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Ultra-high-pressure liquid chromatography (UHPLC) in method development

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ABSTRACT

In recent years, ultra-high-pressure liquid chromatography (UHPLC) became the modern standard HPLC platform. UHPLC, with its shorter analysis time and quicker column equilibration, is ideally suited to rapid method development. This article provides a critical review of the current status, the benefits and the limitations of UHPLC in method development. We use case studies to describe best practices and recent advances. Examples include conversion of existing HPLC methods to faster analysis, rapid column/mobile-phase screening, and automated method optimization. While we focus on the development of reversed-phase methods for assay and impurity analysis of small-molecule pharmaceuticals, our insights and conclusions can be extended to other applications and sample types. Besides generating faster analysis (when used with short, small-particle columns), the higher pressure limits of UHPLC also allow the effective use of longer columns for superior routine analysis of complex samples.

Contents

1. Introduction

There is no shortage of books [1,2], book chapters [3–6] and journal articles [7,8] on HPLC method development. Literature searches indicated hundreds of references on UHPLC method development, though relatively few are specifically for UHPLC methods in pharmaceutical analysis of drug substances and drug products, a major and demanding application that is the focus of this review [9–15].

Most HPLC instrument and column manufacturers introduced UHPLC product offerings in recent years [16–19]. UHPLC delivers substantial performance enhancements over conventional HPLC (lower system dispersion and dwell volumes) and is particularly attractive for method-development situations, where quick run time and rapid responses to changes in column/mobile-phase conditions are desirable. A time saving of 3-to-10 fold is not unusual while maintaining a high level of performance in resolution, sensitivity, and precision [19–21]. This benefit of UHPLC in high-throughput analysis is particularly significant for rapid column and mobile-phase screening and method optimization.

Table 1 summarizes the main features, the benefits and the limitations of UHPLC in method development. Most of these

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Table 1
System characteristics, benefits and limitations of UHPLC in method development

System characteristics	Comment
High-pressure limit	9000–20,000 psi (600–1400 bar) for effective use with sub-2-μm-particle columns [9,22].
Low dispersion	Instrumental bandwidths of 5–20 μL (4 σ with UV detector) for compatibility with columns of 2–3 mm i.d. [23–25].
Low dwell volume	100–500 μL for reduced gradient delay time and rapid column equilibration [9,22].
Others	Excellent system performance (precision, sensitivity, carryover) allowing the development of highly sensitive stability-indicating methods for regulatory testing [18,26].
Additional enhancements	Systems can be enhanced with automated valving for column/mobile-phase screening. Many are compatible with automated method-development software. Some have quaternary pumps that support "auto-blend" for more convenient method optimization [27].
Benefits	**Comment**
Rapid method development	Increase throughput by 3–10 fold *versus* conventional HPLC. Fast analysis with short columns for rapid column and mobile-phase screening and for method optimization [26,28].
Very high-resolution separations	Increase resolution by up to 3-fold with use of longer columns packed with small particles to generate peak capacities (P_c) of 400–800 for analysis of complex samples [29,30].
Can be combined with other approaches and detection modes [31,32]	UHPLC is compatible with high-temperature LC [33], core-shell columns [25]or 2D-LC [34], individually or in combination [19]. These approaches are options rather than alternatives. UHPLC is amenable to all common HPLC detection modes, such as UV, RI, MS, ELSD and CAD. Newer UHPLC-compatible detectors are increasingly available to handle the smaller peak volumes without significant band broadening.
"Greener" technology	Uses less organic solvents and smaller sample amount due to lower flow rates and faster analysis time because of the use of shorter, smaller i.d. columns.
Limitations	**Comment**
Equipment cost and compatibility with existing HPLC methods	The relative higher equipment cost (20–50%) and the backward compatibility of some UHPLC systems to run existing HPLC methods can be potential issues (limitations of flow-rate range, size of sample loop or column oven) [35].
Limited selection of stationary phases	The availability of UHPLC columns, while growing rapidly, is still somewhat limiting, particularly for modes other than reversed-phase, such as ion-exchange and size-exclusion chromatography [36].
Method transferability	UHPLC methods must be "converted" to conventional HPLC method conditions for laboratories without UHPLC equipment. Method transfer between different laboratories can be comparatively more difficult across different UHPLC system platforms due to differences of dwell volumes and system dispersion, and viscous heating, particularly for high-resolution methods [30].

characteristics have been well reviewed [9,18,19], and we do not elaborate on them, except with the following observations.

(1) Today's UHPLC equipment represents both revolutionary and evolutionary progressions in HPLC performance. Current system characteristics (dispersion, dwell volumes and pressure limits) [9,37], are fairly well suited to columns packed with sub-2-μm or sub-3-μm particles in 2-mm or 3-mm inner-diameter (i.d.) format. UHPLC-compatible detectors [i.e., UV, MS, refractive index (RI), evaporative light scattering (ELSD) and charged aerosol detection (CAD)] are increasingly available to handle the smaller peak volumes without significant band broadening [23,24]. Nevertheless, maintaining high column efficiency for very small columns (e.g., 50 × 2.1 mm) under isocratic conditions remains difficult for most UHPLC systems [37].

(2) The addition of high-pressure-compatible switching valves with different configurations can turn any UHPLC into an automated column/mobile phase-screening system. Intelligent method-development software capable of UHPLC system control or simulation modeling and statistical data analysis/display can further facilitate the optimization of critical separations [19,38–40].

(3) Quaternary UHPLC pumps, despite having larger dwell volumes than high-pressure mixing binary pumps [5], are offered by most vendors. At least two manufacturers offer an automated feature, called "auto-blend" or a "buffer advisor", to generate mobile phases in a defined pH range from buffer concentrates for more convenient mobile-phase optimization [27].

(4) UHPLC offers superior high-resolution analysis of complex samples [29,30,41] and is highly compatible with columns packed with superficially porous (core-shell) materials, high-temperature LC and two-dimensional LC (2-D LC) for further resolution enhancements [25,33,34,42–44]. Peak capacities in the range 400–800 (e.g., from one or more 150-mm long columns packed with sub-2-μm particles) are achievable under ambient or elevated temperatures.

(5) Many potential issues of earlier UHPLC systems [9,26,35] have been eliminated by improved designs, while others can be mitigated through judicious selection of system configurations or options [9,35]. Still other potential issues often mentioned are the relatively higher equipment cost and the backward compatibility of some UHPLC systems to conventional HPLC columns and existing methods (e.g., limitations of flow-rate range, size of sample loop or column oven) [35]. Another well-recognized issue for using UHPLC in method development is the need for method "conversion" to conventional HPLC method conditions to allow for universal adoption of the method to laboratories without UHPLC equipment [21,35,45,46].

This article provides an overview of the current status, the benefits and the limitations of using UHPLC in method development. The long-term benefits of moving to UHPLC have been well documented elsewhere and we do not discuss them further [18,19,22,26]. Table 2 summarizes the typical steps used in the development of critical HPLC methods (e.g., impurity methods for a drug substance), where selectivity, sensitivity, reproducibility, and robustness are all critical. Case studies to illustrate the best practices and recent advances in several areas are taken from the literature or examples in our own laboratories. While the focus is on the development of reversed-phase LC methods for small organic molecules and impurity testing of pharmaceuticals using UV detection, the findings and the discussions are generally applicable to other chromatographic modes and sample types.

