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Advances in LC: bioanalytical method transfer

There are three main reasons for transferring from an existing bioanalytical assay to an alternative chromatographic method: speed, cost and sensitivity. These represent a challenge to the analyst in that there is an interplay between these three considerations and one factor is often improved at the expense of another. These three factors act as drivers to encourage technology development and support its uptake. The more recently introduced chromatographic technologies may show significant improvements against one of more of these factors relative to conventional 4.6-mm id reversed-phase HPLC. In this article, some of these new chromatographic approaches will be considered in terms of what they can offer the bioanalysts.

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Reasons for changing chromatography method

Although a detailed theoretical consideration of chromatography will not be undertaken here, a simple outline of chromatographic parameters will help to contextualize how technology developments can deliver improvements over conventional assays. Chromatographic resolution is described by the following equation:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{k'}{(k' + 1)} \cdot \frac{(\alpha - 1)}{\alpha}$$

The column plate count is denoted by N , the peak capacity factor by k' and the selectivity by α . Selectivity is compound specific, requiring careful optimization on a compound-by-compound basis, and so will not be discussed.

It is the plate count and analysis time that are influenced most by new technologies. Plate count indicates the efficiency of the column. A higher plate count results in sharper peaks and greater sensitivity by vir-

tue of improved S/N. The plate count is itself dependent on a number of factors, such as column particle size, column length and flow rate. Capacity factors would be unchanged for a given analyte in a specific assay. A chromatographic column contains a fixed volume of solvent, the void volume. An unretained analyte elutes from the column if a volume of solvent equal to the void volume flows through it. For retained analytes, the capacity factor is the number of additional void volumes that are required to elute the peak. While the capacity factor does not change, the time taken to pass these void volumes through the column is inversely related to the flow rate. The retention times and, consequently analysis times, can be reduced by technologies which allow increased flow rate to be used.

The relationship between plate count and flow rate is complex. It depends on mass transfer between the mobile and stationary phases, the degree of compound diffusion in the mobile phase and the tortuosity of the flow path through the column. The tortuos-

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ity of the flow path is a constant for the column, the degree of diffusion is inversely proportional to the flow rate and mass transfer effects are directly proportional to the flow rate. These are combined to describe the relationship between the flow rate and plate count. A plot of column efficiency (expressed as the height equivalent to a theoretical plate) against linear flow velocity describes as a 'U'-shaped profile with the minima corresponding the greatest column efficiency. This is the widely reported van Deemter curve [1]. This is most important in understanding why different chromatographic technologies offer advantages to bioanalysis. There is an optimum flow rate for a column. Flow rates below the optimum result in lower efficiencies and increased analysis times, while above optimum the efficiency is lower but analysis times are shorter. Technologies which move the van Deemter minima to higher flows or that flatten the profile offer the potential for faster, cheaper and more sensitive analysis.

Developments in chromatographic technologies

Ultra-HPLC

Developments in the production of column packing materials have led to small particle sizes being available, while improvements in the mechanical performance of

chromatography systems allow higher pressure solvent flows. Adopting packing materials of less than 2- μm diameter moves the minima of the van Deemter profile to higher flows (Figure 1). They also flatten the curve, so that flow rates beyond the optimum reduce efficiency to a lesser extent. The flow rates that can be used with sub 2 μm particle packings are beyond the pressure limits of traditional HPLC systems. New systems, capable of operating at two- to three-times the pressure of conventional HPLC pumps, were introduced under the banner of ultra-HPLC (UHPLC).

Small particle sizes have the added advantage of increasing column efficiency. UHPLC offers increases in speed and sensitivity as analysis can be completed more quickly with greater column efficiency [2-5] (Figure 2).

