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ORIGINAL

Using Superficially Porous Particles and Ultrahigh Pressure Liquid Chromatography in Pharmacopeial Monograph Modernization of Common Analgesics

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Abstract

The higher pressures and flow rates needed to increase throughput in ultrahigh pressure liquid chromatography (UHPLC) can lead to thermal broadening due to viscous friction. The use of superficially porous particles and still air thermal environments can help reduce this broadening, but this has not yet been studied for applied, isocratic methods. Here, the use of five columns with varying lengths, diameters, and particle sizes were investigated for increased analytical throughput of pharmacopeial monograph methods for two over-the-counter analgesic drugs. System suitability parameters (resolution and peak asymmetry) and temperature changes across the axial length of the column were monitored at conditions near column or system pressure limits. Results from these investigations indicated that a 2.1 × 50-mm column packed with 2.6 µm particles provides the best opportunity for increased throughput, which was demonstrated with a 20-s cycle time method for the separation of four compounds (two active pharmaceutical ingredients, one impurity, and one internal standard) while maintaining a baseline resolution of 1.5 between all peaks.

Keywords Ultrahigh pressure liquid chromatography (UHPLC) · Viscous friction · Superficially porous particles · Monograph · Thermal environment

Introduction

Since 2005, the United States Pharmacopeia (USP) has identified over 2600 monographs that need revision to meet the modern demands of the pharmaceutical industry [1]. In a push for updates to these monographs, the USP has partnered with industry, academic, and government labs to replace older methods that rely on outdated technology or techniques in a "modernization" process [2]. One key area in monograph modernization is the analysis of over-the-counter (OTC) drugs [3]. Much of the motivation for these updates has focused on replacing older qualitative tests with instrument-based quantitative analyses, including utilizing more chromatographic methods. Additionally, changes to monographs that already require LC can be made by following the guidelines listed in Chapter 621 of the USP-NF, which details permitted modifications to mobile phase composition, column length, column diameter, and particle size [4, 5]. Thus, methods can be updated to utilize state-of-the-art LC columns and instrumentation, but there are still limits to how far the throughput and performance can be increased within these guidelines.

Innovations in ultrahigh pressure liquid chromatography (UHPLC) over the past 10–15 years [6, 7] have led to higher speed and efficiency in the analysis of pharmaceutical compounds, achieved through the use of higher pressure instrumentation and columns packed with smaller particles [8–14]. Additionally, this same period has seen increased use of superficially porous particles (SPPs) to improve the

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performance of pharmaceutical analyses by LC [15–19]. These advances have improved the quality of high-throughput, or ultrafast, LC techniques [20–26] where columns are shortened and flow rates are increased to reduce separation times below 1 min. Although such methods have recently been demonstrated for chiral separations [27–30], ultrafast techniques have not yet been widely applied to the modernization of OTC monographs using reversed-phase LC.

When increasing the flow rate of a chromatographic method to reduce the analysis time, heating due to viscous friction can diminish column performance [31–34]. Because increasing the flow rate (F, in $m^3\ s^{-1}$) also leads to higher backpressures (ΔP, in Pa), the rate of heat generation (or, power) can increase further as this power is calculated by [35]:

$$\text{Power} = F\Delta P. \quad (1)$$

As the pressure limits of commercial UHPLC instruments have continued to rise [36, 37], understanding the impact of viscous friction on separation performance has become an important step in chromatographic method development [38–48]. In conditions where heat is generated due to viscous friction, both axial and radial thermal gradients form within the column [38, 49, 50]. The axial thermal gradient forms along the length of the column and reduces analyte retention as its magnitude increases. The radial thermal gradient exists between the center and the wall of the column and is more detrimental in most chromatographic methods because it reduces the overall separation efficiency. Although both of these gradients are present at high flow rates and pressures, the relative magnitudes can be changed based on the thermal environment of the column [51–55]. In more adiabatic conditions (insulated column or still air oven), the axial gradient is maximized which increases the temperature of the mobile phase along the column but minimizes efficiency losses [52, 56, 57]. In more isothermal conditions (water bath or forced air oven), the radial gradient is maximized decreasing the magnitude of the temperature increase along the column at the expense of decreased plate counts [38, 40, 53, 54, 58]. One way that the negative effects of the radial thermal gradient can be reduced is by increasing the thermal conductivity of the packed bed. With SPPs, the nonporous core increases the total amount of silica in the bed (relative to FPPs), raising the overall thermal conductivity of the column and decreasing thermally induced band broadening [54, 59–61]. Thus, not only does the particle structure of SPPs enhance performance over FPPs due to mass transfer and diffusional band broadening [62], but additional efficiency gains are made as thermal gradient magnitudes are reduced due to higher thermal conductivity.

