Evaluation of Acclaim® HPLC Columns using the National Institute of Standards and Technology Standard Reference Material® 870

INTRODUCTION

The National Institute of Standards and Technology (NIST) Standard Reference Material 870 (SRM 870) is a mixture of five organic compounds dissolved in methanol that was formulated for characterizing general aspects of HPLC column performance, including efficiency, void volume, methylene selectivity, retentiveness, and activity toward chelators and organic bases.[1] This standard was designed primarily for testing C18 columns, but can be used to characterize C8 and some polar-embedded phases. Other possible uses include column classification to aid column selection during method development, as a control material for monitoring LC column performance over time, and as a standard for lot-to-lot and column-to-column comparisons. The five organic compounds in SRM 870 are uracil, toluene, ethylbenzene, quinizarin, and amitriptyline. These compounds are used to evaluate the void volume, hydrophobic retention, methylene selectivity, activity toward chelating reagents, and activity toward bases, respectively.

In this application update, SRM 870 was used to evaluate the Acclaim 120 C18, 120 C8, PA, PA II, and 300 C18 columns. These columns are prepared from high purity type B silica and use advanced, and in some cases patented, bonding chemistries. The NIST formulation of SRM 870 was also reformulated so that the five compounds had similar peak heights at 254 nm. Standards with one half and one fifth concentration of each compound in SRM 870 were prepared. Different separation conditions are also shown (i.e., a different mobile phase concentration for the Acclaim 300 C18 column and a different column temperature for the Acclaim 120 C18 column). These are believed to be more valuable for making lot-to-lot and column-to-column comparisons.

EQUIPMENT

Dionex Summit® HPLC system consisting of:

- LPG P680 pump or HPG P680 pump
- SOR-100 solvent rack
- ASI-100™ Auto Sampler
- TCC-100 Thermostatted Column Compartment
- UVD 170 UV-Vis Detector and UVD 340 Photodiode Array Detector
- Chromeleon® Chromatography Management Software, Version 6.70

CONDITIONS

Columns and Samples

Columns

Acclaim 120 C18 (P/N 059149), 120 C8 (P/N 059141), PA (P/N 061321), and PA II (P/N 063199), all 4.6 × 250 mm packed with 5-µm particles having a pore size of 120 Å; and 4.6 × 150 mm Acclaim 300 C18 column (P/N 060266) packed with 3-µm particles having a pore size of 300 Å.

Samples

The five-component standard was prepared based on the SRM 870 document with the concentration of each compound the same as SRM 870 (uracil 28 µg/g, toluene 1400 µg/g, ethylbenzene 1700 µg/g, quinizarin 94 µg/g and amitriptyline 2800 µg/g, five compounds all dissolved in methanol). Alternatively, this standard can be purchased from the NIST (*). Another standard with an amitriptyline concentration that was tenfold less than that in SRM 870 with the other concentrations unchanged was also prepared. These concentrations yield similar peak heights for all five compounds on the Acclaim 120 C8 column with detection at 254 nm.

* Order number: 870, LC performance, set (5), (https://srmors.nist.gov/pricerpt.cfm)

Source of Chemicals

HPLC-grade toluene, ethylbenzene, and quinizarin were purchased from Fluka, HPLC-grade uracil was purchased from Aldrich, and HPLC-grade amitriptyline was purchased from Sigma.

Chromatographic Conditions

Eluent:	80% methanol, 20% buffer [50% 20 mM KH_2PO_4 and 50% 20 mM K_2HPO_4 (v/v)], adjust pH to 7.00 accurately, as described in the SRM 870 documentation.
Flow Rate:	1.0 mL/min
Temperature:	23 ºC and 30 ºC
Inj. Volume:	2 µL
Detection:	210 nm, 254 nm, and 480 nm

RESULTS AND DISCUSSION

The Acclaim 120 C18, 120 C8, PA, PA II, and 300 C18 columns were evaluated with SRM 870 at 23 ºC. These evaluations used both a standard with the specified concentrations of each of the five compounds in SRM 870 and a standard prepared with concentrations that yielded a similar peak height for all five compounds at 254 nm when analyzed with an Acclaim 120 C8 column. Four injections were performed for each sample using the separation conditions specified on the certificate of analysis for SRM 870, and were averaged for all calculations. All five peaks were separated on Acclaim C8, PA, and PA II columns; however, quinizarin and amitriptyline were not baseline resolved on the Acclaim 120 C18 and 300 C18 columns. Therefore, single component standards of amitriptyline and quinizarin were injected to calculate their associated column parameters.

