Journal of Pharmaceutical and Biomedical Analysis 187 (2020) 113371

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Updating the European Pharmacopoeia impurity profiling method for terazosin and suggesting alternative columns

Dániel Enesei[a], Imre Kapui[a], Szabolcs Fekete[b], Róbert Kormány[a,*]

[a] Egis Pharmaceuticals Plc., Keresztúri út 30-38, 1106 Budapest, Hungary
[b] School of Pharmaceutical Sciences, University of Geneva, CMU - Rue Michel-Servet, 1, 1206 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 19 February 2020
Received in revised form 6 May 2020
Accepted 14 May 2020
Available online 19 May 2020

Keywords:
Column comparison
Design of experiments
Method validation
Quality by design
Robustness
Terazosin

ABSTRACT

This work was motivated by the demand of European Directorate for the Quality of Medicines and HealthCare (EDQM). A new liquid chromatographic (LC) method was developed for terazosin impurity profiling to replace the old European Pharmacopoeia (Ph. Eur.) method. This new method is published as part of the new Ph. Eur. monograph proposal of terazosin in Pharmeuropa issue 32.2.

The aim of the method renewal was to cut the analysis time from 90 min (2 × 45 min) down to below 20 min. The Ph. Eur. monograph method is based on two different chromatographic separations to analyze the specified impurities of terazosin. The reason for the two methods is that two of the impurities are not sufficiently retained in reversed phase (RP) conditions, not even with 100% water as eluent. Therefore, next to RP, an ion-pair (IP) chromatographic method has to be applied to analyze those two impurities.

With our new proposed method it was possible to appropriately increase the retention of the two critical compounds using alternative stationary phases (instead of a C18 phase which is suggested by the Ph. Eur. method). Applying a pentafluoro-phenyl (PFP) stationary phase, it was feasible to separate and adequately retain all the impurities. The detection wavelength was also changed compared to the Ph. Eur. method and is now appropriate for the detection and quantification of all impurities using perchloric acid in the mobile phase at low pH.

Another goal of the present study was to develop a generic workflow and to evaluate the chromatographic resolution in a wide range of method variables and suggest some replacement columns for terazosin impurity profiling. Retention modeling was applied to study the chromatographic behavior of the compounds of interest and visualize resolution for the different columns, where a given criterion is fulfilled. A zone (set of chromatographic conditions) of a robust space could be then quickly identified by the overlay of the individual response surfaces (resolution maps).

It was also demonstrated that two columns from different providers (Kinetex F5 and SpeedCore PFP) can be used as replacement columns, providing sufficient resolution at the same working point and a high degree of robustness.

© 2020 Elsevier B.V. All rights reserved.

1. Introduction

Thousands of liquid chromatographic (LC) columns are commercially available today. On one hand, this can make the method development easier since chromatographers can select the most suitable stationary phase for a given separation. On the other hand, it can be a difficult and time consuming task to find an appropriate replacement (alternative) column, which provides a very similar separation compared to the original column. Today, it is indeed required to suggest an alternative column in pharmaceutical analytical laboratories, and to prove its equivalency during the method validation process. In fact, the pharmaceutical regulatory guidelines mention that method robustness has to be checked on columns from different batches and also on other manufacturer's column providing similar separation quality [1]. The column interchangeability in the U.S. Pharmacopeia Convention is quite straightforward. The LC columns are classified in' L' groups according to their chemical modification [2]. As an example, columns with pentafluorophenyl (PFP) groups chemically bonded belong to the L43 group, which is defined as: "pentafluorophenyl groups chemically bonded to silica particles 5–10 μm in diameter". This definition is rather inaccurate, since it contains all the

* Corresponding author.
E-mail address: kormany.robert@egis.hu (R. Kormány).

https://doi.org/10.1016/j.jpba.2020.113371
0731-7085/© 2020 Elsevier B.V. All rights reserved.

phases with irregular silica particles, high metal ion content as well as the widely used hybrid silica. It does not distinguish between different particle morphologies (e.g. fully porous or superficially porous), furthermore does not allow the use of sub-5 μm particles.

To compare or characterize RP columns, various tests have been developed and proposed in the past [3–8]. Databases are also available based on those tests. The limitation of such tests is that they provide information only on a limited number of test compounds, measured under "one constant set" of particular conditions. Those tests cannot predict the applicability of columns for impurity or degradation profiling, assays or other separations, which are the most common applications in pharmaceutical analysis. In previous studies, a so-called simulated robustness testing and other more common design of experiments (DoE) based approaches have been applied to select alternative LC columns for a given separation [9,10]. An efficient way of finding alternative columns is to perform retention modeling at the early stage of method development on several columns simultaneously and then – if required –, the selectivity can be further adjusted on any columns by the modification of method variables (e.g. gradient steepness, mobile phase temperature, mobile phase pH. . .). With that approach, it was feasible to perform appropriate separation on several columns. It worth mentioning that the different columns may require for different working points to attain the highest resolution [10]. Commercial software is also available today to help in finding a replacement column. Very recently a provider offers a software package (column comparison module) enables to overlay the resolution maps obtained on different columns and can easily point out the conditions where all columns fulfill a pre-defined resolution criterion [11]. Such an approach is used in this current study to identify alternative columns for terazosin impurity profiling.

Next to retention modeling, analytical quality by design (AQbD) is also often applied in liquid chromatography as a systematic approach to reduce the number of out-of-trend (OOT) results and out-of-specification (OOS) results due to the robustness of the method within a given region of method variables [12–15]. It is a current trend among pharmaceutical industry to implement AQbD in method development process as a part of risk management, pharmaceutical development, and pharmaceutical quality system.

Kormány et. al have already developed a very fast ultrahigh-pressure liquid chromatographic (UHPLC) method using a 50 × 2.1 mm (1.7 μm) column to separate 11 terazosin related compounds and compared it to the Ph. Eur. conventional LC method [16,17]. The UHPLC method was able to separate those solutes within 6 min while it takes 90 min with the Ph. Eur method. However, it has to be noticed that the developed UHPLC method uses a mobile phase with high pH which may decompose terazosin. It was another reason to reconsider and rework the previously developed UHPLC method.

This current work was initiated by the European Directorate for the Quality of Medicines and HealthCare (EDQM) by requesting for a fast and robust method which can be performed either on a HPLC or UHPLC system without the need for very high pressure operation. Moreover there was also a need for suggesting alternative columns. Therefore a method was developed to separate 16 compounds (all possible impurities and the API) and validated for the 10 specified impurities (Imp A, Imp B, Imp C, Imp E, Imp J, Imp K, Imp L, Imp M, Imp N and Imp O, see in Sections 2.1. and 2.6). The goal of this study was to model the changes in selectivity that can be helpful for better column selection and to reduce the analysis time and preferably to use only one method—instead of the two suggested ones by the pharmacopoeia monograph.

