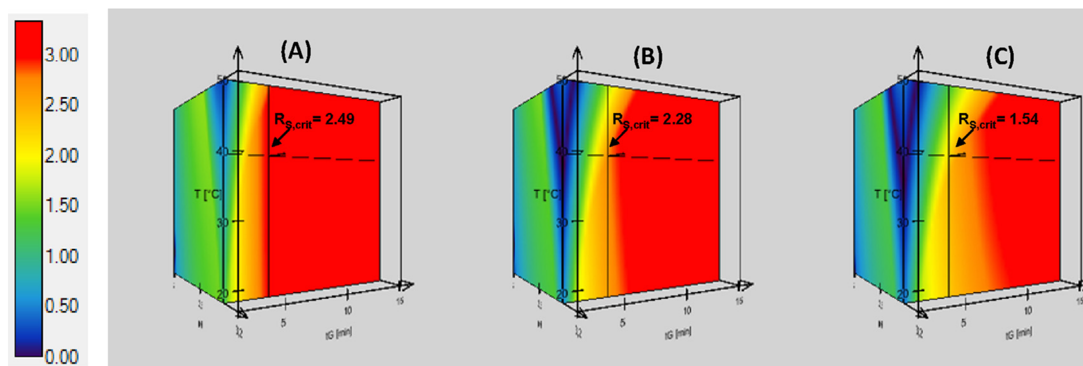

Fig. 2. Design spaces for the 3 models (A: Kinetex C18 1.3 μm, B: Kinetex C18 1.7 μm and Kinetex C18 2.6 μm columns of 50 mm × 2.1 mm). The mobile phase pH range: 2.0, 2.6 and 3.2 (*y*-axis). The red zones indicate the design space where the critical resolution was the highest ($R_{s,crit}$ > 3). The $R_{s,crit}$ is indicated at the selected working point: t_g = 5 min (10%B → 90%B), T = 40 °C, mobile phase pH = 3.0. Flow rate: 0.5 ml/min, injected volume: 1 μl. Mobile phase "A": 30 mM sodium dihydrogen phosphate buffer, mobile phase "B": acetonitrile. Instrument: Waters Acquity I-Class system (V_D = 0.1 ml), sample: a mixture containing loratadine and its seven related PhEur impurities. (For interpretation of the references to color in this legend, the reader is referred to the web version of the article.)

The MP 225 pH-metre was purchased from Mettler-Toledo (Mettler-Toledo, Greifensee, Switzerland).

Method development and method transfer were performed using DryLab 4.1 optimization software (Molnár-Institute, Berlin, Germany).

2.3. Apparatus and methodology

The selected example describes a fast and efficient method development and transfer for the determination of impurities and degradation products of a histamine antagonist active pharmaceutical ingredient (loratadine), using the high resolving power of state-of-the-art sub-2 and sub-3 μm core–shell packings. Due to the basic character of the solutes (basic *pKa* of 4.27), the mobile phase pH should play an important role in tuning the selectivity; therefore this variable was considered as a significant factor and its impact was studied at three levels (non-linear effect). When dealing with low molecular weight analytes, the most common method development strategy consists in selecting suitable stationary phase chemistry, organic modifier nature and mobile phase pH [15]. In a second instance, the gradient programme and mobile phase temperature are optimized as complementary parameters, for fine tuning the optimum after selection of the correct combination of stationary phase, organic modifier and mobile phase pH.