Figure 1 shows typical chromatograms for the evaluation of the Acclaim 120 C8 with SRM 870. Using these chromatograms and the equations below, the column parameters that SRM 870 was designed to produce were calculated. The data for all four injections are shown in Table 1. Table 2 shows the average values for all five columns. The tailing factor for amitriptyline is higher than we normally observe for Acclaim columns. This is due to the column temperature of 23 ºC recommended by NIST. Acclaim columns are tested and exhibit their best peak shapes at 30 ºC as shown in Table 2 by the tailing factor for amitriptyline at 30 ºC. Therefore for optimum chromatography, a 30 ºC operating temperature is recommended. Figures 2 through 6 show the five columns evaluated with the standard that yields similar peak heights for the five test compounds at 210, 254, and 480 nm. Dionex recommends this concentration for column-to-column and lot-to-lot comparisons. To baseline resolve quinizarin and amitriptyline, the column temperature was changed to 30 ºC for the Acclaim 120 C18 column and the proportion of methanol and buffer was changed for the Acclaim 300 C18 column. Figures 7 and 8 show the chromatography with the altered conditions on the 120 C18 and 300 C18 columns, respectively.

Chromatography and Calculations

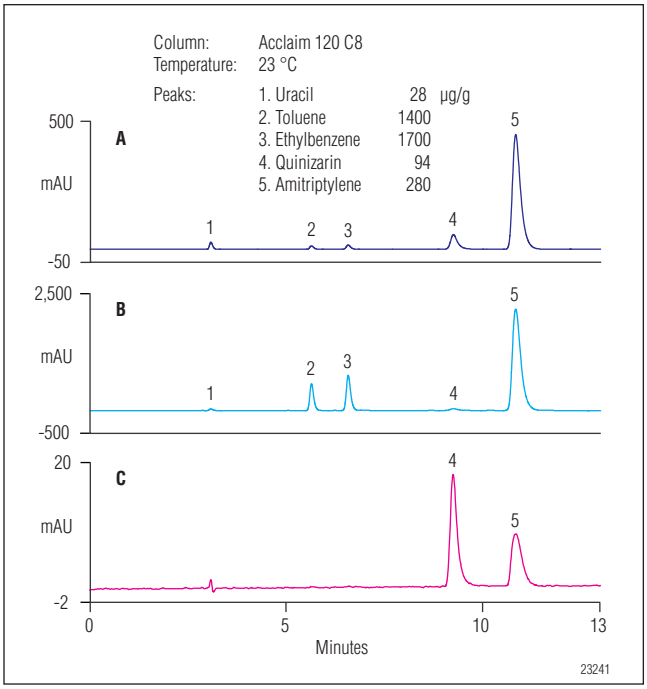

Figure 1. Chromatography of SRM 870 with an Acclaim 120 C8. A) 254 nm, B) 210 nm, C) 480 nm.

Parameters for an Acclaim 120 C8 using SRM 870

Calculations

1. k' (ethylbenzene) = $(t_R - t_0) / t_0 = (6.575 - 3.077)/3.077 = 1.14$
   k' = Capacity factor
   t_R = 6.575 (Retention time of ethylbenzene)
   t_0 = 3.077 (Dead time: retention time of uracil)

2. Asymmetry (quinizarin) = $(LW_{5\%}+RW_{5\%}) / (2 \times LW\ 5\%) = (0.16 + 0.32) / (2 \times 0.16) = 1.5$
   Asymmetry: Peak tailing factor
   $LW_{5\%}$: 0.16 (left peak width in 5% of the peak height)
   $RW_{5\%}$: 0.32 (right peak width in 5% of the peak height)

3. k' (amitriptyline) = $(t_R - t_0) / t_0 = (10.844 - 3.077) / 3.077 = 2.52$
   k' = Capacity factor
   t_R = 10.844 (Retention time of amitriptyline)
   t_0 = 3.077 (Dead time: retention time of uracil)