In this study, we tested a series of SPP columns to determine which column dimensions and particle sizes provided the greatest increase in throughput for USP monograph methods for common OTC analgesics. As these methods required higher flow rates and pressures that lead to viscous friction effects, the potential impact of thermal broadening on the chromatographic performance of these methods was also investigated. Few studies have focused on the impact of frictional heating with non-ideal samples [43, 55]. Those reports utilized gradient conditions that can reduce thermal-induced effects on retention and efficiency due to gradient focusing [55]. As these experiments focus on isocratic monograph methods, the impact of the column thermal environment was examined as its effects should be more prominent without gradient elution. Finally, the potential for developing ultrafast techniques for OTC analgesic analysis using UHPLC and SPPs is explored.

Materials and Methods

Sample Preparation

As the sample and mobile phase preparation guidelines are based directly on the procedures outlined by the USP monographs for naproxen [63], ibuprofen [64], and acetaminophen and aspirin [65, 66], a full description of the critical parameters is detailed in Table 1 and a full description can be found in the Supporting Information.

Chromatographic Methods and Instrumentation

All chromatographic methods were run on a Vanquish Horizon UHPLC system (Thermo Fisher Scientific, Germering, Germany) equipped with a diode array detector (DAD). Five columns packed with superficially porous Accucore C18 particles provided by Thermo Fisher Scientific (Bellefonte, PA) were compared in this study: 2.1 × 50 mm and 2.1 × 100 mm columns containing 1.5 µm diameter particles and 2.1 × 50 mm, 2.1 × 100 mm, and 4.6 × 50 mm columns containing 2.6 µm diameter particles. Each column was connected to the injector with 0.1 × 380 mm Viper tubing and then from the column outlet to the detector with 0.1 × 445 mm tubing (these were the mobile phase preheater and post-column cooler, 3.0 and 3.5 µL, respectively, each used without temperature control). Further information on this instrument can be found in [37]. An inline filter was also used at the inlet of each column. The injection volume was set to 0.1 µL to reduce broadening effects due to large injection volume (1.0 µL for the 4.6 mm i.d. column to ensure sufficient signal for the impurity trace peak in Method 2). For each column and monograph method, the highest flow rate possible while maintaining a minimum resolution between peaks and limiting the asymmetry below a listed maximum USP tailing factor was used (see Tables 1, 2 for details). All methods used in this study were isocratic, with column

Table 1 Description of chromatographic methods listed in USP monographs

Method designation	Analytes of interest	Other compounds	Minimum resolution	Maximum tailing factor	Mobile phase
Method 1[a]	Naproxen	Ethylparaben	3.0	2.0	50:50 water:methanol with 30 mM sodium acetate (pH 5.8 ± 0.2)
Method 2[b]	Ibuprofen	Valerophenone, ibuprofen-related compound C	2.5	2.5	40:60 water:acetonitrile with 0.4% chloroacetic acid (pH 3.0 ± 0.2)
Method 3[c]	Acetaminophen, aspirin	Benzoic acid, salicylic acid	N/A	N/A	75:12.5:12.5 water:methanol:acetonitrile with 0.1% glacial acetic acid and 1.24 mM tetramethylammonium hydroxide pentahydrate
Method 4[d]	Acetaminophen, aspirin, caffeine	Benzoic acid, salicylic acid	1.4	1.2	28:3:69 methanol:glacial acetic acid:water
Method 5[e]	Acetaminophen, aspirin	Benzoic acid, salicylic acid	N/A	N/A	90% 28:3:69 methanol:glacial acetic acid:water and 10% acetonitrile

[a]Reference [63], [b]Reference [64], [c]Reference [65], [d]Reference [66], [e]Combined method for high-throughput analysis based on Methods 3 and 4

Table 2 Columns used in the current study

Column designation	Diameter (mm)	Length (mm)	Particle size (μm)	Method 1 flow rate (mL min^{-1})	Method 1 still air pressure (bar)	Method 2 flow rate (mL min^{-1})	Method 2 still air pressure (bar)
Column A	2.1	50	2.6	1.10	670	1.10	320
Column B	2.1	100	2.6	0.70	730	1.50	820
Column C	4.6	50	2.6	2.80	650	5.00	740
Column D	2.1	50	1.5	0.40	750	0.80	740
Column E	2.1	100	1.5	0.33	1160	0.75	1420

equilibration conducted at a low flow rate and pressure (*see* "Temperature measurements" section) and then increased to the method flow rate as the first injection began. The flow rate ramp for all methods was set to 6.0 mL min^{-2}. For each method, fifteen consecutive injections from a prepared sample were made, with relevant figures of merit averaged over the final ten runs after thermal equilibrium had been reached (see Supporting Information). The column oven compartment was maintained at 303 K (318 K for Method 5 based on [66]), with Methods 1 and 2 tested in both the still air (no convective fan) and forced air oven modes (the forced air oven mode with the fan turned on was conducted with the maximum instrument fan speed setting of 7). Data acquisition and analysis of USP tailing factor ($A_{s,USP}$), USP resolution ($R_{s,USP}$), and USP plate count (N_{USP}) were completed using the included Chromeleon 7.2 software based on the following equations listed in Chap. 621 of the USP-NF [4]:

$$A_{s,USP} = \frac{w_{5\%}}{2f_{5\%}}, \tag{2}$$

$$R_{s,USP} = \frac{2\left(t_{r,2} - t_{r,1}\right)}{\left(w_{b,1} + w_{b,2}\right)}, \tag{3}$$

$$N_{USP} = 16\left(\frac{t_r}{w_b}\right)^2, \tag{4}$$

where w is the peak width (at either the peak base b or 5% of the peak height), $f_{5\%}$ is the distance between the leading edge of the peak and the peak maximum at 5% of the peak height, and t_r is the peak retention time. UV-DAD data acquisition (0.8 μL flow cell) was conducted at the wavelength described by the USP monograph, with an acquisition rate of 50 Hz to ensure that at least 40 data points were collected per peak. All calculations were performed in Microsoft Excel (Redmond, WA) and all data were plotted using Excel and Igor Pro 6.0 (Wavemetrics Inc., Lake Oswego, OR).

Temperature Measurements

Previously reported methods for the measurement of mobile phase temperature at the column outlet were implemented here [52, 54, 55] utilizing a Type-T (Copper–Constantan) HYP-0 hypodermic (0.008″ diameter) thermocouple probe from Omega Engineering (Stamford, CT). The probe was inserted into a 1.5-cm piece of 0.015″ inner diameter PEEK tubing (Idex Health and Science, Oak Harbor, WA) and connected to a OM-EL-USB-TC Thermocouple Data Logger interfaced to Easy-Log USB software from Omega for temperature data acquisition at a rate of 1 Hz. For each chromatographic method and column, baseline readings were made at a low flow rate and pressure (under a maximum viscous friction level of 1 mW) for 30 min, followed by 30 min at the higher flow rate of each method described above, and then a final 30 min at the initial baseline level. Reported temperature values are described as a change in temperature from the initial level where negligible column heating occurred (303 K, the set oven temperature) to the final temperature at the method flow rate and pressure. Thermal friction levels were calculated using Eq. 1 and then normalized by the column mobile phase volume, which was determined by averaging the elution times of an unretained marker (uracil) at four different flow rates (including the analysis flow rate) and correcting for extra-column volume by measuring its elution time with a zero-dead volume Viper union (Thermo Scientific, Germering, Germany) in place of the column.

Results and Discussion

Increasing Method Speed

For common pharmaceutical drugs that are produced in very high volumes, such as OTC analgesics, rapid chromatographic methods are useful to increase the throughput of required assay and impurity analyses. However, many current USP monographs for these drugs are longer methods that rely on older LC column technology and are in need of modernization. As an initial investigation into the use of SPPs and ultrahigh pressures to increase throughput for USP methods, the naproxen oral solution monograph [63] was studied. This method consists of the separation of naproxen and an ethylparaben internal standard, with a required minimum resolution between the peaks of 3.0 and a maximum peak tailing factor of 2.0. As described above, the goal for these studies was to determine the shortest time that could be achieved when considering these parameter restrictions, column and instrument pressure limits, and potential repeatability issues that can arise due to thermal gradients caused by viscous friction. On each separate column, the sample was injected 15 consecutive times, with various instrument parameters tracked as both the flow rate and temperature increased in the column. Because of the initial instrument flow rate ramp required when first going to the higher flow rate levels used in these measurements, as well as the time needed for thermal gradients to form, results from the first five injections demonstrated lower efficiency and were not included in calculations. A comparison of the relevant method parameters (plate counts, tailing factors, peak resolution, magnitude of the axial thermal gradient, pressure, and retention time for the last eluting peak) calculated over the final ten injections are shown in Fig. 1 for Columns A and E (plots for Columns B, C, and D, and full experimental values can be found in the Supporting Information).

The radar plots in Fig. 1 (and the Supporting Information) were designed to compare various method and system suitability parameters, with the reciprocal of some positive attributes (plate count and resolution) depicted so that a smaller enclosed area demonstrates a faster, more efficient separation with lower pressure and frictional heating. By examining Fig. 1, the column properties that might be most useful in reducing method time (based on the retention time for the last eluting peak) while still maintaining required monograph performance levels can be determined. As expected, the 2.1×100 mm column packed with 1.5 µm particles provides the highest chromatographic efficiency of the columns tested. However, the higher efficiency comes at the cost of a much higher required pressure (1160 bar) and longer method time (3.6 min) than the other four columns. The higher pressure also results in the magnitude of the axial thermal gradient being noticeably larger (10.5 K) for this column. Using the same particle type and reducing the column length in half (see Supporting Information) decreases the required pressure (760 bar) and method time (1.3 min), at the expense of higher peak asymmetry (USP tailing factor of 1.2–1.3) due to the extra-column dispersion having a larger impact on peak shape with this lower volume column. With a larger particle diameter the column efficiency is lower, which limited the maximum flow rate that could be used to increase speed since the resolution between peaks suffered from kinetic broadening effects. Still, the 50 mm long columns with 2.6 µm particles had the shortest methods (0.45 min) even when decreasing the flow rate to ensure that the minimum resolution between naproxen and ethylparaben was achieved for both oven modes. In general, the asymmetry factor was lower with the larger particles as the 2.1×100 mm column with 2.6 µm particles had the most symmetric peaks for these analytes. The larger asymmetry factors with the 1.5 µm particles are most likely a result of the greater impact of extra-column volumes on more efficient peaks, but could also arise from packing differences, surface silanol differences between the two particle sizes, or thermal mismatch between the mobile phase at the inlet and the column temperature (higher in these columns with