2. Discussion

Since UHPLC represents an improved, modernized version of HPLC, most strategies and discussions pertaining to HPLC method development are generally applicable to UHPLC. However, the use of smaller i.d. columns packed with sub-2 μm or sub-3 μm particles under very high pressures does present some challenges and operational nuances for new users of UHPLC [9,35]. The systematic method-development strategies described in most HPLC books are quite effective for the development of methods for complex samples [1,3,5]. A three-pronged approach for rapid HPLC method development [8] offers a useful template for developing simple or complex methods.

Table 2
Steps in HPLC method development for assays and impurity methods

Steps	Comment
Defining method types	Analytical HPLC methods can be broadly categorized as simple (potency of method to measure one or a few components) or complex (impurity or stability-indicating methods). The complexity of the method generally dictates the method-development strategy and the amount of time needed for development and validation [1,2,5].
Gathering sample and analyte information	For the development of critical methods, such as impurity methods for new drug substances, the first step is typically the gathering of pertinent physicochemical properties of the molecules, such as chemical structure, molecular weight, number of acidic/basic/neutral functional groups and chiral centers, pK_a, and reactivity. The certificate of analysis (COA) and technical package from the manufacturer of the new drug substance can sometimes supply some of these useful data [4,5].
Initial method development	Initial method development typically means conducting some "scouting" runs and getting the first chromatograms. Reversed-phase LC with broad gradients (e.g., C18 column with an acidic mobile phase) with UV detection (at λ_{max} of the analyte or a low UV wavelength) remains a very common choice in many situations. An automated systematic screening strategy of different columns and mobile phases has been widely implemented. Samples used can be a mixture of the main components with expected impurities and degradants (if available as reference standards) or forced degradation samples [3,5]. Automated column/mobile-phase screening can also be used in this step to allow selection of parameters for the best selectivity and resolution.
Method fine-tuning and optimization	This is the most time-consuming step of the method-development process involving fine-tuning the separation by adjusting the mobile phase (pH, buffer, organic solvent), or other parameters (flow rate, column temperature, gradient time and range). After ensuring that all critical analytes are separated, further improvements for sensitivity, peak shape and analysis time can be made [3,5].
Method pre-qualification	This is the last step of the method-development process to ensure that the method is "validatable" by checking out method specificity, precision, linearity, and sensitivity [3,5].

Content of this table is extracted from published ideas [1,5].

The use of UHPLC will significantly enhance productivity in all phases of method development from initial scouting, column/mobile-phase screening, systematic optimization, to method qualification/validation. We discuss five areas with case studies to illustrate the best practices and fundamental concepts:

(1) HPLC/UHPLC method conversion;
(2) rapid column screening;
(3) development of fast, mid- or high-resolution methods;
(4) rapid mobile-phase screening using low-pH and high-pH mobile phases; and,
(5) automated method development and optimization.

2.1. HPLC/UHPLC method conversion using geometrical scaling

There are three typical scenarios for method "conversions" between HPLC and UHPLC [35,45]:

(1) use of conventional HPLC methods on UHPLC equipment;
(2) newly developed UHPLC methods "back transferred" or converted to HPLC conditions (commonly practiced in pharmaceutical laboratories to support global manufacturing); and,
(3) existing HPLC methods converted to UHPLC methods to reduce analysis time (a common strategy that has become a driver for purchasing UHPLC).

Since a UHPLC system generally yields performance equal to or better than HPLC in running existing HPLC methods, the first scenario is relatively straightforward after compensating for differences in dwell volumes for gradient methods [35]. A geometrical scaling approach is typically used to accomplish the second and third scenarios [45,46], and is demonstrated in the case study for a drug-product sample in Fig. 1 [21], where reductions in analysis time of 3–5 fold with similar resolution are not unusual (~10 fold in this case using an optimum flow rate for smaller particles).

Some ground rules for geometrical scaling are:

(1) column length is scaled to particle size keeping the column-length to particle-size ratio the same (thereby yielding the same plate count);
(2) flow rate is scaled to cross-sectional area of the column (since optimum flow rates are inversely proportional to particle size, use of higher flow rates is warranted if the system-pressure limit permits);
(3) gradient time (t_G) is scaled to column length; and,
(4) injection volume is scaled to column void volume.

One important requirement for critical assays is that the new UHPLC column used must contain identical bonded-phase materials to eliminate any selectivity differences. Also, mobile phases used should be identical (e.g., buffer type and strength, pH, and organic modifier). Calculator programs are available on various vendors' websites and from other sources [47].

UHPLC can offer 3–10-fold increases in analysis speed with similar resolution using the geometrical scaling approach. This is a compelling reason for new users to adopt UHPLC technologies.

2.2. Rapid column screening

A recent comparative study of three emergent separation techniques [UHPLC, high-performance supercritical fluid chromatography (UPSFC) and non-aqueous capillary electrophoresis (NACE)] for the analysis of closely-related pharmaceuticals found UHPLC to be the most versatile, and particularly desirable for quality-control (QC) applications [48]. The use of short, small-particle UHPLC columns is particularly productive for rapid column screening in the development of stability-indicating methods for pharmaceutical analysis, as illustrated by a recent reference [28] and also in the example shown in Fig. 2a–c in our laboratory for a new chemical entity (NCE). A forced degradation sample, containing the active pharmaceutical ingredient (API), a process impurity (an aniline analog), two degradants (Deg A and Deg B) and other minor impurities, was used on a UHPLC system equipped with a four-position column-selection valve. The screening experiment lasted only 30 min using a broad-gradient (5–95% B in 5 min), and allowed the identification of a suitable column (a polar-embedded C18, 50 x 2.1 mm, 1.7 µm), which resolved all four major peaks (Fig. 2b). Deg B is a photo-degradant and tends to coelute with the aniline analog or the API on C18 and phenyl columns, respectively (Fig. 2a and c). This polar-embedded C18 bonded phase separates all four components and was selected for further UHPLC method development using longer columns.

As illustrated in this case study, rapid column screening can be accomplished easily on a UHPLC system equipped with a multi-position column-selection valve. For example, a four-column screen can be accomplished in 30 min, allowing identification of a suitable column showing the best selectivity for critical pairs for further method development. Note that similar screening with columns