Based on preliminary measurements, it seemed that the combination of Kinetex C18 stationary phase and acetonitrile as organic modifier provides appropriate selectivity and separation. Therefore, the two stages strategy was simplified to one stage method development as described below. Experimental design for simultaneous optimization of gradient time (t_g), temperature (T) and pH requires 12 experiments [8–10]. Two linear gradients from 10 to 90%B in 3 and 9 min were carried out at two different column temperatures, namely 20 and 50 °C on 50 mm × 2.1 mm columns. The mobile phase "A" consisted of 30 mM sodium dihydrogen phosphate buffer with pH set at three values equal to 2.0, 2.6 and 3.2. Please note, that current chromatographic practice explores mobile phases generally in the pH range 2–8, for basic drugs. Since the U.S. Pharmacopoeia suggests a mobile phase with pH = 3 for loratadine related compounds [16], therefore we focused on this low pH range to explore the most appropriate pH to attain suitable selectivity. Moreover the ionic state of residual silanols can change in this pH range providing possible changes in selectivity and by adding organic modifiers, a change (shift) in mobile phase pH and solute *pKa* values are also expected. Mobile phase "B" was acetonitrile, because of its low viscosity and favourable UV cut-off. The flow-rate was set at 0.5 ml/min and injection volume was 1 μL. Detection was carried out at 230 nm. The twelve initial experiments were performed on the Acquity I-Class system, with three columns possessing the same dimensions and chemistries, but packed with 1.3, 1.7 and 2.6 μm particles. Then the obtained 3-dimensional resolution maps, peak capacities and column permeabilities (operating pressure) achieved with the three different particle sizes were compared.

After finding the optimal conditions, the final method was transferred on a 100 mm × 3 mm (2.6 μm) and a 150 mm × 4.6 mm (5 μm) conventional columns and alternative instruments. The geometrical method transfer was calculated using DryLab software, on the basis of generic rules [17–19] and taking into account the system dwell volumes.

3. Results and discussion

3.1. Determining the optimal working point

Initially, the optimal working points on the three 50 mm × 2.1 mm columns were determined. Fig. 2 shows the corresponding 3-dimensional resolution maps. The red zones indicate the highest critical resolution, while the blue colour indicates co-elution. At first sight, the colour distribution seems to be comparable for the three columns, suggesting similar selectivity. Despite the fact that the same column chemistry was applied, in practice slight – or sometimes significant – changes in selectivity could be observed due to differences in packing quality (especially for columns having different internal diameter or particle sizes), amount of residual silanols (silanol activity) and flow rate/pressure related effects as described in Section 3.3. The main difference is that the smaller the column particle size, the larger the red zones surface is in the resolution maps, indicating higher resolution power for the smaller particles. Taking into account the critical resolution and analysis time, a working point at t_g = 5 min, T = 40 °C and at pH = 3.0 was selected. This working point provides a robust and high throughput analysis on all the three columns (last peak eluted at ~4 min) where the influence of method parameters on the response function is not significant. Fig. 2 shows the critical resolutions predicted at the working points. The column packed with the smallest particles (1.3 μm) provided $R_{s,crit}$ = 2.49, while the 1.7 and 2.6 μm packings performed $R_{s,crit}$ = 2.28 and 1.54, respectively.

3.2. The impact of column efficiency

Peak capacity is a measure of the separation power that includes the entire chromatographic space together with the variability of the peak width over the chromatogram [20,21]. In this study, peak capacities were experimentally determined based on the gradient time (t_g) and the average peak width measured at 50% height ($w_{50\%}$). The following simple equation was used to estimate the peak capacity, corresponding to a resolution of R_s = 1 between consecutive peaks [22]:

$$n_c = 1 + \frac{t_g}{1.699 \cdot w_{50\%}} \quad (1)$$

To avoid the imprecision associated with the measurement of peak widths at baseline, the peak widths at half height were measured since in some cases small trace peaks eluted close to the peaks of interest could disturb the determination of baseline width.

A peak capacity of n_c = 203 was observed when operating the 1.3 μm Kinetex C18 column at the working point (5 min long gradient), while the 1.7 and 2.6 μm Kinetex C18 columns provided n_c = 181 and 159 under the same conditions. Peak capacity depends on various experimental parameters, among them the column length (L), particle size (d_p) and gradient time (t_g) play important role. The following expression is valid when assuming the same packing quality and particle structure (e.g. core–shell particles with the same volume fraction) [23]:

$$n_c \approx \frac{t_g}{t_0}\sqrt{\frac{L}{d_p}} \quad (2)$$

where t_0 is the column dead time. Our observed peak capacity values are in agreement with theoretical expectations. The results suggest two important findings: (1) the column packing quality of the 50 mm × 2.1 mm Kinetex C18 columns packed with 1.3, 1.7 and 2.6 μm particles is quite similar and (2) the impact of extra-column band broadening when operating these very efficient small columns in gradient elution mode on very low dispersion system (such as Acquity I-Class in our case) is negligible.

