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Effect of high-temperature on high-performance liquid chromatography column stability and performance under temperature-programmed conditions

Stephanie J. Marin*, Brian A. Jones, W. Dale Felix, Jody Clark

Selerity Technologies Inc., 2484 W. Custer Road, Salt Lake City, UT 84104, USA

Abstract

Six commercially available analytical (4.1 or 4.6 mm i.d.) columns were evaluated under temperature-programmed high-temperature liquid chromatography (HTLC) conditions to access their stability and performance at extreme temperatures. Seven components consisting of acidic, basic and neutral compounds were analyzed under temperature-programmed conditions and solvent gradient conditions using three different mobile phase compositions (acidic, basic and neutral). Each column was checked with a two-component test mix at various stages of the evaluation to look for signs of stationary phase collapse. Three zirconia based stationary phases studied exhibited column bleed under temperature-programmed conditions. The other three columns, a polydentate silica column, a polystyrene-divinylbenzene (PS-DVB) polymeric column, and a graphitic carbon column performed well with no evidence of stationary phase degradation. The R.S.D. for the retention times and efficiencies were less than 10% for most conditions, and not more than 15% during the course of the evaluation for each column. The polydentate silica stationary phase was temperature programmed to 100 °C, the PS-DVB stationary phase was temperature programmed up to 150 °C, and the graphitic carbon column was used with temperature programming up to 200 °C. Comparable peak capacities and similar retention behaviors were observed under solvent gradient and temperature-programmed conditions. Temperature programming with dynamic mobile phase preheating can replace solvent gradient analysis without a loss of peak capacity when used with 4.1 or 4.6 mm columns.

Keywords: Temperature effects; Temperature programming; Column performance; Column stability; Stability studies

1. Introduction

High-temperature liquid chromatography (HTLC) has had limited use in the laboratory due to instrument and column limitations. New instrumentation is available that allows operation at temperatures up to 200 °C with mobile phase preheating to eliminate thermal mismatch. This has generated increased interest in utilizing high-temperatures in separation work on a more routine basis. Column selection, however, is still rather limited; no stationary phases other than those based on zirconia have been used at these extreme temperatures for routine work. Stationary phases based on graphitic carbon, rigid polystyrene-divinylbenzene polymeric particles, and polydentate silica phases should be stable at much higher temperatures than the traditional limits of 50 or 60 °C. One of the most intriguing aspects of HTLC is the ability to perform temperature programming.

The advantages of utilizing elevated temperatures in HPLC analysis are well documented in the literature [1–4]. HTLC offers several distinct advantages to the separation scientist. Back pressure is reduced as the temperature is increased, allowing the use of stationary phases with smaller particle sizes for increased efficiency. The analyst can also operate at higher flow rates because of lower back pressure. The Van Deemter curve "flattens out" as a result of increased diffusion rates within the stationary phase and the mobile phase as the temperature is increased, allowing operation at flow rates that are many times the optimal velocity without the sacrifice in efficiency that is found at ambient temperature [5]. The net result is faster and more efficient separations.

A recent review outlines the use of temperature programming with capillary and microbore columns [6]. Temperature programming with microbore columns was reported in the literature as early as 1983 [7]. Instrument limitations

* Corresponding author. Tel.: +1-801-978-2295; fax: +1-801-978-2298.
E-mail address: stephaniemarin@selerity.com (S.J. Marin).

