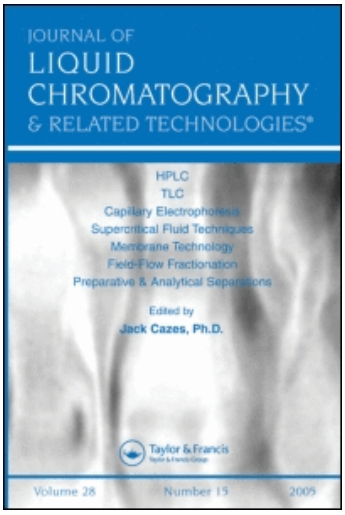

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Comparison of Multiparallel Microfluidic HPLC Instruments for High Throughput Analyses in Support of Pharmaceutical Process Research

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Comparison of Multiparallel Microfluidic HPLC Instruments for High Throughput Analyses in Support of Pharmaceutical Process Research

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Abstract: The Eksigent Express 800 8 channel and the Nanostream Veloce 24 channel microfluidic HPLC systems were compared for their utility in providing high throughput chromatographic analysis to support a growing demand in pharmaceutical process research. Criteria for success are discussed, a comparison of the two instruments is made, and results from a head to head evaluation using a variety of different analyte mixtures are presented.

Keywords: Microfluidic HPLC, Multiparallel, Pharmaceutical, Process research

INTRODUCTION

The increasing use of multiparallel experimentation in pharmaceutical process research has led to a need for high throughput analytical techniques, without which, assessing experimental outcome soon becomes a bottleneck.[1,2] Two approaches can be pursued in the quest for high throughput analysis: fast analysis and parallel analysis. We have developed or utilized a number of fast analytical methodologies in our laboratories for use in high throughput process research, including fast SFC analysis,[3] flow injection analysis using LC with MS,[4] or chiroptical detection,[5] LC-MS deconvolution of overlapping peaks,[6] and the use of isotopically differentiated 'pseudoenantiomers' for resolution screening.[7] Significant

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improvements in analysis speed are sometimes achieved by such approaches, with two or three analyses per minute being possible in the best cases. Nevertheless, not all experimental situations are amenable to the use of such fast analysis 'tricks', and analytical methods to support actual process research problems can sometimes exceed twenty minutes per sample, making the evaluation of a single 96 well microplate an arduous and lengthy experimental challenge. Consequently, there is a growing interest in multiparallel analysis approaches, where many samples are analyzed concurrently.

Planar chromatography and electrophoresis are inherently multiparallel techniques that have long been available. Although TLC has in the past been used to support process research investigations, quantitation is somewhat problematic, and the technique is poorly suited for enantiopurity analysis, a key focus area within process research. Both of these shortcomings have been addressed to some extent by recent technical improvements,[8,9] and further progress in this area may make planar chromatography once again a competitive technique.

The CombiSep 96 channel multiparallel CE instrument has been available for several years.[10] Despite the demonstrated utility of CE for general analysis,[11] including enantiopurity analysis,[12] we chose to initially pursue the avenue of multiparallel HPLC.[13–16] HPLC has been the preferred separation technique within the field of process research for a number of years, and most problems coming forward for high throughput analysis in our laboratory already have an associated HPLC method of some sort. Therefore, when faced with the challenge of developing a general high throughput analytical capability to support process research investigations, we naturally gravitated toward a multiparallel HPLC approach.

A commercial multiparallel HPLC system has been available for several years, dating from the introduction of the SepiaTec 8 channel shared flow HPLC instrument.[17–19] Recently, two different multiparallel microfluidic HPLC systems have appeared on the market: a 24 channel shared flow microfluidic system from Nanostream[20–22] and an 8 channel individual flow HPLC system from Eksigent.[23]

The qualities desired in a system for high throughput analysis may be different in different research areas. For our work in pharmaceutical process research, we are dealing almost exclusively with small molecules in organic solvents in fairly simple sample matrices. As opposed to laboratories in the drug discovery area, most of our samples for analysis contain the same components, but at different levels. Thus, we may evaluate a large number of samples, typically in 96 well microplates, in order to determine which of a variety of enzymes, catalysts, or separation conditions results in a product with the highest yield, lowest impurity level, or best isomer ratio, etc. The purpose of high throughput screening in the process research environment is to rapidly explore the landscape of reagents and conditions, so as to identify 'hits' that can be investigated in more detail using more conventional

experimental approaches. Thus, a lower level of accuracy and precision may sometimes be allowable, so long as 'hits' can convincingly be rank ordered and distinguished from 'misses'.