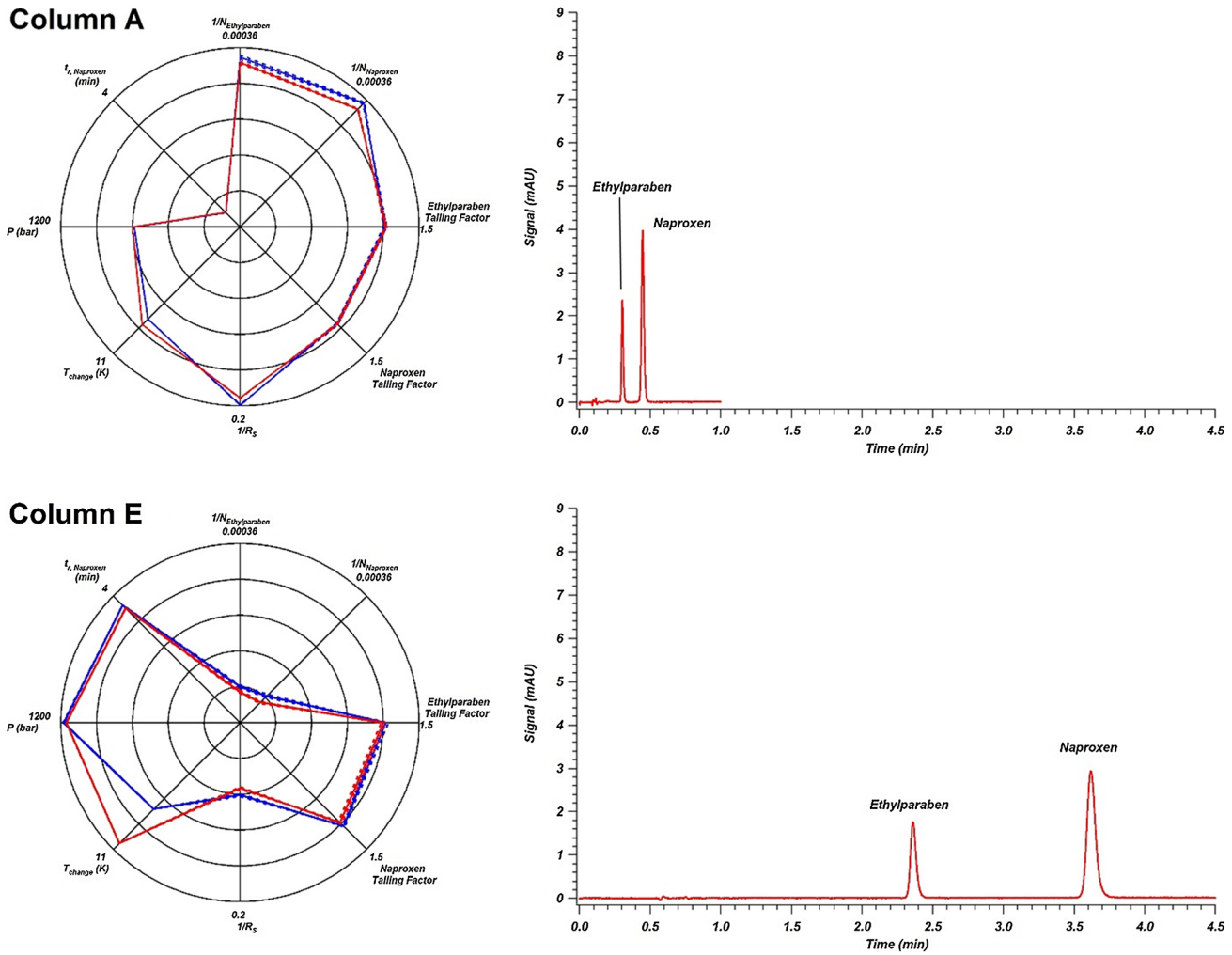

Fig. 1 Radar plots and representative chromatograms describing relevant performance parameters for the Method 1 USP monograph with Columns A and E listed in Table 2 (figure panel designations match column designations). Clockwise from top (maximum value for each radial axis listed in parentheses): inverse plate count for ethylparaben peak (0.00036), inverse plate count for naproxen peak (0.00036), USP tailing factor for ethylparaben peak (1.5), USP tailing factor for naproxen peak (1.5), inverse resolution between ethylparaben and naproxen (0.2), magnitude of the axial thermal gradient across the column (11 K), system pressure (1200 bar), and the retention time of the last eluting peak (naproxen, 4 min). Values are based on an average of ten final injections from a set of fifteen, with dotted lines indicating a range of ±1 standard deviation. Red traces indicate still air oven mode and blue traces indicate forced air oven mode. Chromatogram mobile phase, column, and instrument conditions can be found in Tables 1 and 2. Radar plots and representative chromatograms for Columns B, C, and D, as well as full data for all five columns, can be found in the Supporting Information

a larger magnitude for the axial thermal gradient) [67, 68]. As other monographs have different resolution and asymmetry requirements, the selection of a column in monograph modernization should include consideration as to which of these requirements is more stringent and will be the limiting factor in increasing throughput.

A comparison of the magnitude of the axial thermal gradient across different columns and thermal environments provides another set of interesting observations that can potentially impact method performance at higher mobile phase velocities. As noted above, the 2.1 × 100 mm column packed with 1.5 µm SPPs had the highest measured increase in outlet mobile phase temperature (10.5 K) in the still air oven mode. Switching to a forced air oven decreased this amount by nearly 30%, with a comparable drop only seen in the other 100 mm long column. The higher magnitude of the axial thermal gradient is a combination of the higher viscous friction effects due to higher pressure from the longer column and the increased column length ensuring that a sufficient thermal entrance length is present for the gradient to develop [42]. The greater length also means that the columns have a larger surface area in which temperature exchange with the surrounding environment can occur [50], causing the larger difference between still and forced air oven modes

that is observed in these columns. The forced air oven mode does increase the magnitude of the radial thermal gradient, which lowers the efficiency (and thus, resolution) for each method compared to the still air mode (resolutions of 12.4 and 13.7, respectively), suggesting the use of still air mode when resolution is the critical parameter limiting higher throughput in a method. Further discussion of viscous friction effects in high-throughput isocratic monograph methods can be found in the next section.

