Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 66 (2007) 475–482
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Method transfer for fast liquid chromatography in pharmaceutical analysis: Application to short columns packed with small particle. Part I: Isocratic separation

Davy Guillarme, Dao T.-T. Nguyen, Serge Rudaz, Jean-Luc Veuthey *

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland

Received 22 September 2006; accepted in revised form 29 November 2006
Available online 13 December 2006

Abstract

Liquid chromatography (LC) is considered to be the gold standard in pharmaceutical analysis. Today, there is a need for fast and ultra-fast methods with good efficiency and resolution for achieving separations in few minutes or even seconds. The present work describes a simple methodology for performing a successful method transfer from conventional LC to fast and ultra-fast LC.

In order to carry out fast separations, short columns (20–50 mm) packed with small particles (3.5 and 1.7 μm) were used and their chromatographic performance was compared to that of a conventional column (150 mm, 5 μm). For that purpose, an optimized LC system was employed to limit extra-column volumes which can have a dramatic impact on efficiency and resolution.

This paper reports the fundamental equations used for transferring an isocratic chromatographic separation performed with a given column geometry and chemistry to a smaller column packed with similar or identical stationary phase, without influence on chromatographic performance. For this purpose, the flow rate and the injected volume need to be adapted.

The effect of column length and particle size reduction on chromatographic resolution and analysis time was described for an isocratic separation. Using the method transfer equations, it is possible to predict the new conditions to be used, for fast and ultra-fast separations. In this work, ultra-fast separations were achieved thanks to a new generation of instrumentation (ultra performance liquid chromatography, UPLC) which uses simultaneously short column packed with sub-2 μm particles and ultra-high pressure (up to 1000 bar). This work demonstrates an analysis time reduction up to a factor 12, compared to a conventional LC separation, without affecting the quality of separation. Therefore, the complete resolution of a pharmaceutical formulation was achieved in only a few seconds.

Keywords: Fast liquid chromatography; Isocratic transfer; Short columns; UPLC; Sub-2 μm particles

1. Introduction

The development of a drug product is a long and tedious task, taking about 10–15 years from the synthesis of a lead compound to its commercialisation. One of the main objectives of the pharmaceutical industry is to reduce this time period by using high-throughput discovery and screening methods [1]. Therefore, the analytical laboratory has to manage a great number of samples and must reduce the time response delivery at each step during drug development [2]. A solution for this problem is to develop new rapid and efficient procedures for qualitative and quantitative analysis.

The analytical techniques commonly used for screening and performing quantitative determinations are immunoassay, spectroscopy, and separation tools such as chromatography

* Corresponding author. Laboratory of Analytical Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Bd d'Yvoy 20, 1211 Geneva 4, Switzerland. Tel.: +41 22 379 63 36; fax: +41 22 379 68 08.
E-mail address: jean-luc.veuthey@pharm.unige.ch (J.-L. Veuthey).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.11.027

and electrophoresis. Liquid chromatography (LC) is often considered most suitable for the quantitative determination of drugs and related substances. However, conventional analyses usually need between 10 and 45 min. As reported in the literature [3], rapid or fast methods are relative terms depending on the analyst and the requirement. Ultra-fast or ultra-rapid methods can be defined by cycle times inferior to 1 min (including column reconditioning), and fast or rapid methods by cycle times inferior to 5 min. In order to carry out rapid and ultra-rapid analyses, different strategies can be applied.

The use of monolithic instead of packed columns containing porous particles is an innovative approach. Monolithic rods, made of silica or polymeric material, contain a porous structure, providing lower flow resistances and backpressure than conventional columns [4,5]. Monolithic columns can operate at high flow rates (typically 3–10 times larger than conventional LC) without generating high backpressure and with efficiency and resolution comparable to those of silica particles of ca. 3–3.5 μm [6]. This behaviour is due to its bi-modal structure with macropores and mesopores. However, these supports made of silica present several drawbacks in terms of low resistance to high pH values (pH > 7), as well as limited choice of surface chemistry and column dimension [7].

High temperature liquid chromatography (80 °C < T < 200 °C) allows the use of high flow rates without loss in efficiency or increase in backpressure. Indeed, at high temperature, the mobile phase viscosity is reduced and the diffusion coefficients of the solutes in the mobile phase are increased [8]. However, this strategy suffers of limitations such as the small number of stable packing materials at temperatures higher than 80 °C as well as the potential degradation of thermolabile compounds and the need to have a constant temperature along the chromatographic system. Therefore, until now, the pharmaceutical industry has not considered this approach routinely [9].

The use of short columns (20–50 mm) to decrease the analyte retention volume and increasing the flow rate are the simplest approaches to reduce analysis time while limiting the generated backpressure [10]. However, both of these can compromise the chromatographic performance. Therefore, particle size should be simultaneously decreased or other chromatographic materials such as monoliths may be developed. The last decade, new supports appeared on the market using small (3–3.5 and sub-2 μm) silica-based particles. According to the theory, efficiency and optimal velocity are increased with small particles, allowing a separation with good resolution in a short analysis time [11]. Furthermore, due to a better mass transfer, velocity can be increased beyond its optimal value maintaining a good efficiency. However, a high flow rate applied on small particles, particularly with sub-2 μm particles, can generate a high backpressure (>400 bar), which is not compatible with conventional instrumentation. Thus, new equipments have been recently commercialised to perform analyses at high pressure (up to 1000 bar) such as the UPLC™ (ultra performance liquid chromatography from Waters). With this approach, the columns must be able to operate at high pressure [12,13] and new silica-based materials have been recently developed for this purpose [14]. Finally, when the same stationary phase chemistry is used, it is possible to easily transfer existing methods to fast chromatography. By applying scaling factors, the modifications of the column length and/or particle dimension allow us to obtain fast analytical methods without losing resolution or sensitivity [15].