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have prevented the use of temperature programming with standard 4.6 mm i.d. columns. Block column heaters are not capable of rapidly transferring heat into the mobile phase or the interior of a 4.6 mm diameter column efficiently and reproducibly during temperature programming. One aspect critical to successful HTLC analysis with 4.6 mm i.d. columns is adequate preheating of the mobile phase. This is not an issue with capillary or microbore columns. Heat from a forced-air oven alone is sufficient to warm the mobile phase to the same temperature as the column because the columns used are lower in mass and operated at lower flow rates. The mass of the hardware and packing of 4.6 mm i.d. columns, and the higher flow rates employed make it impossible for the oven alone to heat the fluid sufficiently to match the column temperature. If the mobile phase is not preheated, the cool mobile phase entering the heated column will warm up faster along the walls of the column than in the center. The warmer mobile phase in this region will flow faster than that in the column center and lead to band broadening. This "thermal mismatch" band broadening is eliminated if the mobile phase is preheated [8]. Thermal mismatch band broadening can occur at temperatures as low as 80 °C with 4.6 mm i.d., columns [9]. Past attempts at HTLC have involved long coils of stainless steel tubing in either a forced air oven [10–12] or a liquid bath to preheat the mobile phase [13]. This is not very efficient and adds a large dwell volume to the HPLC system. More recent designs incorporate a passive heat exchanger [14]. None of these approaches will provide the fast response required for temperature-programmed HTLC with 4.6 mm i.d. columns. The HTLC oven used in this study was equipped with a low mass, low volume preheater that was incorporated into the column inlet tubing [9]. It heated the mobile phase independently of the forced air oven, and was capable of preheating the mobile phase during fast temperature ramps used in temperature-programmed HTLC.

New instrumentation is now available to perform temperature-programmed HPLC at temperatures up to 200 °C with 4.6 mm i.d. columns. This technique allows the user to perform a temperature program to alter retention and selectivity in place of a solvent gradient. This is possible because hydrogen bonding effects in water are reduced as the temperature is increased, making it less polar so that water behaves like a moderately polar organic solvent like methanol or acetonitrile during the separation process [15–19]. This means that many separations requiring a binary solvent gradient can be separated isocratically using a temperature program.

Although a number of column heaters have been available for several years that are capable of operation at temperatures up to 100 °C, traditional silica based column packings were only stable to about 60 °C when used with aprotic solvents [20]. It was not until the creation of zirconia based stationary phases that high-temperature liquid chromatography was seriously investigated as a routine laboratory technique [21]. Although these zirconia stationary phases are most often the only ones that come to mind for high-temperature use, there are other commercially available columns that can be used at temperatures up to 200 °C. This work involved evaluating a number of different stationary phases under HTLC conditions using temperature programming. Six columns were initially chosen as candidates for temperature-programmed HTLC evaluation. The ZirChrom PBD, CARB, and DiamondBond columns from ZirChrom Separations, a Selerity Technologies Blaze C_8 polydentate silica column, a Hamilton PRP-1 polymeric column, and a Thermo Hypersil-Keystone HyperCarb column consisting of a graphitic carbon stationary phase, were evaluated to assess their stability and performance under temperature-programmed HTLC conditions.

2. Experimental

2.1. Instrumentation and reagents

An HPLC system consisting of an Alltech vacuum degasser (Alltech Associates, Deerfield, IL, USA), Milton Roy CM4000 pump (Thermo Electron, Waltham, MA, USA), Thermo Separations Spectrasystem UV2000 detector (Thermo Electron), and Alcott 708 AL autosampler (Alcott Chromatography, Norcross, GA, USA) combined with a Selerity Series 8000 HTLC oven with temperature programming and independent mobile phase preheating (Selerity Technologies, Salt Lake City, UT, USA) was used for all analyses. The Series 8000 is a forced air oven with dynamic mobile phase preheating and post-effluent cooling.

A Barnstead Nanopure II water system was used to generate 18 megaohm water. HPLC grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium hydroxide and trifluoroacetic acid (TFA) were purchased from Aldrich (Milwaukee, WI, USA). All analytes used were reagent grade or better quality and purchased from either Aldrich or Fisher Scientific.