Speed to an analytical result is of great importance in high throughput process research, with the time required to analyze a 96 well microplate, the 'plate time', being a key metric. A range in stationary phase and mobile phase choice is another attractive feature, especially the ability to use chiral stationary phases in the normal phase mode. Of course, general robustness and reliability of operation of the instrument are also important, with minimum 'down time' a desirable feature. Finally, simple and easy to use software, as always, is highly desirable in any analytical instrumentation.

We foresaw two divergent needs for this equipment when we initiated acquisition of a multiparallel HPLC capability several years ago. In addition to the routine situation in which all channels run the same chromatographic method, with the same column and mobile phase, we envisioned that running different methods/columns/mobile phases on each channel could speed up the process of method development. Such multiparallel method development approaches require the ability to utilize different eluents, flow rates, or gradient profiles on each channel, and are, therefore, not suited to a shared flow approach in which flow from a single set of pumps is split among a number of different columns. It was primarily this factor that led us choose to develop the individually controlled 8 channel system with Eksigent, rather than pursue existing shared-flow multiparallel HPLC.[17] During the course of the development of the Eksigent Express system, another microfluidic system, the 24 channel shared flow system from Nanostream, became commercially available. While not meeting our needs for method development, we reasoned that a 24 channel shared flow instrument might afford significantly shorter plate times than an 8 channel system, and therefore, acquired a system for evaluation. We herein present a comparative evaluation of the two instruments.

EXPERIMENTAL

Chemicals

Ortho phosphoric acid (85%) was purchased from Fisher Scientific (Fair Lawn, PA, USA). Acetonitrile and HPLC grade water were obtained from EM Science (Gibbstown, NJ, USA). (R,R)-(+)-hydrobenzoin, (S,S)-(−)-hydrobenzoin, meso-hydrobenzoin, naphthalene, anthracene, benzoin, bis-naphthol, bisphenol, and ortho/para-chloro/bromo phenols were purchased from Aldrich (Aldrich Chem. Co, Milwaukee., WI, USA). Trifluoromethyl-9-anthrylcarbinol (9-Ac) and the homologous series of diketones from sample K were available from previous studies.

Preparation of Materials

The mobile phases were 0.1% (v/v) aqueous phosphoric acid and acetonitrile. Samples were prepared in 50% (v/v) acetonitrile in water. For the more non-polar samples, e.g., anthracene, 100% acetonitrile was used as diluent.

HPLC Instrumentation

Two parallel HPLC systems investigated were used in the comparison study, an Express-800 (Eksigent Technologies, LLC, Livermore, CA, USA) and a Veloce μPLC (Nanostream, Inc., Pasadena, CA, USA).

The Veloce μPLC system is equipped with a binary gradient module, 24 UV absorbance detectors, 24 fluorescence detectors, and a 6-head autosampler for the use of 96 well plates. The column used in the system consists of a cartridge (Brio cartridge) that has 24 microfluidic channels packed with stationary phase. The flow is shared between those channels. The dimensions of the 24 columns are each 80 × 0.5 mm. The Brio cartridge was packed with C_{18} stationary phase, 7 μm.

The Eksigent Express 800 system is equipped with eight HPLC channels that can be operated independently. The HPLC columns used with the system were a Zorbax 300SB-C18 150 × 0.300, 3 μm particles (part number 5064-8300, Agilent Technologies, Palo Alto, CA, USA) and an ACE 3 C18 150 × 0.3 mm column (part number ACE-111-15003, MAC-MOD Analytical, Inc., Chadds Ford, PA, USA).

RESULTS AND DISCUSSION

Basic Characteristics of the Eksigent and Nanostream Systems

The basic features the Eksigent Express 800 and the Nanostream Veloce μPLC systems are summarized in Table 1. The Eksigent system devotes two miniaturized pneumatically driven pumps to each of the eight channels, for a total of 16 pumps. Consequently, varying flow rates, gradients, or eluents can be run concurrently on different channels. In contrast, the Nanostream 24 channel instrument is a shared flow system, with two standard HPLC pumps controlling flow to a manifold of 24 channels.

The Eksigent system uses conventional microbore HPLC columns (typically 0.3 mm i.d.) while the Nanostream 24 channel system uses a 24 channel microfabricated cartridge (the Brio™ cartridge) containing microfluidic flow dispersion channels and 24 square profile 'columns', each with 0.5 mm effective i.d. A variety of commercial microbore columns are available for use with the Eksigent system, and column packing with stationary phases that are not available in microbore format is relatively straightforward.

