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ORIGINAL

Robust UHPLC Separation Method Development for Multi-API Product Amlodipine and Bisoprolol: The Impact of Column Selection

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Abstract This paper describes a new and fast ultra-high pressure liquid chromatographic separation of amlodipine and bisoprolol and all their closely related compounds, for impurity profiling purposes. Computer-assisted method development was applied and the impact of several state-of-the-art stationary phase column chemistries (50 × 2.1 mm, sub-2 μm, and core–shell type materials) on the achievable selectivity and resolution was investigated. The work was performed according to quality by design principles using design of experiment with three experimental factors; namely the gradient time (t_G), temperature (T), and mobile phase pH. Thanks to modeling software, it was proved that the separation of all compounds was feasible on numerous column chemistries within <10 min, by proper adjustments of variables. It was also demonstrated that the reliability of predictions was good, as the predicted retention times and resolutions were in good agreement with the experimental ones. The final, optimized method separates 16 peaks related to amlodipine and bisoprolol within 7 min, ensuring baseline resolution between all peak-pairs.

Keywords UHPLC · Method development · Quality by design (QbD) · DryLab · Amlodipine · Bisoprolol

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Introduction

When dealing with reversed-phase liquid chromatographic (RPLC) method development, computer modeling programs can be employed to improve the analysis throughput as well as maximize information about method selectivity. The most successful and widespread modeling program (DryLab, Molnar-Institute, Berlin, Germany) optimizes the Design Space mainly by measuring and visualizing the effects of mobile phase conditions: gradient time and shape, pH, ionic strength, ternary eluent composition, additive concentrations, or temperature [1]. For this purpose, the program suggests a relatively well-defined number of experiments on a particular stationary phase; furthermore it can predict the separation inside the Design Space, based on changes in the mobile phase composition, mode of elution (either isocratic or gradient), temperature, pH or column parameters such as column length, internal diameter, particle size, and flow-rate [2]. The retention mechanism in RPLC can be explained by the solvophobic theory that gives a guidance for planning the experiments for RPLC method development and optimization [3]. The theory describes the effects on the chromatographic behavior of components, when varying different parameters. DryLab chromatographic optimization software is mostly based on this theory [4], and its three-dimensional (3D) application helps to understand the peak movements and

the selectivity or resolution changes within the Design Space [5, 6].

Searching for alternative columns, while keeping the quality of a given separation is always one of the key purposes of method robustness testing, but finding the alternative column for a given separation (column interchangeability) is often complicated. Generally, the method is developed using one given column and then, an alternative column can be considered during the validation procedure under the optimized conditions. Since the alternative column probably has not the same working point (optimal conditions in a robust zone) as the primary column, this "trial and error" like approach often fails at the end of method development. Column databases could be helpful for selecting an alternative column but common stationary phase tests are not always able to predict certain column similarity for particular separations. Numerous papers dealing with stationary phase characterization procedures, developed by Snyder, Dolan, Tanaka, Euerby, and Petersson are available and could be helpful for users, in finding a similar column during the method development and validation [7–10]. One of our previous work illustrated that the baseline separation of amlodipine impurities was feasible on nine different 50 × 2.1 mm columns packed with sub-2 μm fully porous and core–shell particles [11]. In that work, the authors compared the selectivity and achievable analysis time when selecting the condition that ensures the highest possible resolution. Another recent study showed that if column was not directly interchangeable, it was still possible to achieve very similar separations by adjusting the chromatographic conditions [12]. The study suggested that the evaluation of column interchangeability should be a part of early stage method development and not of the method validation.

In this current study, our aim was to develop a fast and robust ultra-high pressure liquid chromatographic (UHPLC) method for the separation of amlodipine and bisoprolol-related impurities. Amlodipine is a long-acting calcium channel blocker dihydropyridine and acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance, thereby reducing blood pressure. Bisoprolol belongs to the group of beta-blockers and is used primarily in cardiovascular diseases. The combination of these two active drugs is applied for the treatment of chronic stable angina pectoris and hypertension. Previous works described the spectrophotometric and conventional high-performance liquid chromatographic determination of amlodipine and bisoprolol from pharmaceutical preparations and plasma [13–15]. To the best of our knowledge, no UHPLC separation of all the related impurities was reported up to now.

In this study, a novel and fast UHPLC impurity profiling method is reported for amlodipine and bisoprolol combined active pharmaceutical ingredients (API), and the benefits of computer-assisted method development is discussed. Moreover, the impact of RP stationary phase selection on the selectivity is studied and reported in details.