Whatever the selected strategy, fast or ultra-fast analyses present some constraints in terms of extra-column volumes, injection time, and detector response [16,17].

This paper describes the equations used for transferring an isocratic chromatographic method from a given system to another one (i.e. with different column geometry but similar stationary phase) with limited influence on chromatographic resolution. This methodology was applied to the transfer of conventional methods toward fast and ultra-fast chromatographic separations. To speed up the chromatographic separation, two procedures were implemented, namely the reduction of column length and particle size. The advantages/drawbacks and impact of both strategies on chromatographic performance (efficiency and resolution) were briefly discussed. As example and following these simple rules, it was possible to analyze a pharmaceutical formulation (Rapidocaïne®) containing lidocaïne, a local anaesthetic used for its propriety of cardiac antiarrhythmia, in only few seconds instead of minutes in conventional conditions with the same performance.

2. Theory

When dealing with geometrical method transfer, stationary phase nature has to be identical in the original and final method. Then it is of prime importance to check the compatibility of the chromatographic system with the new column dimensions. In isocratic mode, an additional band broadening, due to the instrumentation (known as "extra-column effect" or "extra-column band broadening"), could occur and become predominant when the column volume is reduced. Equations allowing to better understand the influence of extra-column effects according to the column dimensions are presented (Section 2.1),

Abbreviations: d_c, column internal diameter; D_m, diffusion coefficient of solute at very low concentration in solvent; d_p, particle size in packed bed; F, mobile phase flow rate; k, retention factor; K_{cell}, constant linked to UV cell configuration; K_{inj}, constant linked to injection mode; L, column length; L_c, connection tubing length; N, plate number; N_{col}, theoretical column plate number; N_{obs}, experimentally observed number of column plates; r_c, connection tubing radius; t_0, column dead time; t_r, analysis time; u, mobile phase linear velocity; v, reduced mobile phase linear velocity; V_0, column dead volume; V_{cell}, UV cell volume; V_{inj}, injected volume; V_R, retention volume; σ^2_{col}, column dispersion; σ^2_{det}, variance due to detector; σ^2_{ext}, extra-column dispersion; σ^2_{inj}, variance due to injector; σ^2_{tot}, observed peak variance; σ^2_{tub}; variance due to tubing; τ, detector constant time.

and are discussed on the basis of experimental data in Section 4.1.

In a second step, some adaptations are mandatory on injected volume and mobile phase flow rate to maintain chromatographic performance during the method transfer process. Corresponding equations are given in Section 2.2 and experimental results obtained in fast-LC with shorter columns or columns packed with smaller particles are discussed in Sections 4.2 and 4.3.

2.1. Extra-column band broadening

With small column dimensions, it is of prime importance to avoid any detrimental extra-column volumes. Indeed, the observed peak variance (σ^2_{tot}) is linked to the chromatographic column itself (σ^2_{col}) and all the extra-column volumes of the chromatographic system (σ^2_{ext})·σ^2_{tot} could be expressed as:

$$\sigma^2_{tot} = \sigma^2_{col} + \sigma^2_{ext}. \quad (1)$$

For a good separation, the ratio between extra-column variance σ^2_{ext} and total variance should be less than 10%.

Dispersion linked to the chromatographic column itself (σ^2_{col}) could be given by:

$$\sigma^2_{col} = \frac{V_R}{\sqrt{N}} = \frac{V_0 \cdot (1+k)}{\sqrt{N}}, \quad (2)$$

where σ^2_{col} is the column variance (in μL^2); N is the number of plates and V_R is the retention volume which is a function of the column's dead volume V_0 and the retention factor k ($V_R = V_0 \cdot (1+k)$).

Extra-column band broadening could be expressed as the sum of three main dispersion sources:

$$\sigma^2_{ext} = \sigma^2_{inj} + \sigma^2_{det} + \sigma^2_{tub}, \quad (3)$$

where σ^2_{ext} is the extra-column variance, while σ^2_{inj}, σ^2_{det} and σ^2_{tub} are variances due to the injector, the detector and tubing, respectively. Thus, σ^2_{ext} depends on the injected volume V_{inj}, the tubing radius r and length l, the flow-cell volume V_{cell}, the detector time constant τ and the flow rate F according to the equation given below:

$$\sigma^2_{ext} = K_{inj} \cdot \frac{V^2_{inj}}{12} + K_{cell} \cdot \frac{V^2_{cell}}{12} + \tau^2 \cdot F^2 + \frac{r^4_c \cdot l_c \cdot F}{7.6 \cdot D_m}, \quad (4)$$

in which case K_{inj} and K_{cell} are constant (generally between 1 and 3 [18]) and linked to the injection mode and the UV cell geometry, respectively.