The cost-reduction implications are more involved. Instrument costs are higher but more samples can be analyzed in a specified time. Solvent costs are unchanged as the same eluent volume passes through the column in less time. A reduction in column length and/or diameter reduces solvent usage and, hence, costs. This is true for all HPLC systems including UHPLC as these new chromatographic instruments have been designed with minimal dispersion as a critical requirement. As UHPLC is more efficient than HPLC, comparable separations can be produced in

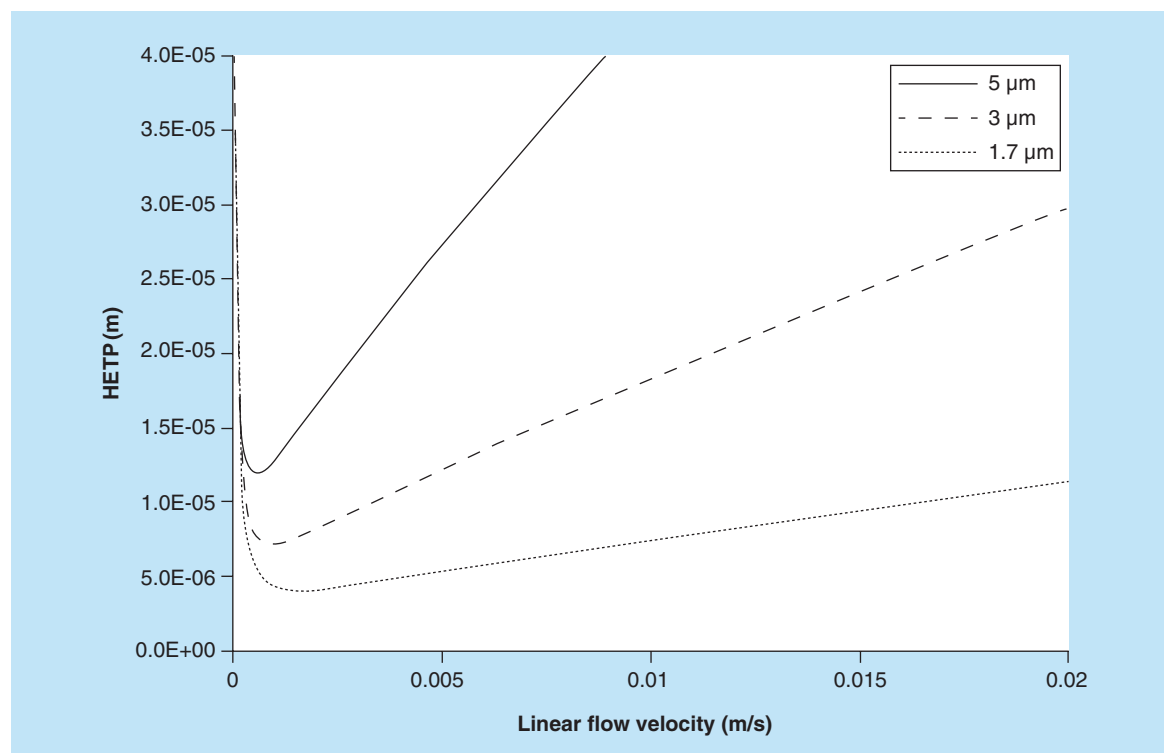


Figure 1. Theoretical van Deemter curves of the height equivalent to a theoretical plate against linear flow velocity for columns of 5-, 3- and 1.7- μm diameter packing materials. The curves were generated using Knox's equation $h = Av^{1/3} + B/v + Cv$; the constants were appropriate for a well-packed column.

