Accuracy and Precision of Temperature Programmed HPLC

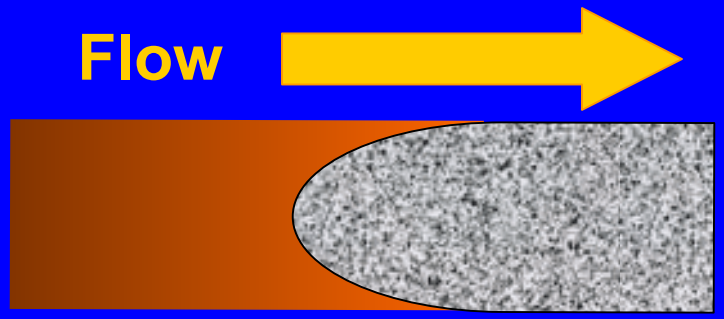
Stephanie J. Marin

Brian Jones, Dale Felix, Nathan Porter

Selerity Technologies, Inc.
Salt Lake City, UT
www.selerity.com

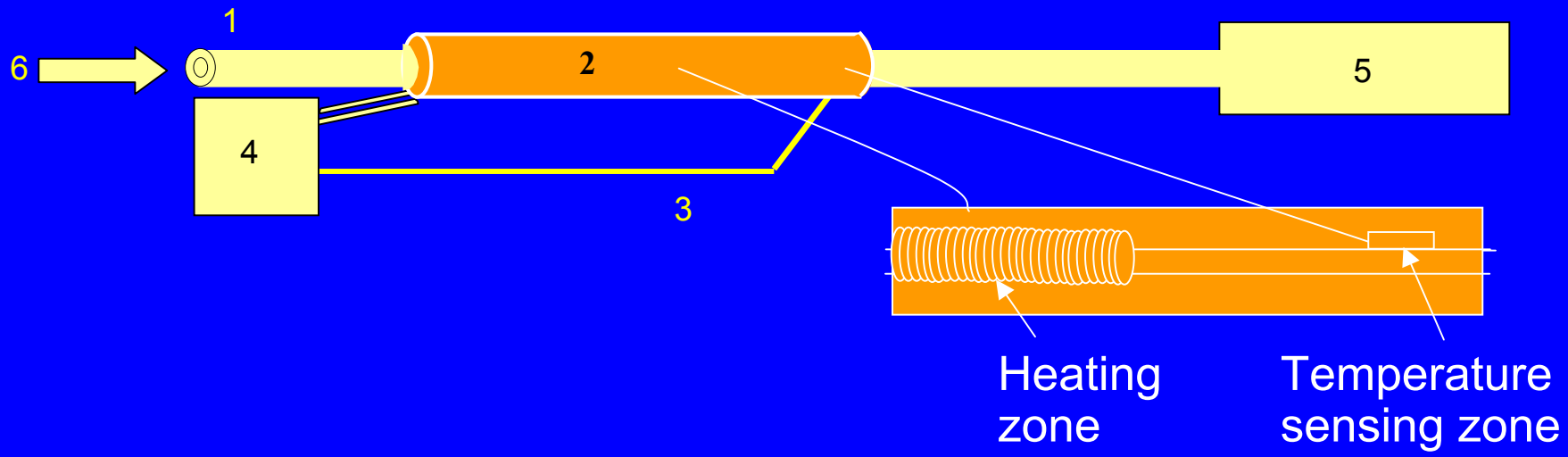
Why is Mobile Phase Preheating so Important?

No Preheating



With Preheating

Preheater Design



(1) stainless steel tubing, (2) heater, (3) thermocouple sensor, (4) temperature controller, (5) column, (6) from pump

Patent pending Selerity Technologies, Inc.