Table 1. Comparison of features of Eksigent Express™ and Nanostream Veloce™ microfluidic HPLC systems

	Eksigent	Nanostream
Channels	8	24
Pumps	Individual channel control	Shared flow
	16 pumps	2 pumps
Columns	Microbore columns	Brio 24 column disposable cartridge
	Typical (o.3 mm i.d.)	0.5 mm i.d. square profile channels
	Many columns available	Few columns available
	Compatiple with NPLC	Not compatible with NPLC
	Small particle/high pressure	Larger particles/lower pressure
Injection	Loop injection, 10–100 nL	Filled 'pit' injection, 500 nL
Dwell volume	Small gradient dwell volume	Large gradient dwell volume
Autosampler	Dual LEAP, 18 microplates	8 or 12 head injector, single microplate
Detection	8 individual detectors	Shared UV detector
	Diode array	Variable wavelength
	No fluorescence	Fluorescence detector available

In contrast, the selection of stationary phases for use with the Nanostream instrument is controlled by the vendor, and at the time of this writing, only a few options are available (C_{18}, C_{18}-AQ, C_8, Phenyl). Self packing the Brio™ cartridges with alternative stationary phases is not feasible, nor is packing of different materials into each of the 24 channels. The Brio™ cartridge is disposable, and is limited to 100 injections by instrument software, whereas the conventional microbore columns used by the Eksigent system can be used as long as desired. Finally, the materials of construction of the Nanostream Brio cartridge place some constraints on mobile phases, pressures, and chromatographic media that can be used. The cartridge is formed from two sheets of etched plastic, bonded together by an adhesive. Consequently, normal phase solvents cannot be utilized, and even in the reversed phase mode, it is suggested that pure acetonitrile be avoided. High backpressure on the Brio™ cartridge can lead to separation of the two plastic sheets, with resulting column failure. Consequently, only moderate backpressures can be used, which limits flow rate relative to conventional HPLC. In addition, this backpressure limitation has led to the selection of relatively large 7 μm i.d. stationary phase materials as the packing material of choice.

The injection size (500 nL–1000 nL) of the Nanostream system is quite large relative to the column size used, corresponding proportionally to the injection of more than 40 μL on a 4.6 mm i.d. column. The ability to adjust the injection size is quite limited, and dictated by the volume of small 'pits' located on the surface of the plastic Brio cartridge. Furthermore, flow must be stopped during the injection cycle, then restarted to carry

out elution. In contrast, the Eksigent system uses a conventional (albeit miniaturized) loop injector, with a proportionally more typical injection size of 20–100 nL, corresponding to 5–25 μL injection on a 4.6 mm i.d. column.

Gradient dwell volume, or dwell time measures the volume (or time) required for a change in mobile phase composition at the pumps to reach the head of the column. It is an important parameter determining the speed with which gradient elutions can be executed. For the Eksigent system, the gradient delay volume is ~0.4 μL, which corresponds to only about 10% of the volume of the 50 × 0.3 mm column typically used with the system. In contrast, the gradient delay volume of the Nanostream instrument is proportionally much larger, although exact measurement is difficult owing to the inability to bypass the column. From a practical perspective, the difference in dwell volumes is readily apparent when one compares the significant difference in time needed to equilibrate the two systems.

The Eksigent Express system comes with a dual head CTC LEAP autosamler, allowing injection from 96 or 384 well microplates, in addition to standard autosampler vials. The system is quite versatile, but in the standard configuration, as many as 18 microplates can be loaded at a given time. The Nanostream instrument can be configured to inject from either a single 96 or 384 well plate, using either a 6 or 8-head multipipettor head. While the baseline system is limited to injection from a single microplate, a plate changer option is available. Injection from autosampler vials is not possible at this time.

The Eksigent system uses individual diode array UV detectors for each channel, while the Nanostream instrument uses a variable wavelength UV detector shared among the 24 channels. A fluorescence detector is available for the Nanostream instrument, but not for the Eksigent system. At the time of this writing, neither instrument is available with multichannel MS detection, although both vendors suggest that this capability may be available at some point in the future.