2. Experimental

2.1. Chemicals

Eluents: The mobile phase was a mixture of purified water, acetonitrile (ACN) and methanol (MeOH). Each eluent contained 0.1 v/v% cc. perchloric acid solution ($HClO_4$-solution), which provided a slightly acidic eluent (pH of 0.1% $HClO_4$ in water is about 2.0). Under these conditions, the residual silanol groups of a silica based column are in ion suppressed form, therefore no electrostatic interaction is expected with basic solutes. ACN (gradient grade), MeOH (gradient grade) and $HClO_4$-solution (analytical grade) were purchased from Merck (Darmstadt, Germany). Water was prepared freshly, using ELGA Purelab UHQ water (ELGA, Lane End, UK). The model sample contained 2 mg/mL terazosin and its Ph. Eur. impurities at limit level (0.5 % for IMP A, IMP C, IMP E, IMP K and 0.1 % for IMP B, IMP J, IMP L, IMP M, IMP N, IMP O) (see Fig. 1). They were purchased from EDQM and Egis Pharmaceuticals Plc. chemical standard store. The sample solvent was MeOH/water = 70/30 v/v%.

Chemicals for Tanaka test (uracil, pentylbenzene, butylbenzene, o-terphenyl, triphenylene, baffeine, phenol, benzylamine), phosphoric acid, sodium hydroxide for buffers and standard reference buffers (pH 2.00, 4.01, 7.00 and 9.21) were purchased from Merck (Darmstadt, Germany). The MP 225 pH-meter was purchased from Mettler-Toledo (Mettler-Toledo, Greifensee, Switzerland). The buffers were filtered before use on regenerated cellulose filter membrane, 0.2-μm pore size (Sartorius, Goettingen, Germany).

2.2. Equipment, software

Three different liquid chromatographic systems were used during the study: (1) Acquity UPLC I-Class system equipped with binary solvent delivery pump (dwell volume, V_D was measured as 0.1 mL), an auto sampler with flow-through-needle (FTN) sample injector, a column thermostat, a photo diode array (PDA) detector and an (2) Acquity UPLC H-Class system equipped with quaternary solvent delivery pump (V_D was measured as 0.4 mL), an auto sampler with flow-through-needle (FTN) sample injector, a column thermostat, a photo diode array (PDA) detector were purchased from Waters Corporation (Milford, USA). An (3) 1290 Infinity II UHPLC system equipped with quaternary solvent delivery pump (V_D was measured as 0.5 mL), an auto sampler with flow-through-needle (FTN) sample injector, a column thermostat, and a diode array detector (DAD) was purchased from Agilent Technologies (Santa Clara, USA).

All chromatographic data were acquired and processed by Empower3 software (Waters Corporation, Milford, USA). UHPLC method development and modeling were performed by using DryLab4, v.4.3.1 software (Molnár-Institute, Berlin, Germany).

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

2.3. Stationary phases

Ph. Eur. declares 15 impurities of terazosin [16]. Based on preliminary experiments, it seemed that the appropriate separation of all solutes was more easily feasible on other than C18 RP phases (e.g. on perfluoro-phenyl). The columns used in this study were selected on the basis of the following criteria: all of them should be based on superficially porous particles (to neglect differences in morphology), with similar particle size (to have comparable specific surface area and efficiency) and perfluorophenyl surface modification.

The Kinetex F5 columns (100 × 3 mm, 2.6 μm, two different batches) were purchased from Phenomenex (Torrance, USA), SpeedCore PFP column (100 × 3 mm, 2.6 μm) was purchased from Fortis Technologies (Neston, UK), Poroshell 120 PFP column

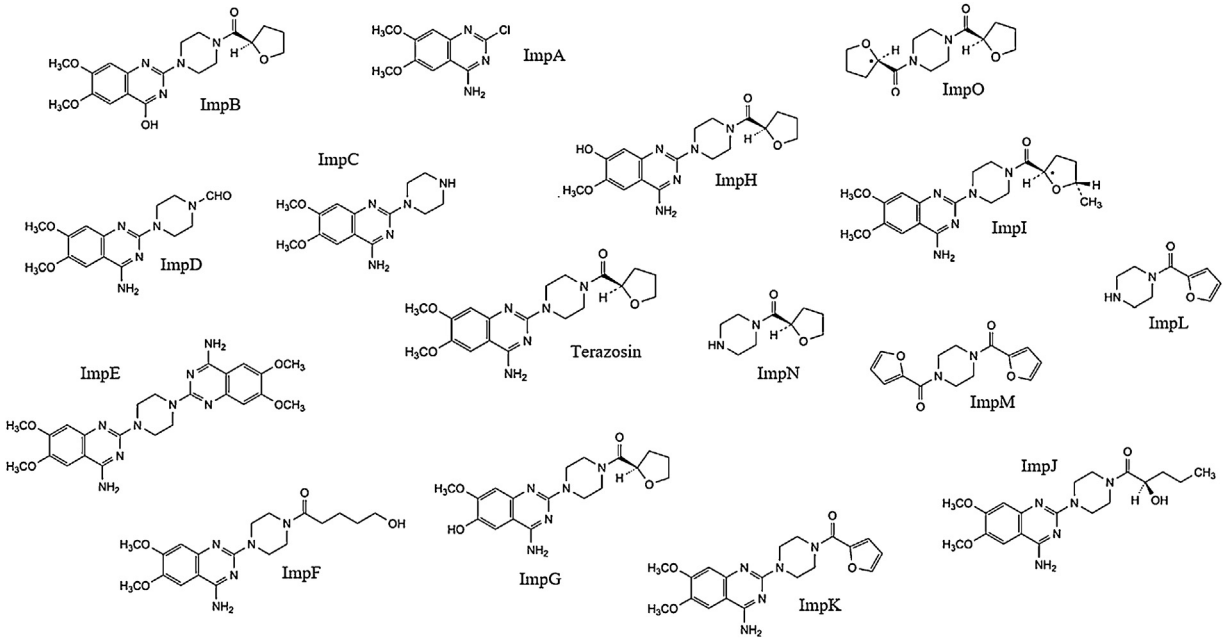


Fig. 1. Terazosin and its Ph. Eur. impurities which specified are A, B, C, E, J, K, L, M, N and O [15].

(100 × 3 mm, 2.7 μm) was purchased from Agilent Technologies (Santa Clara, USA).

2.4. Characterization of the stationary phases

Our goal was to introduce a strategy where – besides method optimization – a substitution (alternative) column can be offered as part of the robustness testing. About robustness, the ICH Q2 (R1) guideline contains the following "*The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. . . . In the case of liquid chromatography, examples of typical variations are . . . different columns (different lots and/or suppliers)*" [1].

Stationary phases are classified into groups by Pharmacopoeias based on their surface modification [18,19]. This means that a C18 column is not allowed to be replaced directly by a C8, phenyl or perfluorophenyl column. However in practice, columns with different surface chemistry can be quite similar in terms of overall selectivity [20] and – on the contrary – columns of the same group can provide substantial differences in selectivity. The procedure proposed by Tanaka offers a correct comparison of column selectivity [3,4]. The perfluorophenyl columns used in this study were compared by means of the protocol proposed in the Tanaka test.

