Reproducibility of Temperature Programmed HPLC Using 4.6 mm ID Columns

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Introduction

Temperature is an overlooked variable in most HPLC separations. Performing HPLC analyses at higher temperatures results in faster and more efficient separations. Instrumentation that allows isothermal and temperature programmed HPLC at temperatures up to 200°C has been available for several years.

Mobile phase preheating is critical to good chromatography at elevated temperatures. Thermal mismatch band broadening, which occurs when ambient temperature mobile phase contacts the heated column, causes poor peak shape and loss of resolution and efficiency. During a temperature program, the reproducibility depends upon how well the column compartment controls the temperature and proper function of the mobile phase preheater.

Introduction

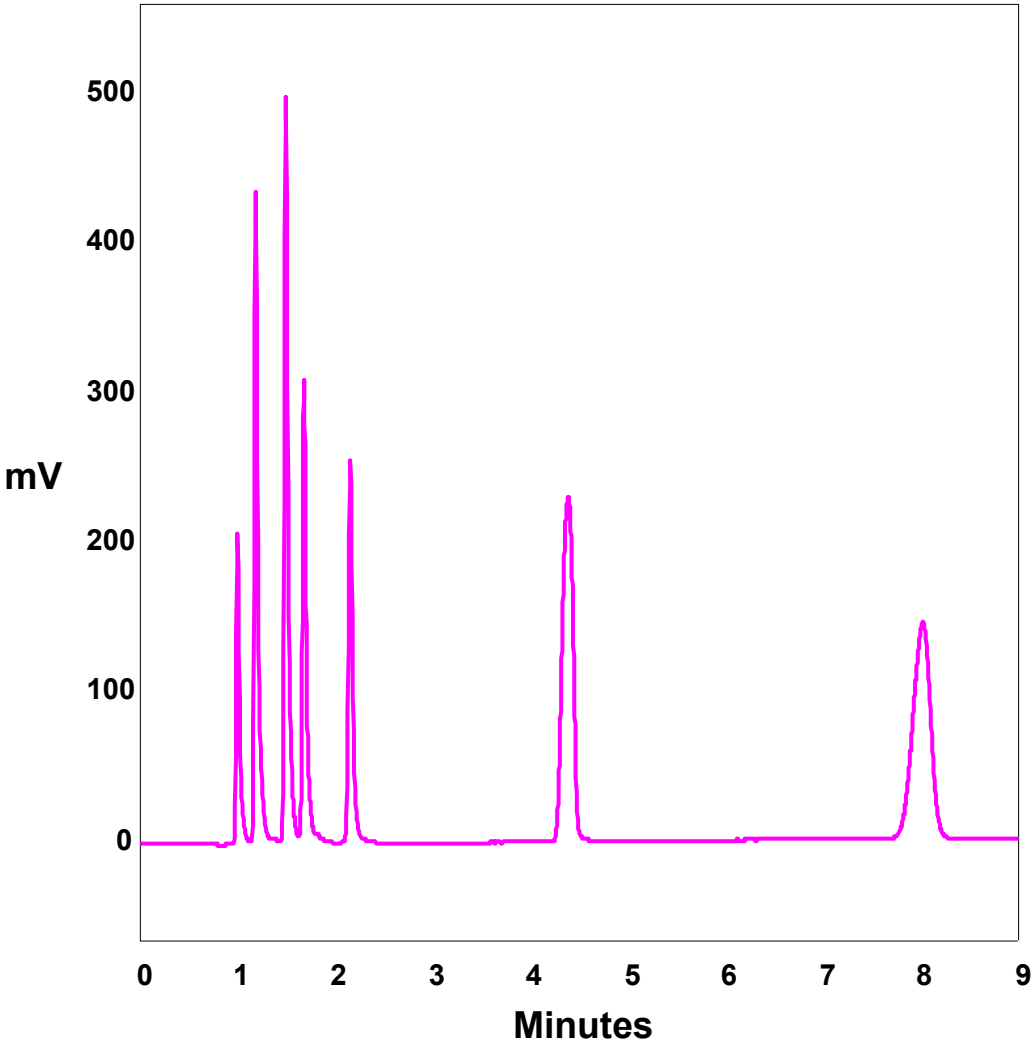
This work evaluated the reproducibility of temperature programmed HPLC analyses using a dynamic mobile phase preheater. This preheater can be programmed independently of the oven program. This means that the mobile phase temperature can be programmed to lead, match or lag the oven temperature. Three different preheaters were evaluated using temperature programs where the mobile phase temperature matched the oven, lagged the oven temperature by 10°C, and led the oven temperature by 10°C. Ten injections of an analgesic mix were separated using a temperature program. Retention time, theoretical plates, and peak area were recorded for two peaks in each run.