Following the determination of the σ^2_{col} and σ^2_{ext} contributions, the observed number of plates (N_{obs}) can be estimated, taking into account the extra-column band broadening, by the equation below:

$$N_{obs} = N_{col} \cdot \frac{1}{1 + \frac{\sigma^2_{ext}}{\sigma^2_{col} + \sigma^2_{ext}}}, \quad (5)$$

where N_{col} is the theoretical number of plates of the considered chromatographic support.

2.2. Transfer rules in isocratic mode

Two important parameters, the injection volume and the mobile phase flow rate, have to be modified for transferring an isocratic method in a column packed with identical stationary phase but other dimensions.

In order to avoid a detrimental extra-column band broadening (Eq. (4)) and maintain an equivalent level of sensitivity, it is necessary to adapt the injection volume to the column dimension. The new injected volume (V_{inj_2}) can be determined using the dead volumes of the original and transferred method, V_{0_1} and V_{0_2}, respectively, according to the following equation:

$$V_{inj_2} = V_{inj_1} \cdot \frac{V_{0_2}}{V_{0_1}}. \quad (6)$$

The column dead volume can be expressed as:

$$V_0 = \pi \cdot \frac{d^2_c}{4} \cdot L \cdot \varepsilon, \quad (7)$$

where d_c is the column internal diameter, L is the column length and ε is the support porosity with the same stationary phase. Therefore, the injected volume can be calculated according to the following equation:

$$V_{inj_2} = V_{inj_1} \cdot \frac{d^2_{c_2}}{d^2_{c_1}} \cdot \frac{L_2}{L_1}. \quad (8)$$

For a successful method transfer, the reduced linear velocity of the mobile phase (v) must be kept constant, since this value is independent of the column geometry or mobile phase flow rate:

$$v = \frac{u \cdot d_p}{D_m}, \quad (9)$$

with u as the mobile phase linear velocity (which depends on the column diameter), d_p the particle diameter, and D_m the solute diffusion coefficient. Therefore, for a geometrical transfer, the product ($u \cdot d_p$) remains constant and the new flow rate (F_2) is calculated with the following equation:

$$F_2 = F_1 \cdot \frac{d^2_{c_2}}{d^2_{c_1}} \cdot \frac{d_{p_1}}{d_{p_2}}. \quad (10)$$

Finally, the total analysis time of the transferred method, maintaining retention factors constant, can be estimated according to:

$$t_{r_2} = t_{r_1} \cdot \frac{F_1}{F_2} \cdot \frac{V_{0_2}}{V_{0_1}}. \quad (11)$$

All these equations can be easily used for determining the new parameters of a transferred isocratic method. A freely usable Excel® program called "HPLC calculator", automatically establishing optimal conditions for method transfer in isocratic mode, has been distributed on an internal website [19].

3. Experimental

3.1. Materials

Lidocaine hydrochloride, methylparaben, and propylparaben were obtained from Sigma–Aldrich (Steinheim, Germany) and 2,6-dimethylaniline from Fluka (Buchs, Switzerland). The pharmaceutical formulation (Rapidocaïne 0.5%) was provided by Sintetica SA (Mendrisio, Switzerland).

HPLC-grade acetonitrile was from Panreac Quimica (Barcelona, Spain) and water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Aqueous buffer was prepared with anhydrous di-potassium hydrogen phosphate and potassium di-hydrogen phosphate (Fluka) by measuring the pH with a Metrohm pH meter (Herisau, Switzerland).

Columns used were all provided by Waters and are reported in Table 1.

3.2. Samples and mobile phases

An aqueous reconstituted solution of Rapidocaïne® containing 0.5 mg/mL lidocaïne hydrochloride, 0.08 mg/mL methylparaben, and 0.01 mg/mL propylparaben was prepared by appropriate dilution of 1 mg/mL standard solution of each component in acetonitrile–water (50:50, v/v). The degradation product of lidocaïne (2,6-dimethylaniline) was added to the reconstituted solution of Rapidocaïne® at a concentration of 0.005 mg/mL, after dilution of 1 mg/mL standard solution in acetonitrile–water (50:50, v/v). The mobile phase was constituted of a mixture with appropriate proportion of aqueous phosphate buffer, pH 7.2, 50 mM and acetonitrile.

Buffers were prepared by dissolving the appropriate amount of KH_2PO_4 and K_2HPO_4 in water and by mixing these two solutions to attain pH 7.2. In all cases, the pH was measured before adding the organic modifier.

3.3. Apparatus

3.3.1. Conventional HPLC

Separations were performed on a Merck LaChrom system (Merck, Darmstadt, Germany) constituted of L-7100 programmable pumps, a L-7200 auto-sampler with a 100 μL loop, a L-4250 UV–VIS programmable detector and a L-7300 column oven. The UV–VIS detector contained a 14 μL standard flow cell, the time constant was set at 0.1 s and data sampling rate at 20 Hz. Data acquisition, data handling, and instrument control were performed by D-7000 HPLC System Manager Software. Extra-column band broadening of this instrument was estimated (Eq. (4)) at approximately $\sigma^2_{ext} = 200\ \mu L^2$.