Experimental

Chemicals

Acetonitrile (gradient grade), phosphoric acid, and natrium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). For the measurements, water was prepared freshly using ELGA Purelab UHQ water (ELGA, Lane End, UK).

Amlodipine and its Ph.Eur. impurities (A, B, D, E, F, G, H) and bisoprolol and its Ph.Eur. impurities (A, G, L, R) were purchased from European Directorate for the Quality of Medicines and HealthCare (EDQM). The structure of the compounds is shown in Fig. 1.

Preparation of Solutions

The mobile phase used in this work was a mixture of acetonitrile and 30 mM phosphate buffer (pH 2.0, 2.6, and 3.2).

The buffers were prepared by mixing the appropriate amount of 30 mM phosphoric acid and 30 mM sodium dihydrogen phosphate. Buffers were filtered before use on regenerated cellulose filter membrane, 0.2 μm pore size (Sartorius, Goettingen, Germany).

Mobile phase "A" was 30 mM phosphate buffer (pH 2.0, 2.6, and 3.2) and mobile phase "B" was acetonitrile.

Sample solvent was a mixture of acetonitrile:water 10:90 (V:V).

Representative real-life sample of amlodipine, bisoprolol, and their Ph.Eur. impurities contained 1 mg mL^{-1} amlodipine besilate and bisoprolol fumarate and their impurities at 0.1 % level was prepared by spiking all the impurities to the API solution.

Chromatographic System

UHPLC experiments were performed on a Waters Acquity UPLC system (Milford, USA) equipped with binary solvent delivery pump, auto sampler, photodiode array detector, and empower software. This UHPLC system had 5 μL injection loop and 500 nL flow cell. The dwell volume of the system was measured as 125 μL. The column compartment of the system is equipped with a CM-A column manager that enables the use of four columns and programmable switching of the mobile phase among the columns.

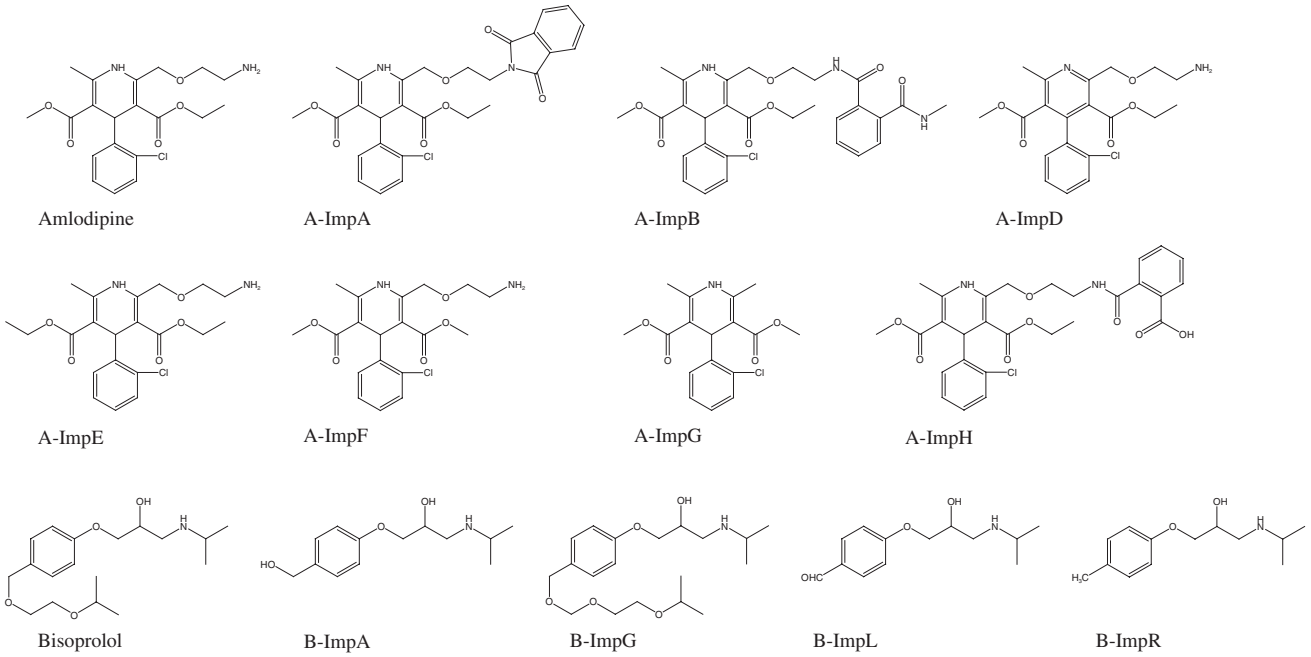

Fig. 1 Structure of Amlodipine, Bisoprolol and their impurities

For the initial model runs, the mobile phase flow rate was set to 0.5 mL min^{-1} and gradients were run from 10 to 90 %B. The injection volume was set to 1 μL.