Combining Multiple Analyses into Single Chromatographic Separations

In addition to decreasing the time for a single method, another aspect that can be explored in monograph modernization to increase method throughput is the combination of multiple analysis steps into single runs. The monograph describing the analysis of ibuprofen requires two steps: the separation of ibuprofen and a valerophenone internal standard (as a content assay) and the separation of the same internal standard with ibuprofen-related compound C (for impurity analysis). However, modern column technology and UHPLC instrumentation makes the integration of these two separations into a single method a simple task. Again, the goal was to increase method speed while still maintaining the required resolution between each pair of peaks and ensure that the asymmetry was below a maximum threshold. Similar to the naproxen method, comparison of the figures of merit for the five columns tested are shown in Fig. 2 and the Supporting Information.

Compared to the naproxen method, the biggest limitation in increasing the speed of the combined ibuprofen method was maintaining the minimum resolution between the analytes. This was made more difficult by the increased impact of extra-column broadening since these analytes are retained less than naproxen and ethylparaben. Here, both 50 mm columns packed with 2.6 µm particles were the fastest methods (retention time for ibuprofen related compound C of 0.33 min), but further reductions in time were limited by the lower efficiency (achieving a resolution of at least 2.6 to exceed the minimum limit of 2.5) compared to the other columns rather than any column or instrument pressure limits. With these two columns, the 2.1 mm diameter column had similar resolution between the critical pair (valerophenone- and ibuprofen-related compound C) in both oven modes, but the 4.6 mm diameter column had more measurable effects from viscous friction and demonstrated 10% higher resolution for this pair in the still air oven mode. Doubling the column length required higher flow rates and pressures approaching the column limits (increasing the viscous friction), but the retention time of the last eluted analyte was only slightly higher (0.5 min) because the higher column efficiency ensured that the minimum resolution was still achieved. As this method utilized acetonitrile as the organic component rather than methanol (as in the naproxen method), the lower viscosity of the mobile phase meant that when column or instrument pressure limits were reached, a higher flow rate was achieved and higher temperatures were measured at the outlet. Some preliminary results were collected (see Supporting Information) that indicate that increasing the mobile phase strength within USP guidelines can improve throughput by reducing analyte retention and raising the flow rate, but this may not work with every method since composition changes can reduce resolution based on varying retention changes and an overall loss in selectivity due to reduced retention at higher temperatures.