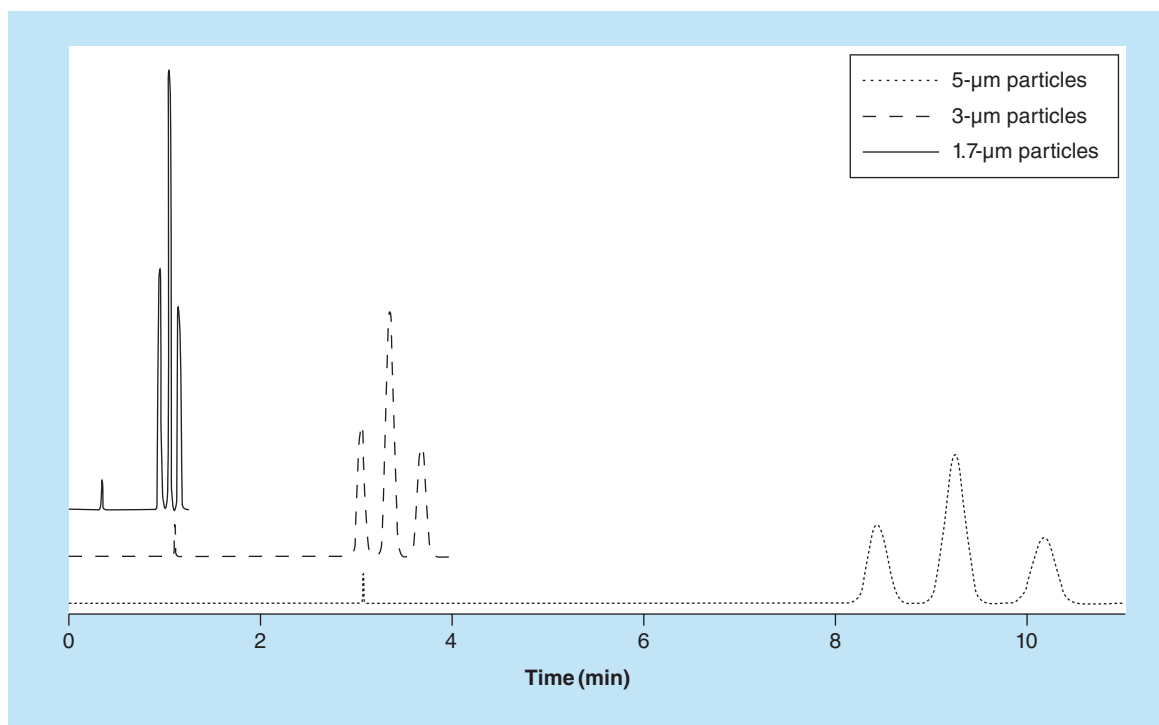


Figure 2. Computer-generated chromatograms for 5-, 3- and 1.7- μ m diameter particulate packing materials.

All columns were operating at their optimum flow rates. The 5- μ m particle column was 10-cm long. The column lengths for 3 and 1.7 μ m were selected, so all produced an identical plate count; the lengths were 6.0 and 3.4 cm, respectively. Injection volumes were adjusted to maintain a fixed injection volume to column volume ratio. Peak capacity factors and resolution between adjacent peaks were identical in each chromatogram.

much less time. Equivalent column efficiencies can be produced from shorter columns in UHPLC than HPLC, although the optimum flow rate is higher. Higher flows and shorter columns reduce retention times, producing sharper peaks. Greater sensitivity results from an improved S/N. Improvements in time, cost and sensitivity are possible if method equivalence is the objective.

The mobile phases for UHPLC require more care in their preparation than those of conventional HPLC. It is important to use high-quality solvents and water which are both filtered and degassed as UHPLC is particularly sensitive to problems resulting from particulates and dissolved gases as a result of the very high pressure of the system. If sufficient attention is paid the manufacturers' recommendations on operation, UHPLC proves to be robust and suited to routine use.

Fused-core columns

HPLC and UHPLC utilize the same technology in the packed columns; a chemical reaction between a porous silica particle and an organo-silane reagent produces the stationary phases used in LC. In most cases, the silica particle is spherical and eluent flows in gaps between the spheres, which are packed tightly together. The porosity of the silica also means that elu-

ent flows through the particle. The restriction to flow is greater through particles than between them. A large proportion of column-back pressure results from flow through the silica particles. It is this phenomena that results in the tortuosity of flow and reduces column efficiency.

Several types of column have been produced to reduce the effects of silica particle porosity and to reduce the influence of tortuosity on column efficiency. One of these columns utilizes a spherical packing material with a solid core of silica and a thin outer layer of porous silica onto which the stationary phase is reacted (Figure 3). These are known as fused-core or superficial porous packing materials [2–7]. The flow path for eluent is predominantly through spaces between particles. With less restriction to flow, the pressure generated by the column is reduced. A less tortuous flow path also results in greater column efficiency. Fused-core particles generate greater column efficiencies than HPLC or UHPLC packings of comparable diameter. They yield column efficiencies equivalent to those of smaller porous packing materials but with lower back pressures. Fused-core particles have been produced with diameters intermediate between existing HPLC packings and the new UHPLC materials. These can be considered as a viable alternative to UHPLC as they