Generally, column packing quality becomes worse when decreasing the particle size and column internal diameter [24]. In addition, it was shown that the 1.3 μm Kinetex columns were very efficient but their potential cannot be utilized in isocratic mode even when the extra-column peak variance was lower than $\sigma^2_{ec} < 2\,\mu l^2$ [25]. In gradient elution mode, the samples concentrate at the inlet of the column, therefore only the connecting tube after the column and the detector cell contribute to peak broadening. Based on the observed peak capacity values, it seems that this extra-column peak variance in gradient elution mode is negligible when operating these very efficient columns on the Acquity I-Class system ($\sigma^2_{ec} = 1 - 3\,\mu l^2$). When transforming these peak capacity values into isocratic plate heights (procedure not shown), reduced plate heights h of less than 2 was calculated under the applied conditions, for all the three columns. These reduced plate height values are in agreement with some results reported elsewhere and make core–shell particles highly attractive in terms of achievable column efficiency [25].

3.3. The impact of pressure and frictional heating on selectivity

A possible complication of UHPLC is related to the effect of pressure and mobile phase velocity on the retention properties of the analytes [5]. Pressure can have significant effects on (i) physical properties of the mobile phase (density, viscosity, dielectric constant...), (ii) physicochemical properties of solutes (molar volume, conformation, pressure induced change in *pKa*...) and (iii) the stationary phase (its compressibility, phase ratio) [5,26,27]. Generally, the "pressure-related changes" in retention can be explained by the effect of pressure alone or by the additional effect of frictional heating if pressure is increased by using high flow rates. When applying a column that possesses low permeability, the generated high pressure increases the solute retention in most cases under reversed phase conditions. This phenomenon can be explained by the change of molar volume [28]. The dependence of the retention factor (k), of a compound on the pressure (P) can be derived from the Gibbs free energy as follows [28]:

$$k = k_0 \exp\left[-\frac{\Delta V_m}{RT}P\right] \quad (3)$$

where ΔV_m is the change of molar volume of solute during adsorption, R the universal gas constant, T the absolute temperature and k_0 is the limiting value of the retention factor at zero pressure. This equation clearly shows the expected increase of retention with pressure.

On the other hand, longitudinal frictional heating effects generally decrease solute retentions due to the temperature increase along the column [6]. The temperature increase causes the improvement of molecular diffusion and the reduction of mobile phase viscosity. Therefore, some variation of resolution is also expected since the two effects mentioned above affect the peak widths. The heat power generated by friction depends on the superficial linear velocity (u_s) and the generated pressure gradient ($\Delta P/L$) [26]:

$$P_f = u_S \frac{\Delta P}{L} \quad (4)$$

When working in real UHPLC conditions, there is a combined effect of pressure and frictional heating effects. Since both effects influence solute retention in different way (antagonist effects), the overall result on retention is expected to be limited [5]. In gradient elution mode (systematically employed in this study), the effects related to pressure and frictional heating seems to be negligible. Fig. 3 illustrates the experimentally observed chromatograms obtained on the three 50 mm × 2.1 mm columns, when operating them at the working point. As shown the 1.3 μm packing generates two times higher pressure than the 1.7 μm packing and around 3 times higher than the 2.6 μm material. Therefore, 2 and 3 times higher impact of pressure related molar volume change (retention increase) can be expected but the generated frictional power is also 2 and 3 times higher, respectively (see Eqs. (3) and (4)). These two effects seem to completely cancel out each other since the observed retention times and selectivities are practically the same on the three columns independently on their permeability. The differences in retention times (and retention factors) were systematically evaluated under the 12 conditions applied for creating the initial 3-D model for the method development. The deviation of retention times for each solutes scattered between −3.5 and +2.5%. No systematic change was observed, the retention times varied randomly. Another possible explanation for the negligible pressure related effects is that closely related compounds were separated in this study. Since their structures were very similar, both the pressure and friction affect their retentions to the same degree, therefore no change in selectivity was observed.