2.2. Column selection

The Blaze C_8 column (prototype) was manufactured by Selerity Technologies. The PRP-1 column (part number 79479, serial number 26) was provided by Hamilton (Reno, NV, USA). The HyperCarb column (part number 35007-104646, serial number 1123021A) was provided by Thermo Hypersil-Keystone (Bellefonte, PA, USA). The ZirChrom DiamonBond (part number DB01-1046, serial number OD052302H), CARB (part number ZR01-1046, serial number CARB061802O), and PBD (ZR03-1046, serial number PBD012502V) columns were provided by ZirChrom Separations (Anoka, MN, USA). Before the extensive column evaluation began, all six columns were tested by analyzing a two-component test mix consisting of uracil and phenol. A flow rate of 1.0 ml/min, acetonitrile–water (50:50) mobile phase, and UV detection at 254 nm were used. The amount of sample injected was 5 μl. The retention

Table 1
HPLC columns evaluated using temperature-programmed HTLC

Manufacturer	Column	Packing	Particle size (μm)	Maximum temperature (°C)
Thermo Hypersil-Keystone	HyperCarb	Graphitic carbon pH 0–14	7	200
Hamilton Company	PRP-1	PS-DVB polymer pH 0–14	5	150
Selerity Technologies	Blaze C_8	Polydentate silica pH 2–8	3	100
Zirchrom separations	PBD	Bonded zirconia pH 0–14	3	150
Zirchrom separations	CARB	Zirconia–graphite pH 0–14	3	200
Zirchrom separations	DiamondBond	Zirconia–carbon pH 0–14	3	200

All columns were 10 cm in length with an i.d. of 4.6 mm, except the Hamilton PRP-1, which had an i.d. of 4.1 mm.

time, peak area, theoretical plates and asymmetry for each peak were recorded. This analysis was repeated throughout the evaluation to monitor the columns and look for signs of column degradation. Next, a blank temperature-programmed run (no sample injected) starting at 40 °C and ramping at 15 °C/min using an acetonitrile–water (50:50) mobile phase was performed with each column. The maximum temperature for the temperature program was chosen based on recommendations from the manufacturer for the Blaze, HyperCarb and ZirChrom columns. The maximum temperature for the Hamilton column was determined from a previous study in our Laboratory using isothermal conditions (see Table 1). Chromatograms were generated using a UV detector set at 254 and 220 nm with a range setting of 2 AUFS (absorbance units full scale).

2.3. Column evaluation conditions

The columns selected for evaluation and their characteristics are summarized in Table 1. Seven analytes consisting of acidic, basic, and neutral compounds were analyzed using a binary solvent gradient at 35 °C, and also using under temperature-programmed conditions with an isocratic mobile phase (acetonitrile–aqueous, 50:50). The seven analytes were aniline, acetophenone, amitriptylene, ibuprofen, 2-phenyl-2-propanol, salicylic acid and styrene glycol. Solutions were prepared in 50:50 acetonitrile–water. The concentration of each sample was 2.50 ± 10% mg/ml except for ibuprofen which was prepared at 1.30 mg/ml. Five microlitres of solution were injected for each chromatographic run. A flow rate of 1.0 ml/min was used for all chromatographic runs.

Three different mobile phases were used: (1) acetonitrile–water, (2) acetonitrile–water with 0.1% TFA (pH ≈2), and (3) acetonitrile–20 mM ammonium hydroxide (pH 10). A blank temperature program and a blank solvent gradient with each mobile phase were run for each column. Each component was analyzed individually, so eight solvent gradient and eight temperature-programmed runs were conducted with each mobile phase using each column.

Analysis conditions are summarized in Table 2. A solvent gradient and temperature program were chosen to give approximately the same retention time for each component so a comparison between solvent gradient analysis and temperature-programmed HTLC analysis could be made. Each column was checked between each different mobile phase by analyzing the two-component test mixture consisting of uracil and phenol under the same conditions as the initial run performed on each column. Retention time, efficiency and peak shape for these components were monitored to look for any indication of column degradation.