4. Asymmetry (amitriptyline) = $(LW_{5\%} + RW_{5\%}) / (2 \times LW_{5\%}) = (0.35 + 0.17) / (2 \times 0.17) = 1.53$
   Asymmetry: Peak tailing factor
   $LW_{5\%}$: 0.17 (left peak width in 5% of the peak height)
   $RW_{5\%}$: 0.35 (right peak width in 5% of the peak height)

Table 1. Test Results for an Acclaim 120 C8 Evaluated With SRM 870

Samples	Hydrophobicity Capacity Factor (k') for Ethylbenzene	Chelating Tailing Factor (A_s) for Quinizarin	Silanol Activity	
			Capacity Factor (k') for Amitriptyline	Tailing Factor (A_s) for Amitriptyline
1	1.14	1.50	2.52	1.53
2	1.14	1.51	2.53	1.54
3	1.13	1.51	2.52	1.54
4	1.14	1.51	2.52	1.54
Average	1.14	1.51	2.52	1.54

Evaluation of Five Acclaim Columns with SRM 870

Table 2. Test Results for Acclaim Columns Evaluated With SRM 870

Column	Hydrophobicity Capacity Factor (k') for Ethylbenzene	Chelating Tailing Factor (A_s) for Quinizarin	Silanol Activity			Shape Selectivity Bonding Density (µmols/m²)
			Capacity Factor (k') for Amitriptyline	Tailing Factor (A_s) for Amitriptyline		
				23 °C	30 °C	
Acclaim 120 C18	2.57	1.04	6.35	1.31	1.28	3.2
Acclaim 120 C8	1.14	1.51	2.52	1.54	1.23	3.4
Acclaim PA	1.47	1.51	3.16	1.64	1.43	2.6
Acclaim PA II	1.33	1.38	2.99	1.94	2.00	N/A
Acclaim 300 C18	0.67	1.22	1.67	1.30	1.21	3.3

Chromatography of the Modified Standard on the Five Acclaim Columns

The concentration of the amitriptyline in NIST SRM 870 does not yield ideal chromatography for column-to-column and lot-to-lot comparisons. This is due to column overloading by amitriptyline, as seen with the Acclaim PA and PA II phases, which have lower capacities than the other phases tested. To remedy this situation, a standard was prepared with the amitriptyline concentration reduced 10-fold while the concentrations of the other four compounds were kept the same. This reformulated standard produced chromatograms with comparable peak heights for all five components at 254 nm. Figures 2 thorough 6 show chromatography of this modified standard on each of the five Acclaim columns listed in Table 2.

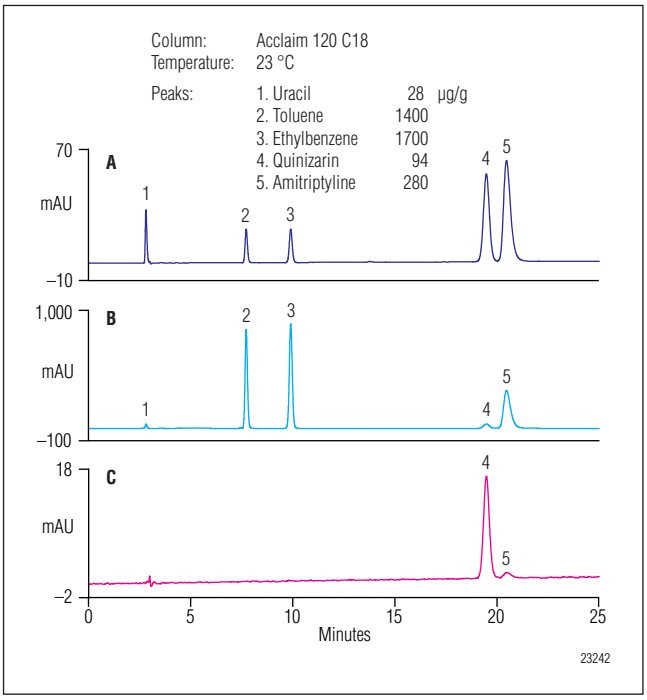

Figure 2. Chromatography of the modified standard with the Acclaim 120 C18 column. A) 254 nm, B) 210 nm, C) 480 nm.