To conclude on the pressure related effects, no significant change in retention and selectivity was observed under gradient elution conditions in the pressure range between 246 and 779 bar for sample containing closely related compounds.

3.4. Method transfer from UHPLC to conventional HPLC

The method developed on 50 mm × 2.1 mm columns was transferred to conventional column dimensions (100 mm × 3 mm and 150 mm × 4.6 mm), considering the generic rules of geometrical method transfer. The flow rate was adjusted to 1.0 and 2.4 ml/min

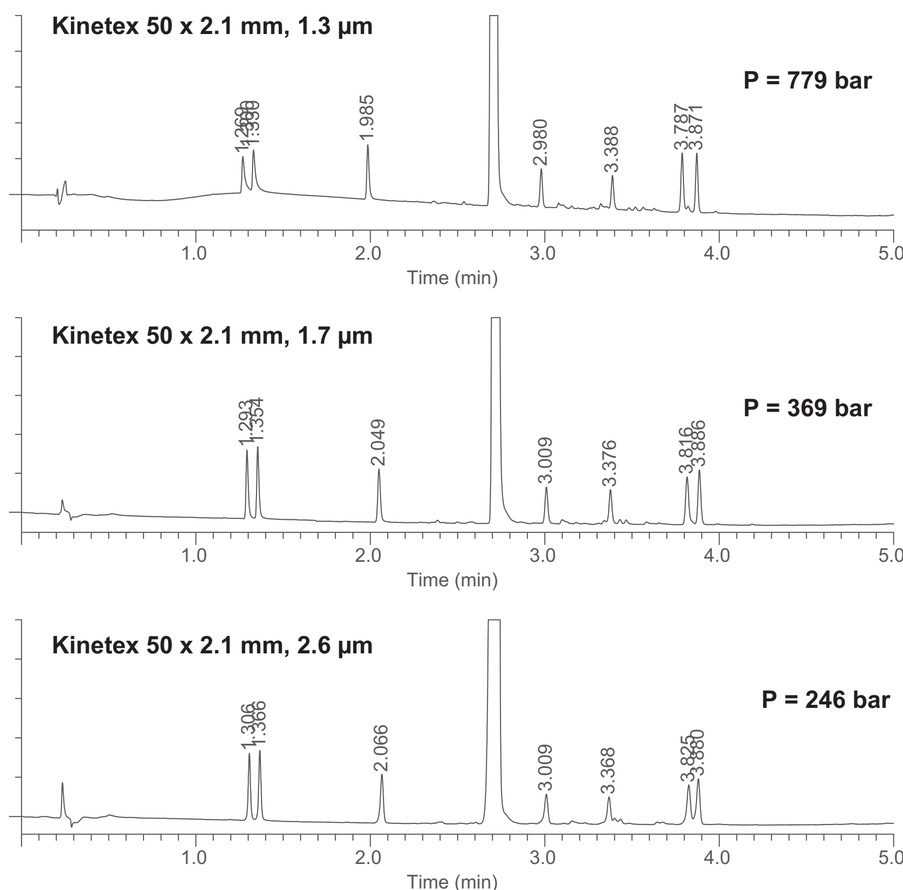

Fig. 3. Experimental chromatograms obtained with 50 mm × 2.1 mm Kinetex C18 columns (1.3, 1.7 and 2.6 μm packing). Sample: loratadine API (1 mg/ml) spiked with all the impurities at 0.1% level. Elution order: (1) ImpD, (2) ImpG, (3) ImpB, (4) Loratadine, (5) ImpE, (6) ImpF, (7) ImpA and (8) ImpC. Chromatographic conditions as defined in Fig. 2. Detection was carried out at 230 nm.