Table 1
Chromatographic columns

Column	Particle size (μm)	Dimensions (mm)	Abbreviation
XTerra RP_{18}	5.0	150 × 4.6	XT150
XTerra RP_{18}	3.5	50 × 4.6	XT50
XTerra RP_{18}	3.5	30 × 4.6	XT30
XTerra RP_{18}	3.5	20 × 4.6	XT20
Acquity Shield BEH RP_{18}	1.7	50 × 2.1	ACQS

3.3.2. Optimized (Rapid) HPLC

Separations were performed on the Waters® Acquity UPLC system. This instrument included a binary solvent manager, an auto-sampler with a 2 μL loop, a UV–VIS programmable detector and a column oven set at 30 °C. The UV–VIS detector contained a 500 nL flow cell, the time constant was set at 25 ms and data sampling rate at 40 Hz. Data acquisition, data handling, and instrument control were performed by Empower Software. Extra-column band broadening of this instrument was estimated (Eq. (4)) at about $\sigma^2_{ext} = 5\ \mu L^2$.

3.4. Methodology

The conditions used for carrying out experiments with conventional and optimized systems are given in Table 2. Reported values were calculated with the ''HPLC calculator'' and using equations described in Section 2.

4. Results and discussion

4.1. Influence of extra-column band broadening on the quality of the transfer

As reported in Eq. (1), the peak variance (σ^2_{tot}) is related to the column itself (σ^2_{col}) and to extra-column volumes (σ^2_{ext}). As an example, 150 × 4.6 and 20 × 4.6 mm columns

Table 2
HPLC conditions

	Conventional HPLC	Optimized HPLC
Column	XT150, XT20	XT50, XT30, XT20, ACQS
Flow rate	1000 μL/min – XT150	1430 μL/min – XT50, XT30, XT20
	1430 μL/min – XT20	610 μL/min and 1000 μL/min – ACQS
Injection volume	20 μL – XT150	7 μL – XT50
	3 μL – XT20	4 μL – XT30
		3 μL – XT20
		1.4 μL – ACQS
Mobile phase (A:B)	50:50	50:50
UV at 230 nm	Flow cell: 14 μL	Flow cell: 500 nL
	Time constant: 100 ms	Time constant: 25 ms
	Sampling rate: 20 pts/s	Sampling rate: 40 pts/s

A: phosphate buffer pH 7.2, 50 mM.
B: acetonitrile.

have an approximate dead volume of 1.6 and 0.2 mL, respectively. The column dead volume (V_0) being directly proportional to the column length (L), a low value generates low column dispersion. Therefore, with small σ^2_{col} values, it is of prime importance to reduce all extra-column band broadening contributions for maintaining a good efficiency. Fig. 1a presents the remaining efficiency N_{obs} (estimated with Eq. (5) and a retention factor of 3) according to the column geometry and extra-column band broadening values. Fig. 1b gives the same information in a relative scale. For drawing these plots, the theoretical efficiency (N_{col}) was estimated with an average value of reduced height equivalent to a theoretical plate, $h = 2.5$. It was demonstrated that short columns (20 and 50 mm) packed with 3.5 μm particles possessed lower efficiencies than the conventional column (150 mm, 5 μm), while a short column (50 mm) packed with sub-2 μm particles presented an equivalent efficiency. However, depending on the chromatographic system (i.e. σ^2_{ext}), the loss in efficiency could be detrimental.

For example, the conventional HPLC system ($\sigma^2_{ext} = 200\ \mu L^2$) was well adapted to 150×4.6 mm columns, but became unsuitable for smaller columns (50 and 20 mm length), since efficiency was strongly reduced (up to 35%). As shown in Fig. 2, an isocratic separation of four compounds performed on a conventional LC system with a column of 150×4.6 mm gave satisfactory results, while the same separation (transferred according to Eqs. (8) and (10)) carried out in a shorter column (20×4.6 mm) was unsatisfactory (Fig. 5). The significant loss in resolution was due to the low efficiency of the column and to extra-column volumes (remaining efficiency of ca. 1300 plates). In particular, when column ID is reduced (2.1 mm), an optimized chromatographic system with very low σ^2_{ext} is mandatory, as shown in Fig. 1b.

An optimized HPLC system should possess low-volume connecting tube and detector cell (500 nL–2 μL). The detector time constant τ could also affect the separation at high flow rates (Eq. (4)) and should be decreased as much as possible without generating excessive noise. Because, the main goal of this study was to compare chromatographic performance in terms of efficiency and/or analysis time and not sensitivity, the lowest time constant value was always selected. Finally, the injected volume was adapted to the column geometry according to Eq. (8). Nevertheless, when sensitivity becomes a critical issue, a larger volume can be injected using the on-column focusing principle [16,20].