The evaluation of the PRP-1 column progressed in this order: initial evaluation at 35 °C with the two-component test mix, solvent gradient and temperature-programmed analyses using acetonitrile–water (50:50), evaluation at 35 °C using the two-component test mix, solvent gradient and temperature-programmed analyses using acetonitrile–water (50:50) with 0.1% TFA, evaluation at 35 °C using two-component test mix, solvent gradient and temperature-programmed analyses using acetonitrile–20 mM ammonium hydroxide pH 10 (50:50), and a final evaluation

Table 2
Analysis conditions for temperature-programmed HTLC column evaluation

Column	Temperature program	Solvent gradient
HyperCarb	50–200 °C at 15 °C/min, hold 5 min	50–100% acetonitrile over 10/min, hold 5/min (all three columns)
PRP-1	50–150 °C at 10 °C/min, hold 5 min	
Blaze	35–100 °C at 10 °C/min, hold 5 min	

Solvent gradient and temperature-programmed runs with three different mobile phases were performed using the PRP-1 and the HyperCarb columns: acetonitrile–water (50:50), acetonitrilie–water (50:50) with 0.1% TFA, and acetonitrile–20 mM ammonium hydroxide pH 10 (50:50). Solvent gradient and temperature-programmed runs with the Blaze column were performed with acetonitrile–water (50:50) and acetonitrile–water with 0.1% TFA (50:50). Flow rate was 1.0 ml/min with UV detection at 254 nm.

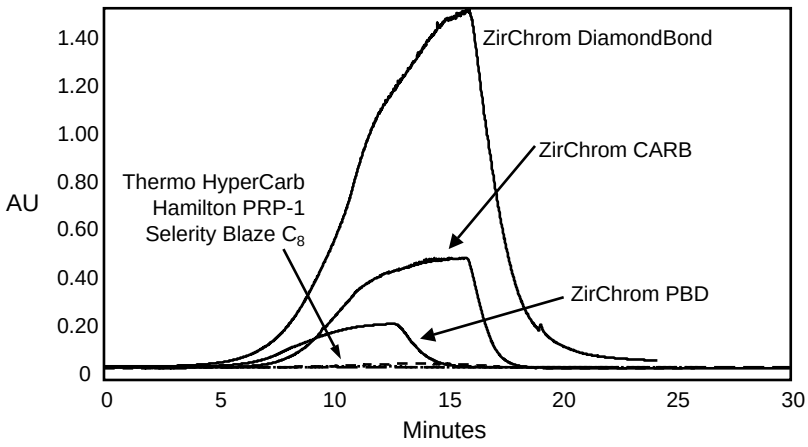

Fig. 1. Blank temperature-programmed runs for six HPLC columns. The PRP-1 and Blaze C_8 have an essentially flat baseline. The HyperCarb column shows a slight baseline rise (approximately 0.01 AU). The three Zirchrom columns show a significant rise in the baseline. Temperature program conditions: PRP-1: 40–150 °C at 15 °C/min, hold 5 min; Blaze C_8: 40–100 °C at 15 °C/min, hold 5 min; HyperCarb: 40–200 °C at 15 °C/min, hold 5 min; ZirChrom PBD: 40–150 °C at 15 °C/min, hold 5 min; ZirChrom CARB: 40–200 °C at 15 °C/min, hold 5 min; ZirChrom DiamondBond: 40–200 °C at 15 °C/min, hold 5 min.

using the two-component test mix at 35 °C. The evaluation of the HyperCarb column was the same except that the acetonitrile–ammonium hydroxide mobile phase was used before the acetonitrile–water with 0.1% TFA. The evaluation of the Blaze C_8 column was conducted in the same order as the PRP-1, except that no ammonium hydroxide at pH 10 was used as a mobile phase. The Blaze column is silica based and is not stable at pH 10. Retention times, peak areas, peak widths, and asymmetry values were recorded for each of the seven analytes under each set of conditions. Peak capacities were calculated for each set of conditions as described in [22].