Columns

Acquity columns (50 × 2.1 mm, 1.7 μm BEH C18, BEH Shield RP 18, BEH C8, BEH Phenyl, CSH C18, CSH Phenyl-Hexyl, CSH Fluoro-Phenyl, 1.8 μm HSS C18, HSS C18 SB, HSS T3, HSS PFP, HSS CN) were purchased from Waters (Milford, USA).

The 50 × 2.1 mm, 1.7 μm Aeris peptide XB-C18, and kinetex columns (XB-C18, C18, C8, Phenyl-Hexyl, PFP) were purchased from Phenomenex (Torrance, USA).

Hypersil columns (50 × 2.1 mm, 1.9 μm Gold, Gold C8, Gold CN) were purchased from Thermo Scientific (Waltham, USA).

The 50 × 2.0 mm, 1.9 μm Triart C18 column was purchased from YMC (Kyoto, Japan).

Zorbax columns (50 × 2.1 mm, 1.8 μm SB-C18, SB-C8, SB-Phenyl) were purchased from Agilent (Santa Clara, CA, USA).

Software

Modeling was carried out using DryLab v.4.0 and the quantitative robustness evaluation of generated models was performed with the latest DryLab Robustness Module v.1.0. (Molnár-Institute, Berlin, Germany).

Results and Discussion

Design of Experiments (DoE)

The selected example describes a fast and efficient method development for the determination of impurities and degradation products of combined active pharmaceutical ingredients, utilizing the separation power of state-of-the-art columns. A general methodology is to simultaneously model the effect of temperature and gradient steepness on selectivity with a given RP column. Thanks to the current developments in chromatographic modeling software products, it is now possible to model the effect of three variables simultaneously for a given separation. In our case, gradient steepness (t_G), temperature (T), and mobile phase pH were selected as model variables to create a cube resolution map, showing the critical resolution of the peaks to be separated against the three factors. Probably, these selected variables have the most significant effect on the selectivity and resolution for such analytes. In most cases, the retention can be described as a function of gradient steepness, with the linear solvent strength theory and its temperature dependence following a van't Hoff type relationship. Both relationships can be transformed to linear dependencies. When separating ionizable compounds, strong pH-related changes in retention occur for pH values within ±1.5 units of the *p*Ka value. Outside this range, the compound is considered as mostly ionized or non-ionized, and its retention is not significantly altered with pH. In a relatively small

pH range—within the ±1.5 units of the *p*Ka value—the dependence of retention on the mobile phase pH can generally be described using quadratic polynomials.

Therefore, in the proposed final model, two variables (t_G and T) were set at two levels ($t_{G1} = 3$ min, $t_{G2} = 9$ min, and $T_1 = 20$ °C and $T_2 = 50$ °C) while the third factor (pH) was set at three levels ($pH_1 = 2.0$, $pH_2 = 2.6$, and $pH_3 = 3.2$). This full factorial experimental design required 12 experiments (2 × 2 × 3) on a given column.

Column Screening

In a first instance, several state-of-the-art columns were evaluated by performing the 12 experiments and creating the corresponding 3D resolution maps. By utilizing the benefits of the column manager unit and small columns (50 × 2.1 mm), the column screening procedure requires only 4–5 days for testing 25 columns, since a lot of work can be automated. Based on the resolution maps, the peak movements and the change in selectivity/resolution were assessed and the columns were ranked in terms of achievable resolution.

Table 1 shows the achievable maximum critical resolution ($R_{s,crit}$) on all the 25 columns, when operating them at their own optimal working point.