In order to reduce the importance of extra-column volumes and avoid an unacceptable efficiency loss (higher than 10%), chromatographic conditions leading to high retention factors could be considered (i.e. high σ^2_{col} values). As shown in Fig. 3, for a separation achieved with the conventional system ($\sigma^2_{ext} = 200\ \mu L^2$), retention factors should be close to 2, 4, and 8 for 150, 50, and 20 mm column lengths, respectively. Fig. 4 demonstrates the separation obtained in the analysis of Rapidocaïne® formulation on a conventional HPLC with the minimal considered column length, i.e. 20 mm. Organic modifier was reduced to 20% for obtaining a better resolution with a simultaneous improvement in selectivity, retention, and efficiency. With 20% ACN, retention factors were between 6 and 30. These values were not

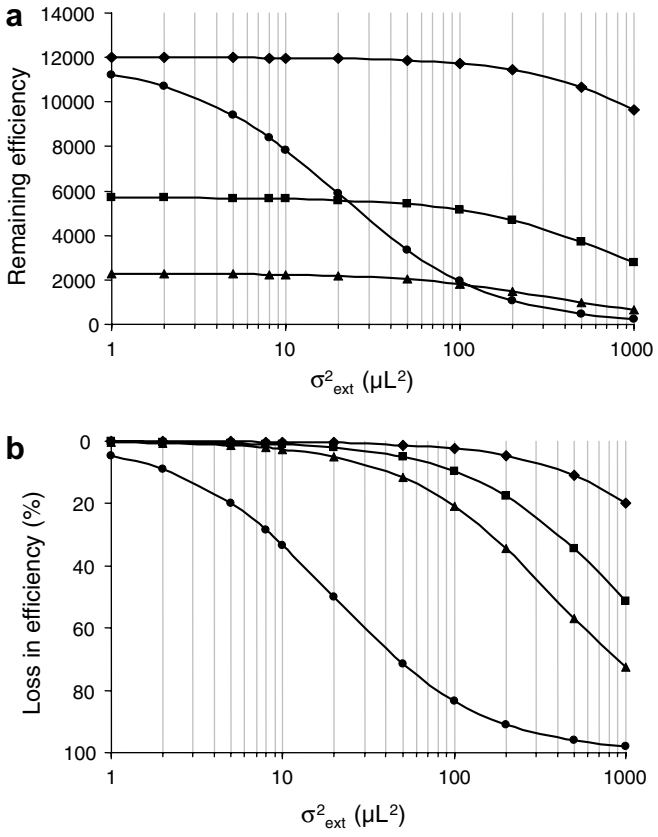

Fig. 1. Estimation of the remaining efficiency as a function of extra-column dispersion (σ^2_{ext}) for a retention factor $k = 3$. Column geometry: ◆ = 150×4.6 mm, 5 μm; ■ = 50×4.6 mm, 3.5 μm; ▲ = 20×4.6 mm, 3.5 μm; ● = 50×2.1 mm, 1.7 μm. (a) Absolute remaining efficiency as a function of σ^2_{ext}. (b) Relative remaining efficiency (%) as a function of σ^2_{ext}.

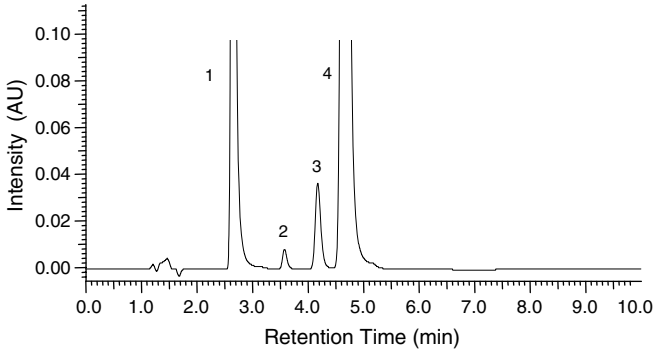

Fig. 2. Analysis of Rapidocaïne®: 1, methylparaben; 2, 2,6-dimethylaniline; 3, propylparaben; 4, lidocaïne hydrochloride, on conventional LC system. Column, XTerra RP_{18} 150×4.6 mm, 5 μm; mobile phase, phosphate buffer pH 7.2 – acetonitrile (50:50, v:v); flow rate, 1000 μL/min; injection volume, 20 μL; detection, UV at 230 nm; flow cell, 14 μL, constant time, 100 ms; temperature, 30 °C.

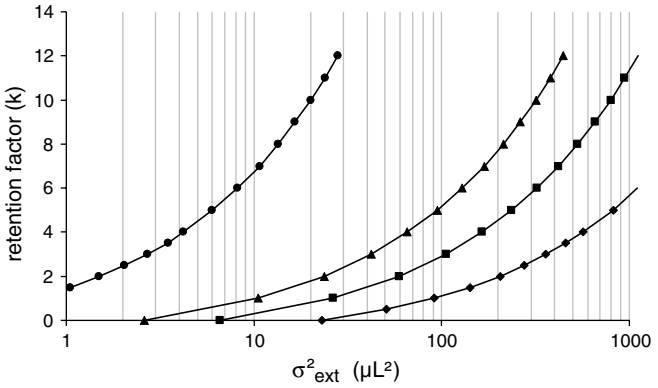

Fig. 3. Estimation of retention factor values leading to an efficiency loss inferior to 10%, according to the extra-column dispersion (σ^2_{ext}) of the chromatographic system. Column geometry: ◆ = 150 × 4.6 mm, 5 μm; ■ = 50 × 4.6 mm, 3.5 μm; ▲ = 20 × 4.6 mm, 3.5 μm; ● = 50 × 2.1 mm, 1.7 μm.

prohibitive, since the column dead time was only 10 s and the running time remained acceptable (ca. 5.5 min). However, since the mobile phase composition was modified, a direct geometrical transfer could not be applied and the method should be re-optimized.