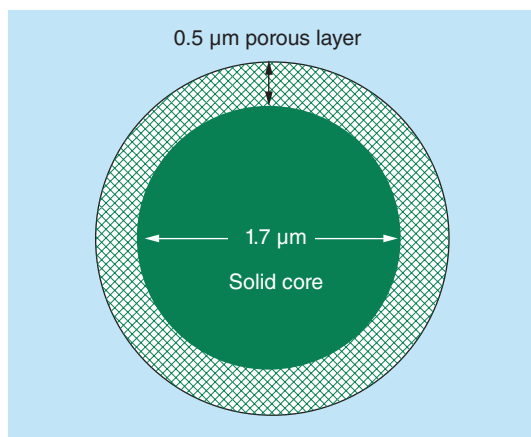


Figure 3. Typical structure of a fused-core particle.

produce comparable chromatography without the need to purchase ultrahigh pressure pumps, existing HPLC instrumentation may be used. Using HPLC instruments does require careful optimization to keep dispersion to a minimum as column efficiency improvements can be quickly lost. Fused-core columns in bioanalysis usually offer an improvement in both cost and speed of analysis.

To maximize the potential of fused-core columns still further, these columns can be used with UHPLC instrumentation. UHPLC instrumentation has been designed from a perspective of dispersion minimization, so fused-core materials have also been produced with comparable particle diameters to UHPLC columns. In this combination, the lower back pressures (at comparable flow rates to UHPLC) allow even shorter analysis times than UHPLC.

Monolithic columns

Another type of column uses a packing material that is a more radical departure from individual particles; it uses monoliths (i.e., rods) composed of silica or polymer that fill the entire column. Silica-based monoliths tend to have higher efficiency than those based on polymers and so silica monoliths have been adopted widely for bioanalysis [5,8–9]. Monolithic materials have two types of pores: large macropores that allow flow through the column, and small mesopores that increase the surface area where the stationary phase is bonded. The tortuosity of flow through monoliths is lower than packed columns, so efficiency suffers less reduction as flow increases. The macropores through a monolithic column are larger than the spaces between particles in a packed column and create less resistance to flow. Monoliths allow high flow rates with low back pressure. The van Deemter profile for monolithic columns is flatter than traditional HPLC, so higher flow rates do not significantly reduce efficiency; sensitivity and peak resolution can be maintained even with short

analysis times. A 4.6-mm id monolithic column can be operated at flow rates as high as 9 ml/min, although 3 ml/min is the more common choice, enabling the formation of ballistic gradients. Ballistic gradients result in short run times and higher sensitivity due to sharper peaks and potentially less mass spectral ionization suppression as a result of separation from endogenous material even over the course of the rapid mobile-phase gradient. A caveat to using ballistic gradients for LC–MS is that a flow splitter may be required post column to make sure the flow is optimal for mass spectral sensitivity.

Turbulent flow chromatography

Turbulent flow chromatography offers the potential for increased speed of analysis in that neat biological samples can be analyzed directly without the need for sample extraction [4–5,10]. In turbulent flow chromatography, a high flow rate is applied to a short microbore (1 mm id) HPLC column with large porous packing. The flow is highly turbulent as the mobile phase eddies around the packing particles, which encourages diffusion of small molecules in to the pores of the packing while larger molecules such as proteins are excluded. It is essentially a size exclusion-based method. This approach has been shown to be suitable for the analysis of three basic compounds in neat dog plasma pharmacokinetic samples [11], with a reported LOQ of 5 ng/ml by LC–MS/MS. However, even with the online removal of the proteins there is still some ionization suppression from the other matrix components, which may limit its use for higher sensitivity mass spectrometric assays.

Supercritical fluid chromatography

The van Deemter profile is influenced by the mass transfer rate as an analyte partitions between eluent and stationary phase. It takes time for an analyte to move between these two phases. The slower rate, the lower the column efficiency. Supercritical fluids as an eluent exhibit a much higher rate of mass transfer than liquid eluents. Supercritical fluid chromatography (SFC) has been adopted to exploit these physicochemical characteristics [12–15]. The influence of the mass transfer term on column efficiency is diminished. Columns produce greater efficiencies in SFC when compared with HPLC or UHPLC.