Chromatographic conditions: Flow rate was 0.5 mL/min, temperature was set as 40 °C, injection volume was set to 0.1 μL (0.1 mg/mL sample concentration). Uracil was used as dead time marker. Five measures were used to characterize column selectivity. (1) Surface area and surface coverage (ligand density) were compared on the basis of retention factors (k) obtained for pentylbenzene (mobile phase: MeOH/water = 80/20 v/v%). (2) Hydrophobicity or hydrophobic selectivity (α_{CH2}) was ranked on basis of the ratio of pentylbenzene and butylbenzene retention, $\alpha_{CH2} = k_{PB}/k_{BB}$ (mobile phase: MeOH/water = 80/20 v/v%). (3) Shape selectivity ($\alpha_{T/O}$) was estimated by the selectivity observed between triphenylene and o-terphenyl, $\alpha_{T/O} = k_T/k_O$ (mobile phase: MeOH/water = 80/20 v/v%). (4) Hydrogen bonding capacity ($\alpha_{C/P}$) was measured by means of selectivity observed between caffeine and phenol, $\alpha_{C/P} = k_C/k_P$. This descriptor is a measure of the number of available silanol groups and the degree of end-capping (mobile phase: water/MeOH = 70/30 v/v%). (5a) Total ion-exchange capacity ($\alpha_{B/P\ pH=7.6}$) was determined by the selectivity observed between benzylamine and phenol, $\alpha_{B/P\ pH=7.6} = k_B/k_P$. This is an estimation of the total silanol activity (mobile phase: 20 mM Potassium-phosphate pH 7.6 in water/MeOH = 70/30 v/v%). And finally (5b) the acidic ion-exchange capacity ($\alpha_{B/P\ pH=2.7}$) was estimated by the selectivity obtained between benzylamine and phenol, $\alpha_{B/P\ pH=2.7} = k_B/k_P$ (mobile phase: 20 mM Potassium-phosphate pH 2.7 in water/MeOH = 70/30 v/v%). Table 1 shows the results of Tanaka tests.

2.5. Preliminary experiments

Based on former experiments, it has been seen that the solutes cover a broad range of lipophilicity therefore linear gradients were run from 5 to 80% MeOH content to sufficiently retain polar compounds and to elute the lipophilic solutes. Since the sample solvent was much stronger than the initial mobile phase composition, low volume injection was applied to avoid solvent mismatch and the early partial elution of the less retained polar solutes.

It worth mentioning that due to the high structural similarity of some compounds (e.g. Imp I, J and K) the baseline separation of all solutes was difficult.

During the preliminary experiments, three perfluorophenyl phases (columns) belonging to the USP L43 group were selected as promising candidates among many other different columns. The Kinetex F5 column was selected as reference column and our goal was to find the most appropriate and similar replacement column.

2.6. Method development

A general methodology of LC method development consists of simultaneously modeling the effect of method variables (typically mobile phase temperature (T) and gradient steepness (t_G)) on selectivity (or resolution) for a given column [20–23]. Today, it is also possible to simultaneously model more than two variables (up to three measured and other three calculated variables). In our work, gradient steepness (t_G), mobile phase temperature (T) and ternary mobile phase composition (t_C) were selected to build up resolution cubes (model response surfaces) in three dimensional space, show-

Table 1
Tanaka test results for characterization of column selectivity.

		Kinetex F5	SpeedCore PFP	Poroshell PFP
	t_0 (min)	0.82	0.83	0.83
(1) Retention factor for pentylbenzene	$t_{R,PB}$ (min)	2.03	2.10	1.93
	k_{PB}	1.48	1.53	1.31
	$t_{R,BB}$ (min)	1.73	1.82	1.73
(2) Hydrophobicity or hydrophobic selectivity	k_{BB}	1.12	1.19	1.08
	$\alpha_{CH2} = k_{PB}/k_{BB}$	1.32	1.29	1.22
	$t_{R,T}$ (min)	3.41	3.81	3.72
	k_T	3.18	3.60	3.46
(3) Shape selectivity	$t_{R,O}$ (min)	1.96	2.00	1.91
	k_T	1.40	1.41	1.29
	$\alpha_{T/O} = k_T/k_O$	2.27	2.55	2.68
	$t_{R,C}$ (min)	2.29	2.62	2.48
	k_C	1.80	2.16	1.97
(4) Hydrogen bonding capacity	$t_{R,P}$ (min)	2.83	3.39	3.65
	k_P	2.46	3.09	3.39
	$\alpha_{C/P} = k_C/k_P$	0.73	0.70	0.58
	$t_{R,BA}$ (min)	1.70	3.92	2.24
	k_{BA}	1.08	3.73	1.69
(5a) Total ion-exchange capacity	$t_{R,P}$ (min)	2.80	3.37	3.65
	k_P	2.43	3.06	3.38
	$\alpha_{BA/P} = k_{BA}/k_P$	0.45	1.22	0.50
	$t_{R,BA}$ (min)	1.06	1.46	0.87
	k_{BA}	0.29	0.76	0.04
(5b) Acidic ion-exchange capacity	$t_{R,P}$ (min)	2.82	3.39	3.60
	k_P	2.45	3.10	3.32
	$\alpha_{BA/P} = k_{BA}/k_P$	0.12	0.25	0.01

ing the critical resolution of the peaks to be separated against the three variables. Based on the preliminary experiments, it seemed that these selected variables have the most significant effect on selectivity and resolution for such analytes.

For the final optimization, two variables (t_G and T) were set at two levels (t_{G1} = 10 min, t_{G2} = 30 min and T_1 = 20 °C and T_2 = 40 °C), while the third one (t_C) was set at three levels (the mobile phase A consisted of 0.1 v/v% $HClO_4$-solution, and mobile phase B was varied as ACN (B1), MeOH (B2) and as their 50/50-mixture (B1–B2)). This full factorial design (virtual design of experiments to decrease the number of experiments to build up the retention model) required for 12 initial experiments ($2 \times 2 \times 3$) on a given column. Such experiments have been performed on all the selected columns. Detection wavelength was set at 220 nm, flow rate was 0.5 mL/min and injection volume was 0.5 μL.

2.7. Method validation

The developed method was validated according to the ICH guidelines [1] and the EDQM Techniqual Guide for the Elaboration of Monographs (part III. Analytical Validation) [24,25] for the following performance characteristics: selectivity, specificity, limit of detection, limit of quantification, linearity, precision (system-, method- and intermediate-), accuracy, stability of sample solutions and robustness.

The following solutions and samples were prepared and used for the validation procedure:

Blank solution and sample solvent was MeOH/water = 70/30 (v/v%).

Test solution was ~2 mg/mL terazosin hydrochloride working standard dissolved and diluted in sample solvent.

To evaluate method selectivity, the following sample was prepared (selectivity sample): Imp A, Imp B, Imp C, Imp D, Imp E, Imp F, Imp G, Imp H, Imp I, Imp J, Imp K, Imp L, Imp M, Imp N, Imp O impurity standards and terazosin hydrochloride working standard were dissolved and diluted in sample solvent to obtain a final concentration of ~10.0 μg/mL Imp A, Imp C, Imp E, Imp K, ~3.0 μg/mL Imp B, Imp D, Imp F, Imp G, Imp H, Imp I, Imp J, Imp L, Imp M, Imp N, Imp O and terazosin hydrochloride.

A so-called limit solution, Imp A, Imp B, Imp C, Imp E, Imp J, Imp K, Imp L, Imp M, Imp N, Imp O impurity standards and terazosin hydrochloride working standard were dissolved and diluted in sample solvent to obtain a final concentration of ~10.0 μg/mL Imp A, Imp C, Imp E, Imp K, ~ 3.0 μg/mL Imp B, Imp J, Imp L, Imp M, Imp N, Imp O, and terazosin hydrochloride.