3. Results and discussion

Blank temperature-programmed analyses for all six columns are shown in Fig. 1. The Blaze C_8 and PRP-1 columns had an essentially flat baseline. The HyperCarb column exhibited a slight rise in the baseline (approximately 0.01 AU) starting at about 150 °C continuing to 200 °C. The zirconia based columns exhibited excessive column bleed under temperature-programmed HTLC conditions. The ZirChrom PBD column had a steep rise in the baseline with a maximum absorbance of almost 0.20 AU at 220 nm. The ZirChrom CARB column also had a steep rise in the baseline with a maximum absorbance of close to 0.45 AU at 220 nm. The ZirChrom DiamondBond column had the largest baseline rise with a maximum absorbance of almost 1.5 AU at 220 nm. This large baseline rise was not observed with the Blaze C_8, PRP-1 or HyperCarb columns or when the columns were replaced with a stainless steel union in the instrument. This suggests that the observed baseline rise was caused by some material leaching from the packing of the zirconia columns during a temperature-programmed run. This material "bleeding" from the column absorbs in the UV at 254 and 220 nm. To verify this, two or three (depending on availability in our laboratory) of each of the ZirChrom columns were tested and the column bleed was observed with each column. A methanol–water mobile phase was also used to conduct temperature-programmed runs using the zirconia columns. There was a slight reduction in the baseline rise when compared to the acetonitrile–water chromatograms, but it was still significant. The column bleed would be significant enough to interfere with analyte quantitation.

Table 3
Average column retention and efficiency during the course of the HTLC evaluation

Column	Component	Retention time		Theoretical plates		Peak area	
		Average	R.S.D. (%)	Average	R.S.D. (%)	Average	R.S.D. (%)
Blaze C_8	Uracil	1.05	0.61	4577	12.99	2940408	8.94
Blaze C_8	Phenol	1.86	2.31	7295	3.52	191753	11.68
PRP-1	Uracil	0.83	3.41	1374	11.73	3007136	3.29
PRP-1	Phenol	1.81	3.58	1275	4.45	197540	3.41
HyperCarb	Uracil	1.45	1.11	4753	14.31	2937874	6.43
HyperCarb	Phenol	2.49	5.04	5159	6.40	188944	6.79

Acetonitrile–water (50:50) (isocratic), 1.0 ml/min, 35 °C, 254 nm. Average values for each component and each column were calculated from values determined initially, after each mobile phase change, and upon conclusion of the evaluation.

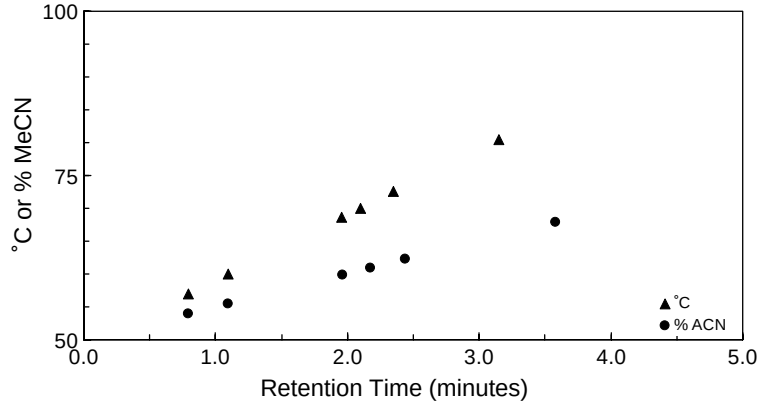

Fig. 2. Retention time vs. °C and retention time vs. percent MeCN for the PRP-1 column using acetonitrile–water as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the temperature program. Elution order was salicylic acid, styrene glycol, 2-phenyl-2-propanol, aniline, 2-phenyl-2-propanol, acetophenone. Ibuprofen and amitriptylene did not elute using either the solvent gradient or the temperature program within the designated run time.