Higher flow rates in SFC produce a much lower column-back pressure relative to UHPLC or HPLC. Supercritical fluids have physical characteristics intermediate between those of liquids and gases. These intermediate properties mean SFC is able to analyze some samples normally run on GC, as well as many samples usually analyzed by LC. The phrase ‘conver-

gent chromatography' has been coined to reflect this intermediate behavior.

The mass transfer advantage and the low viscosity enable significantly higher flow rates to be applied without impacting column efficiency. SFC can have a significant positive impact in reducing analysis times. SFC has, in the past, used modified HPLC equipment. As a result, the S/N was poor and the back-pressure regulation was less than optimum. SFC had not achieved its potential and it was largely reserved for a niche application in chiral separation and purification. Recent mechanical engineering advances in pumping and control of back pressure have allowed a new generation of instrument able to deliver ultra-performance SFC, or convergent chromatography. The step change in instrumentation exceeds that for HPLC to UHPLC. SFC separations can be achieved on HPLC and UHPLC columns with high efficiency but even shorter analysis times.

Cost reductions arising from using SFC are considerable. The supercritical fluid adopted for chromatographic separation is carbon dioxide; a by-product of fermentation processes. The cost of carbon dioxide is extremely low, unlike the organic liquids used with UHPLC or HPLC. It is regarded as a green reagent and carries no disposal costs. In the cases of organic solvents such as methanol or acetonitrile, the disposal costs may exceed purchase costs by a factor of 2 or 3. A comparison of SFC analysis and UHPLC or HPLC analysis for the same analyte can show a cost reduction of tenfold or more. An application note shows costs reducing to \$0.05 from \$5.89 on switching from a normal-phase method to an SFC one.

Miniaturization: microbore & nanobore chromatography

Improvements in sensitivity can be achieved by miniaturization of column dimensions and matching the flow rate accordingly. The benefits of reducing solvent costs through lower flow rates have been mentioned earlier, so the focus will be on sensitivity. This approach has been applied to LC technologies for a long time. Narrow-bore, microbore and nano- or capillary HPLC are well-established terms, although there is probably no precise definition of the column diameters corresponding to the different terms. In reality, the terms represent a reduction of diameter from the traditional 4.6 mm [16,17]. Advances in instrumentation and optimization of liquid flow paths for columns of smaller dimensions mean that UHPLC and SFC technologies have not been subdivided in the same way.

The sensitivity enhancement on reducing column diameter reflects a reduction in the minimum detectable mass, m . The value of m is directly proportional

to the column radius squared as shown in the equation below. This means that a 1-mm column has a mass sensitivity 20-times greater than a 4.6-mm column. Also, if the same volume of sample could be injected onto the two columns, then the detection limit would be around 20 lower for the 1-mm column.

$$m = \frac{2\pi r^2 l \Psi (1 + k') C}{\sqrt{N}}$$

where r is the column radius, l is its length, Ψ is the fraction of volume occupied by mobile phase, k' in the capacity factor, C is the sample concentration and N is the column plate count.

For reduced diameter columns, the injection volume, if it is too large, can cause peak broadening and some loss of column efficiency. In this case, the sensitivity enhancement may not be as expected. Bioanalysis samples are usually aqueous-based and large injection volumes have less detrimental effects. Sensitive detection limits may be realized by using large injection volumes, even with traditional column diameters. These smaller diameter columns are often applied for analyses where the available volume is limited such as for pediatric studies. High sensitivity may be achieved on small sample volumes. The widespread adoption of mass spectrometers as the detection method of choice for many assays is also supported a move to smaller diameter columns. Mass spectrometers are extremely sensitive and well suited to being coupled to chromatographic methods with low flow rates. The innate mass sensitivity advantage can be exploited most effectively using MS as the detection option.