A sample including the impurities in limit concentration and the API in nominal concentration was also prepared (limit spiked test solution). It consisted of ~10.0 μg/mL Imp A, Imp C, Imp E, Imp K, ~3.0 μg/mL Imp B, Imp J, Imp L, Imp M, Imp N, Imp O, and ~2.0 mg/mL terazosin hydrochloride (dissolved and diluted in sample solvent).

3. Results and discussion

3.1. Method development

3.1.1. Method optimization, finding the working point

Computer assisted method development becomes today a standard procedure [20–23]. Chromatographic modeling, including the possibility to calculate a model chromatogram, requires a decent peak tracking. This means that we need to know, where each peak elutes in a chromatogram during the initial runs (12 runs/conditions in this case) required building up the model. Peak tracking was done by matching peak areas (since peak areas are expected to remain constant at a given flow rate and detection wavelength).

Fig. 2 shows the resolution map (cube: response surface) obtained on the Kinetex F5 column and built up from the 12 initial experiments. Color codes indicate the critical resolution as function of method variables (t_C, t_G and T). Three different planes of the resolution cube are shown corresponding to 100% ACN (Fig. 2a), ACN/MeOH = 90/10 v/v% (Fig. 2b) and 100% MeOH (Fig. 2c) as mobile phase organic modifier (ternary) composition. The larger the red zone the more robust the condition is. As can be seen, the maximal robust space can be obtained with ACN/MeOH = 90/10 v/v%, thus ternary composition is preferred. Using 100% ACN or MeOH as eluent B, somewhat less robust separation can be performed. The highest resolution can be achieved at t_G = 50 min (5–80%B), T = 25 °C

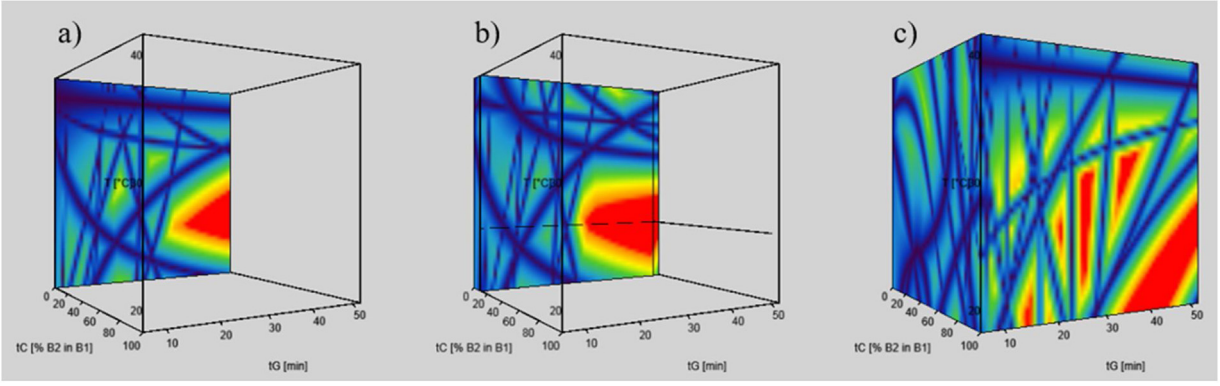


Fig. 2. 3D-critical resolution cube on Kinetex F5 column: a) 100% ACN, b) ACN/MeOH = 90/10 v/v% and c) 100% MeOH as mobile phase B. Red colour corresponds to conditions where $R_{s,crit} > 1.0$ (resolution of the critical peak pair) while blue color indicates coelution ($R_{s,crit} = 0$) of the closest ("critical") peaks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Final UHPLC method conditions.

Instrument	UHPLC/HPLC system with $t_D < 1$ min at 0.5 mL/min ($V_D < 0.5$ mL)		
Column	Kinetex 2.6 m F5, 100 × 3.0 mm		
Sample solvent	Methanol / Water = 70:30 (V/V%)		
Sample concentration	2.0 mg/mL		
Mobile phase			
"Eluent A"	0.1% Perchloric acid solution Perchloric acid Preparation: dissolve 1.0 mL cc. Perchloric acid (70%) in 1000 mL purified water		
"Eluent B"	Acetonitrile/Methanol = 90/10 (v/v%)		
Method	Gradient		
Gradient table	t [min]	Eluent A [%]	Eluent B [%]
	0	95	5
	$1-t_D$	95	5
	$20+(1-t_D)$	65	35
	t_D: dwell time (min)		
Equilibration time	4 min (start eluent composition)		
Flow rate	0.5 mL/min		
Column temperature	25 °C		
Sample temperature	20 °C		
Detection	220 nm		
Injected volume	0.5 μL		

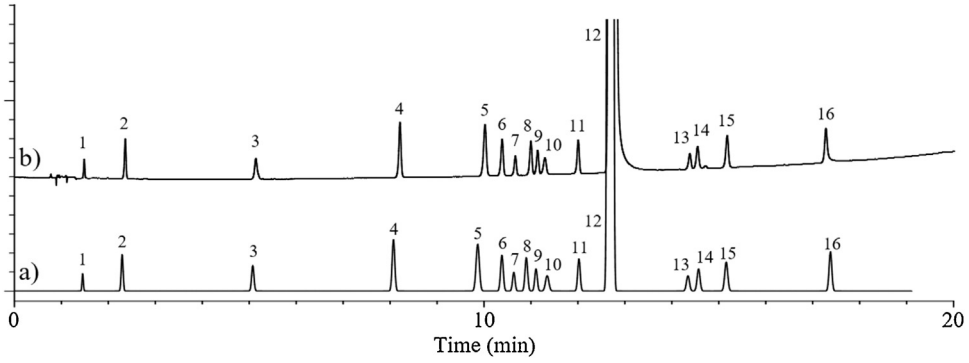


Fig. 3. Predicted (a) and experimental (b) chromatograms. Between ImpI and ImpJ $R_{s,crit} = 1.65$ was predicted while experimentally $R_{s,crit} = 1.61$ was measured. Peaks: 1: ImpN, 2: ImpL, 3: ImpO, 4: ImpC, 5: ImpA, 6: ImpB, 7: ImpG, 8: ImpD, 9: ImpH, 10: ImpM, 11: ImpF, 12: Terazosin, 13: ImpI, 14: ImpJ, 15: ImpK, 16: ImpE.

and t_C = ACN/MeOH = 90/10 v/v% as working point. These conditions correspond to a gradient steepness of 1.5%B/min. Under these conditions, the most retained solute elutes at 17.5 min. Therefore there is no need to run the gradient until 80%B. By maintaining the gradient steepness, the analysis can be stopped at 20 min which corresponds to 35%B eluent. Table 2 contains the final method conditions while Fig. 3 shows the experimental verification of the working point by comparing the predicted and the experimentally measured chromatograms to separate all the possible 16 compounds. Very good agreement was observed between the predicted and measured chromatograms.