As with the naproxen method, the 2.1×100 mm column packed with 1.5 µm particles had the largest temperature increase across the column, with a measured magnitude of 18 K for this method. In a perfectly adiabatic system, the magnitude of this axial thermal gradient is dependent on the physical properties of the mobile phase and the pressure drop across the column (see Supporting Information) [41, 69]. In the still air oven mode, the magnitude of the thermal axial gradient was approximately half of that calculated for an adiabatic thermal environment for all five columns. This indicates that some of the heat is being lost from the column due to natural convection from the column walls and endfittings [41], with the forced air mode increasing the convection levels due to the fan and further reducing the measured temperature at the column outlet. Because neither oven mode is completely adiabatic, radial thermal gradients form in both column compartment environments, although they are magnified in the forced air mode as the measured temperature increases are diminished and the observed efficiencies are lower (see Supporting Information). In this study, the focus was on maximizing the throughput in each column until USP performance thresholds, column pressure limits, or instrument flow rate limits were exceeded. This led to various pressures, flow rates, and mobile phase linear velocities for each column that may prevent a more direct comparison between each column in terms of the formation of each of the thermal gradients, especially as different column dimensions play a significant role in the magnitude of radial thermal gradients and the thermal entrance length needed for both gradients to develop [42, 50]. Future studies will focus on differences in these effects between various column dimensions with more consistent operating conditions than permitted with the USP guidelines discussed here.

Although small variations occur between the naproxen and combined ibuprofen methods because of differences in the performance requirements and the conditions that were selected to achieve them, sub-2 µm SPPs and longer columns provided higher chromatographic efficiency at the expense of higher temperature changes across the column and longer method times in both monographs. Increasing