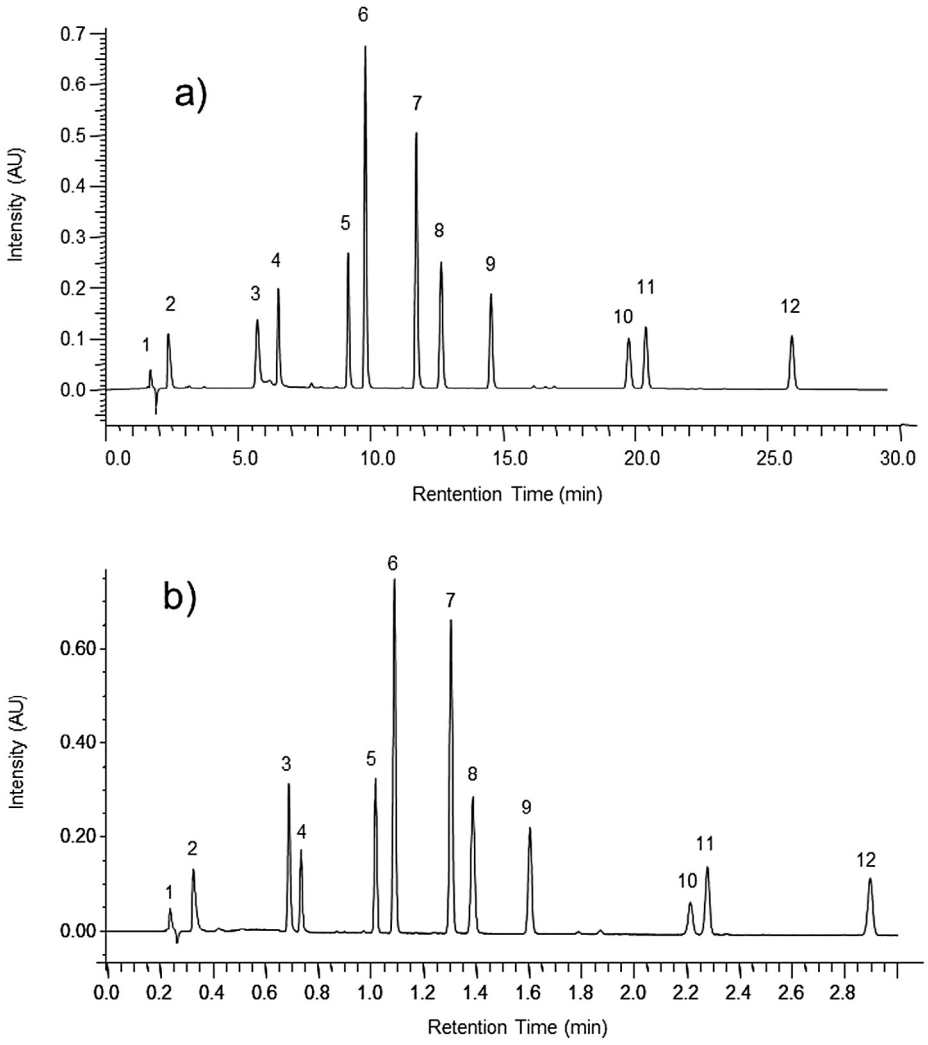

Fig. 1. An example of a method conversion for a quality-control gradient impurity assay of a pharmaceutical formulation using a geometrical scaling approach from conventional HPLC to UHPLC. Peak identification: Peak 6 is the active pharmaceutical ingredients, while others are expected impurities. HPLC conditions: Column C18 150 × 4.6 mm, 5 µm, Flow rate, F = 1 mL/min, Injection volume, V_{inj} = 20 µL, total run time, 45 min. UHPLC conditions: Column C18 50 × 2.1 mm, 1.7 µm, F = 0.61 mL/min, V_{inj} = 1.4 µL, total run time, 5.1 min. A nine-fold reduction of analysis time was realized as the optimum flow rate was used here. {Reprinted with permission from [21]}.

packed with 3-µm or 5-µm particles would be significantly longer and less effective.

2.3. Developing fast, medium- or high-resolution separations using sub-2-µm columns

A major benefit of UHPLC with increased pressure limits is versatility for the development of ultrafast or very high-resolution methods. Fig. 3a–c shows three chromatograms of the same forced degradation sample (shown in Fig. 2) conducted with a narrower gradient range of 20–60% B (with gradient time t_G 3–20 min) to expand the region around the API peak [8]. These examples illustrate the versatility of UHPLC to provide ultrafast, mid-resolution and very high-resolution analysis:

(a) a fast 6-min analysis with good resolution using a 50-mm column;
(b) a 16-min analysis with excellent resolution using a 100-mm column; and,
(c) a 30-min analysis with very high resolution using a 150-mm column.

The flow rate and gradient times (t_G) were adjusted to yield similar gradient volumes for the three chromatograms, normalized for column-void volumes. Not surprisingly, the resolutions between the four major peaks increased substantially with the longer columns at longer t_G [29]. This high-resolution capability is useful for separation of closely-eluting peaks (Fig. 3c). Note that each of the last two minor impurity peaks in the inset of Fig. 3a (fast method) were subsequently resolved into two distinct peaks (possibly isomers) as shown in the inset of Fig. 3c (high-resolution method).

UHPLC allows versatility to develop a wide variety of methods – from fast analysis with good resolution to very high-resolution methods for detailed profiling of complex samples – thus extending the capability of HPLC in analysis speed and/or resolution. The effective analysis of very complex samples is a hitherto unmet need of conventional HPLC [30].

2.4. Rapid mobile-phase screening

Mobile-phase screening (pH, buffer type and strength, and organic solvent) can be a time-consuming task in method development.