Nevertheless, it is not possible with an acceptable k range ($2 < k < 20$) to obtain satisfactory results using a column of 50 × 2.1 mm with a conventional system. With the optimized instrumentation ($\sigma^2_{ext} = 5\ \mu L^2$), low retention factors ($k < 2$) are compatible with all column lengths of 4.6. mm I.D., while a 50 × 2.1 mm column requires retention factors higher than 4.

4.2. Method transfer with short columns packed with 3.5 μm particles

A method was originally developed for the analysis of Rapidocaïne 0.5% on a 150 × 4.6 mm, 5 μm column using conventional HPLC instrumentation (Fig. 2). This method was altered (Eqs. (8) and (10)) for the use of shorter columns, packed with XTerra RP_{18}, 3.5 μm, of various geometries (50, 30, and 20 × 4.6 mm) with an optimized UPLC system (Fig. 5). The analysis time was reduced using these short columns. As indicated by Eq. (11), a reduction by a factor of 10 was observed between the conventional and the smaller column. However, as expected by theory, efficiencies significantly decreased with the column length leading to a significant and critical loss in resolution and sensitivity with 30 and 20 mm columns. Peak height was reduced 3- to 4-fold with columns of 150 and 20 mm.

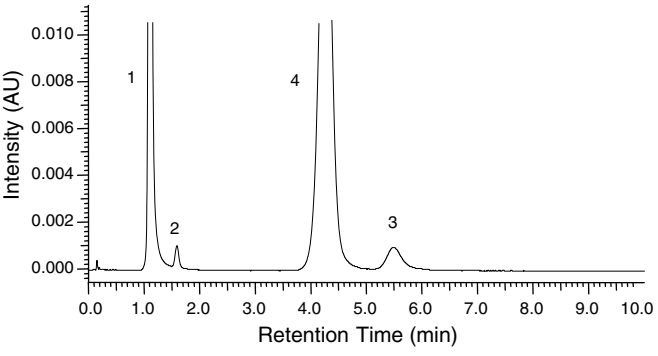

Fig. 4. Separation of Rapidocaïne® (1, methylparabene; 2, 2,6-dimethylaniline; 3, lidocaïne; 4, propylparabene) on conventional HPLC system: column, XTerra RP_{18} 20 × 4.6 mm, 3.5 μm; mobile phase, isocratic phosphate buffer pH 7.2 – acetonitrile (80:20); flow rate, 1430 μL/min; detection, UV at 230 nm; flow cell, 14 μL, constant time, 100 ms; temperature, 30 °C; injection volume, 3 μL.

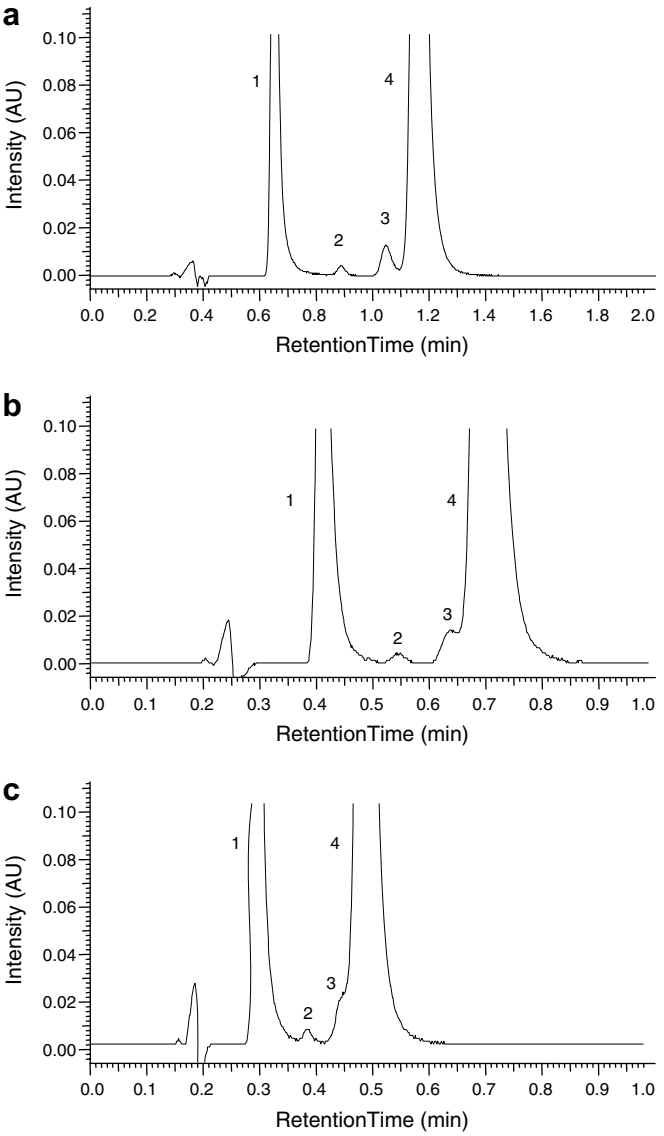

Fig. 5. Analysis of Rapidocaïne®: 1, methylparaben; 2, 2,6-dimethylaniline; 3, propylparaben; 4, lidocaïne hydrochloride, on optimized LC system. Detection, UV at 230 nm; flow cell, 500 nL; constant time, 25 ms; temperature, 30 °C; mobile phase, phosphate buffer pH 7.2 – acetonitrile (50:50, v/v). (a) Column, XTerra RP_{18} 50 × 4.6 mm, 3.5 μm; flow rate, 1430 μL/min; injection volume, 7 μL. (b) Column, XTerra RP_{18} 30 × 4.6 mm, 3.5 μm; flow rate, 1430 μL/min; injection volume, 4 μL. (c) Column, XTerra RP_{18} 20 × 4.6 mm, 3.5 μm; flow rate, 1430 μL/min; injection volume, 3 μL.