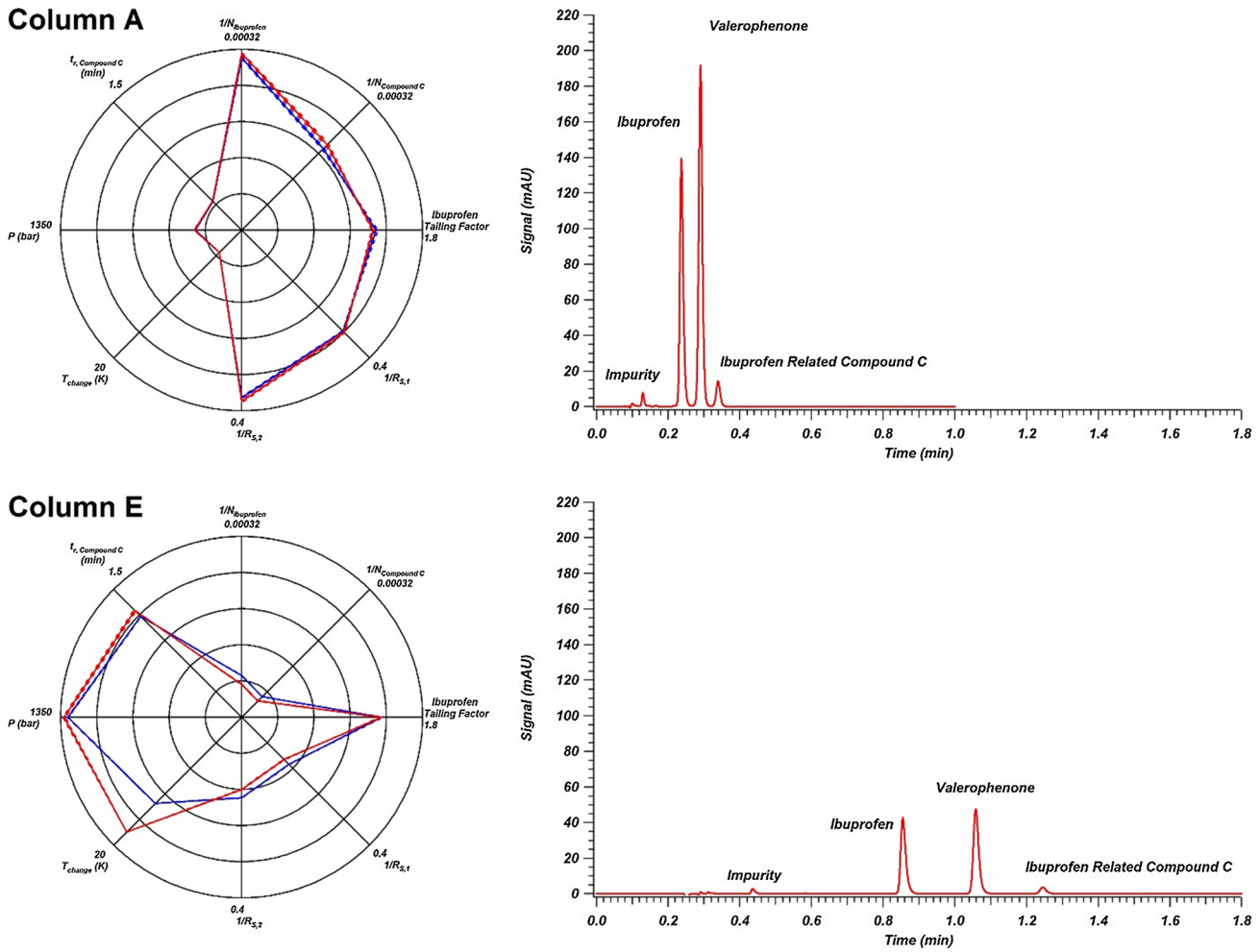

Fig. 2 Radar plots and representative chromatograms describing relevant performance parameters for the Method 2 USP monograph with Columns A and E listed in Table 2 (figure panel designations match column designations). Clockwise from top (maximum value for each radial axis listed in parentheses): inverse plate count for ibuprofen peak (0.00032), inverse plate count for ibuprofen-related compound C peak (0.00032), USP tailing factor for ibuprofen peak (1.5), inverse resolution between ibuprofen and valerophenone (0.4), inverse resolution between valerophenone- and ibuprofen-related compound C (0.4), magnitude of the axial thermal gradient across the column (20 K), system pressure (1350 bar), and the retention time of the last eluting peak (ibuprofen-related compound C, 1.5 min). Values are based on an average of ten final injections from a set of fifteen, with dotted lines indicating a range of ±1 standard deviation. Red traces indicate still air oven mode and blue traces indicate forced air oven mode. Chromatogram mobile phase, column, and instrument conditions can be found in Tables 1 and 2. Radar plots and representative chromatograms for Columns B, C, and D, as well as full data for all five columns, can be found in the Supporting Information

the column diameter marginally improves tailing factors, but does not provide a large improvement in overall efficiency compared to lower diameter columns with the same particle diameter and length. The maximum speed when using larger column diameters can also be impacted by instrument flow rate limits, which can be reached before the actual instrument or column pressure limit is exceeded, as was the case here for the ibuprofen method. Thus, shorter columns with larger SPPs can provide the best opportunity for higher throughput methods under these various monograph-related restraints. This potential was further explored in the following section to determine how far instrument operating limits can be pushed for monograph modernization.

Future Prospects for Monograph Modernization

In the process of increasing the speed of the previous methods, the main focus relied on changing the column dimensions, particle size, and mobile phase flow rate, albeit slightly beyond some of the suggested guidelines (as explained in detail in [5]) described in the general 'Chromatography' section 621 of the USP-NF [4] through the flexibility enabled by monograph modernization

(a comparison of these methods to previously reported methods for the same monographs can be found in the Supporting Information). However, other methods exist where requirements for asymmetry and resolution are not explicitly stated, permitting even higher throughput beyond what has been described above. Two USP monographs have previously been published for tablets containing acetaminophen and aspirin—one also containing caffeine [66] and the other without [65]. Only the method for tablets that contain caffeine lists performance thresholds, so the focus here was to maximize the speed of the non-caffeine method that would not be limited by the lower efficiencies observed at very high flow rates. For this purpose, the 2.1 × 50 mm column containing 2.6 µm particles was selected since the usual limitation for this column with the other methods was due to chromatographic performance rather than flow rate or pressure. Because the still air oven mode provided higher plate counts than the forced air oven mode for throughout the previous two methods, it was the only thermal environment utilized here to try and limit further increases to plate height at such high mobile phase velocities. Additionally, a hybrid mobile phase based on the composition listed in each separate monograph was used to help reduce viscosity so that higher flow rates could be obtained before the column pressure limit was reached, while still maintaining resolution between the four peaks at these higher flow rates. To further increase method speed, the injection preparation process was changed so that the sample to be injected for the next method in a sequence was prepared during the current run rather than in between each run as is standard in most instrument operating procedures. By maximizing the speed of the method and eliminating any time associated with the injection between consecutive runs, a complete cycle time of 20 s was achieved so that fifteen runs could be completed in 5 min (Fig. 3).

In this set of fifteen consecutive 20 s runs, there was some performance loss in the first few injections due to the flow rate ramp and initial formation of thermal gradients, similar to the previous experiments. Across these final ten injections, the critical pair (benzoic acid and salicylic acid) maintained a resolution exceeding 1.5, indicating baseline resolution between all compounds. Efficiency was rather low for all compounds because of the high mobile phase velocity, especially for the least retained compound (acetaminophen) which had an average plate count of 1400, but this did not diminish the resolution between any two peaks below 1.5. Specialized autosampler and injection schemes can be implemented to increase the speed slightly more [25], but these 20 s cycle times are readily achievable with currently available commercial instrumentation. Although this method was developed empirically based on a combination of USP monographs, increasing the speed of these analyses can also be explored through the use of online chromatographic modeling tools [70–72]. The kinetic plot method is another powerful tool that could be used for predictive determination of efficiency based on column and instrument operating limits [73–77], although Chap. 621 of the USP-NF indicates that direct measurement of resolution is needed rather than just a calculated value based on efficiency.