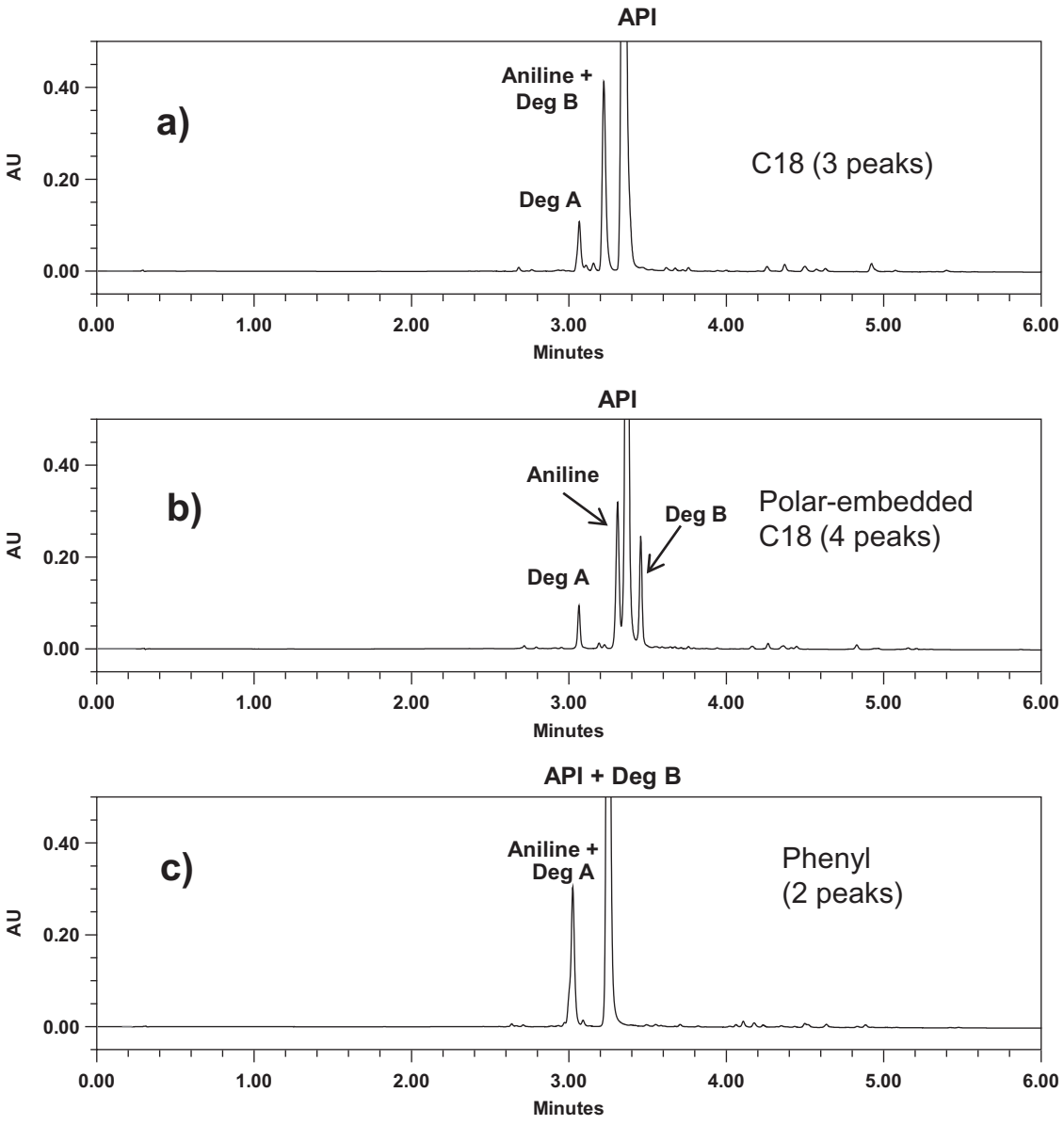

Fig. 2. A case study using a forced degradation API sample to illustrate rapid column screening. Mobile phase A (MPA) = 0.05% formic acid, mobile phase B (MPB) = acetonitrile, Detection = 254 nm, Gradient: 10–90% B in 5 min, F = 0.5 mL/min, T = 30°C, pressure, ΔP = 530 bar. Columns used were: (a) C18, (b) polar-embedded C18, and (c) phenyl. All columns were 50 × 2.1 mm, 1.7 µm. The polar-embedded C18 column was clearly the best, as it was able to resolve all four peaks. Total screening time for four columns lasted 30 min (results obtained on another C18 type of column were not shown).

An exceptionally simple, but effective, generic mobile-phase-screening strategy from a column manufacturer is shown [49] with the following observations and comments.

(1) Each screening run was completed in 5 min using a generic broad gradient (5–95% B in 5 min) with acidic or basic mobile phase (pH 3 or 10) coupled with methanol or acetonitrile.
(2) The column used in this example (C18, 50 × 2.1 mm, 1.7 µm) [49] was derived from a hybrid support with a net positive surface charge, and designed to yield more symmetrical peak shapes for basic analytes using low ionic strength mobile phases (e.g., 0.1% formic acid). This family of charged surface columns are usable in a pH range 1–12 and are available in different particle sizes (1.7 µm, 2.5 µm, 3.5 µm and 5 µm) in highly "orthogonal" bonding chemistries [C18, phenyl and pentafluorophenyl (PFP)].
(3) These columns have quickly become very popular in many pharmaceutical laboratories because they minimize peak-shape issues that are particularly problematic for many NCEs with multiple ionizable nitrogen atoms [50].

Fig. 4 shows four chromatograms of a seven-component test mixture consisting of neutral, basic and acidic analytes using both acidic and basic mobile phases with acetonitrile or methanol. Note that the retentions of the neutral analytes (components 4 and 7) are relatively unaffected by mobile phase pH while those of the acidic (components 3, 5, 6) and basic analytes (components 1 and 2) are controlled primarily by the ionization states of the molecules. The use of a high-pH mobile phase is particularly attractive for the analysis of water-soluble bases, yielding excellent retention and peak shape [51]. Retention times using methanol are appreciably longer due to its lower solvent strength compared to acetonitrile. The use

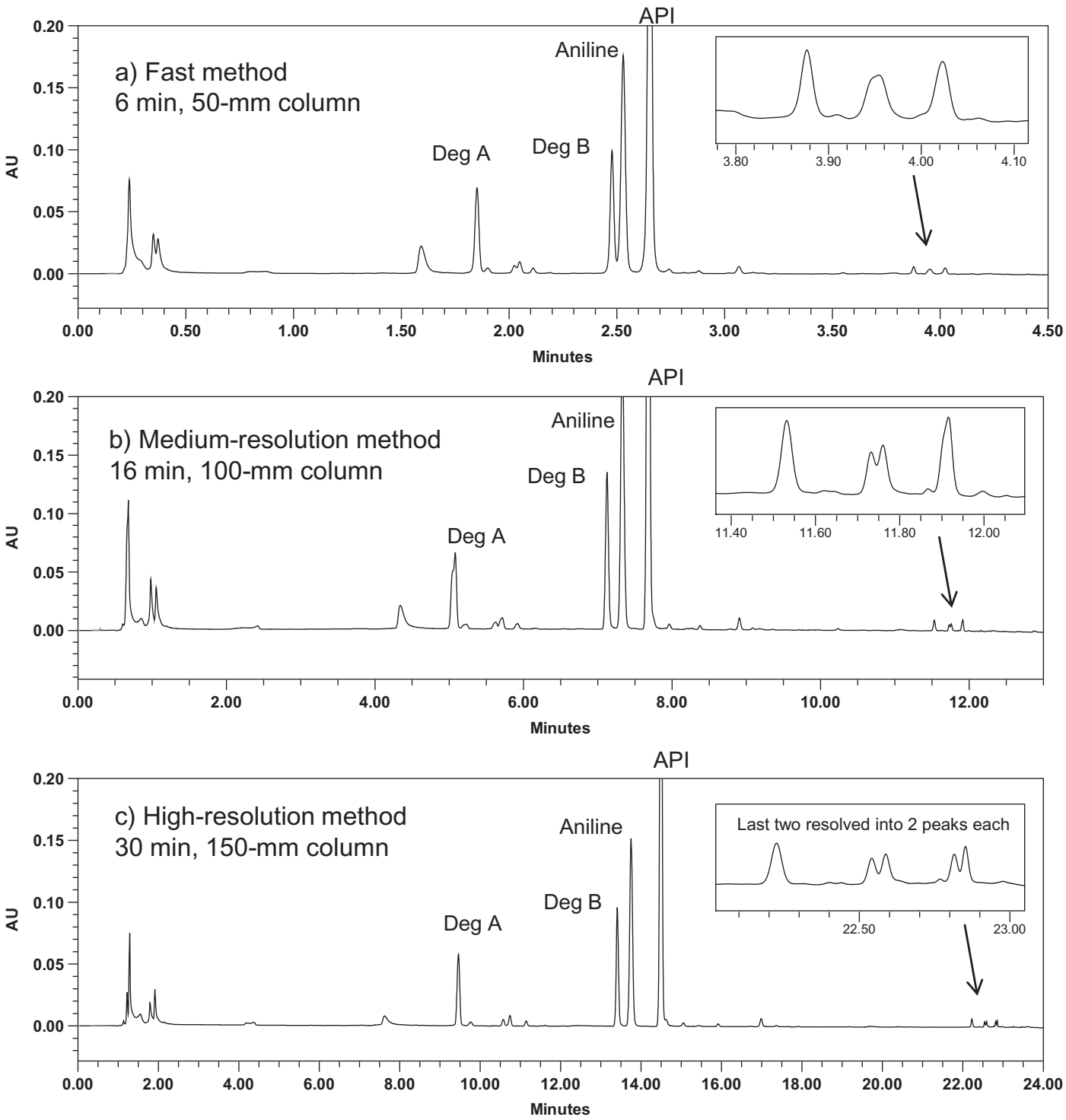

Fig. 3. A case study using a forced degradation API sample to illustrate the flexibility of UHPLC to deliver fast, mid- and high-resolution methods using sub-2-μm columns of various lengths and gradient time, t_G of 3–20 min. MPA = 0.05% formic acid, MPB = acetonitrile, Detection = 254 nm, gradient: 20–60% B (t_G of 3–20 min), 60–95% B (t_G of 1–3 min), T = 30°C. Note that the last two impurity peaks shown in the inset in Fig. 3a, were resolved to be two distinct peaks each in the inset in Fig. 3c. (a). Fast method: polar-embedded C18 column (50 × 2.1 mm, 1.7 μm), Gradient time, t_G = 3 min, F = 0.6 mL/min, Run time = 6 min, pressure ΔP = 550 bar. (b). Medium-resolution method: polar-embedded C18 column (100 × 2.1 mm, 1.7 μm), t_G = 10 min, F = 0.4 mL/min, Run time = 16 min, ΔP = 630 bar. (c). High-resolution method: polar-embedded C18 column (150 × 2.1 mm, 1.7 μm), t_G = 20 min, F = 0.3 mL/min, Run time = 30 min, ΔP = 760 bar.

of acetonitrile or methanol in conjunction with additional "orthogonal" columns (CSH phenyl or CSH PFP) can help increase selectivity differences for difficult separations of critical pairs [49].