Experimental

Three different mobile phase preheaters were used with one Polaratherm™ Total Temperature Controller. Each preheater was programmed to track the column compartment temperature, lag behind the column compartment temperature by 10°C, and lead the column compartment temperature by 10°C. A seven component analgesic mixture was analyzed using a temperature program Two peaks were chosen to collect reproducibility data. Salicylic acid was baseline resolved from the other components and eluted during the temperature ramp. Naproxen was also well separated from the other components and eluted during the 100°C hold (see Figure 1). Ten injections under each set of conditions were conducted. HPLC conditions are summarized in Figure 1. Data were also collected with and without mobile phase preheating to compare peak quality.

Figure 1

Separation of Analgesics on a Selerity Blaze C_8 Using a Temperature Program



Column: Selerity Blaze C_8, 3 μm 100 x 4.6 mm
Mobile Phase: 40:60 acetonitrile:water with 0.1%TFA
Flow Rate: 1.5 mL/min
Detection: UV 220 nm
Temperature Program: hold at 50°C for one minute, ramp to 100°C at 30°C/min, hold six min.

Elution Order:
Acetaminophen
Caffeine
Salicylamide
Aspirin
Salicylic acid
Ibuprofen
Naproxen

Results

Table 1 shows the retention time, theoretical plates, and peak area data for preheater 1 under all three programmed conditions (lead, lag or match oven temperature). Table 2 lists the reproducibility data for preheater 2, and Table 3 lists the data for preheater 3. All three tables show the average, standard deviation, and %RSD for ten injections for the two peaks of interest, salicylic acid and naproxen. Table 4 is the combined data of all three preheaters.

Figure 2 shows chromatograms collected with and without mobile phase preheating. Table 5 shows the average retention time, theoretical plates and peak area of salicylic acid and naproxen with and without mobile phase preheating.

Discussion

Most of the %RSD values are less than 2%. Preheater 3 showed higher standard deviations and %RSD values for peak area data collected when the preheater temperature lagged by 10°C. This was attributed to a detector failure. This detector has not been repaired, and so the experiment could not be repeated.

There was a dramatic difference in the data collected with and without mobile phase preheating. There was a significant loss of efficiency for both peaks when mobile phase preheating was not used. The poor peak shape and lower plate count was typical of thermal mismatch band broadening, which is caused when cold mobile phase enters the heated column. This was eliminated with dynamic mobile phase preheating.

Table 1

Reproducibility Data for Preheater 1

Salicylic Acid									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	1.67	1,572,882	10,732	1.64	1,544,477	8,785	1.67	1,557,821	9,946
SD	0.00	5,755	71	0.00	16,966	43	0.00	12,553	53
%RSD	0.13	0.37	0.66	0.20	1.10	0.49	0.14	0.81	0.53
Naproxen									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	6.06	1,919,329	21,681	5.92	1,912,860	18,833	6.16	1,927,804	22,614
SD	0.01	33,457	321	0.02	18,941	440	0.01	15,736	331
%RSD	0.17	1.74	1.48	0.34	0.99	2.34	0.16	0.82	1.46

Average of ten injections

Salicylic acid elutes during the temperature ramp

Naproxen elutes during the temperature hold

Table 2

Reproducibility Data for Preheater 2

Salicylic Acid									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	1.69	1,564,457	10,874	1.64	1,566,420	9,316	1.67	1,571,646	10,712
SD	0.01	20,581	110	0.00	6,791	114	0.00	3,603	111
%RSD	0.32	1.32	1.01	0.20	0.43	1.22	0.12	0.23	1.04
Naproxen									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	6.14	1,930,635	20,959	5.87	1,935,474	21,319	6.10	1,949,225	18,402
SD	0.02	24,584	377	0.02	9,951	590	0.01	4,794	397
%RSD	0.33	1.27	1.80	0.26	0.51	2.77	0.19	0.25	2.16

Average of ten injections

Salicylic acid elutes during the temperature ramp

Naproxen elutes during the temperature hold

Table 3

Reproducibility Data for Preheater 3

Salicylic Acid									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	1.68	1,552,521	10,541	1.64	1,575,138	9,113	1.68	1,411,456	10,768
SD	0.00	7,814	84	0.00	4,681	75	0.00	209,057	278
%RSD	0.15	0.50	0.79	0.20	0.30	0.83	0.11	14.81	2.58
Naproxen									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	6.23	1,907,942	25,513	5.99	1,939,382	20,351	6.25	1,735,040	24,784
SD	0.01	9,556	133	0.01	4,017	814	0.01	254,336	228
%RSD	0.09	0.50	0.52	0.18	0.21	4.00	0.14	14.66	0.92

Average of ten injections

Salicylic acid elutes during the temperature ramp

Naproxen elutes during the temperature hold

Table 4
Combined Preheater Data

Salicylic Acid									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	1.68	1,556,557	10,733	1.64	1,562,012	9,071	1.68	1,509,991	10,486
SD	0.00	8,096	57	0.00	6,336	70	0.00	255,056	310
%RSD	0.15	0.52	0.53	0.25	0.41	0.78	0.11	16.89	2.96
Naproxen									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	6.14	1,912,514	22,693	5.92	1,928,654	20,120	6.17	1,865,845	22,035
SD	0.00	8,213	82	0.01	3,356	676	0.01	198,876	260
%RSD	0.07	0.43	0.36	0.19	0.17	3.36	0.11	10.66	1.18