Peak area reproducibility for the two systems is sufficient for use in reaction screening in process research, although variation in peak area with the Nanostream instrument (2–10% RSD vs. typically <1% for the Eksigent system) may be problematic for more demanding analyses. Retention time variation within a given channel is fine with both systems (~0.3% RSD), although channel to channel reproducibility can be problematic with both instruments. However, good reproducibility is obtained if Nanostream cartridges are properly aligned and if Eksigent columns are well matched. Linearity of UV detector response is good with both instruments, although the shortened detector light path makes the limit of quantification somewhat less than that typically obtained with conventional HPLC systems.

Chromatographic Evaluation of the Eksigent and Nanostream Systems

A fair comparison between two different chromatographic systems with different stationary phases is naturally somewhat problematic. We opted to compare the Eksigent and Nanostream systems using C_{18} stationary phases and acetonitrile/water with phosphoric acid eluents, but the fact that the adsorbents are not identical means that care must be taken in drawing conclusions from any single experiment. We, therefore, assembled a variety of different sample mixtures (Figure 1) for evaluation. Sample mixtures A–E are binary mixtures of substituted phenols, sample F represents a potential reaction mixture, i.e., hydrolysis of aspirin to afford salicylic acid, and samples G and K contain previously studied homologous series. Sample H is a commonly used test mixture for assessment of performance of chiral stationary phases under normal phase conditions. In the reversed phase mode, the tri-*tert*-butyl benzene component is very strongly retained, and is, thus, a useful probe. Sample L contains several polycyclic aromatic hydrocarbons, all of which have distinctive UV/Vis spectra and are useful probes for characterizing the capabilities of the UV detectors of the two instruments.

The comparative evaluation of the two instruments was conducted as a competition, with the objective of creating the shortest possible method to afford baseline resolution of the sample components. Changing any instrument or chromatographic parameter was permitted, so long as a C_{18} column with an acetonitrile/aqueous phosphoric acid mobile phase was used. In an effort to guard against bias, operations specialists from both vendors were allowed to see optimized chromatograms and attempt further changes to reduce analysis time. For the Nanostream system a flow rate of 300 μL/min was chosen and an injection volume of 0.5 μL. A Brio Cartridge column with 24 microfluidic channels was used (C_{18}, dimension of the channels 8 × 0.5 cm, particle size 7 um, 1 μL injection pits). For the Eksigent system, flow rates of 15 to 30 μL/min were used, with an injection volume of 20–50 nL. Two different C_{18} columns were evaluated, an ACE-C18 (0.3 × 150 mm) and a Zorbax SB-C18 column (0.3 × 50 mm). The packing materials in these columns are fairly standard and commonly used in the pharmaceutical industry. The results of the evaluation are illustrated in Table 2.

The results of this study show dramatically faster run times with the Eksigent system for each sample mixture studied, including the Nanostream test mixture (sample I) where a >10× faster resolution was obtained with the Eksigent system (0.35 min vs. 4 min.). A 5–15 fold advantage in analysis time is typical for the Eksigent system, as illustrated by two examples illustrated below. Figure 2 shows optimized separation for the separation of *para*- and *ortho*-chlorophenol isomers (sample D), where a >14× faster resolution was obtained with the Eksigent system. In general, relatively poor chromatographic peak shape is observed with the Nanostream system. Indeed, the two chlorophenol isomers are not entirely baseline resolved even at 12 min.