Chromatography of the Optimized Conditions on Acclaim 120 C18 and 300 C18 Columns

As shown in Figures 2 and 6, quinizarin and amitriptyline were not baseline separated on Acclaim 120 C18 and 300 C18 columns both at the recommended temperature of 23 ºC and at an 80:20 ratio of methnol/buffer. As mentioned above, Acclaim columns are tested at 30 ºC and, for optimum chromatography, a 30 ºC operating temperature is recommended. Therefore the standard was also analyzed at 30 ºC. Experiments demonstrated that SRM 870 could be separated on all of the five evaluated Acclaim columns at 30 ºC. Figure 7 shows the chromatography of SRM 870 with Acclaim 120 C18 column, from which we can see that baseline separation

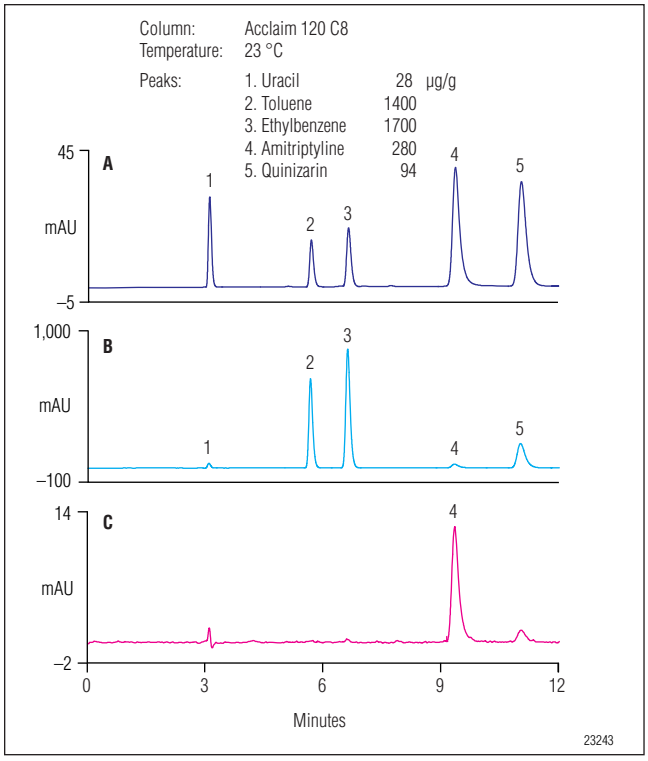

Figure 3. Chromatography of the modified standard with the Acclaim 120 C8 column. A) 254 nm, B) 210 nm, C) 480 nm.

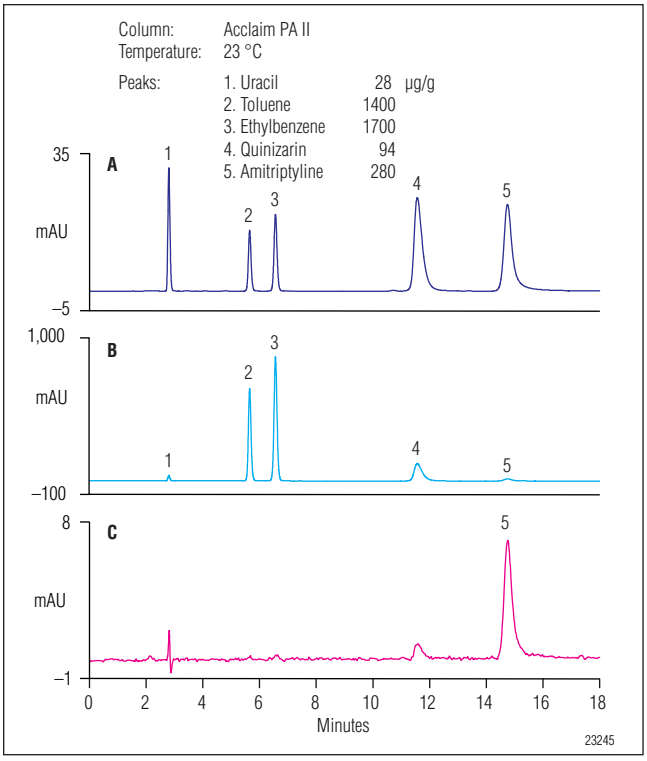

Figure 5. Chromatography of the modified standard with the Acclaim PA II column. A) 254 nm, B) 210 nm, C) 480 nm.