In this study, we also compared the selectivity of the columns based on the snyder–dolan hydrophobicity subtraction (SDHS) database that is available in the column match tool of DryLab. This model takes into account the hydrophobicity (*H*), hydrogen bond basicity (*B*), ionic interactions at two pH (*C*(2.8) and *C*(7.0)), hydrogen bond acidity (*A*), and steric selectivity (*S*). The degree of selectivity similarity can be obtained on the basis of *H*, *B*, *C*, *A,* and *S* values. The resulting similarity factors (Fs) were also reported in Table 1, when available. Fs < 3 means excellent similarity of selectivity between the compared columns; between 3 < Fs < 5, the selectivity similarity is moderate, and between 5 < Fs < 10, there is a questionable but still fair comparability of selectivity. As shown in Table 1, this SDHS-based ranking sometimes resulted in unexpected results. As an example, the Hypersil Gold C8 column that is the third most similar (Fs = 6.3) to the reference BEH C18 phase gave completely different working point. This column has to be operated at $T = 42$ °C, to reach the highest possible resolution while the BEH C18 requires low operating temperature ($T = 13.5$ °C). Moreover, the critical peak pair was ImpD and ImpF on the Hypersil Gold C8 while it was the ImpG–ImpH pair on the BEH C18 Phase. On the contrary, the Kinetex PFP phase appears as the most different stationary phase on the basis of its Fs value (Fs = 81.6). However, its working point was found to be very close to the BEH C18 material and possesses the same critical peak pair. To conclude on the SDHS-based column comparison approach, it gives some useful idea for selecting a similar or diverse stationary phase in terms of interaction mechanisms but does not give information about the achievable resolution and analysis time when separating a specific complex mixture. The other disadvantage of the SDHS approach is that the database is not regularly updated and it does not include data on several popular state-of-the-art stationary phases.

Our 12 experiments based approach seems to be a more reliable procedure when comparing the achievable analysis time, resolution, and working point. By applying 50 × 2.1 mm columns, it takes approximately only 2–3 h of experimental work for one given column. The advantage of this column screening approach is that the suitability of a column—for a given application—can be evaluated at the very early stage of the method development. In addition, the column interchangeability can also be estimated during the method development. Based on our experience, it appears that most of the columns can provide sufficient resolution within an acceptable analysis time, by adjusting properly the chromatographic conditions. In this example, only one column among the 25 ones tested failed to achieve $R_{s,crit} > 1.5$ (see table 1).

To conclude on our column screening approach, a promising method development strategy consists in performing initial runs and building up 3D models using different columns at the early phase of method development.

Finding the Optimal Conditions

For the mixture of compounds, the highest resolution could be performed on the Acquity CSH C18 material. Therefore, this column was selected for the final method (Table 1). It is also worth mentioning that this column also provided the highest peak capacity ($P = 201$ with a 10 min long gradient).

First, the criteria for the minimum required resolution were set. The impurities have to be separated from (a) each other, (b) the APIs, and (c) other possible disturbing compounds such as the fumaric acid and benzenesulfonic acid. For the baseline separation of the critical peak pairs, the value of $R_{s,crit}$ should be higher than 1.5. But considering that impurities are present in small concentrations (at ~0.1 %), and have to be separated from the APIs at high concentration, the $R_{s,crit} > 1.5$ might not be enough. In this case, it is better to select $R_{s,crit} > 2.5$ as criteria. Figure 2 shows the 3D resolution map obtained with the Acquity CSH C18 material. Red color represents the regions inside the Design Space where the resolution criteria is fulfilled, while blue colors indicate co-elutions ($R_s = 0$). There are four robust spaces that meet the criteria (Fig. 2b). At low pH (pH < 2.5), and at low temperature (below 30 °C) or at high temperature (above 40 °C) the resolution between fumaric acid and bisoprolol-ImpA was the lowest one, while at higher mobile phase pH (pH > 2.5) and at low

Table 1 List of columns used in the study, the conditions where the highest critical resolution can be reached, the critical peak-pairs, selectivity similarity (Fs), and the average of retention time relative errors