3.1.2. Impact of the dwell volume, method transfer

A validated chromatographic method often needs to be transferred to other laboratories or to other chromatographic systems. Therefore it is important to take the gradient delay time/volume into account. For gradient separations, a good practice is to insert a relatively short (e.g. 1 min) isocratic segment at the very beginning of the separation before the gradient starts. Then, when transferring between different systems, by adjusting the length of the initial isocratic segment, the differences between gradient delay volumes can easily be corrected. In our method, a 1 min long initial isocratic

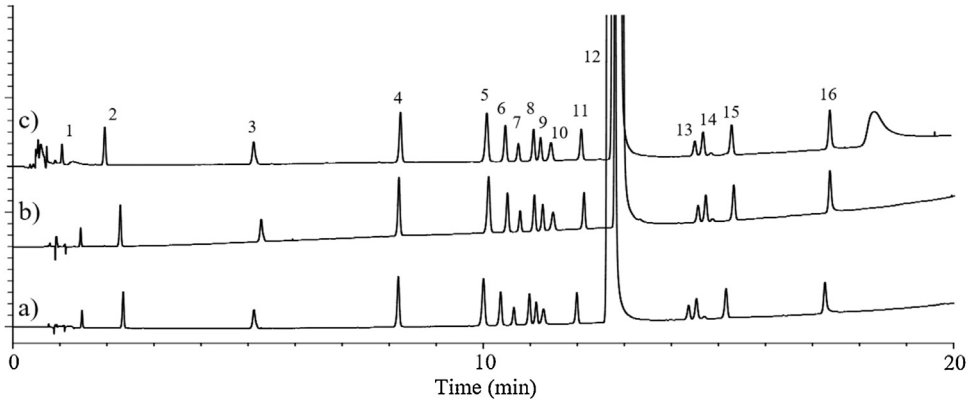


Fig. 4. Experimental chromatograms using Kinetex F5 column and different UHPLC systems. Systems: (a) Acquity UPLC I-Class, (b) Acquity UPLC H-Class, (c) Agilent 1290 Infinity II Quaternary. Peaks: 1: ImpN, 2: ImpL, 3: ImpO, 4: ImpC, 5: ImpA, 6: ImpB, 7: ImpG, 8: ImpD, 9: ImpH, 10: ImpM, 11: ImpF, 12: Terazosin, 13: ImpI, 14: ImpJ, 15: ImpK, 16: ImpE. Differences in gradient delay volumes have been taken into account.

segment is applied. At 0.5 mL/min flow rate, it corresponds to 0.5 mL delay volume.

When running this method on a system possessing V_D = 0.1 mL (Acquity I-Class) then its delay volume corresponds to t_D = 0.2 min. Therefore the gradient should start after t_{iso} = 1–0.2 = 0.8 min (t_{iso} is the length of the initial isocratic segment). When running the method on systems with V_D = 0.4 mL (Acquity H-Class) or V_D = 0.5 mL (1290 Infinity II) then accordingly t_{iso} = 0.2 min and t_{iso} = 0 min have to be set, respectively.

Fig. 4 shows three chromatograms measured on three systems possessing different gradient delay volumes, where the differences have been taken into account and the length of the initial isocratic segment was adjusted properly. The chromatograms show very similar profile and selectivity. The minor differences in retention times are probably due to the differences between the extra-column volumes and electronics (e.g. length and diameter of connecting tubes, delay of data acquisiton. . .).

3.1.3. Column comparison

The results of the Tanaka test already indicated some differences between the stationary phases, especially in ion-exchange capacity. The SpeedCore phase shows significantly higher ion-exchanger affinity ($\alpha_{BA/P}$ = 1.22) than the Kinetex and Poroshell phases ($\alpha_{BA/P}$ = 0.45 and 0.50, respectively) which can be important for the separation of ionizable compounds. Otherwise, slight differences in hydrophobicity, shape selectivity as well as in hydrogen bonding capacity were observed. Based on such test, it is hard to predict whether the columns are interchangeable or not for terazosin and related compounds. Therefore, the same 12 initial runs were also performed on the SpeedCore PFP and the Poroshell PFP columns similarly to the reference Kinetex F5 column (discussed in Section 3.1) in order to compare them for the separation of terazosin and related products. Retention model and resolution maps were built for each column. Each stationary phase was able to separate all the compounds however at different working points and the retention order of the solutes also changed. At gradient steepness of 1.5%B/min (t_G = 50 min, 5–80%B), the following working points were found: (1) T = 25 °C, t_C = ACN/MeOH = 90/10 v/v% on the Kinetex F5 column, (2) T = 28 °C, tC = ACN/MeOH = 74/26 v/v% on the SpeedCore PFP and (3) T = 33 °C, tC = ACN/MeOH = 60/40 v/v% on Poroshell PFP column. Fig. 5 shows the experimentally observed chromatograms on the three columns, operating them under their own working points (optimal conditions where the best separation can be achieved). Significant differences can be seen in both retentivity and selectivity. The Poroshell PFP column is clearly more diverse than the SpeedCore PFP, as compared to the Kinetex F5 phase, multiple retention order change is found. Between the Kinetex F5 and SpeedCore PFP phases, only one peak pair showed retention order change (Imp B and Imp G). When setting the same conditions on all columns (as reference condition, the working point obtained on the Kinetex F5 column was applied) it was not possible to perform a good separation on the Poroshell PFP column. Co-elution and not appropriate retention were observed on the Poroshell PFP phase. However, the SpeedCore column provided very similar retention window and elution order compared to the reference Kinetex F5 column. Therefore at the end, the SpeedCore PFP stationary phase was selected as a suitable alternative column to Kinetex F5 for the separation of terazosin and the 10 specified related impurities.

Table 3
Correction factors for impurities of Terazosin hydrochloride.

Component	CF
IMP A	0.46
IMP B	0.79
IMP C	0.66
IMP E	1.05
IMP J	1.20
IMP K	0.97
IMP L	1.27
IMP M	1.39
IMP N	3.97
IMP O	1.61

Besides finding an alternative column, batch to batch comparison is also required today. To compare two batches of the Kinetex F5 column and the alternative SpeedCore PFP column, their response surfaces and robust zones have been compared by means of their resolution cubes (calculated for the 10 specified impurities for which the method needs to be validated). Fig. 6a, b and c show the resolution cubes obtained with Kinetex F5 columns (two batches) and the SpeedCore PFP material, respectively. In addition, Fig. 6 d illustrates the common robust space of the resolution cubes as an overlay of the three cubes ($R_{s,crit}$ > 1.5). The stack plot (Fig. 6d) clearly illustrates the high degree of robustness at the working point on all three columns.