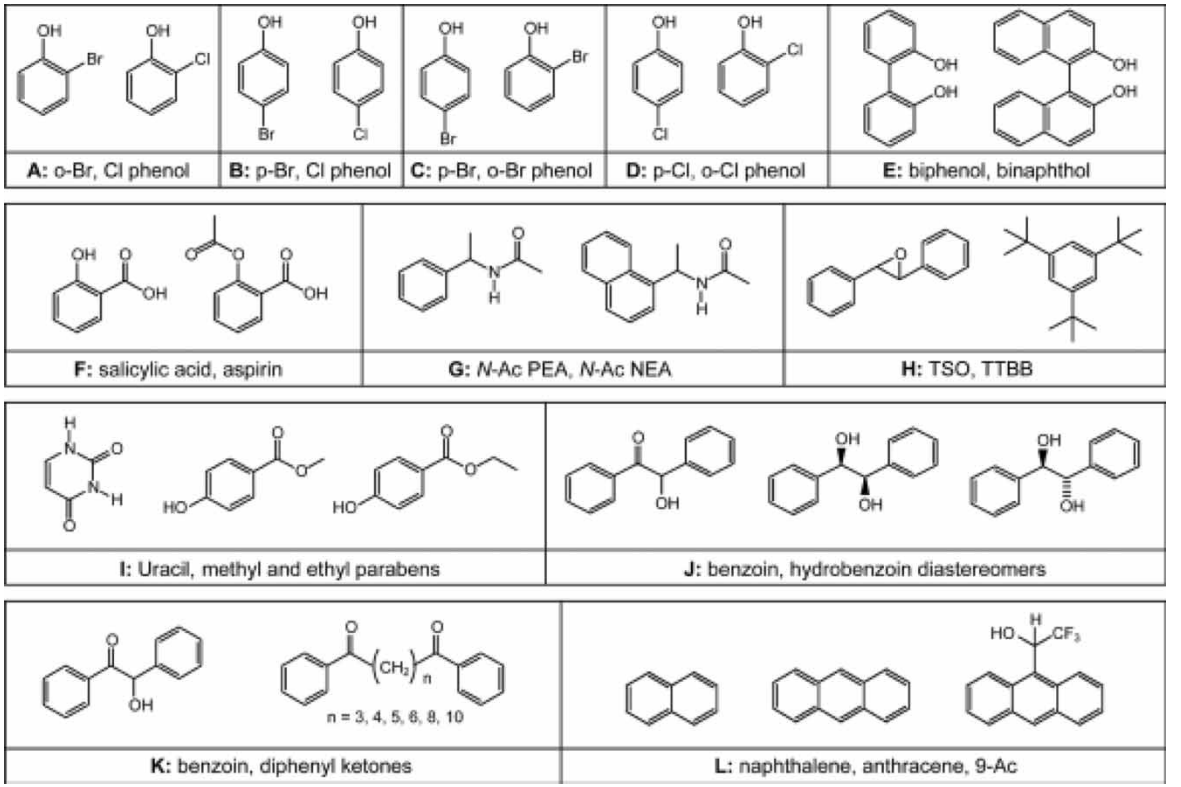

Figure 1. Test mixtures utilized in the comparison of the Eksigent Express and Nanostream Veloce microfluidic HPLC systems.

Table 2. Minimum analysis time to baseline resolution. Experimental conditions: Gradient elution with A = 0.1%H_3PO_4 and B = Acetonitrile as mobile phases. Method conditions nanostream: Brio Cartridge Vydac C18, 7 μm particle size, 0.5 × 8 mm column length, 0.5 μL injection, 214 nm. Method conditions Eksigent: Column Ace (Ace3-C18, 3 μm, 0.3 × 50 mm) or Zorbax (Zorbax SB-C18, 3.5 μm, 0.3 × 50 mm), 20–50 nL injection; UV at 210 nm

	Nanostream Veloce	Conditions		Eksigent Express	Conditions	
Sample	Separation time (min)	Gradient	Flow rate (μL/min)	Separation time (min)	Gradient	Flow rate (μL/min)
A	13	40 to 60%B in 10 min	360	0.6[a]	30–50%B in 0.5 min with hold	15
B	10	30% B	360	0.6[a]	30–50%B in 0.5 min with hold	15
C	13	40 to 60% B in 10 min	300	0.6[a]	20–90% B in 1.8 min with hold at 100% to 2 min	15
D	13	40 to 60% B in 10 min	300	0.9[b]	10 to 30% B in 0.05 min then to 40% in 1 min	20
E	4.5	30 to 90% B in 2.5 min	300	0.3[b]	61 to 67% in 0.3 min	20
F	3.0	40% B	300	0.4[a]	Ace 25–70% B in 0.25 min with hold	15
G	5.5	10 to 100% B in 5 min	300	0.3[b]	50–70% B in 0.3 min	20

(*continued*)

Table 2. Continued

Sample	Nanostream Veloce Separation time (min)	Conditions		Eksigent Express Separation time (min)	Conditions	
		Gradient	Flow rate (μL/min)		Gradient	Flow rate (μL/min)
H	No separation	10 to 100% B in 5 min	300	0.2[b]	99% B	30
I	4	30 to 90% B in 2.5 min	300	0.35[b]	40 to 90% B in 0.25 min with hold to 0.25 min	20
J	16	40 to50% in 8 min, to 95%in 2 min, hold 3 min	300	0.6[b]	30 to 35% B in 0.2 min, to 95% B in 0.1 min, hold 0.3 min	20
K	8.5	50 to 100% B in 7 min	300	1.5[a]	90–100% B in 0.25 min with hold at 100% to 1 min	15
L	>23, Co-elution of 9AC and Naphthalene	60 to 90% B in 15 min, hold 12 min	300	1.0[a]	100% B	15

[a]Using ACE-C18 150 × 0.3 mm column.
[b]Using Zorbax 50 × 0.3 mm column.