Columns	pH	T (°C)	t_G (min)	$R_{s,crit}$	Critical peak pair	Fs	Average of retention time relative errors (%)
Acquity BEH C18	2.1	13.5	8.1	2.54	ImpG–ImpH	0.0	0.23
Acquity BEH Shield RP 18	2.0	38.3	9.8	2.16	ImpB–ImpG	–	−0.79
Acquity BEH C8	2.5	33.0	9.8	2.27	ImpD–ImpF	8.0	−0.85
Acquity BEH Phenyl	2.0	29.3	9.8	2.32	ImpG–ImpB	27.7	0.41
Acquity CSH C18	3.0	13.5	9.8	3.13	ImpD–ImpF	–	0.88
Acquity CSH Phenyl-Hexyl	2.1	13.5	2.9	1.92	ImpD–ImpF	–	0.60
Acquity CSH Fluoro-Phenyl	3.0	13.5	2.7	1.22	ImpD–ImpF	–	−0.55
Triart C18	3.0	13.5	7.4	2.49	ImpD–ImpF	–	0.57
Acquity HSS C18	2.1	24.0	9.8	2.50	ImpG–ImpH	–	−1.95
Acquity HSS C18 SB	2.0	30.0	9.8	2.04	ImpD–ImpF	–	−0.37
Acquity HSS T3	2.0	31.5	9.8	2.16	ImpG–ImpH	–	−0.94
Acquity HSS PFP	2.0	19.5	9.8	1.58	ImpD–ImpF	–	−0.27
Acquity HSS CN	3.0	13.5	7.9	1.95	ImpD–ImpF	–	−0.15
Hypersil gold	3.0	41.3	9.8	2.72	ImpD–ImpF	20.5	−0.10
Hypersil gold C8	2.7	42.0	9.8	2.55	ImpD–ImpF	6.3	−0.24
Hypersil gold CN	2.9	27.8	9.0	1.67	ImpG–ImpB	–	0.56
Zorbax SB-C18	2.2	29.3	9.8	2.13	ImpG–ImpH	53.6	−0.36
Zorbax SB-C8	2.8	13.5	6.1	2.03	ImpD–ImpF	52.6	1.09
Zorbax SB-Phenyl	2.0	13.5	8.9	1.52	ImpD–ImpF	–	−2.42
Aeris peptide XB-C18	3.0	15.0	9.8	2.50	ImpG–ImpH	–	0.35
Kinetex XB-C18	2.2	13.5	9.8	2.24	ImpD–ImpF	–	0.81
Kinetex C18	2.5	20.3	9.8	2.38	ImpD–ImpF	4.0	−0.54
Kinetex C8	2.4	13.5	9.8	2.52	ImpD–ImpF	–	0.14
Kinetex Phenyl-Hexyl	2.2	33.8	9.8	2.22	ImpD–ImpF	–	−0.28
Kinetex PFP	2.4	16.5	9.8	2.44	ImpG–ImpH	81.6	1.67

Difference (min): Predicted Retention Time − Experimental Retention Time

% error: [(Predicted Retention Time − Experimental Retention Time)/Experimental Retention Time] × 100

temperature (<30 °C), bisoprolol and bisoprolol-ImpG were considered as the critical peak pair. Furthermore, a steeper gradient decreases the resolution between bisoprolol and its impurity-G. Taking all these observations into account, the best working point is located into the robust space at high pH (pH > 2.5) and at high temperature (T > 40 °C). The final conditions were set as t_G = 10 min starting from 10 %B up to 90 %B (slope = 8.0 %B min^{-1}), column temperature T = 45 °C and mobile phase pH 3.0. Please note that the selected 10 min long gradient is outside the 3- and 9-min calibrated model, but the accuracy of the extrapolation is valid in this range [1]. Moreover, the reliability of the model was verified (see later).

Simulated Robustness Testing

The reliability of DryLab's new simulated robustness testing feature was recently reported [12]. Similarly to this previous work, the robustness of the optimized method was also assessed by the built-in robustness module. Beside the three model variables (t_G, T, pH), the flow rate, as well as initial, and final compositions of the mobile phase represent the investigated factors in the built in model. The effect of these six factors was evaluated at three levels. The modeled deviations from the nominal values were the following: the gradient time was set to 9.9, 10, and 10.1 min; temperature was set to 44, 45, and 46 °C; mobile phase pH was set to 2.9, 3.0, and 3.1; flow rate was set to 0.495, 0.500, and 0.505 mL min^{-1}; initial mobile phase composition was set to 9.5, 10, and 10.5 %B and its final composition was set to 89.5, 90, and 90.5 %B. Then, the 729 experiments (3^6) were simulated in <1 min, thanks to the software. A criterion of $R_{s,crit}$ > 1.5 was considered. Figure 3a shows the results of the experiments expressed in frequency as a function of critical *Rs*. As shown, the most frequent resolution value was $R_{s,crit}$ = 2.55 (20 conditions provided this *Rs* value), while the lowest predicted resolution was $R_{s,crit}$ = 2.21. Therefore, the method can

be considered as robust, since the failure rate was 0 % in the studied Design Space. Another feature of the modeling software employed in this study is the calculation of individual and interaction parameter effects. Figure 3b describes the importance of each parameter, related to the selected deviation from the nominal value, for the critical resolution. This figure indicates that the column temperature has the most important influence on the critical resolution while the mobile phase pH plays the less important role.