for the 3 and 4.6 mm columns, while the injected volume was set to 4 and 15 μl, respectively. Then, the gradient programmes were adjusted considering the following relationships [29]:

$$t_{r,grad} = \frac{t_0}{b}\log(2.3k_{ini}b + 1) + t_0 + t_D \quad (5)$$

where $t_{r,grad}$ is the retention time of a compound in gradient elution mode, t_0 is the column dead time, b is the gradient steepness, k_{ini} is the retention factor at a mobile phase composition corresponding to the beginning of the gradient and t_D is the system dwell time. To consider the geometrical ratio between different column dimensions, the gradient programme should be set according to the next equation [19,29]:

$$t_{g2} = t_{g1}\frac{F_1}{F_2}\frac{L_2}{L_1}\left(\frac{d_{C2}}{d_{C1}}\right)^2 \quad (6)$$

where t_{g2} and t_{g1} are the lengths of the linear gradient programmes, F_1 and F_2 are the flow rates on the two columns and d_{c1} and d_{c2} are the columns internal diameters.

By utilizing HPLC modelling software, it is possible to automatically calculate and predict the effect of column parameters (length, diameter, particle size) and system dwell volume/time. Moreover, experimentally observed column porosity can also be considered for making the model more accurate. For the simulated method transfer, the initial data acquired with the 50 mm × 2.1 mm, 1.7 μm column were used and the column dimensions were virtually changed to 100 mm × 3 mm, 2.6 μm and 150 mm × 4.6 mm, 5 μm. Beside the column parameters, the system dwell volumes were also adjusted to 0.4 ml (Acquity H-Class system applied for the 100 mm × 3 mm, 2.6 μm column) and 1.0 ml (Waters Alliance system applied for the 150 mm × 4.6 mm, 5 μm column). Then, the predicted chromatograms were experimentally verified (see Section 3.5). Fig. 4 shows the experimentally observed chromatograms on the three different columns and systems. The separations were equivalent, as they provided equivalent selectivities and ensure baseline resolutions between all peak pairs. A peak capacity of n_c = 181 was achieved when operating the 1.7 μm Kinetex C18 column (5 min long gradient), while the 100 mm × 3 mm, 2.6 μm Kinetex column provided n_c = 246 (10 min long gradient) and finally the 150 mm × 4.6 mm, 5 μm Kinetex column performed n_c = 199 (15 min long gradient), when operating them under geometrically scaled conditions. These results are in agreement with theoretically expected peak capacities when considering the impact of column length and particle size (see Eq. (2)). To sum up, these results suggest the equivalency of 1.3, 1.7, 2.6 and 5 μm Kinetex C18 families independently on column dimensions.

3.5. Reliability of simulated method transfer

The accuracy of virtually transferred methods was experimentally verified. Table 1 shows the selectivity observed on the three columns. The differences in individual selectivity values were between −1.76 and 2.08% while the average difference between columns was lower than 1%. To establish the accuracy of this simulated method transfer, the predicted and experimentally derived chromatograms (retention times) were compared when transferring the method from 50 mm × 2.1 mm to 100 mm × 3 mm and 150 mm × 4.6 mm columns on different systems (Table 2). The system dwell volumes were accounted by the modelling software. The