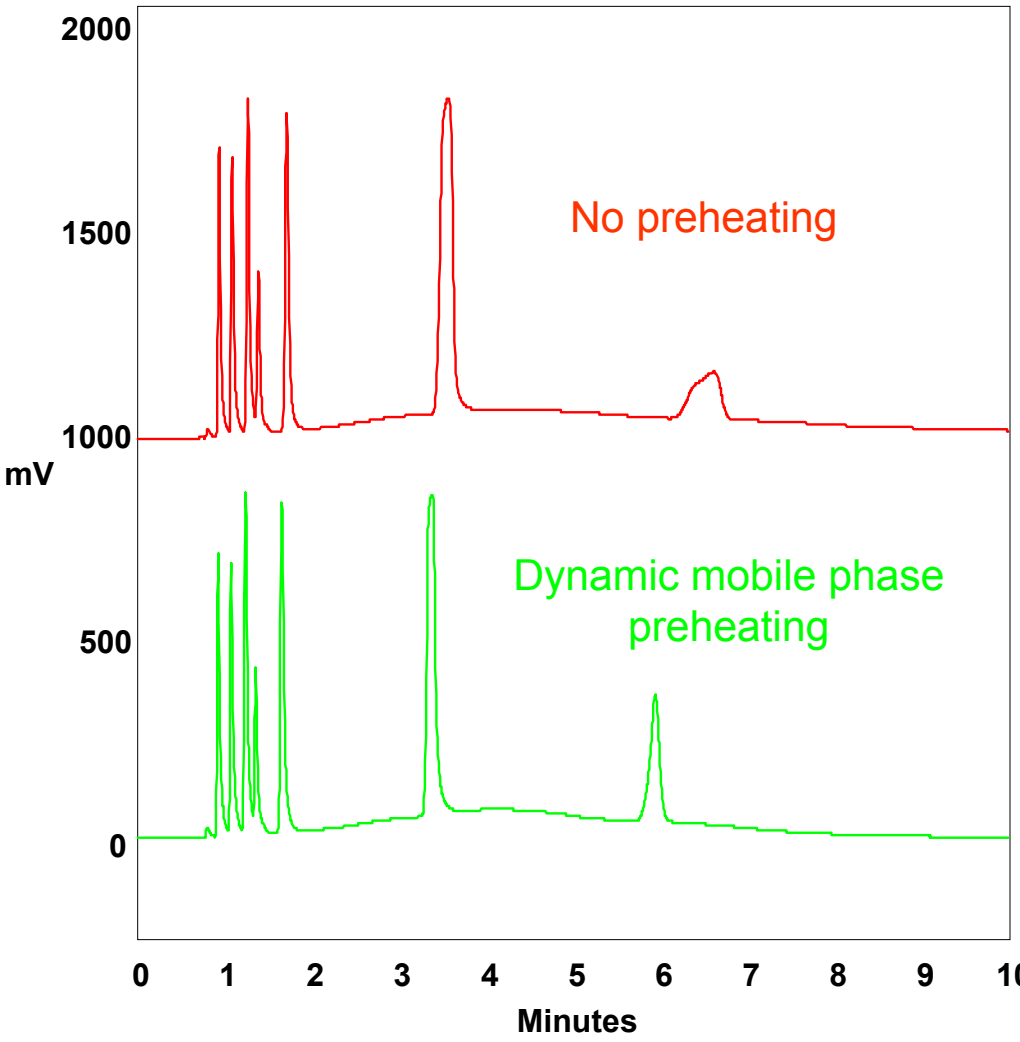
Average of ten injections

Salicylic acid elutes during the temperature ramp

Naproxen elutes during the temperature hold

Figure 2

Separation of Analgesics With and Without Mobile Phase Preheating



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Caffeine
Salicylamide
Aspirin
Salicylic acid
Ibuprofen
Naproxen

Table 5

Preheating vs No Preheating

Salicylic Acid				
	PH1=oven		no PH	
	RT	plates	RT	plates
average	1.65	8,911	1.72	8,265
SD	0.01	251	0.00	74
%RSD	0.51	2.82	0.12	0.89
Naproxen				
	PH1=oven		no PH	
	RT	plates	RT	plates
average	5.90	13,375	6.62	2,237
SD	0.03	214	0.01	16
%RSD	0.43	1.60	0.12	0.70

Average of five injections – different detector was used

Salicylic acid elutes during the temperature ramp

Naproxen elutes during the temperature hold

Conclusions

- Mobile phase preheating eliminates thermal mismatch band broadening
- Dynamic mobile phase preheating makes temperature programmed HPLC possible with 4.6 mm ID columns.
- Good chromatographic reproducibility was attained during temperature programmed runs.
- Good reproducibility was observed when different preheaters were used.

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