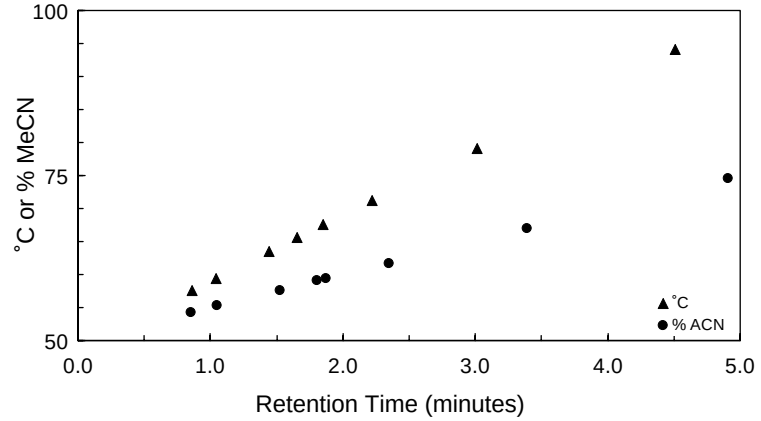

Fig. 3. Retention time vs. °C and retention time vs. percent MeCN for the PRP-1 column using acetonitrile–water with 0.1% TFA as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the temperature program. Elution order was aniline, styrene glycol, amitriptylene, salicylic acid, 2-phenyl-2-propanol (two peaks), acetophenone, ibuprofen.

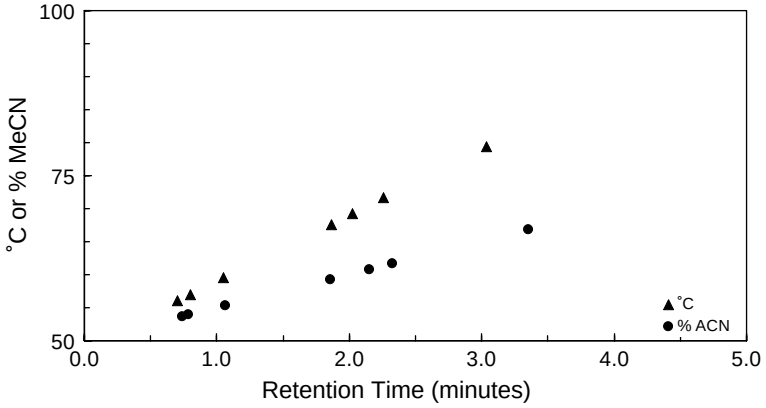

Fig. 4. Retention time vs. °C and retention time vs. percent MeCN for the PRP-1 column using acetonitrile–20 mM ammonium hydroxide (pH 10) as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the temperature program. Elution order was salicylic acid, ibuprofen, styrene glycol, 2-phenyl-2-propanol, aniline, 2-phenyl-2-propanol, acetophenone. Amitriptylene eluted at 11 min with the solvent gradient but did not elute under temperature program conditions within the designated run time.

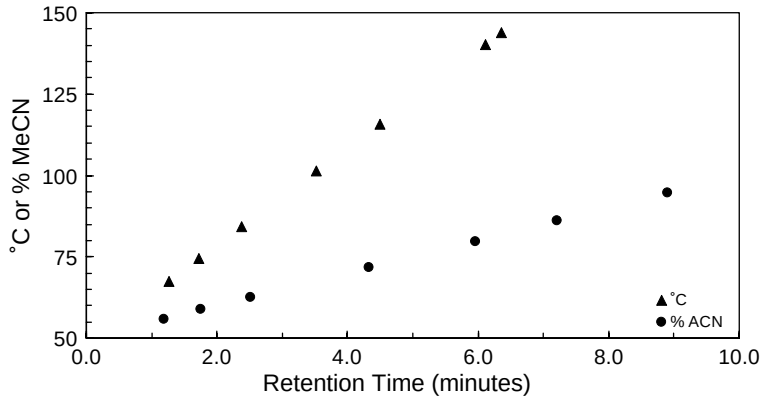

Fig. 5. Retention time vs. °C and retention time vs. percent MeCN for the HyperCarb column using acetonitrile–water with 0.1% TFA as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the temperature program. Elution order was aniline, styrene glycol, 2-phenyl-2-propanol (one peak), amitriptylene, acetophenone, salicylic acid, ibuprofen.