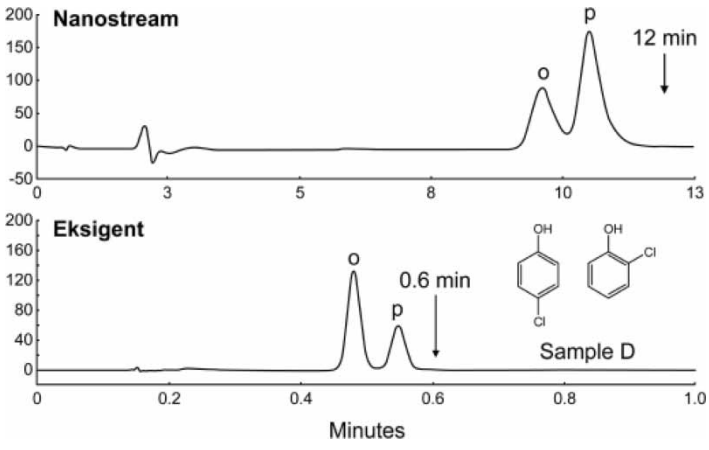

Figure 2. Representative chromatograms showing optimized methods for rapid resolution of test mixtures D using Eksigent and Nanostream microfluidic HPLC systems. Nanostream conditions: Brio C18, 0.5 × 80 mm, 300 uL/min, 500 nL injection, UV@214 nm, gradient elution 40–60% acetonitrile in 0.1% H_3PO_4 in 10 min. Eksigent conditions: Zorbax SB C18, 0.3 × 50 mm, 3 μm particles, 20 uL/min, 30 nL injection, detection UV@254 nm, Gradient elution 10–30% acetonitrile in 0.1% H_3PO_4 over 0.5 min, then 40% in 1 min.

The phenomenon of poor peak shape with the Nanostream instrument seems to be general. Figure 3 illustrates the resolution of a multicomponent mixture of aryl ketones (sample K) with the two systems. Again, the Eksigent system provides much faster resolution, approximately five-fold faster in this example. As in the previous case, the peaks on the optimized Nanostream chromatogram are not quite baseline resolved.

The observation that the Eksigent system offers faster resolution on a per channel basis than the Nanostream system is not entirely unexpected, although the magnitude of the difference between the systems was more dramatic than anticipated. The Eksigent system can be used with highly efficient columns containing small particle stationary phases. In addition, the system has been engineered with a view to keep extracolumn volume to an absolute minimum. As a result, the Eksigent system has a gradient dwell volume that is considerably smaller than a conventional HPLC system. This low gradient dwell volume affords considerable advantage in executing fast gradients, and faster chromatography relative to conventional HPLC systems is often observed. In contrast, the Nanostream system has a large gradient dwell time, presumably owing to the shared flow configuration and proportionally larger volume devoted to mixing. In addition, the Nanostream columns are much less efficient, presumably owing to the large diameter particles used. Finally, the lower pressure limitation of the Nanostream system means that proportionally lower flow rates