The above described 12 initial experiments based column comparison (screening) approach has the advantage that suitability of a column – for a given application – can be evaluated quickly at the very early stage of the method development. In addition, column interchangeability can also be estimated during the robustness study. To help in the selection of the most promising alternative column, the new version of a commercial software allows the users to directly compare (match) the parts of the resolution cubes where a required resolution criterion (e.g. $R_{s,crit}$ > 1.5) is fulfilled. However,

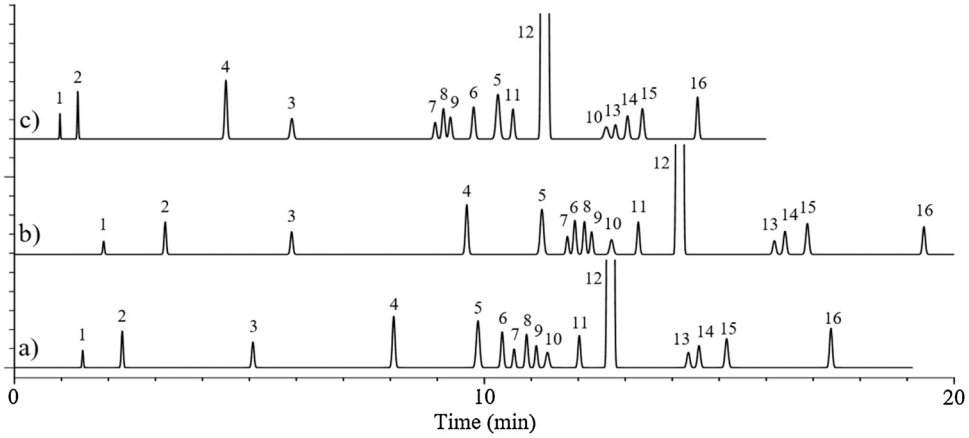


Fig. 5. Chromatograms measured at the optimal conditions on Kinetex F5 (a), SpeedCore PFP (b) and Poroshell PFP (c) columns. Peaks: 1: ImpN, 2: ImpL, 3: ImpO, 4: ImpC, 5: ImpA, 6: ImpB, 7: ImpG, 8: ImpD, 9: ImpH, 10: ImpM, 11: ImpF, 12: Terazosin, 13: ImpI, 14: ImpJ, 15: ImpK, 16: ImpE.

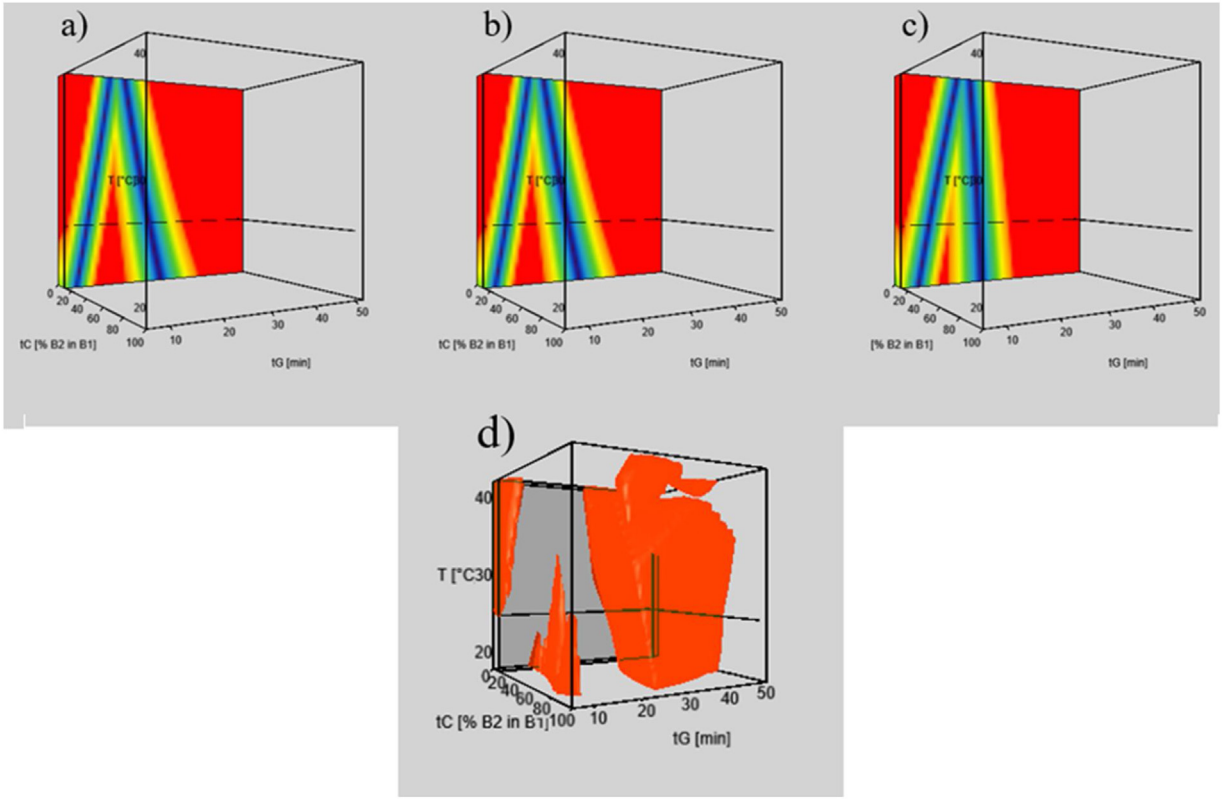


Fig. 6. 3D-critical resolution cubes with the chosen working point obtained on Kinetex F5 batch1 (a), Kinetex F5 batch2 (b), and SpeedCore PFP (c). Baseline resolution regions for the 10 certified impurities are shown in red. Panel (d) shows the common robust space on the overlay of the three resolution cubes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

it needs to be mentioned that the retention order of the solutes should be checked carefully (change in elution order may happen).

3.2. Method validation

3.2.1. Validation protocol

Details of method validation are included in the EDQM Technical Guide for the Elaboration of Monographs (part III. Analytical Validation) [24].

3.2.2. Selectivity, specificity

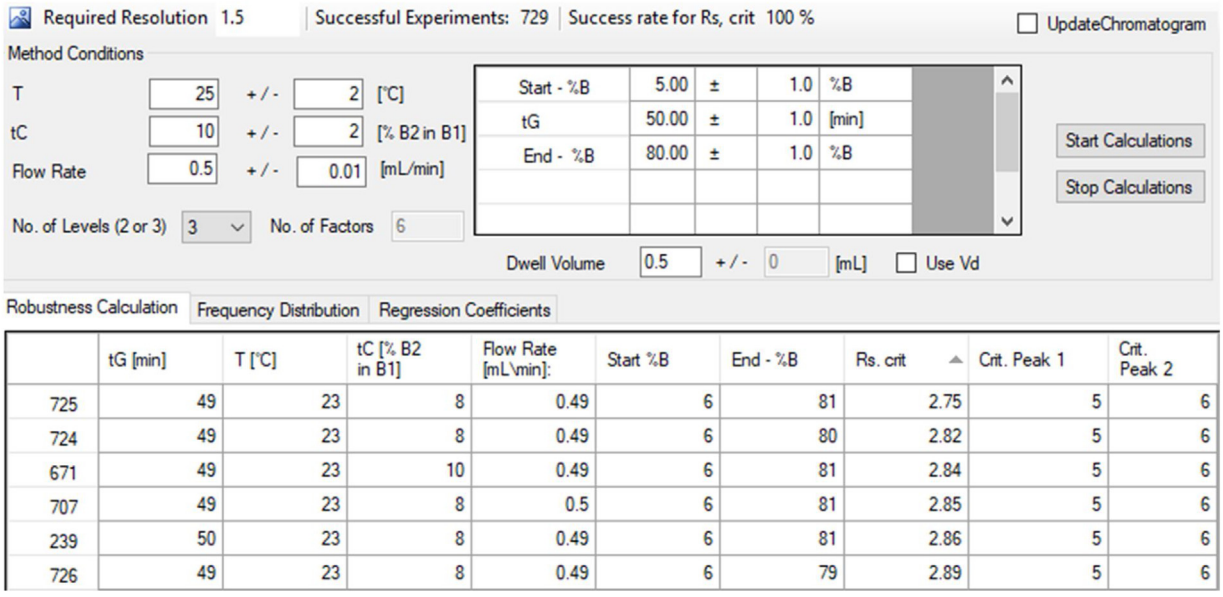
Selectivity and specificity of the method was checked by injecting blank solution and "selectivity solution". No interference was detected at the retention time of terazosin hydrochloride and its impurities. All peaks were separated. The method is considered then as being selective for the determination of impurities in terazosin hydrochloride API.