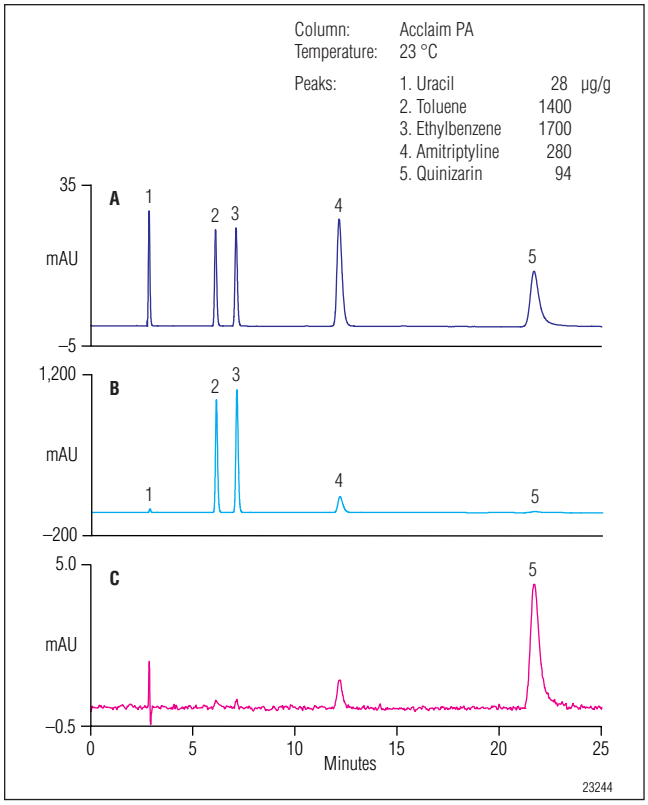

Figure 4. Chromatography of the modified standard with the Acclaim PA column. A) 254 nm, B) 210 nm, C) 480 nm.

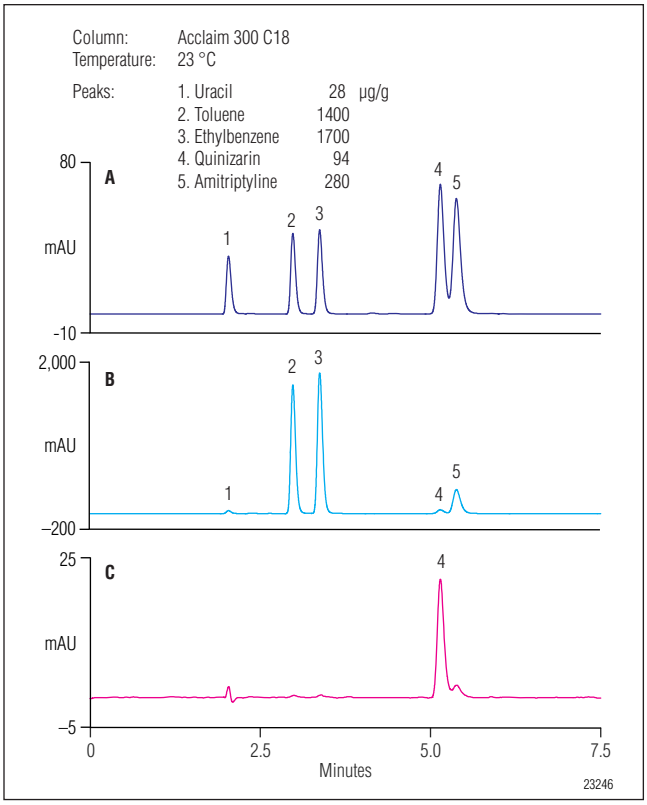

Figure 6. Chromatography of the modified standard with the Acclaim 300 C18 Column. A) 254 nm, B) 210 nm, C) 480 nm.