This example illustrates the utility of performing a rapid initial mobile-phase screening using low-pH and high-pH mobile phase A with acetonitrile or methanol. The total screening time for four conditions was only 30 min [49]. Note that the use of high-pH mobile phases in method development is increasingly due to the availability of high-pH-compatible silica-based phases. However, this practice may come with some potential issues for specific analytes, such

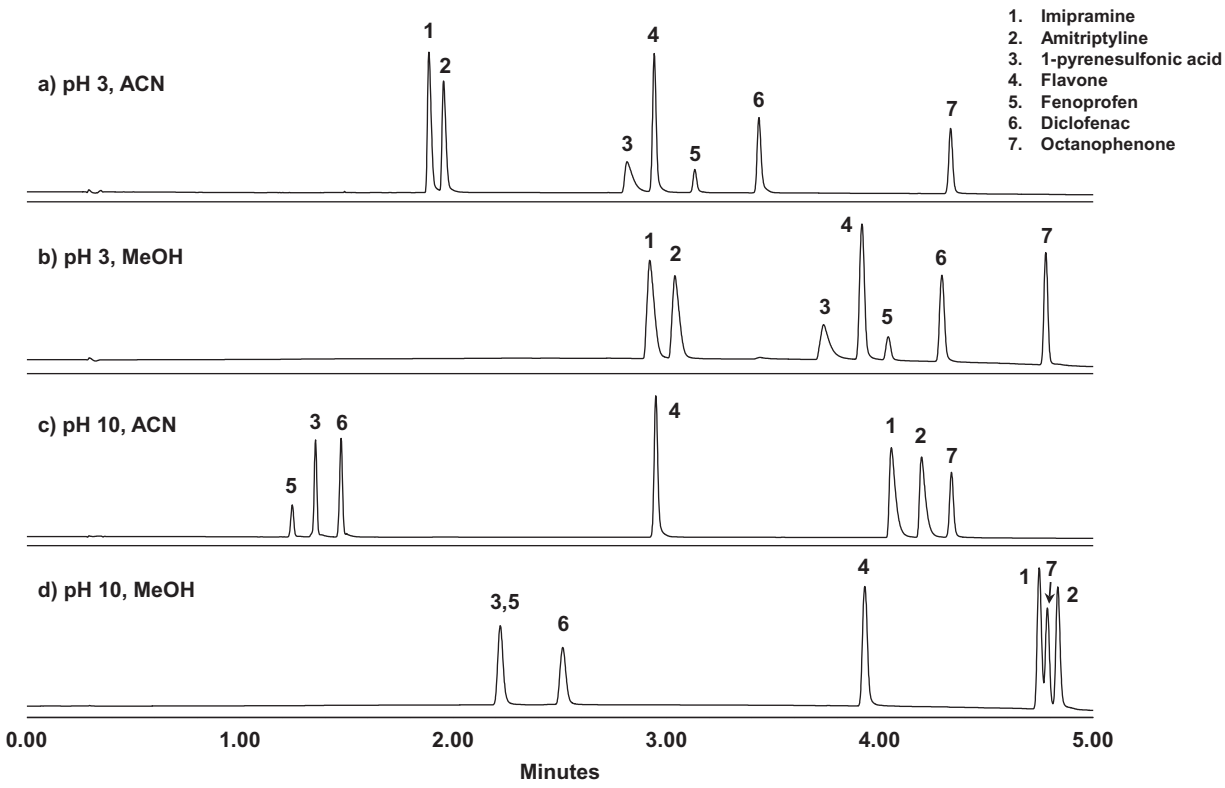

Fig. 4. Case study illustrating rapid mobile-phase screening of a seven drug-component mixture using low-pH and high-pH MPA with acetonitrile (ACN) or methanol (MeOH). Column = C18 (50 × 2.1 mm, 1.7 μm), MPA = 0.1% formic acid (pH 3.0) or 0.1% ammonia (pH 10.0), gradient = 5–95% B in 5 min, F = 0.5 mL/min, Detection = 254 nm. T = 30°C. Total mobile-phase screening time for all four conditions was 30 min. (Chromatograms reprinted with permission from Waters Corporation).

as the formation of artifacts under these analytical conditions [51].

2.5. The use of an automated method-development system in the optimization of a faster UHPLC method for a complex multi-chiral molecule

Method optimization can be the most time-consuming step in the development of critical methods [3,5,6]. The use of automated method-development systems with systematic experimental design can accelerate these processes, leading to more robust methods. In this case study, we describe a UHPLC method-development scenario of a drug candidate with three chiral centers and an absolute configuration of SRR (Sinister, Rectus, and Rectus). The original HPLC method took 42 min and separated all known impurities and degradants, including all expected diastereomers of the API (SRS, RRR and SSR) [30,52]. A UHPLC method was developed with an automated method-development system using the principles of Quality by Design (QbD) and Design of Experiments (DoE) [19,39] with the intention to reduce analysis times and to improve further the resolution of the diastereomers. Note that the use of QbD principles for developing HPLC methods is becoming a major trend and is cited in recent draft guidance for method validation by the US Food and Drug Administration [53].

Fig. 5 shows results from a fractional factorial design in a DoE study (30 experiments in the design space) with several input variables [t_G, Final Percentage of mobile phase B in the gradient segment, % $B_{final,}$ and two different columns (C18 and polar-embedded C18)]. The sample used was a mixture of the four diastereomers (critical pairs in the method). A mobile phase A (MPA) of 20 mM ammonium formate at pH 3.7 and a mobile phase B (MPB) of acetonitrile were used, as in the original HPLC method. These mobile phases were confirmed to be optimum by the same UHPLC system in a prior mobile-phase-screening run using MPA at pH 2, 2.8, 3.7, 5, 7 and 10 (data not shown here). The overlaid chromatograms in Fig. 5 (results from a DoE study of 30 experiments) show baseline resolution of all four diastereomers in several conditions. The optimum set of conditions (C18, t_G = 17 min and % B_{final} =35%) was found and was based on assessment of the baseline resolution of all four isomers in the shortest analysis time.

Automated method-development systems with UHPLC can greatly increase the productivity in the systematic method-development process for critical methods required in regulatory testing of pharmaceuticals, where all impurities and degradants must be resolved and accurately determined to ensure product safety. Such systematic studies using principles of QbD are becoming an expectation from regulatory agencies for HPLC method development [53].