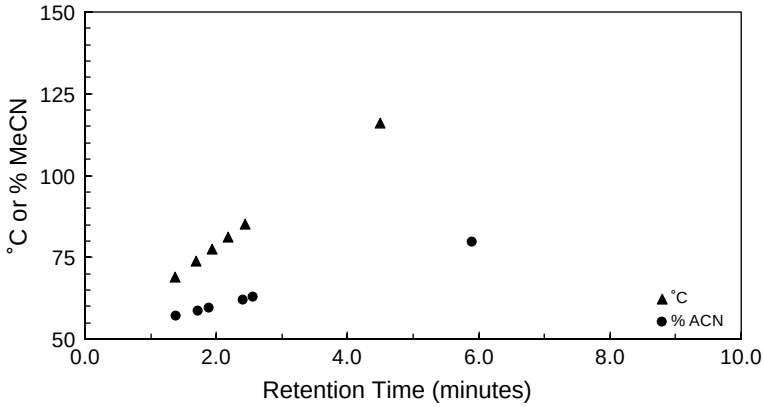

Fig. 6. Retention time vs. °C and retention time vs. percent MeCN for the HyperCarb column using acetonitrile–20 mM ammonium hydroxide (pH 10) as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the temperature program. Elution order was salicylic acid, styrene glycol, ibuprofen, aniline, 2-phenyl-2-propanol (one peak), acetophenone. Amitriptylene did not elute under either solvent gradient or the temperature program conditions within the specified run time.

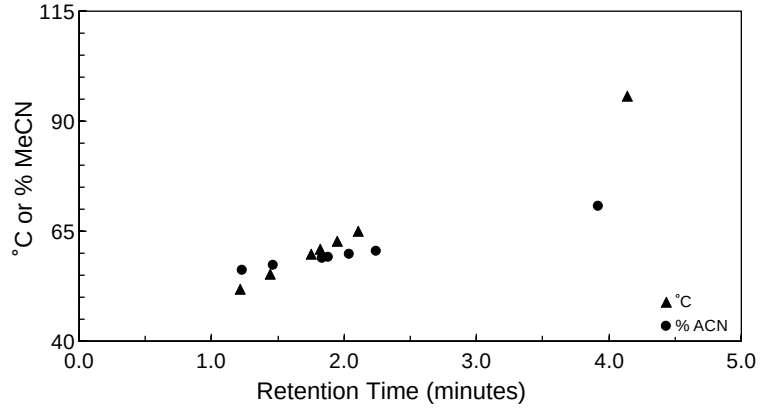

Fig. 7. Retention time vs. °C and retention time vs. percent MeCN for the Blaze C_8 column using acetonitrile–water as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the temperature program. Elution order was styrene glycol, salicylic acid, aniline, 2-phenyl-2-propanol (two peaks), acetophenone, ibuprofen. Amitriptylene did not elute under either solvent gradient or the temperature program conditions within the designated run time.

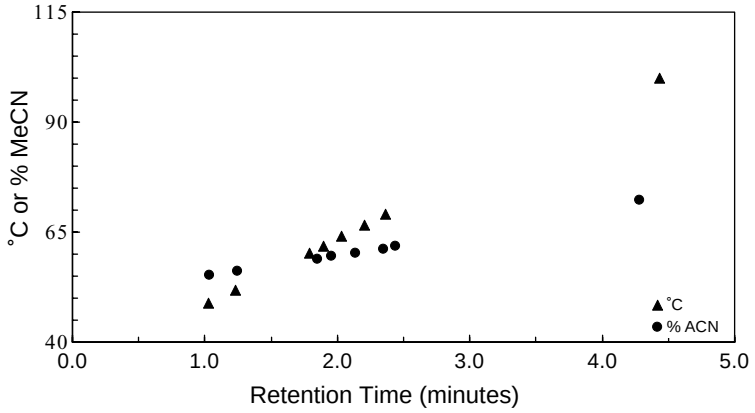

Fig. 8. Retention time vs. °C and retention time vs. percent MeCN for the Blaze C_8 column using acetonitrile–water with 0.1% TFA as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the temperature program. Elution order was aniline, styrene glycol, salicylic acid, 2-phenyl-2-propanol (two peaks), amitriptylene, acetophenone, ibuprofen.