Mobile Phase Preheater

- Very responsive and non-invasive
- Low-mass and low-volume: <2 grams mass (including the tubing),<1 μL totally swept volume
- 0.005”, 0.007” and 0.010” ID available

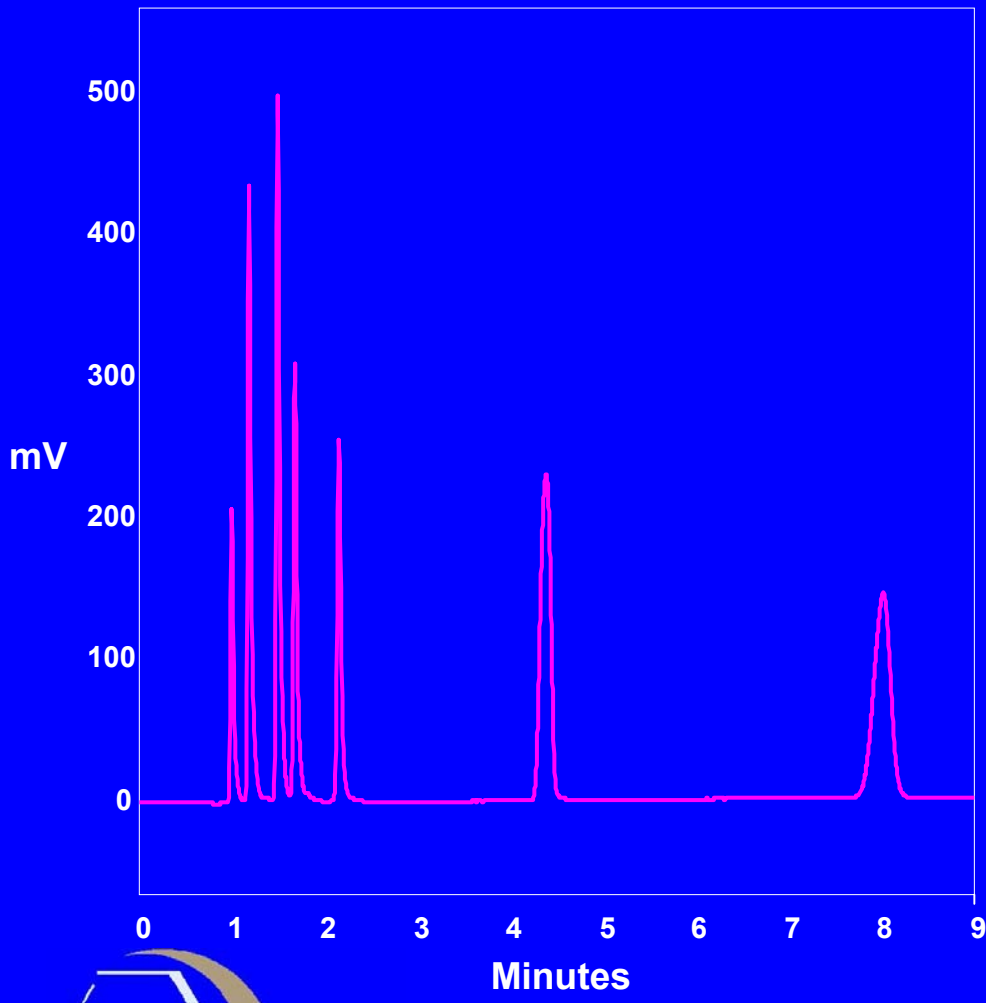
Determining Preheater Reproducibility

- Determine reproducibility of dynamic mobile phase preheater
- Three different preheater evaluated under the same conditions
- Preheater set to match, lead and lag the oven temperature
- Ten injections under each set of conditions

The Selerity Polaratherm Total Temperature Controller

- Forced air oven and chiller
- Isothermal and thermal gradient operation
  - Sub-zero to 200°C
  - Flow rates up to 10.0 mL/min
  - Thermal gradient up to 30°C/min
- Mobile phase preheating
- Peltier effluent cooling
- Vapor sensor
- Compatible with any HPLC system

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

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

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

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

***detector malfunctioned with grating error which affected intensity and peak areas, but did not effect retention time or plates**