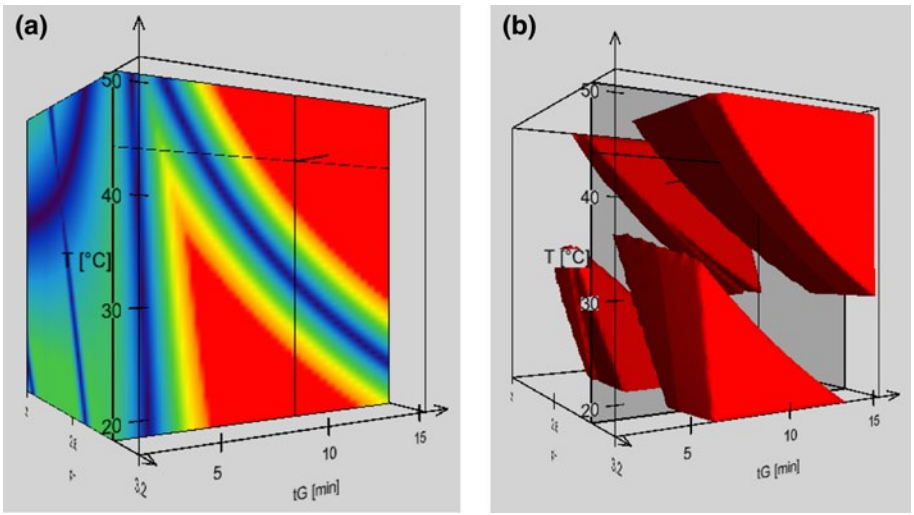

Fig. 2 Three-dimensional resolution map based on t_G-T-pH model, showing the influence of t_G, T, and pH on the critical resolution. *Red* indicates baseline separation while *blue* indicates co-elution ($R_{s,crit} = 0$) (**a**), and showing only the robust zones (**b**)

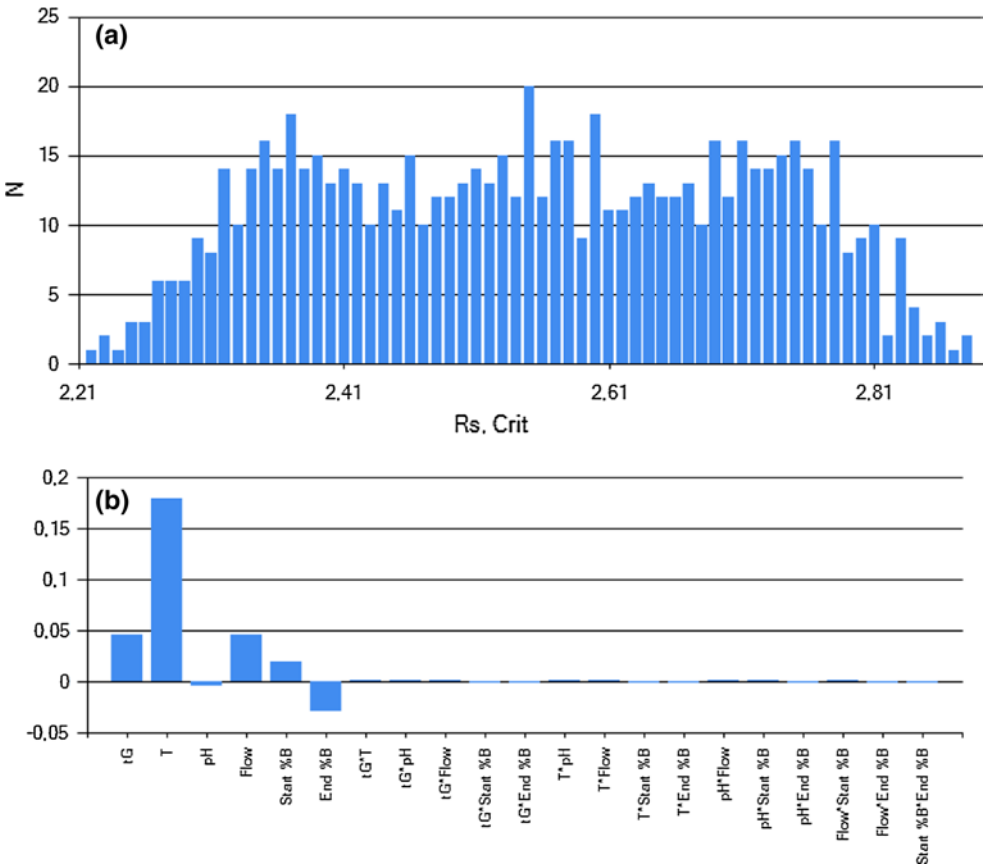

Fig. 3 Results of simulated robustness testing. Frequency of critical resolution (**a**) and the relative effects of the chromatographic parameters on separation (**b**)