In conclusion, short columns packed with particles of 3.5 μm are interesting for simple separations carried out in isocratic mode (e.g. quality control of a pharmaceutical formulation). However, it is necessary to use high retention factors ($k > 3$) for avoiding detrimental effect of extra-column band broadening. It must be noted that these short columns can be used for more complex separations when coupled to mass spectrometry (MS) [21,22]. With this selective detector, satisfactory results can be obtained even if all compounds were not baseline resolved. However, the detector's sampling rate must be adapted for performing these fast-LC separations.

4.3. Method transfer with short columns packed with 1.7 μm particles

As mentioned above, the original separation of lidocaïne and related compounds was performed on an XTerra RP_{18}, 5 μm (Fig. 2). In order to transfer the method to fast-LC, an Acquity BEH Shield RP_{18}, 1.7 μm was selected since both stationary phases were similar in chromatographic selectivity (data not shown). The method was geometrically transferred to a 50×2.1 mm column, taking into account the described transfer rules (Eqs. 8 and 10). Under these conditions, the analysis time was reduced to only 40 s (Fig. 6a) at a flow rate of 610 μL/min. As expected, selectivity was only slightly modified and resolution remained almost constant. It is noteworthy that efficiency was even slightly improved (about 10%) with the short column, due to a better chromatographic behaviour of the embedded stationary phase for analyzing basic compounds.

According to the literature [11 and references therein], sub-2 μm particles can be used at higher flow rates than their optimal values without any significant change in efficiency. Therefore, the same analysis was performed at 1000 μL/min corresponding to the maximal pressure compatible with the UPLC system (Fig. 6b). With these conditions, the ultra-fast separation was achieved in less than 30 s with equivalent chromatographic performances (retention factor and selectivity) and a small, but acceptable, loss in efficiency (<10%).

In term of sensitivity, no significant modifications of peak heights were observed, but the signal-to-noise ratio was reduced by a factor 3 with the transferred method. This phenomenon was attributed to the low time constant and the higher acquisition rate of the UV detector (25 ms and 40 Hz, respectively) which generated higher background noise. In conclusion, short columns packed with sub-2 μm particles are compatible with fast and ultra-fast analyses maintaining good chromatographic performances, even in isocratic conditions.

5. Conclusion

This paper described the fundamental chromatographic equations used for transferring an isocratic separation carried out with a given stationary phase and column geometry

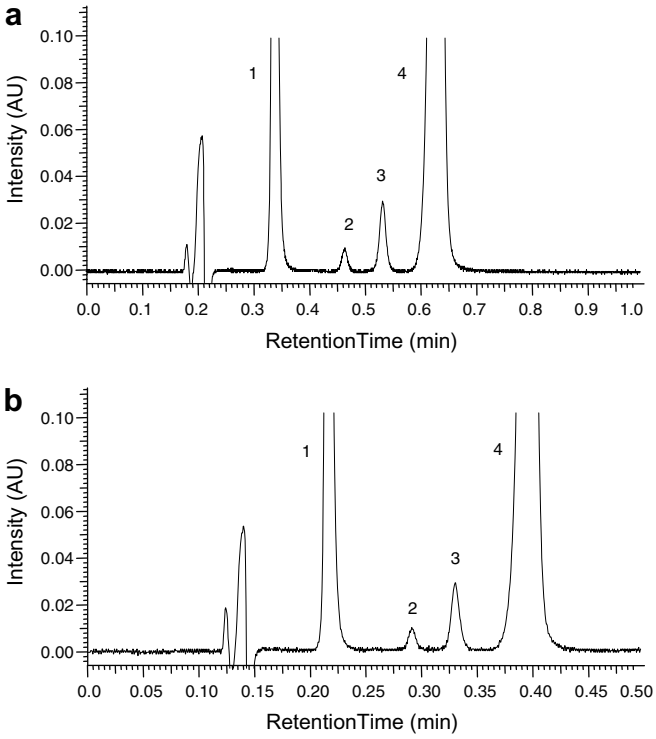

Fig. 6. Analysis of Rapidocaïne®: 1, methylparaben; 2, 2,6-dimethylaniline; 3, propylparaben; 4, lidocaïne hydrochloride, on an optimized LC system. (a) Column, Acquity Shield BEH RP18 50×2.1 mm, 1.7 μm; mobile phase, phosphate buffer pH 7.2 – acetonitrile (50:50, v/v); flow rate, 610 μL/min; temperature, 25 °C; detection, UV at 230 nm; injected volume, 1.4 μL. (b) Identical conditions except: flow rate, 1000 μL/min and injected volume, 1.4 μL.

to a smaller column packed with similar or identical stationary phase with limited influence on chromatographic resolution. For this purpose, the flow rate and the injected volume were adapted and an optimized LC system was used.