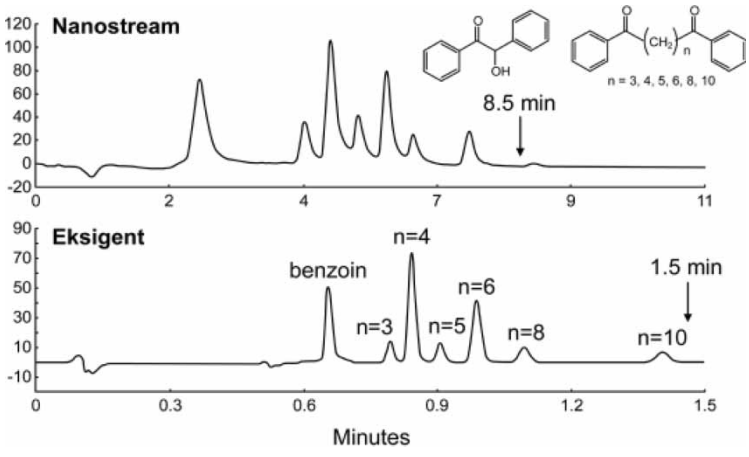

Figure 3. Representative chromatograms showing optimized methods for rapid resolution of test mixtures K using Eksigent and Nanostream microfluidic HPLC systems. Nanostream conditions: Brio C18, 0.5 × 80 mm, 300 uL/min, 500 nL injection, UV@214 nm, gradient elution 40–60% acetonitrile in 0.1% H_3PO_4 in 10 min. Eksigent conditions: Ace3 C18, 0.3 × 150 mm, 3 μm particles, 15 uL/min, 30 nL injection, detection UV@254 nm, Gradient elution 90–100% acetonitrile in 0.1% H_3PO_4 over 0.25 min, then 100% in 1 min.

must be used, naturally leading to longer chromatographic runs. The ability to operate the Eksigent system at higher flow rates and back pressures offers a decided advantage, especially coupled with use of more efficient columns.

Plate Time vs. Run Times

It is important to consider that when analyzing microplates, the critical parameter is not the individual chromatographic run time, but the plate time, i.e., how long it takes to run a single assay on all the wells of a 96 well microplate. Autosampler delay and column equilibration times can contribute substantially to plate time, and must be taken into consideration. The situation is illustrated schematically in Figure 4. If autosampler delay, equilibration time, and run time are assigned the terms x, y, and z, respectively, then the plate time for a single channel instrument can be given by the expression 96 $(x + y + z)$. Plate time for a multichannel instrument is given by the expression $(96/M) \times (x + y + z)$, where M is the multiplex number (the number of channels). For many instruments, column reequilibration is taking place during the course of autosampler delay, leading to an effective decrease in the value of y, the equilibration term. Owing to the unusual injection style of the Nanostream instrument,

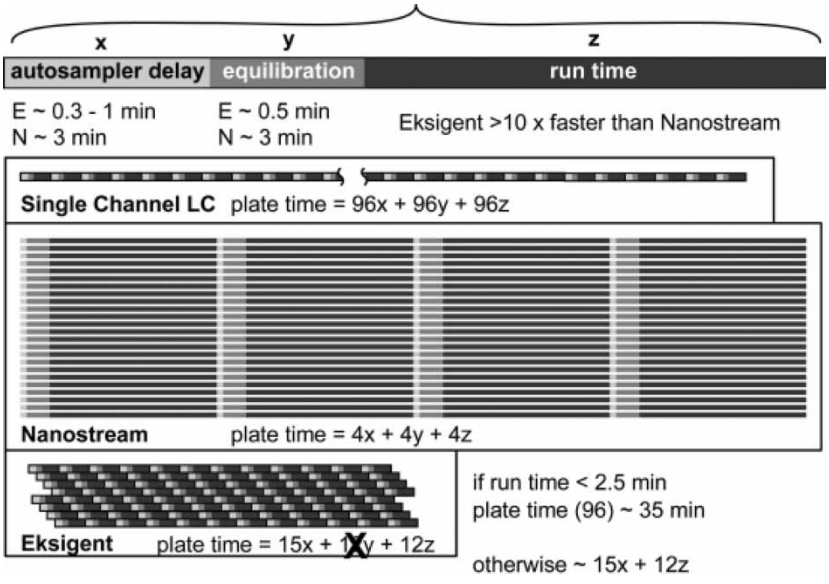

Figure 4. Comparison of times to run a 96 well plate on the Eksigent and Nanostream systems.

column equilibration and autosampler delay cannot take place simultaneously. Consequently, the expression $4x + 4y + 4z$ is an accurate predictor of actual plate time with the Nanostream system. With typical values for x and y (autosampler delay and equilibration time) being 3 minutes each, it can readily be appreciated that 24 min of delay are involved in the analysis of each plate, even before any consideration is made of the actual chromatographic run times!.

The situation is somewhat more complex for the Eksigent system, since samples are not injected simultaneously into all channels, as with the Nanostream system, but are sequentially injected as autosampler delay permits, using two injector heads. The result is a staggered time profile, as illustrated in Figure 4, and as described by the expression $15x + 12y + 12z$. Typical autosampler delay (x) for the Eksigent system is 0.3–1 min, and typical equilibration time (y) is 0.5 min. Owing to the fact that column equilibration can be carried out during autosampler delay, the contribution of the y term typically drops out, giving the expression $15x + 12z =$ plate time. This formula can accurately predict plate times >35 min, which is the limit of the system imposed by the speed with which the two autosampler heads can operate. Thus, methods with run times shorter than 2 min do not result in an improved overall plate time, i.e., it makes no difference whether the run time is 2 min, 1 min, or 0.5 min; plate time in each case will be 35 min given the current instrument configuration.