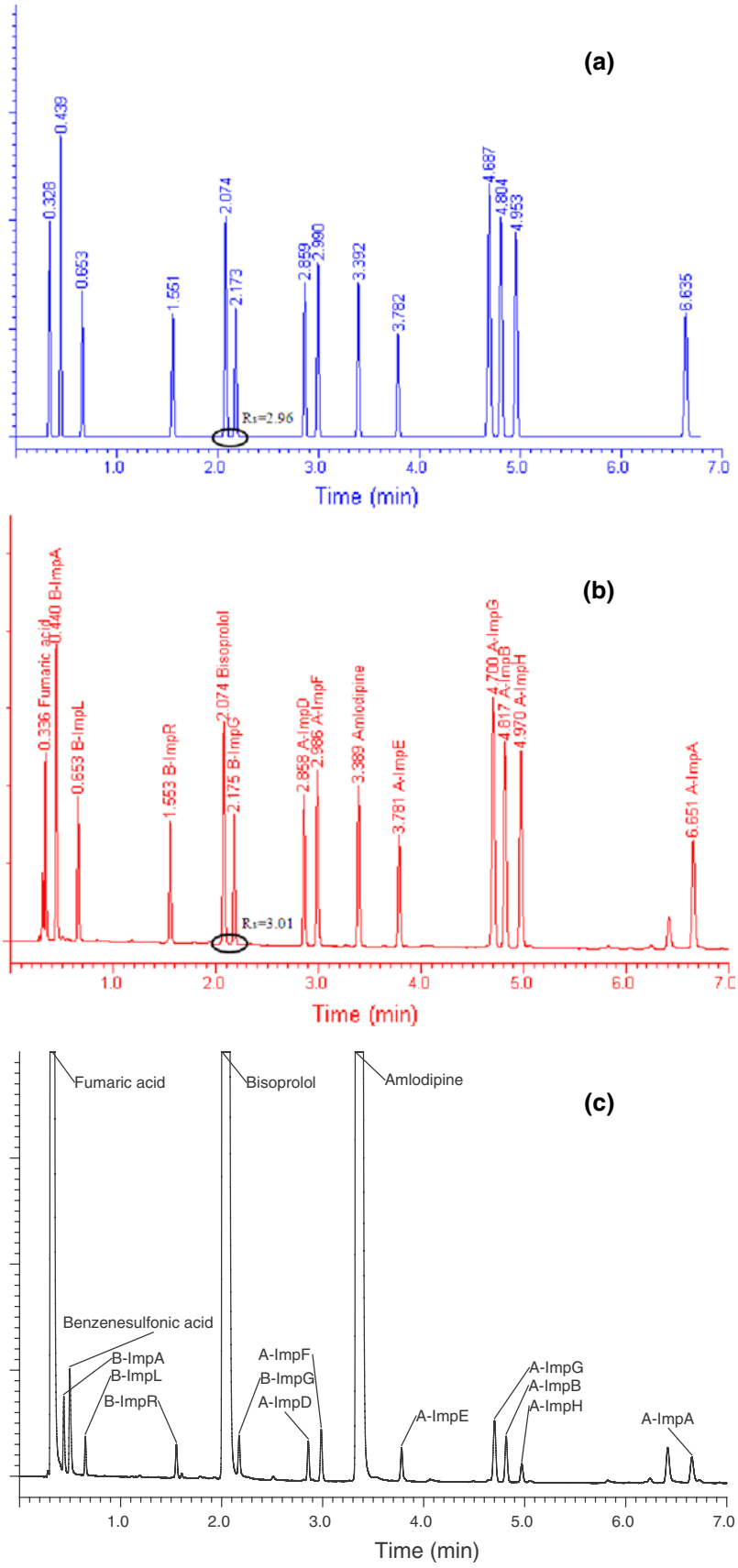

Fig. 4 Predicted (**a**) and experimental (**b**) chromatograms of the model reference solution and of a real sample spiked with 0.1 % impurities (**c**). Column: Waters CSH C18 50 × 2.1 mm (1.7 µm), mobile phase "A": 30 mM phosphate buffer pH 3.0, mobile phase "B": acetonitrile, gradient time = 10 min, starting from 10 % B up to 90 % B, flow rate 0.5 mL min^{-1} and column temperature $T = 45$ °C

Reliability of the Modeled Results

As a final step, the accuracy of the predicted results was evaluated. Experimental verifications of predicted chromatograms were performed. First, the optimal method was verified. Figure 4 shows the predicted and experimentally observed chromatograms when operating the column at the optimal working point. The predicted retention times were in good agreement with the experimental ones, since the average retention time relative errors were <1.0 % (see Table 2), which can be considered as an excellent prediction for such a fast gradient. The accuracy of critical resolution prediction was also assessed. As illustrated in Table 2, the predicted critical resolution was also in good agreement with the experimental one (2.55 versus 2.52). Table 1 also provides the average relative error of retention time predictions for all the tested 25 columns, when operating them at their own optimal working point.