2.6. Comments on method validation (qualification) and transfer

Method validation is generally considered to be a Good Manufacturing Practice (GMP) activity, conducted under a validation protocol with pre-defined acceptance criteria [3–5]. However, method development is considered a non-GMP activity [4,5]. With more UHPLC equipment being used in QC applications, reports on UHPLC method validation are beginning to show up in the literature and in regulatory filings [13,15,22,30,54]. In the development of any regulatory methods, the last step is usually a method-prequalification step (specificity, sensitivity, and linearity) to ensure that the newly-developed method is "validatable". This is a risk-mitigation step to ensure that the new development is more likely not to fail pre-determined acceptance criteria in the method-development protocol.

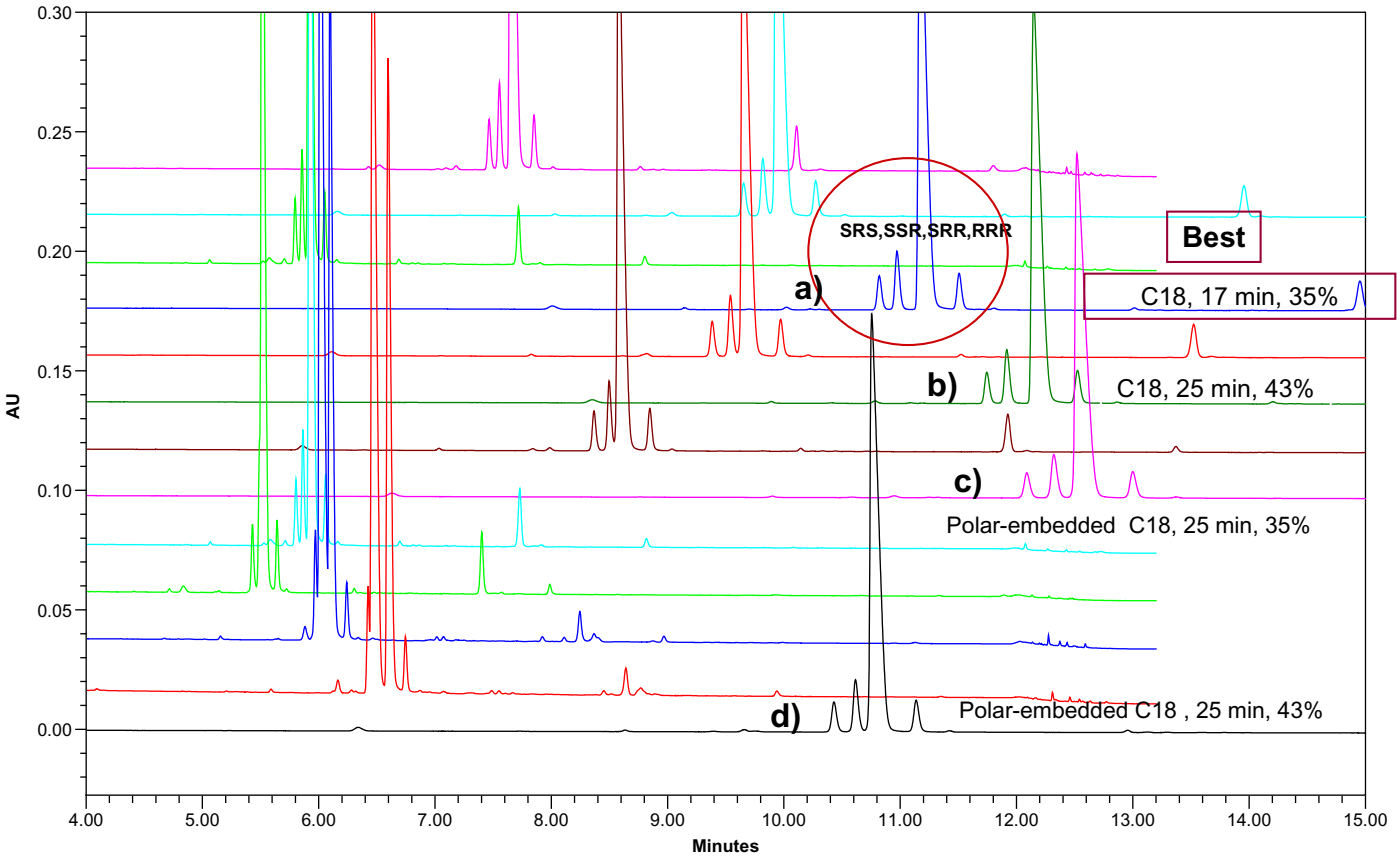

Fig. 5. Overlaid chromatograms from results generated from an automated method-development system using S-Matrix Fusion QbD software. A fractional factorial DoE design of 30 experiments in the design space with three input variables: t_G (10–25 min), % B_{final} (35–50% ACN) and column type (C18 or polar-embedded). Responses (resolution, retention time and tailing factors) were tallied and used to determine the optimum method for the separation of the four diastereomers of a multi-chiral NCE [SRS, SSR, SRR (API) and RRR]. UHPLC conditions: Column = C18 or polar-embedded C18 100 × 2.1 mm, 1.7 μm), MPA = 20 mM ammonium formate (pH 3.7), MPB = ACN, gradient = 5% B to % B_{final} (35–50% B) in t_G (10–25 min) as defined in the DoE study, F = 0.5 mL/min, Detection = 280 nm. T = 30°C. Of the 30 chromatograms of the DoE experiments, 13 are shown with four more optimum separations (chromatograms a to d) are labeled with operating conditions (column, t_G and % B_{final}). The best set of UHPLC conditions (circled, chromatogram a) based on the achievement of robust baseline separation of all isomers in minimum time were found to be (C18 columns, % B $_{final}$ = 35% B, and t_G = 17 min).

We believe that there is some confusion between method conversion and method transfer, as these two terms are often used interchangeably in the literature. As mentioned earlier, newly developed UHPLC methods are often converted to HPLC methods using geometrical scaling to ensure than the final GMP methods can be implemented globally, including at facilities where UHPLC equipment is not yet available. The three scenarios in method conversions earlier have been described elsewhere [35]. Method transfer is the formal process of demonstrating that a validated method, developed or validated in one laboratory, can be properly executed by another laboratory operating under a GMP regime. Transfers of UHPLC methods can be more challenging across different UHPLC system platforms due to differences of dwell volumes, system dispersion and the effect of viscous heating, particularly for high-resolution separations, as discussed in some recent references [9,30,35,45,46].

2.7. UHPLC methods using other chromatographic modes or sample types

At the debut of the first commercial UHPLC equipment (Waters Acquity UPLC) in 2004, the choice of available columns was limited to C18 and C8 reversed-phase columns. While reversed-phase LC remains the most dominant mode in UHPLC, this limitation is easing as other chromatographic modes, such as normal phase, chiral [55], ion-exchange (IEC), size exclusion chromatography (SEC) [56] and hydrophilic interaction chromatography (HILIC) [57] are catching on as more sub-2-μm or sub-3-μm materials and columns become available [36]. Methods for other sample types, such as biomolecules (proteins, monoclonal antibodies, peptides, and polynucleotides), metabolites, polymers, biofluids, and ions are also appearing in the literature as appropriate columns and instruments are introduced [18,56,58–61].