Average of ten injections

Salicylic acid elutes during the temperature ramp

Naproxen elutes during the temperature hold

Combined Preheater Data

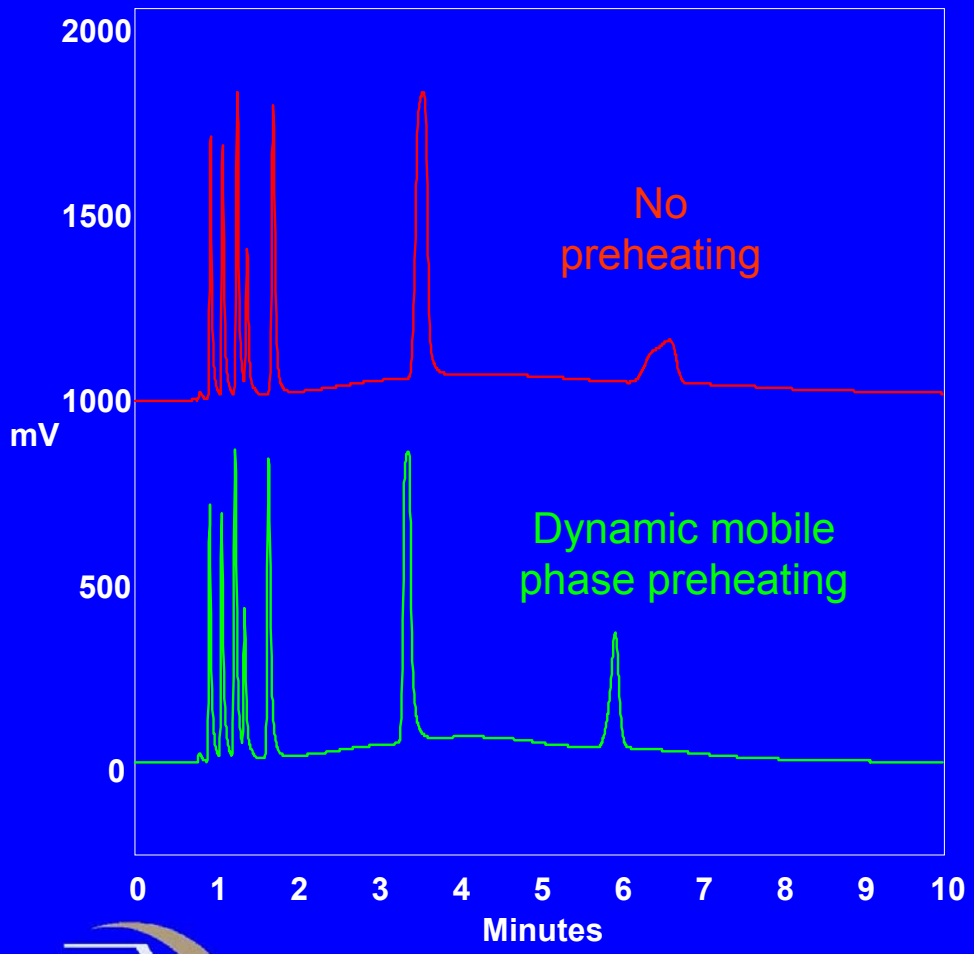
Salicylic Acid									
	PH=oven			PH +10			PH -10		
	RT	area	plates	RT	area	plates	RT	area	plates
average	1.68	1,556,557	10,733	1.64	1,562,012	9,071	1.68	1,509,991	10,486
SD	0.00	8,096	57	0.00	6,336	70	0.00	255,056	310
%RSD	0.15	0.52	0.53	0.25	0.41	0.78	0.11	16.89	2.96
Naproxen									
	PH=oven			PH +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

Separation of Analgesics With and Without Mobile Phase Preheating



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

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.

Striking When Hot

Visit us at Booth 2739

Selerity Technologies Inc.

801-978-2295

www.selerity.com