We performed the blank temperature-programmed runs using the maximum temperature recommended by the manufacturer. This means that not all columns were programmed to the same temperature. We thought that this would be the most useful since the most interesting effects of temperature programming often occur at the higher temperatures. We also planned to evaluate the columns by programming to their maximum temperatures. The bleed observed with the zirconia columns was an unexpected result of our work. The observed bleed would probably be reduced if all of the columns were programmed to only 100 °C as the Blaze column was. We did include two other columns that were programmed to 150 and 200 °C. It is important to note that the Hamilton PRP-1 and the Thermo HyperCarb column did not show the excessive bleed at 150 and 200 °C, respectively, so the bleed does seem to be unique to the Zirchrom columns, at least within the scope of this study.

Average retention times, theoretical plates, and peak areas during various stages of the evaluation for the three columns chosen for further study are summarized in Table 3. The variation in retention time and efficiency was ±10% in most cases for each set of conditions. No loss of efficiency or retention was observed after exposure to high-temperatures even when combined with pH extremes. Stationary phase collapse due to extreme temperature or temperature programming should have resulted in a loss of retention and efficiency. Although not recorded, no significant change in back pressure was observed during the course of the evaluation with any of the columns during analysis of the two-component test mix. Since this evaluation, several hundred injections have been made on each of these columns while conducting application work using temperature programming up to their maximum temperature with a number of different mobile phases. All three columns are still performing well with no evidence of stationary phase damage.

Peak capacities for each column under both solvent gradient and temperature-programmed conditions are listed in Table 4. Comparable peak capacities were observed for solvent gradient and temperature-programmed analyses for each column and mobile phase composition. This indicated that the quality of the chromatographic peaks were similar for the two techniques. It is widely believed that temperature programming is not feasible with 4.6 mm i.d. columns because thermal gradients across the column would cause band broadening and a loss of peak capacity. Perhaps increased diffusion rates compensate for the loss of efficiency and yield separations of comparable quality, or perhaps the thermal gradient across the column does not have a negative effect on the quality of the separation. This work demonstrates that separations using temperature programming with an isocratic mobile phase can be used in place of solvent gradients without a loss of peak capacity.

Figs. 2–8 show plots of retention time versus °C or percent acetonitrile at time of elution. These plots demonstrate that similar retention was achieved using a temperature program and an isocratic mobile phase compared to a solvent gradient. It also gives some indication of the solvating strength of the temperature program (using an isocratic mobile phase of

Table 4
Peak capacity comparison for solvent gradient and temperature-programmed analysis

Mobile phase	Blaze C_8		PRP-1		HyperCarb	
	Temperature program	Solvent gradient	Temperature program	Solvent gradient	Temperature program	Solvent gradient
Acetonitrile–water	44.8	39.7	15.8	15.2	32.7	32.5
Acetonitrile–water with 0.1% TFA	45.4	43.7	22.1	20.8	31.7	27.9
Acetonitrile–20 Mm ammonium hydroxide, pH 10	–	–	15.0	14.6	32.4	36.4

acetonitrile–aqueous, 50:50) compared to the mobile phase gradient. The use of temperature programming instead of solvent gradients provide new opportunities for altering retention and selectivity using the full range of isocratic mobile phases from 0 to 100% organic modifier in addition to the solvating power of the temperature program.

4. Conclusions

The Selerity Blaze C_8, Hamilton PRP-1, and Thermo Hypersil-Keystone HyperCarb columns can be used at maximum temperatures between 100 and 200 °C with temperature programming without evidence of column degradation. Acidic and basic pH conditions combined with high column temperatures did not appear to cause any collapse of the stationary phase. Calculation of peak capacities indicated that comparable peak quality is attained when a temperature program and an isocratic mobile phase are used in place of a solvent gradient to perform a separation. Although zirconia based stationary phases are routinely used isothermally at temperatures up to 200 °C, they are not good candidates for temperature-programmed high-temperature liquid chromatography due to a significant rise in the baseline during temperature programming.

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