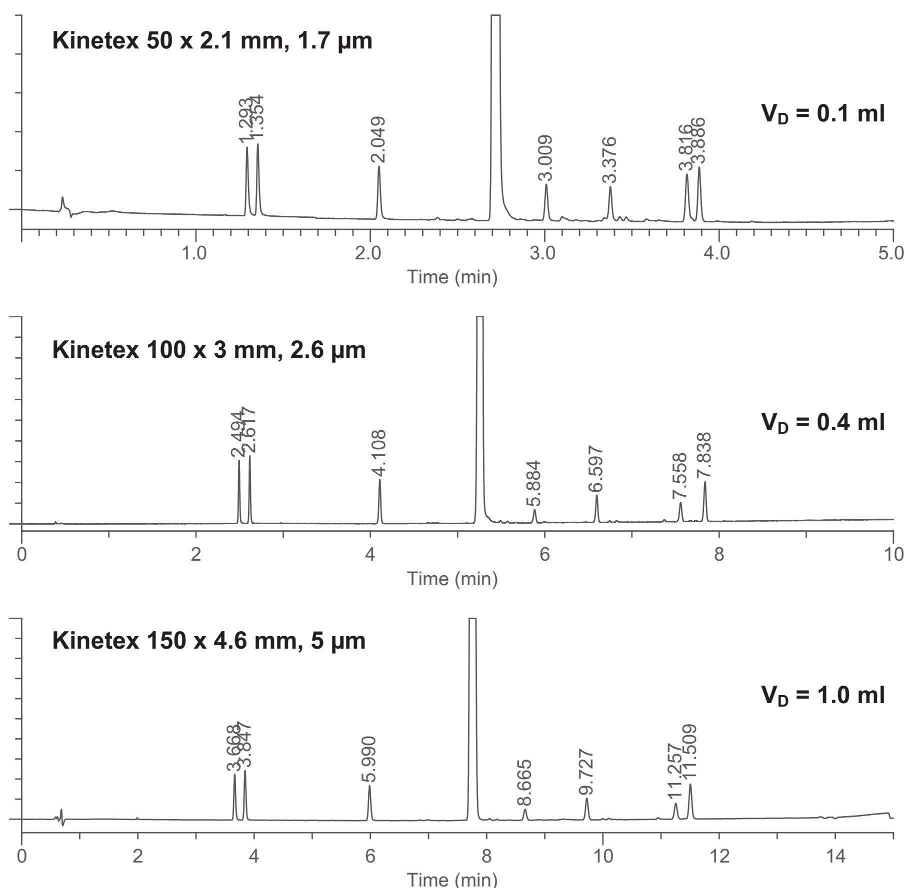

Fig. 4. Comparison of separations performed on 50 mm × 2.1 mm, 1.7 µm, 100 mm × 3 mm, 2.6 µm and 150 mm × 4.6 mm, 5 µm Kinetex C18 columns. Sample and chromatographic conditions as defined in Figs. 2 and 3. The flow rate is set to 0.5 ml/min, 1 ml/min and 2.4 ml/min on the 50 mm × 2.1 mm, 100 mm × 3 mm and 150 mm × 4.6 mm columns, respectively. The gradient times are set accordingly to 5, 10 and 15 min.

Table 1
Comparison of selectivities between different column dimensions.

50 mm × 2.1 mm, 1.7 µm column (reference column)		100 mm × 3 mm, 2.6 µm column		150 mm × 4.6 mm, 5 µm column	
Peak pair	Selectivity	Selectivity	% Difference[a]	Selectivity	% Difference[a]
1–2	1.056	1.059	0.28	1.059	0.24
2–3	1.643	1.677	2.08	1.665	1.34
3–4	1.333	1.309	−1.76	1.330	−0.22
4–5	1.115	1.131	1.44	1.127	1.07
5–6	1.131	1.130	−0.06	1.132	0.09
6–7	1.139	1.155	1.45	1.162	2.02
7–8	1.019	1.039	1.94	1.039	1.88
Average			0.77		0.92

[a] %Error = [(actual column − reference column)/reference column] × 100.

Table 2
Experimental retention times vs. predicted from the three-dimensional gradient time–temperature-pH model when transferring the method from 50 mm × 2.1 mm column to 100 mm × 3 mm and 150 mm × 4.6 mm columns.

Peak	Retention time (min)					
	100 mm × 3 mm, 2.6 µm column, H-class system (V_D = 0.4 ml)			150 mm × 4.6 mm, 5 µm column, alliance system (V_D = 1.0 ml)		
	Predicted	Experimental	%Error[a]	Predicted	Experimental	%Error[a]
1	2.479	2.489	0.40	3.549	3.650	2.85
2	2.596	2.611	0.58	3.725	3.829	2.79
3	4.020	4.106	2.14	5.864	5.976	1.91
4	5.360	5.268	−1.72	7.868	7.759	−1.39
5	5.915	5.886	−0.49	8.702	8.652	−0.57
6	6.660	6.601	−0.89	9.817	9.713	−1.06
7	7.637	7.562	−0.98	11.283	11.248	−0.31
8	7.832	7.837	0.06	11.575	11.499	−0.66
Average			−0.11			0.45

[a] %Error = [(experimental − predicted)/predicted] × 100.