Short columns packed with 3.5 μm particles allowed a large decrease in analysis time but did not offer sufficient efficiency even with an optimized LC system, leading to an unacceptable loss in resolution vs. a conventional analysis. In the isocratic mode, these columns are thus only compatible for simple separations and should be used only with mass spectrometry.

On the other hand, short columns packed with sub-2 μm particles reduced more drastically the analysis time while keeping efficiency and resolution constant. Therefore, fast and ultra-fast analyses can be performed, while maintaining good chromatographic performance.

Acknowledgement

The authors greatly acknowledge Waters for the loan of the UPLC system and the gift of the columns.

References

[1] S. Ahuja, Overview: handbook of pharmaceutical analysis by HPLC, Sep. Sci. Technol. 6 (2005) 1–17.

[2] J.N. Kyranos, H. Cai, D. Wei, W.K. Goetzinger, High-throughput high-performance liquid chromatography/mass spectrometry for modern drug discovery, Curr. Opin. Biotechnol. 12 (1) (2001) 105–111.
[3] M. Joseph, Reduce pharmaceutical analysis time – without compromise – with fast and ultra-fast HPLC methods, LCGC North Am. 23 (560) (2005) 3–34.
[4] F. Svec, L. Geiser, Monolithic stationary phases for HPLC and sample preparation, LCGC North Am. 24 (S4) (2006) 22–27.
[5] K. Cabrera, Applications of silica-based monolithic HPLC columns, J. Sep. Sci. 27 (10–11) (2004) 843–852.
[6] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, K. Hosoya, N. Tanaka, Chromatographic properties of miniaturized silica rod columns, J. High Resol. Chromatogr. 21 (8) (1998) 477–479.
[7] S. Rudaz, L. Geiser, D. Guillarme, J.L. Veuthey, Development of rapid analytical methods in the laboratory of pharmaceutical analytical chemistry (LCAP), Chimia 59 (6) (2005) 303–307.
[8] D. Guillarme, S. Heinisch, J.L. Rocca, Effect of temperature in reversed phase liquid chromatography, J. Chromatogr. A 1052 (1–2) (2004) 39–51.
[9] D. Guillarme, S. Heinisch, Detection modes with high temperature liquid chromatography – a review, Sep. Purif. Rev. 34 (2) (2005) 181–216.
[10] I.M. Mutton, Use of short columns and high flow rates for rapid gradient reversed-phase chromatography, Chromatographia 47 (5/6) (1998) 291–298.
[11] D. Nguyen, D. Guillarme, S. Rudaz, J.L. Veuthey, Fast analysis in liquid chromatography using small particle size and high pressure, J. Sep. Sci. 29 (2006) 1836–1848.
[12] J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns, Anal. Chem. 69 (6) (1997) 983–989.
[13] N. Wu, J.A. Lippert, M.L. Lee, Practical aspects of ultrahigh pressure capillary liquid chromatography, J. Chromatogr. A 911 (1) (2001) 1–12.
[14] D.T.T. Nguyen, D. Guillarme, S. Rudaz, J.L. Veuthey, Chromatographic behaviour and comparison of column packed with sub-2 μm stationary phases in liquid chromatography, J. Chromatogr. A 1128 (2006) 105–113.
[15] E. Grumbach, T. Wheat, D. McCabe, D. Diehl, J. Mazzeo, Improving LC separations: transferring methods from HPLC to UPLC, LCGC North Am. (Suppl.) (2006) 80–81.
[16] J.P.C. Vissers, A.H. de Ru, M. Ursem, J.P. Chervet, Optimized injection techniques for micro and capillary liquid chromatography, J. Chromatogr. A 746 (1) (1996) 1–7.
[17] J.P. Chervet, M. Ursem, J.P. Salzmann, Instrumental Requirements for Nanoscale Liquid Chromatography, Anal. Chem. 68 (9) (1996) 1507–1512.
[18] A. Pruss, C. Kempter, J. Gysler, T. Jira, Extracolumn band broadening in capillary liquid chromatography, J. Chromatogr. A 1016 (2) (2003) 129–141.
[19] http://www.unige.ch/sciences/pharm/fanal.
[20] B.L. Ling, W. Baeyens, C. Dewaele, Comparative study of ''on-column focusing'' applied to micro-LC and conventional LC, J. Microcol. Sep. 4 (1) (1992) 7–22.
[21] T. Goto, Y. Ito, H. Oka, I. Saito, H. Matsumoto, H. Sugiyama, C. Ohkubo, H. Nakazawa, H. Nagase, The high throughput analysis of *N*-methyl carbamate pesticides in wine and juice by electrospray ionization liquid chromatography tandem mass spectrometry with direct sample injection into a short column, Anal. Chim. Acta 531 (1) (2005) 79–86.
[22] L. Romanyshyn, P.R. Tiller, R. Alvaro, A. Pereira, C.E. Hop, Ultrafast gradient vs. fast isocratic chromatography in bioanalytical quantification by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 15 (5) (2001) 313–319.