The challenges of miniaturization stem from the performance of the chromatographic system rather than the column itself [18,19]. The laminar flow profiles when an eluent pumps through a tube causes dispersion and reduces the apparent column efficiency. True column efficiency is unaffected but band broadening effects postcolumn results in wider peaks at the detector. Dispersion due to laminar flow profiles is related to the diameter and length of the tube. Reducing both is crucial if the full benefit of miniaturization is to be realized. Connections between tubing, columns, injectors and detectors are equally important as even very small dead volumes cause significant contributions to dispersion. Tubing must reach right to the base of a fitting and must be square cut and polished to avoid any dead volumes. It is also important that the bore of hole in a fitting matches that of the connecting tubing for the same reason. It is also important to realize that the instrumentation must be intended for low flow work as well. For spectrometric detection, flow cells need to be matched to the peak

volumes if dispersion in the cell is to be avoided. If mass spectrometric detection is used, there are specialist interfaces available, such as the Agilent Chip-Cube, the PicoFuze (for the Sciex Turbo probe) and the Waters IonKey [20]. Injectors and their loops must also be appropriate for low flow work. Meeting these exacting requirements requires significant work. It is common for results of switching to miniaturized columns to be disappointing due to insufficient attention being paid to the instrumentation and the fluid path.

HILIC columns

The sensitivity of an analysis can be influenced by the level of background noise and the presence of peaks from the sample matrix. Technologies that can aid the separation of the analyte from these interferences can improve sensitivity. A significant number of bioanalytical methods focus on the measurement of small, polar molecules. Retention of such analytes with traditional HPLC methods is often difficult and they elute in the same region as interfering matrix components. Although selectivity itself is too large a subject to be discussed in detail here, it is worth mentioning one approach which has become popular in bioanalysis for its ability to selectively retain highly polar compounds.

Hydrophilic interaction LC, or HILIC, has become a mechanism for retaining polar analytes in the presence of interferences, achieving a normal phase type retention with an aqueous mobile phase [7]. A polar stationary phase interacts with the polar analyte through a variety of retention mechanisms resulting in much greater retention than with reversed-phase chromatography. In many instances, the elution order is essentially the reverse of that observed with reversed-phase. Analytes are retained at high percentages of organic modifier and elute as aqueous levels increase.

Validation considerations

For regulatory studies, transferring a method from conventional chromatography to an alternative approach may require revalidation of the assay.

The September 2013 US FDA Draft Guidance on Bioanalytical Method Validation [21] states *'FDA encourages the development and use of new bioanalytical technologies'* and *'generally, the use and submission of data based on new technologies should be supported with data generated by established technology, until the new approaches become accepted practice.'* Although what may be considered new technology is, to a certain extent, subjective, this statement does suggest it is best practice that new assay should be cross-validated

with the existing assay. An example of cross-validation cited by the FDA is *"an original validated bioanalytical method serves as the reference, and the revised bioanalytical method is the comparator, comparisons should be done both ways."* It is not always feasible, however, to cross-validate; for example, if the original technology is no longer available or, in particular, if the LOQ has been lowered and the original assay cannot generate LOQ data within the new calibration range.

In addition, the new assay will require some verification or validation in its own right. The extent of validation depends on whether the changes from the original method are considered to be major or minor. The definition of major or minor may be subjective and should be based on a sound scientific understanding of the technology involved and regularity requirements. Although not specifically written for bioanalysis, the US and European Pharmacopoeias both detail 'allowable' adjustments to methods and these may be used as guidelines [22,23]. Comparing the Pharmacopoeia recommendations with the technologies discussed in this chapter, it is only decreasing the HPLC column diameter to change from conventional to microbore, nanobore or capillary HPLC, which may be considered an allowable adjustment. Both the European and US Pharmacopoeias state that the column diameter may be decreased if the flow rate is adjusted to maintain the same linear velocity (the stationary phase remaining the same). Reduction in injection volume is also allowable. Under these circumstances, system suitability samples and quality controls (QCs) may be used to verify the performance of the new method rather than requiring a complete validation.

The other approaches considered here involve fundamental changes in the methodology; alternative stationary phases (e.g., to monolithic or fused-core), significant reduction in particle size (e.g., to 1.7 μm for UHPLC) or different mobile phase (e.g., a supercritical fluid). Therefore, revalidation is likely to be required.