To estimate the reliability of the modeled robustness testing, 3 of the 729 experiments were selected and experimentally performed. In the first case, the conditions that provided the lowest critical resolution were set (t_G = 9.9 min, T = 44 °C, pH 3.1, flow rate = 0.495 mL min^{-1}, start %B = 9.5, and end %B = 90.5). Next, the case where all parameters were set at their lowest levels was evaluated (t_G = 9.9 min, T = 44 °C, pH 2.9, flow rate = 0.495 mL min^{-1}, start %B = 9.5, and end %B = 89.5). Finally, the third case corresponds to all parameters set at their highest levels (t_G = 10.1 min, T = 46 °C, pH 3.1, flow rate = 0.505 mL min^{-1}, start %B = 10.5, and end %B = 90.5). In any of these three cases, the predicted retention times and $R_{s,crit}$ values were in good agreement with the experimental ones, the errors in retention times were <0.05 min, and errors in $R_{s,crit}$ values were <0.03 (see Table 2).

Conclusion

The goal of this contribution was to develop a fast UHPLC separation of amlodipine and bisoprolol (multi-API product) and all their closely related compounds (impurity profiling purpose). For this purpose, computer-assisted method development was employed and a significant amount of experimental work was performed. On the total, 25 UHPLC columns of 50 × 2.1 mm, sub-2 μm were tested and three experimental factors were studied for each stationary phase, including the gradient time (t_G), temperature (T), and mobile phase pH.

Thanks to modeling software, it was possible to find a suitable separation ($R_{s,crit}$ > 1.5) for 24 among the 25 tested columns, by proper adjustments of gradient, temperature and pH, while maintaining analysis time lower than 10 min. The final method for the baseline separation of 16

Table 2 Experimental verification of retention time and resolution predictions

	Original method		"Worst method"		Low parameters		High parameters	
	Predicted t_R (min)	Experimental t_R (min)	Predicted t_R (min)	Experimental t_R (min)	Predicted t_R (min)	Experimental t_R (min)	Predicted t_R (min)	Experimental t_R (min)
Fumaric acid	0.33	0.34	0.33	0.34	0.36	0.35	0.30	0.30
B-ImpA	0.44	0.44	0.48	0.48	0.47	0.46	0.41	0.43
B-ImpL	0.65	0.65	0.72	0.71	0.70	0.68	0.60	0.63
B-ImpR	1.55	1.55	1.63	1.63	1.59	1.60	1.51	1.52
Bisoprolol	2.07	2.07	2.15	2.14	2.11	2.11	2.02	2.04
B-ImpG	2.17	2.18	2.25	2.24	2.21	2.21	2.12	2.14
A-ImpD	2.86	2.86	2.93	2.92	2.89	2.89	2.81	2.82
A-ImpF	2.99	2.99	3.05	3.04	3.03	3.02	2.93	2.94
Amlodipine	3.39	3.39	3.45	3.44	3.42	3.42	3.33	3.35
A-ImpE	3.78	3.78	3.84	3.82	3.81	3.81	3.72	3.75
A-ImpG	4.69	4.70	4.72	4.72	4.74	4.76	4.61	4.64
A-ImpB	4.80	4.82	4.83	4.82	4.85	4.86	4.73	4.77
A-ImpH	4.95	4.97	4.97	4.96	5.01	5.03	4.86	4.91
A-ImpA	6.63	6.65	6.65	6.63	6.67	6.69	6.56	6.61
$R_{subscript}$ A-ImpG–A-ImpB	2.55	2.52	2.22	2.19	2.29	2.29	2.81	2.84

The "original method" corresponds to the optimal method; the "worst case" corresponds to the conditions where the lowest resolution can be achieved, while "Low" and "High parameters" correspond to conditions where all the variables were set at their lower and higher levels

peaks that can be encountered in the amlodipine/bisoprolol formulation was achieved in <7 min.

The reliability of the predictions achieved with the 3D model feature included in Drylab was excellent, as the average difference between predicted and observed retnetion times was less than 2 %. Moreover, by utilizing both the 3D model and the simulated robustness testing, a huge amount of experimental work can be saved and, therefore, the time spent for method development and robustness testing can be drastically shortened. The procedure described in the present paper can obviously be employed for other type of pharmaceutical formulations.

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