3.2.3. Limit of detection (LD), limit of quantification (LQ)

Detection and quantification limits are calculated by diluting method. Stock solution – containing the specified impurities at limit level – have been prepared. Stock solution was diluted until peak heights of the impurities meet the signal to noise ratio requirements of LD and LQ. The highest LQ level was 0.0154% (for impurity E), this value is less than the disregard limit generally applied in pharmacopoeia methods (0.05%), so the criteria was fulfilled. Fur-

Table 4
Comparison of validation criteria and results.

Parameter	Measurement	Requirement	Result	
Selectivity, specificity	–	no interference peak at the retention time of Terazosin or its impurities	no interference peak at the retention time of Terazosin or its impurities	Fulfilled
Limit of quantification (LQ)	signal-to-noise ratio	LQ level NMT 0.03%	0.0043%–0.0154 %	Fulfilled
Linearity	Correlation coefficient	NLT 0.990	0.9980 - 0.9996	Fulfilled
System precision	RSD (%)	NMT 5.0%	0.44%–1.55%	Fulfilled
Method precision	RSD (%)	NMT 5.0%	0.26%–2.99%	Fulfilled
Intermediate precision	Difference (%)	NMT ± 20.0%	−5.58%–4.08%	Fulfilled
Accuracy	Recovery (%)	75.0%–125.0%	86.56%–124.81%	Fulfilled
Stability of sample solutions	Recovery (%)	85.0%–115.0%	92.4%–113.0%	Fulfilled
Robustness	$R_{s,crit}$ between Imp A and Imp B	NLT 1.5	>2.75	Fulfilled



	tG [min]	T [°C]	tC [% B2 in B1]	Flow Rate [mL\min]:	Start %B	End - %B	Rs. crit	Crit. Peak 1	Crit. Peak 2
725	49	23	8	0.49	6	81	2.75	5	6
724	49	23	8	0.49	6	80	2.82	5	6
671	49	23	10	0.49	6	81	2.84	5	6
707	49	23	8	0.5	6	81	2.85	5	6
239	50	23	8	0.49	6	81	2.86	5	6
726	49	23	8	0.49	6	79	2.89	5	6

Fig. 7. Set deviations (levels) of method variables considered for the virtual robustness study and the calculated results (Rs, crit and critical peak pairs) for the six worst separations among the 729 virtual experiments.

thermore, the highest LD value obtained was 0.0046% (for impurity E).

3.2.4. Linearity

The proposed method provides a linear signal response between LQ and the 133% of nominal concentration for all compounds. The correction factors (CF) for impurities of terazosin hydrochloride were calculated from the slopes of the individual calibration curves. The results obtained are summarized in Table 3. All the results fulfilled the criteria.

3.2.5. Precision

3.2.5.1. System precision. Replicate injections (n = 6) of the limit solution were carried out to determine the statistical error parameters (standard deviation (SD), relative standard deviation (RSD) and confidence interval) of the applied method. The calculated statistical parameters have fulfilled the requirements of system precision. See the results in Table 4.

3.2.5.2. Method precision. Six independent limit spiked test solutions were analyzed. One injection was carried out from each solution. The calculated statistical parameters have fulfilled the requirements of method precision. See the results in Table 3.

3.2.5.3. Intermediate precision. The study of intermediate precision was carried out performing the analysis on two different LC systems (Acquity UPLC I-Class and Acquity UPLC H-Class), using two different columns and by two different analysts. Then the measured impurity contents of terazosin hydrochloride "limit spiked test solutions" were compared. The requirements of intermediate precision were fulfilled.

3.2.6. Accuracy (Recovery)

A terazosin hydrochloride API sample was spiked with different quantities of impurities. The measurements were accomplished at three different levels and with triplicate measurements. The three levels were set as limit, 50% of the limit and 0.03% of the limit and measured levels were determined in recovery percentage. All the recovery data ranged between 86.56% and 124.81%, thus the method was found to be accurate.

3.2.7. Stability of sample solutions

The "test solution", the "limit solution" and the "limit spiked test solution" were analyzed over a period of 48 h. The solutions were stored in the autosampler (at 20 °C, protected from light) during that period. The obtained results showed that the solutions were stable for a period of 48 h and all the recovery data ranged between 92.4% and 113.0%, which fulfilled the requirements.

3.2.8. Robustness

Validated analytical methods must be capable of producing adequate results not only for short term and in a limited environment

but also for a long time period and in a wide, often hardly predictable, complex analytical environment (e.g. method transfer across locations and instruments) [26].

In fact, during a daily application of a method, possible alterations of chromatographic parameters and consequently, slight fluctuations of method performance can be expected. On one hand these alterations are caused by normal variations of instrument properties, such as solvent delivery, solvent mixing, and temperature-controlling that all can be characterized with the so called instrument specification limits (ranges). On the other hand, deviations might be caused by some less evident variations, like batch-to-batch differences of columns, of which effects might be less substantial but require a deep understanding and a systematic plan [11,26].

Robustness, i.e., the ability of a method, to resist against these possible alterations is well known but less detailed in the relevant guidelines. As mentioned, while there is a plain description on how to characterize method performance in terms of accuracy, specificity, linearity, precision, detection limit, qualification limit in ICH Q2 (R1) [1], it is less obvious how to evaluate the robustness of a method in practice. Nevertheless, the ICH's Q2 (R1) clearly formulates that *"The evaluation of robustness should be considered during the development phase. . ."* (stage 1). At the same time, consequences of non-systematic method development comes to the front very often only at a later phase, namely at the performance qualification (stage 2) or even later, at stage 3 [27]. This brings to the relevance of an early-stage robustness evaluation.

Software-based robustness calculation has the advantage that not only all single solvent and instrument effects, but also all conceivable combinations of them can be calculated in a model-mediated way [10]. For these purposes, the robustness module of a commercial software was used (Fig. 7). During the virtual robustness study, the impact of the three model variables (t_G, T, t_C), and three additional calculated variables (flow rate, initial- and final mobile phase compositions) was studied around the working point. The effect of these six variables (in this manner they can be considered as factors of a virtual experimental design) was evaluated at three levels (−1, 0, +1). The modeled deviations from the nominal values were the following: gradient time was set to 49, 50 and 51 min, temperature was set to 23, 25 and 27 °C, ternary composition of mobile phase was set to 88/12, 90/10 and 92/8 v/v%, flow rate was set to 0.49, 0.50 and 0.51 mL/min, initial mobile phase composition was set to 4, 5 and 6 %B and its final composition was set to 79, 80 and 81 %B. Then, the 729 experiments (3^6) were performed in silico.

A criterion of $R_{s,crit} > 1.5$ was considered. As shown is Fig. 7, the lowest predicted resolution was Rs = 2.75 between peak 5 and 6 (Imp A and Imp B) which is still acceptable (Rs > 1.5). Therefore, the method can be considered as robust, since the success rate to perform $R_{s,crit} > 1.5$ separation was 100% in the studied range of method variables.