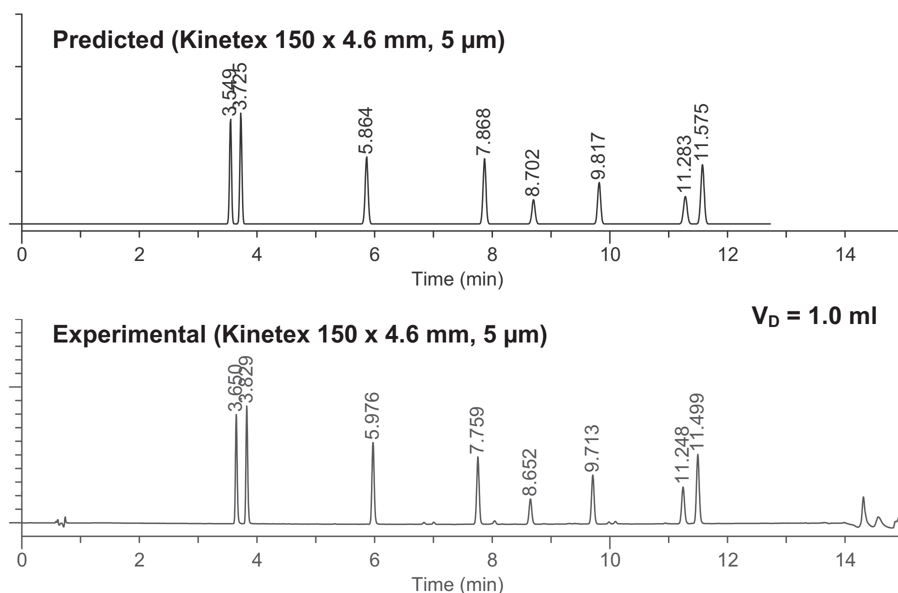

Fig. 5. Comparison of predicted and experimental chromatograms using the 150 mm × 4.6 mm, 5 μm Kinetex C18 column. Prediction is based on data measured on Kinetex C18 50 mm × 2.1 mm, 1.7 μm column.

Table 3
Main characteristics of the method on the different columns.

	t_g (min) 10%B → 90%B	T (°C)	pH	Flow rate (ml/min)	$R_{s,crit}$	Peak capacity	Solvent used for one run (ml)	Systems (Waters)
Kinetex C18 50 × 2.1 mm, 1.3 μm	5	40	3.0	0.5	2.49	203	2.5	Acquity I-Class
Kinetex C18 50 × 2.1 mm, 1.7 μm	5	40	3.0	0.5	2.28	181	2.5	Acquity I-Class
Kinetex C18 50 × 2.1 mm, 2.6 μm	5	40	3.0	0.5	1.54	159	2.5	Acquity I-Class
Kinetex C18 100 × 3 mm, 2.6 μm	10	40	3.0	1.0	4.31	246	10	Acquity H-Class
Kinetex C18 150 × 4.6 mm, 5 μm	15	40	3.0	2.4	2.28	199	36	Alliance e2695

predicted retention times were in excellent agreement with the experimental ones; the average retention time errors were −0.11% and 0.45% when transferring the method to 100 mm × 3 mm and 150 mm × 4.6 mm columns. The largest deviation was 2.85% and occurs for the less retained compound on the 150 mm × 4.6 mm column (the impact of system dwell volume is certainly more important for the less retained compounds). The method transfer prediction can thus be considered as highly accurate. Please note that the error of retention time prediction originated not only from the column dimension and system volume differences but also from the variability of stationary phase chemistry (and other column properties such as packing quality or porosity). Fig. 5 shows the predicted and experimentally observed chromatograms when transferring the method from the 50 mm × 2.1 mm, 1.7 μm column to the 150 mm × 4.6 mm, 5 μm column (from UHPLC to conventional HPLC system). The other important finding again, is that the stationary phase chemistry and therefore the selectivity are practically the same on all the Kinetex C18 columns ranging between 50 mm × 2.1 mm and 150 mm × 4.6 mm and packed with particles of 1.3, 1.7, 2.6 and 5 μm. Hence this Kinetex C18 column family can directly be used for reversed-phase method transfer.