Conclusions

In the modernization of USP monographs for widely used drugs, column selection plays an important role in the throughput that can be achieved. Because performance requirements vary for each method, the critical parameters (resolution, asymmetry, etc.) must be identified to

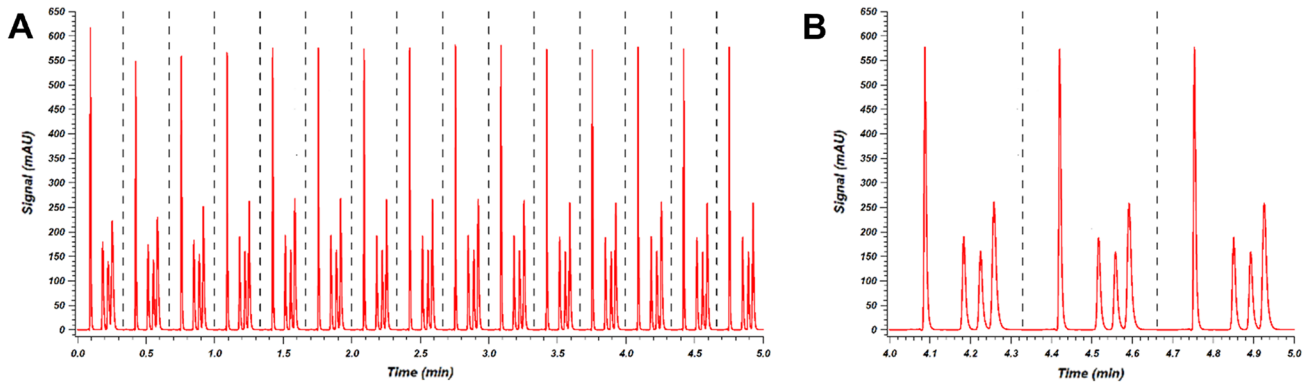

Fig. 3 Fifteen consecutive injections for Method 5 (1.3 mL min^{-1} on Column A) over 5 min coupled on a single chromatogram (from fifteen individually collected chromatograms) with vertical dashed lines designating each injection point are shown in Panel A. Panel B is an expanded view of the final minute (last three runs). Peak order of separation is: (1) acetaminophen, (2) aspirin, (3) benzoic acid, and (4) salicylic acid

determine which column properties are most necessary. Shorter columns enable faster methods but can be limited by the efficiency and peak symmetry that can be achieved. Longer columns improve these parameters but increase the analysis time. Between particle sizes, the efficiency gained by reducing the particle diameter also results in a marked increase in the magnitude of the axial thermal gradient due to viscous friction, especially at longer column lengths. In these cases with larger increases in the mobile phase temperature at the column outlet, the use of the still air oven mode provided much better chromatographic efficiency than more isothermal forced air oven mode. For the two methods examined here (naproxen and ibuprofen) with five different columns, a 2.1 × 50-mm column packed with 2.6 µm SPPs generally provided the highest throughput within the resolution and asymmetry thresholds set by the individual USP monographs. All five columns, however, were significantly faster and required less mobile phase than the listed column types (as described in the Supporting Information) and can be used to improve method throughput. When increasing throughput for isocratic monograph methods within these performance guidelines, the key aspect is determining whether performance criteria, column pressure limits, or instrument pressure/flow limits for a given column type. For shorter, narrower columns, the limit is typically performance, while longer and wider columns are usually limited by pressure and/or flow rate.

As increased focus is placed on achieving method times in the 1–30 s range for pharmaceutical methods, specialized instrument set-ups and injection schemes are being utilized. Here, a standard instrument flow path and readily available column were used to achieve a 20-s cycle time, demonstrating the capability of current technology to be implemented into high-throughput workflows. Beyond the key focus in this study on monograph-specific suitability parameters such as resolution and asymmetry, future work in this area will determine approaches for validating these low cycle time methods (accuracy, robustness, linearity, etc.) and comparing results between the standards measured here and formulated OTC analgesics following dissolution. Because monograph modernization goes beyond the chromatographic parameters detailed in Chapter 621 of the USP-NF, a wider range of experimental conditions (mobile phases, temperatures, etc.) can be implemented to dramatically reduce method time. Here, isocratic methods were studied which eliminates any column equilibration time required between runs that would be necessary for gradient elution. For more complex methods requiring mobile phase gradients, equilibration time and other delays related to instrument dead volume will make achieving throughput levels more difficult without further instrument and column modifications, as others are currently developing [26–28].

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Compliance with Ethical Standards

Conflict of Interest G.A.K. and J.P.G. declare no conflicts of interest. J.-M.T.W., M.D.P., and F.S. are employed by Thermo Fisher Scientific, a company that manufactures some of the technology used in this study.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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