3.2.9. Validation results

Table 4. represents the comparison of method validation criteria for selectivity, limit of quantification, linearity, system precision, method precision, intermediate precision, accuracy, stability and robustness with the results obtained during the validation process. All the results were within the limits of method validation criteria for each parameter.

4. Conclusion

The aim of this work was to actualize the method described for terazosin related substances in the Ph. Eur. monograph. Since the original monograph described two 45 min (=90 min) long HPLC methods, the update of the old method was strongly required to gain in analysis time, separation quality and to simplify the procedure to one method only instead of two.

A workflow was proposed for the first time to compare the resolution of an impurity profiling method in a wide range of three measured and three calculated variables and to find possible replacement columns for the method. Our strategy was based on the use of state-of-the-art chromatographic modeling software, allowing to visually compare the parts of response surfaces (resolution cubes) obtained with different columns, where the criterion for a selected critical resolution is fulfilled. A section of robust spaces can then easily be found by overlaying resolution cubes.

Three pentafluoro-phenyl columns (Kinetex F5, SpeedCore PFP and Poroshell PFP) packed with particles of similar morphology (sub-3 μm, superficially porous particles) were systematically compared. It was found that two of the three columns share the same working point and are robust around this condition. Therefore, these two columns from different providers (Kinetex F5, SpeedCore PFP) were considered as interchangeable columns for this given separation.

Our real life case study seemed to be a good and representative example to illustrate the implementation of an early stage robustness study. Based on 12 initial experiments – on a given column –, robustness could be accurately estimated (on the basis of retention modeling). Six variables have been selected to virtually study their impact on the quality of separation.

Finally, a single robust method was developed and proposed (20 min analysis time) to update the old pharmacopoeia method. The fact, that retention modeling was involved makes the method adjustment more flexible, since only "adjustment" of the method variables is not considered to be a "change". Therefore slight deviations of method variables can be done (allowed) in order to meet the criterion of the separation (e.g. when transferring the method). Thus, method revalidation is not necessary.

This study also demonstrates that common column tests are hardly applicable in practice to find alternative columns.

Author statement

All the authors agreed to submit this work to Journal of Pharmaceutical and Biomedical analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] ICH, Q2 (R1)/Validation of Analytical Procedures: Text and Methology, 2005.
[2] US Pharmacopoeia, United States Pharmacopoeia (USP) XIX Fourth Supplement, 2020.
[3] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, N. Tanaka, Correlation between a preparation method and retention behavior of stationary phase, J. Chromatogr. Sci. 27 (1989) 721–728.
[4] M.R. Euerby, P. Petersson, Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns using principal component analysis, J. Chromatogr. A 994 (2003) 13–36.
[5] M.R. Euerby, P. Petersson, Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing polar embedded groups/amino endcappings using principal component analysis, J. Chromatogr. A 1088 (2005) 1–15.
[6] M.R. Euerby, P. Petersson, Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing phenyl moieties using principal component analysis, J. Chromatogr. A 1154 (2007) 138–151.
[7] G. Galea, D. Mangelings, Y. Vander Heyden, Characterization and classification of stationary phases in HPLC and SFC - a review, Anal. Chim. Acta 886 (2015) 1–15.

[8] U.D. Neue, Stationary phase characterization and method development, J. Sep. Sci. 30 (2007) 1611–1627.
[9] A.H. Schmidt, M. Stanic, I. Molnár, In silico robustness testing of a compendial HPLC purity method by using of a multidimensional design space build by chromatography modeling — case study pramipexole, J. Pharm. Biomed. Anal. 91 (2014) 97–107.
[10] R. Kormány, I. Molnár, J. Fekete, D. Guillarme, S. Fekete, Robust UHPLC separation method development for multi-API product amlodipine and bisoprolol: the impact of column selection, Chromatographia 77 (2014) 1119–1127.
[11] R. Kormány, K. Tamás, D. Guillarme, S. Fekete, A workflow for column interchangeability in liquid chromatography using modeling software and quality-by-design principles, J. Parm. Biomed. Anal. 146 (2017) 220–225.
[12] ICH, Q8 (R2) / Guidance for Industry, Pharmaceutical Development, 2009.
[13] R. Peraman, K. Bhadraya, Y.P. Reddy, Analytical Quality by Design: a tool for regulatory flexibility and robust analytics, Int. J. Anal. Chem. 1 (2015) 1–9.
[14] J. Kochling, W. Wu, Y. Hua, Q. Guan, J. Castaneda-Merced, A platform analytical quality by design (AQbD) approach for multiple UHPLC-UV and UHPLC–MS methods development for protein analysis, J. Pharm. Biomed. Anal. 125 (2016) 130–139.
[15] A. Dispas, H.T. Avohou, P. Lebrun, P. Hubert, C. Hubert, 'Quality by Design' approach for the analysis of impurities in pharmaceutical drug products and drug substances, Trends Analyt. Chem. 101 (2018) 24–33.
[16] European Pharmacopeia (Ph. Eur.) 10.0, Terazosin Hydrochloride Dihydrate 01/, 2017, pp. 3976–3978, 2021.
[17] R. Kormány, I. Molnár, J. Fekete, Renewal of an old European Pharmacopoeia method for Terazosin using modeling with mass spectrometric peak tracking, J. Pharm. Biomed. Anal. 135 (2017) 8–15.
[18] European Pharmacopeia (Ph. Eur.) 10.0, 2.2.46. Chromatographic Separation Techniques 07/, 2016, pp. 80–87, 20246.
[19] United States Pharmacopoeia, USP-NF <621> Chromatography.
[20] R. Kormány, J. Fekete, D. Guillarme, S. Fekete, Reliability of simulated robustness testing in fast liquid chromatography, using state-of-the-art column technology, instrumentation and modelling software, J. Pharm. Biomed. Anal. 89 (2014) 67–75.
[21] R. Komárny, I. Molnár, H. Rieger, Exploring better column selectivity choices in ultra-high perfomace liquid chromatography using Quality by Design principles, J. Pharm. Biomed. Anal. 80 (2013) 79–88.
[22] S. Fekete, J. Fekete, I. Molnár, K. Ganzler, Rapid high performance liquid chromatography method development with high prediction accuracy, using 5 cm long narrow bore columns packed with Sub-2μm particles and design space computer modeling, J. Chromatogr. A 1216 (2009) 7816–7823.
[23] A. Schmidt, I. Molnár, Using an innovative Quality-by-Design approach for development of stability indicating UHPLC method for ebastine in the API and pharmaceutical formulations, J. Pharm. Biomed. Anal. 78-79 (2013) 65–74.
[24] EDQM / Techniqual Guide for the Elaboration of Monographs, 7th edition, 2015.
[25] M.K. Parr, A.H. Schmidt, Life cycle management of analytical methods, J. Pharm. Biomed. Anal. 147 (2018) 506–517.
[26] N. Rácz, R. Kormány, J. Fekete, I. Molnár, Establishing column batch repeatability according to Quality by Design (QbD) principles using modeling software, J. Pharm. Biomed. Anal. 108 (2015) 1–10.
[27] / Stimuli Article on Lifecycle Management of Analytical Procedures Posted for Comment, USP, 2013.