This simulated method transfer procedure can be considered as an accurate support from the modelling software and could be useful for pharmaceutical analytical labs in daily routine work resulting in significant time and solvent saving. The net time spent for method development using a 50 mm × 2.1 mm column – assuming 12 initial runs with 3 and 9 min long gradients – requires 72 min while this procedure takes 144 and 216 min with the 100 mm × 3 mm and 150 mm × 4.6 mm columns. Moreover, the mobile phase volume used for method development is only about 36 ml with the short narrow bore column while it is around 144 and 518 ml with the 100 mm × 3 mm and 150 mm × 4.6 mm columns, respectively. Therefore, it is clearly beneficial to perform the method development on short narrow bore columns and then transfer the optimized method to any column dimension or to any HPLC system in accordance with the requirements of the receiving laboratory.

Finally, Table 3 summarizes the main method characteristics for the different column dimensions. In terms of selectivity, the methods can be considered as equivalent.

4. Conclusion

In this study, a high throughput method was developed for the determination of loratadin and 7 related pharmacopeial impurities, using state-of-the-art stationary phases packed with sub-2 μm superficially porous particles. To achieve the highest possible resolution, HPLC modelling software was employed and a simultaneous optimization of three variables, namely gradient profile, mobile phase temperature and pH was carried out. These 12 initial experiments were performed in about 72 min and the modelling software provides the best analytical conditions for this mixture of compounds. The baseline separation ($R_{s,min}$ of 2.49) was achieved with a column of 50 mm × 2.1 mm, 1.3 μm, using a gradient from 10 to 90% ACN in 5 min, at a temperature of 40 °C, pH of 3, and mobile phase flow rate of 0.5 ml/min. The analysis time was thus drastically reduced compared to the original pharmacopeial method for loratadine (analysis time of 50 min).

Next to this, because the column of 50 mm × 2.1 mm, 1.3 μm could be difficult to employ routinely, due to its high pressure drop and strong contribution to broadening from instrument, the optimal method was tested on columns packed with superficially

porous particles of 1.7 and 2.6 μm. Between these three columns, the selectivities and retentions were found to be identical, proving the congeniality of Kinetex C18 stationary phase chemistry family, independently from particle size. In addition, the effects of frictional heating and pressure (in the range 250–800 bar) on the selectivity were found to be negligible in gradient elution mode. Logically, the peak capacity was decreased from 203 to 181 and 159 due to particle size reduction from 1.3 to 1.7 and 2.6 μm particles, respectively. The resolution was also reduced from 2.49, to 2.28 and 1.54 on the columns packed with 1.3, 1.7 and 2.6 μm superficially porous particles, but the separation remains satisfactory in any cases ($R_s > 1.5$).

In order to also have a method compatible with virtually any HPLC and UHPLC instruments, the method was finally transferred to other columns of different lengths, inner diameters and particle sizes (100 mm × 3 mm, 2.6 μm or 150 mm × 4.6 mm, 5 μm). These two columns were used on other LC instruments possessing higher dwell volumes. The HPLC modelling software was again employed, not for method development but for method transfer between different analytical conditions. The new gradient to be used was easily calculated for the two columns of 3 and 4.6 mm I.D. considering the differences in dwell volume between instruments. The accuracy of prediction was excellent since the average retention time differences between predicted and real chromatograms were equal to −0.11% and 0.45% when transferring the method to 100 mm × 3 mm, 2.6 μm and 150 mm × 4.6 mm, 5 μm columns, respectively. Finally, for these two columns, the minimal resolution was satisfactory and equal to 4.31 and 2.28, respectively.

This work confirms that HPLC modelling software can be successfully employed both for method development and transfer. However, this could also be a useful tool to find out the new analytical conditions in the case of method transfer between columns of different geometry, different mobile phase flow rate or when changing the instrumentation and then its dwell volume.

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