Other Features of the Two Systems

Several other features were noted during the evaluation of the two instruments that are worth consideration. Method development was carried out directly with each instrument. In this respect, the Eksigent system shows a clear advantage because the runtimes are typically very short, allowing the quick optimization of gradient elution conditions. In contrast, method development with the Nanostream system is more problematic. The general slowness of column equilibration, autosampler delay, and chromatographic elution, make manual method development somewhat tedious. In addition, samples must be injected from microplates (not vials), and each cartridge is limited (by instrument software) to only 100 injections, a good number of which are needed for method development.

Software was generally easy to use and reliable with both instruments. Additional modules for carrying out high throughput tasks such as Log P calculations are available from Nanostream. The Nanostream operation software contains a feature that help to adjust variations in run time and peak area between channels, the run time variation being a consequence of the shared flow arrangement, and the peak area variation being a consequence of the pit injection feature. Both systems were acceptably robust, and operated largely as expected. The dual LEAP autosampler of the Eksigent system needed periodic tuning and alignment, and some training and practice was needed to become familiar with making microcapillary connections to the columns.

We undertook a preliminary evaluation into the ability of the Eksigent system to carry out normal phase separations using chiral stationary phases. While the bulk of this study will be reported elsewhere, it is important to consider that the Eksigent system affords very respectable chiral HPLC results, as illustrated in Figures 5 and 6.

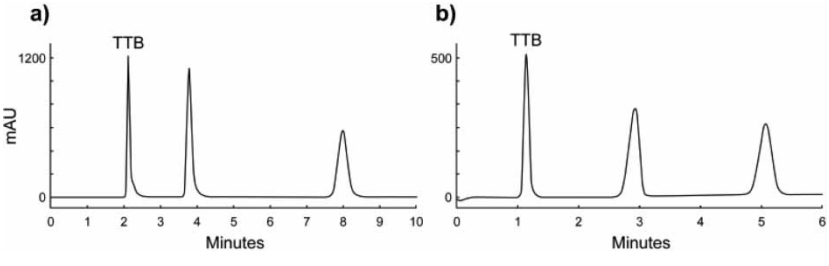

Figure 5. Chiral separtion of a TSO/TTBB test mixture using the Eksigent system. Chiralpak AD-H, 0.3 mm × 15 cm. The column was packed in house using a slurry technique. a) Isocratic Elution. 10/90 IPA/Heptane, 4 uL/min. b) Gradient Elution. 10–90% IPA/heptane over 15 min · 4 uL/min.

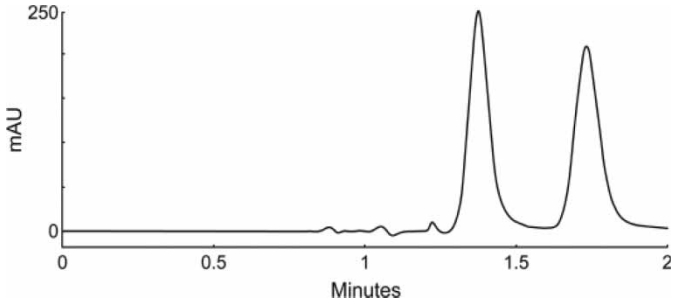

Figure 6. Chiral separtion of Tröger's base using the Eksigent 800 system. Chiralpak AD-H, 0.3 mm × 15 cm, 10/90 IPA/Heptane Isocratic, 10 μL/min.

CONCLUSION

Based on our comparison study, we found that the Eksigent system is the preferred instrument for use in high throughput analysis in support of pharmaceutical process research. The advantages of the Eksigent system include the ability to execute very fast gradients and very fast separations, the ability to use columns containing many different stationary phases, including highly efficient phases or even normal phase chiral stationary phases, and the ability to afford plate times of about 35 min for most separation mixtures studied. The principal disadvantage of the Nanostream Veloce system were the poor peak shapes that were obtained, leading to excessively long chromatograms in order to obtain baseline resolution. Coupled with the somewhat variable sample injection method, and the substantial autosampler and equilibration delays, the system seems poorly suited to the separation of multicomponent mixtures. The Nanostream instrument may be better suited to single component analysis situations, e.g. solubility studies or log P determinations.

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