3. Conclusion

In the past few years, UHPLC has become a modernized standard HPLC platform and an accepted tool in QC. Its impacts on diverse HPLC applications and method development are increasing rapidly as more column types, equipment and software become available. The line between HPLC and UHPLC is getting blurred as this technology becomes mainstream. This article provides a critical review of the application of UHPLC in method-development situations. UHPLC allows for faster method development and more accurate analysis of complex samples due to its high-throughput and high-resolution capabilities. We described case studies in HPLC/UHPLC method conversions, column/mobile-phase screening and automated method optimization to illustrate the best practices and recent advances. The higher cost of UHPLC and backward system compatibility of some UHPC systems are continuing issues. The need for

method conversion between HPLC and UHPLC remains another potential issue, which is expected to ease as older HPLC equipment is phased out.

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References

[1] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, Wiley-Interscience, Hoboken, NJ, 1997.
[2] S. Ahuja, H. Rasmussen, HPLC Method Development for Pharmaceuticals, Academic Press, Amsterdam, 2007.
[3] H.T. Rasmussen, W. Li, D. Redlich, M. Ilias, Jimidar, HPLC method development, in: S. Ahuja, M.W. Dong (Editors), Handbook of Pharmaceutical Anaysis by HPLC, Elsevier., Amsterdam, Netherlands, 2005, pp. 145–190.
[4] R. LoBrutto, Method development, in: Y.V. Kazakevich, R. LoBrutto (Editor), HPLC for pharmaceutical scientists, Wiley-Interscience., Hoboken, NJ, 2007, pp. 347–454.
[5] M.W. Dong, HPLC Method Development, Modern HPLC for Practicing Scientists, Wiley., Hoboken, NJ, 2006, pp. 193–220.
[6] L.R. Snyder, J.J. Kirkland, J.W. Dolan, Introduction to Modern Liquid Chromatography, 3rd ed., Wiley, Hoboken, NJ, 2011.
[7] L.R. Snyder, Efficient HPLC method development and personal reflections, LCGC North Am. 25 (2012) 437–444.
[8] M.W. Dong, A three-pronged template approach for rapid HPLC method development, LCGC North Am. 31 (2013) 612–622.
[9] M.W. Dong, Ultrahigh-pressure LC in pharmaceutical analysis: performance and practical issues, LCGC North Am. 25 (2007) 89–98.
[10] S. Chen, A. Kord, UHPLC method development, in: A.Q. Xu (Editor), Ultra-High Performance Liquid Chromatography and Its Applications, Wiley., Hoboken, NJ, 2013, pp. 1–30.
[11] G. Xue, Y. Zhao, LC method development and strategies, in: B.N.L. Pramanik, S. Mike, C. Guodong (Editors), Characterization of Impurities and Degradants Using Mass Spectrometry, John Wiley & Sons., Hoboken, NJ, 2011, pp. 59–79.
[12] G. Liu, H.M. Snapp, Q.C. Ji, M.E. Arnold, Strategy of accelerated method development for high-throughput bioanalytical assays using ultra high-performance liquid chromatography coupled with mass spectrometry, Anal. Chem. 81 (2009) 9225–9232.
[13] A. Goel, S. Baboota, J.K. Sahni, K.S. Srinivas, R.S. Gupta, A. Gupta, et al., Development and validation of stability-indicating assay method by UPLC for a fixed dose combination of atorvastatin and ezetimibe, J. Chromatogr. Sci. 51 (2013) 222–228.
[14] T.K. Natishan, Developments in fast liquid chromatographic analysis of pharmaceuticals, J. Liq. Chromatogr. 34 (2011) 1133–1156.
[15] P.Y. Sacre, E. Deconinck, P. Chiap, J. Crommen, F. Mansion, E. Rozet, et al., Beer, Development and validation of a ultra-high-performance liquid chromatography-UV method for the detection and quantification of erectile dysfunction drugs and some of their analogues found in counterfeit medicines, J. Chromatogr. A 1218 (2011) 6439–6447.
[16] Q.A. Xu, Ultra-high Performance Liquid Chromatography and Its Applications, John Wiley & Sons, Hoboken, NJ, 2013.
[17] U.D. Neue, M. Kele, B. Bunner, A. Kromidas, T. Dourdeville, J.R. Mazzeo, et al., Ultraperformance liquid chromatography technology and applications, in: S. Fanali, P.R. Haddad, C. Poole, P. Schoenmakers, D.K. Lloyd (Editors), Adv. Chromatogr., Elsevier/CRC, Hoboken, NJ, 2010, pp. 99–143.
[18] D. Guillarme, J.-L. Veuthey, UHPLC in Life Sciences, Royal Society of Chemistry, Cambridge, UK, 2012.
[19] D. Guillarme, M.W. Dong, Newer developments in HPLC impacting pharmaceutical analysis: a brief review, Am. Pharm. Rev. 16 (2013) 36, 38–43.
[20] S.M. Chesnut, J.J. Salisbury, The role of UHPLC in pharmaceutical development, J. Sep. Sci. 30 (2007) 1183–1190.
[21] D. Guillarme, D.T. Nguyen, S. Rudaz, J.-L. Veuthey, Method transfer for fast liquid chromatography in pharmaceutical analysis: application to short columns packed with small particle. Part II: gradient experiments, Eu. J. Pharm. Biopharm. 68 (2008) 430–440.
[22] N.W. Wu, J. Christopher, T.K. Natishan, H. Gao, C. Tilak, Z. Li, Practical aspects of ultrahigh performance liquid chromatography, in: Q.A. Xu (Editor), Ultra-high Performance Liquid Chromatography and Its Applications, John Wiley & Sons., Hoboken, NJ, 2013, pp. 55–94.
[23] N. Wu, A.C. Bradley, Effect of column dimension on observed column efficiency in very high pressure liquid chromatography, J. Chromatogr. A 1261 (2012) 113–120.
[24] F. Gritti, A. Felinger, G. Guiochon, Influence of the errors made in the measurement of the extra-column volume on the accuracies of estimates of the column efficiency and the mass transfer kinetics parameters, J. Chromatogr. A 1136 (2006) 57–72.
[25] S. Fekete, D. Guillarme, M.W. Dong, Superficially porous particles: perspectives, practices and trends, LCGC North Am. 32 (2014) 420–433.
[26] M.W. Dong, Ultra-high-pressure LC in pharmaceutical analysis: benefits, impacts and issues, in: R.L. Wixom, C.W. Gehrke (Editors), Chromatography: A Science of Discovery, Wiley., Hoboken, NJ, 2010, pp. 328–333.
[27] A. Chavali, T.E. Wheat, P.R. McConvile, Systematic scrennning of pH and ionic strength as method development tools for reversed-phase separations. Pittcon Philadelphia, 2013.
[28] M.A. Al-Sayah, P. Rizos, V. Antonucci, M. Wu, High throughput screening of active pharmaceutical ingredients by UPLC, J. Sep. Sci. 31 (2008) 2167–2173.
[29] D. Guillarme, E. Grata, G. Glauser, J.-L. Wolfender, J.-L. Veuthey, S. Rudaz, Some solutions to obtain very efficient separations in isocratic and gradient modes using small particles size and ultra-high pressure, J. Chromatogr. A 1216 (2009) 3232–3243.
[30] M. Dong, D. Guillarme, S. Fekete, R. Rangelova, J. Richards, D. Prudhomme, et al., High-resolution separations of complex pharmaceuticals by UHPLC: case studies and quality control implications, LCGC North Am. (2014, in press).
[31] C. Ghosh, Green bioanalysis: some innovative ideas towards green analytical techniques, Bioanalysis 4 (2012) 1377–1391.
[32] C.J. Welch, N.J. Wu, M. Biba, R. Hartman, T. Brkovic, X.Y. Gong, et al., Greening analytical chromatography, Trac-Trends Anal. Chem. 29 (2010) 667–680.
[33] S. Heinisch, Using elevated temperature in UHPLC: interest and limitations, in: D. Guillarme, J.-L. Veuthey (Editors), UHPLC in Life Sciences, The Royal Society of Chemistry., Cambridge, UK, 2012, pp. 102–128.
[34] M. Gilar, A.E. Daly, M. Kele, U.D. Neue, J.C. Gebler, Implications of column peak capacity on the separation of complex peptide mixtures in single-and two-dimensional high-performance liquid chromatography, J. Chromatogr. A 1061 (2004) 183–192.
[35] M.W. Dong, Myths in ultrahigh-pressure liquid chromatography, LCGC North Am. 31 (2013) 868–880.
[36] R.E. Majors, New chromatography columns and accessories at pittcon 2013, Part I (vol 31, p. 280, 2013), LC GC N. Am. 31 (2013) 537.
[37] S. Fekete, I. Kohler, S. Rudaz, D. Guillarme, Importance of instrumentation for fast liquid chromatography in pharmaceutical analysis, J. Pharm. Biomed. Anal. 87 (2014) 105–119.
[38] J.D. Stafford, T.D. Maloney, D.P. Myers, J.M. Cintron, B.C. Castle, A systematic approach to development of liquid chromatographic impurity methods for pharmaceutical analysis, J. Pharm. Biomed. Anal. 56 (2011) 280–292.
[39] B. Debrus, D. Guillarme, S. Rudaz, Improved quality-by-design compliant methodology for method development in reversed-phase liquid chromatography, J. Pharm. Biomed. Anal. 84 (2013) 215–223.
[40] A.H. Schmidt, I. Molnar, Using an innovative Quality-by-Design approach for development of a stability indicating UHPLC method for ebastine in the API and pharmaceutical formulations, J. Pharm. Biomed. Anal. 78–79 (2013) 65–74.
[41] D. Guillarme, J. Ruta, S. Rudaz, J.-L. Veuthey, New trends in fast and high-resolution liquid chromatography: a critical comparison of existing approaches, Anal. Biochem. Anal. 397 (2010) 1069–1082.
[42] S. Fekete, E. Oláh, J. Fekete, Fast liquid chromatography: the domination of core-shell and very fine particles, J. Chromatogr. A 1228 (2012) 57–71.
[43] A.L. Huidobro, P. Pruim, P. Schoenmakers, C. Barbas, Ultra rapid liquid chromatography as second dimension in a comprehensive two-dimensional method for the screening of pharmaceutical samples in stability and stress studies, J. Chromatogr. A 1190 (2008) 182–190.
[44] K. Zhang, J. Wang, M. Tsang, L. Wigman, N.P. Chetwyn, Two-dimensional HPLC in pharmaceutical analysis, Am. Pharm. Rev. 16 (2013) 39–44.
[45] B. Debrus, E. Rozet, P. Hubert, J.-L. Veuthey, S. Rudaz, D. Guillarme, Method transfer between conventional HPLC and UHPLC, in: D. Guillarme, J.L. Veuthey (Editors), UHPLC in Life Sciences, The Royal Society of Chemistry., Cambridge, UK, 2012, pp. 68–98.
[46] U.D. Neue, D. McCabe, V. Ramesh, H. Pappa, J. DeMuthc, Transfer of HPLC procedures to suitable columns of reduced dimensions and particle sizes, Pharmacopeial Forum 35 (2009) 1622–1626.
[47] D. Guillarme, HPLC calculator : software for chromatographic performance evaluation and HPLC method transfer, U. Geneva. <http://www.unige.ch/sciences/pharm/fanal/lcap/telechargement-en.htm> 2008. (last accessed 14.09.30).
[48] C. Gourmel, A.G.G. Perrenoud, L. Waller, E. Reginato, J. Verne, B. Dulery, et al., Evaluation and comparison of various separation techniques for the analysis of closely-related compounds of pharmaceutical interest, J. Chromatogr. A 1282 (2013) 172–177.
[49] K.J. Fountain, H.B. Hewitson, P.C. Iraneta, D. Morrison, Practical applications of charged surface hybrid (CSH) technology, 720003720EN. Milford MA, 2010.
[50] D.V. McCalley, The challenges of the analysis of basic compounds by high performance liquid chromatography: some possible approaches for improved separations, J. Chromatogr. A 1217 (2010) 858–880.
[51] M.W. Dong, G.D. Miller, R.K. Paul, Mass spectrometry-compatible ICH (International Conference on Harmonization) impurity analysis with a high-pH mobile phase – advantages and pitfalls, J. Chromatogr. 987 (2003) 283–290.
[52] M.W. Dong, The essence of modern HPLC advantages, limitations, fundamentals, and opportunities, LCGC North Am. 31 (2013).
[53] U.S. FDA, Guidance for Industry: Analytical Procedures and Method Validation for Drugs and Biologics, Rockville Maryland, 2014, pp. 1–12.