Conclusion

In order to meet the exacting challenges of bioanalytical research and development, the analyst needs to be flexible, willing to adopt new approaches when necessary. This flexibility comes at a price however, in terms of additional training, time to establish new technology and potentially extra studies to meet regulatory requirements.

Reversed-phase HPLC on 4.6-mm id column, often with C8 or C18 packing, is still popular in bioanalysis. There is good reason for this in that this approach is well understood by practitioners, can be robust and often be directly comparable to historic

Table 1. An overview of technologies in terms of which offer an improvement (✓), some degree of disadvantage (x) or are similar (•) to HPLC with 4.6-mm i.d. column with conventional particulate silica-based packing.

Technology	Speed of analysis	Sensitivity	Cost		
			Hardware	Columns	Solvent
UHPLC	✓	✓	x	x	•
Fused-core HPLC	✓	✓	•	•	•
Monolithic HPLC columns	✓	✓	•	•	•
Turbulent flow chromatography	✓	•	•	•	x
Supercritical fluid chromatography	✓	•	x	•	✓
Microbore/nanobore HPLC	•	✓	x	•	✓
HILIC columns [†]	•	✓	•	•	•

[†]HILIC columns offer potential for highly polar compounds only.

methods. When it is required to transfer a method to a new chromatographic technology platform, the objective for the move should be clear to allow selection of the most appropriate approach. There is no single chromatographic advance that offers improvements in all three of the desirable factors speed, sensitivity and cost. However, there are chromatographic methods, which offer an improvement in at least one of the factors, as summarized in Table 1. In addition, each of the chromatography approaches has their own requirements for sample handling. Successful method transfer requires thorough research and planning, in addition to allocating sufficient time for development of the new method.

Future perspective

It is unlikely that a 'one solution' approach (in terms of obtaining optimal speed and sensitivity at reasonable cost) to bioanalysis will emerge in the near future. The improvements will come from further developments of current technology. Practical design of the instrumentation to make it more user-friendly and guard against potential problems is area where advances are expected. Minimizing (or even removing) connecting tubing and minimizing sources of

dead volume will lead to a redesign of instrument layout. Therefore, direct coupling of column to injector and detector will become common place and tubing of more than a few centimeters a rarity.

A vast array of analytical tools is available to the bioanalyst. That all these techniques have a place is testimony to their effectiveness. A common feature to them all is the manipulation of the van Deemter equation to deliver separation efficiency at higher flow rates. There is potential for further improvement in separation efficiency, even beyond UHPLC, as the limits of performance of very high pressure techniques have not been reached. Advances in instrumentation and column design are required to achieve maximum performance.

Pushing chromatography toward its operational limits, however, introduces potential problems with the sample introduction, particularly for samples derived from biological matrices. Particulates or proteinaceous precipitate run the risk of blocking the tubing or the column. Therefore, there needs to be an increased focus on fast, high-efficiency sample preparation is likely in order to match the advances in analysis methods. This is especially important with SFC where the retention mechanism retains polar endogenous material clogging the column.

Executive summary

- Adopting new technology may be the only way of meeting the demands put on bioanalysis to provide cost-effective, widely applicable methods.
- Appropriate use of new technology is encouraged by the regulatory authorities.
- There is no single chromatographic advance that offers improvements in all three of the factors speed, sensitivity and cost.
- Moving to utilizing new technology requires both training and sufficient time to ensure that the analyst can obtain the best from the equipment. Promising new approaches are often dismissed on the basis of poor results obtained as a result of insufficient resource allocation to establish the technique.
- Transferring an existing assay to a new chromatography method is likely to require full revalidation, with the possible exception of transferring to micro- or nanobore or capillary HPLC by varying the column diameter while keeping the linear velocity constant.

Further miniaturization of technologies, ably supported by highly sensitive detection, is likely in order to increase sensitivity and minimize solvent usage. This brings the possibility of a renaissance in techniques using electrically driven flows in place of high-pressure solvent delivery. The early promise of open-tube electrophoretic or packed-column electrochromatography tools was not maintained as the development of high-pressure methods took over. A similar level of investment could see electroflow technologies added to the bioanalysts' choices.