[54] E. Deconinck, P.-Y. Sacre, S. Baudewyns, P. Courselle, J. De Beer, A fast ultra high pressure liquid chromatographic method for qualification and quantification of pharmaceutical combination preparations containing paracetamol, acetyl salicylic acid and/or antihistaminics, J. Pharm. Biomed. Anal. 56 (2011) 200–209.
[55] D. Fruhauf, M. Juza, Development, optimization and validation of a sub-minute analytical enantioselective high performance liquid chromatographic separation for a folic acid precursor in normal phase mode, J. Chromatogr. A 1269 (2012) 242–254.
[56] E. Uliyanchenko, P.J. Schoenmakers, S. Van der Wal, Fast and efficient size-based separations of polymers using ultra-high-pressure liquid chromatography, J. Chromatogr. A 1218 (2011) 1509–1518.
[57] J. Ruta, J. Boccard, D. Cabooter, S. Rudaz, G. Desmet, J.L. Veuthey, et al., Method development for pharmaceutics: some solutions for tuning selectivity in reversed phase and hydrophilic interaction liquid chromatography, J. Pharm. Biomed. Anal. 63 (2012) 95–105.
[58] S. Fekete, M.W. Dong, T. Zhang, D. Guillarme, High resolution reversed phase analysis of recombinant monoclonal antibodies by ultra-high pressure liquid chromatography column coupling, J. Pharm. Biomed. Anal. 83 (2013) 273–278.
[59] J.C. Rea, Y.J. Wang, T. Zhang, UHPLC for characterization of protein therapeutics, in: Q.A. Xu (Editor), Ultra-High Performance Liquid Chromatography and Its Applications, Wiley, Hoboken, NJ, 2013, pp. 235–252.
[60] E.J. Want, I.D. Wilson, H. Gika, G. Theodoridis, R.S. Plumb, J. Shockcor, et al., Global metabolic profiling procedures for urine using UPLC-MS, Nat. Protoc. 5 (2010) 1005–1018.
[61] J. Jeong, T. Zhang, J. Zhang, Y.H. Kao, Ultra-high-pressure LC (UHPLC) for therapeutic protein characterization, Am. Pharm. Rev. 14 (2011) 44–51.