References

- Billen J, Guilleme D, Rudaz S *et al.* Relation between the particle size distribution and the kinetic performance of packed columns: application to a commercial sub-2 μm particle material. *J. Chrom. A* 1161, 224–233 (2007).
- Fekete S, Olah E, Fekete J. Fast liquid chromatography: the domination of core-shell and very fine particles. *J. Chrom. A* 1228, 57–71 (2012).
- Gritti F, Guiochon G. The current revolution in column technology: how it began, where is it going? *J. Chrom. A* 1228, 2–19 (2012).
- Nunez O, Gallart-Ayala H, Martins CPB, Lucci P, Busquets R. State-of-the-art in fast liquid chromatography-mass spectrometry for bio-analytical applications. *J. Chrom. B* 927, 3–21 (2013).
- Novakova L, Vickova H. A review of current trends and advances in bio-analytical methods: chromatography and sample preparation. *Anal. Chim. Acta* 656, 8–35 (2009).
- Badman ER, Beardsley RL, Liang Z, Bansal S. Accelerating high quality bioanalytical LC/MS/MS assays using fused-core columns. *J. Chrom. B* 878, 2307–2313 (2010).
- Wang X, Barber WE, Long WJ. Applications of superficially porous particles: high speed, high efficiency or both? *J. Chrom. A* 1228, 72–88 (2012).
- Deeb SE. Monolithic silica for fast HPLC: current success and promising future. *Chromatographia* 74, 681–691 (2011).
- Nunez O, Nakanishi K, Tanaka N. Preparation of monolithic silica columns for high-performance liquid chromatography. *J. Chrom. A* 1191, 231–252 (2008).
- Couchman C. Turbulent flow chromatography in bioanalysis: a review. *Biomed. Chromatogr.* 26, 892–905 (2012).
- Chassaing C, Luckwell J, Macrae P, Saunders K, Wright P, Venn R. Direct analysis of crude plasma samples by turbulent flow chromatography/tandem mass spectrometry. *Chromatographia* 53(3–4) 122–130 (2000).
- Farrell WP, Aurigemma CM, Masters-Moore DF. Advances in high throughput supercritical fluid chromatography. *J. Liq. Chromatogr. Relat. Technol.* 32(11–12), 1689–1710 (2009).
- Taylor LT. Supercritical fluid chromatography. *Anal. Chem.* 82(12), 4925–4935 (2010).
- Rios A, Zougagh M, de Andres F. Bioanalytical applications using supercritical fluid techniques. *Bioanalysis* 2(1), 9–25 (2010).
- Lesellier E. Supercritical fluid chromatography for bioanalysis: practical and theoretical considerations. *Bioanalysis* 3(2), 125–131 (2011).
- Arnold DW, Needham SR. Micro-LC–MS/MS: the future of bioanalysis. *Bioanalysis* 5(11), 1329–1331 (2013).
- Christianson CC, Johnson CJL, Needham SR. The advantages of microflow LC–MS/MS compared with conventional HPLC–MS/MS for the analysis of methotrexate from human plasma. *Bioanalysis* 5(11), 1387–1397 (2013).
- Fountain KJ, Neue UD, Grumbach ES, Diehl DM. Effects of extra-column band spreading, liquid chromatography system operating pressure, and column temperature on the performance of sub-2- μm porous particles. *J. Chrom. A* 1216, 5979–5988 (2009).
- Needham SR, Valaskovic GA. Microspray and microflow LC–MS/MS: the perfect fit for bioanalysis. *Bioanalysis* 7(9), 1061–1064 (2015).
- Rainville PD, Langridge JI, Wrona MD, Wilson ID, Plumb RS. Integration of microfluidic LC with HRMS for the analysis of analytes in biofluids: past, present and future. *Bioanalysis* 7(11), 1397–1411 (2015).
- FDA. Guidance for Industry. Bioanalytical Method Validation. www.fda.gov
- Neue U, McCabe D, Ramesh V, Pappa H, DeMuth J. Transfer of HPLC procedures to suitable columns of reduced dimensions and particle sizes. *Pharmaceutical Forum* 35(6), 1622–1626 (2009).
- European Pharmacopoeia 6.0 (2010) Section 2.2.4.6. United States Pharmacopoeia, Section 621. 34 (2011).

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