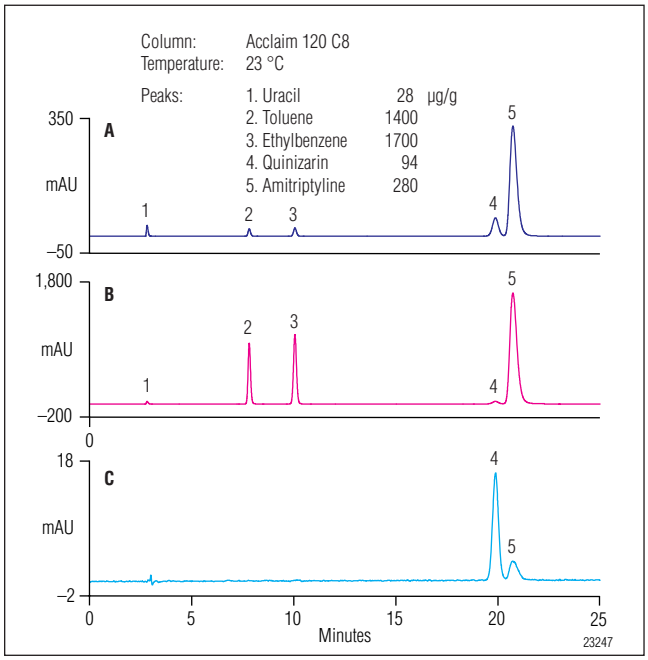

Figure 7A. Chromatography of SRM 870 with the Acclaim 120 C18 at 23 ºC. A) 254 nm, B) 210 nm, C) 480 nm.

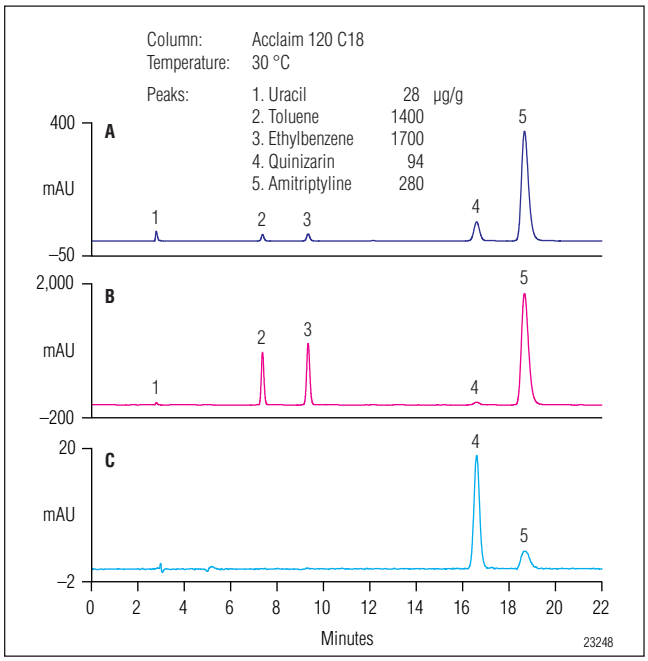

Figure 7B. Chromatography of SRM 870 with the Acclaim 120 C18 at 30 ˚C. A) 254 nm, B) 210 nm, C) 480 nm.

Table 3. Test Results for an Acclaim 120 C18 with Different Concentrations of SRM 870 at 23 ˚C and 30 ˚C

Acclaim 120 C18 Column		Capacity Factor (k´)		Asymmetry (EP)		Plates (EP)	
		23 ˚C	30 ˚C	23 ˚C	30 ˚C	23 ˚C	30 ˚C
SRM 870	Ethylbenzene	2.57	2.34	1.06	1.08	22074	22119
	Quinizarin	6.06	4.94	1.04*	1.06	21286	21703
	Amitriptyline	6.37	5.67	1.31*	1.28	17049	18281
1/2 SRM 870	Ethylbenzene	2.57	2.34	1.08	1.08	21853	22020
	Quinizarin	6.05	4.95	1.02**	1.06	21579	21777
	Amitriptyline	6.38	5.70	1.35**	1.29	17248	18367
1/5 SRM 870	Ethylbenzene	2.57	2.34	1.06	1.08	21791	21838
	Quinizarin	6.05	4.94	1.04**	1.07	21877	21625
	Amitriptyline	6.40	5.71	1.41**	1.32	17066	18327
Adjusted SRM 870 with Comparable Peak Height	Ethylbenzene	2.52	2.32	1.06	1.09	22359	22058
	Quinizarin	5.91	4.73	1.05**	1.08	21207	21613
	Amitriptyline	6.25	5.46	1.36**	1.36	17062	18257

* Obtained by injecting single component standard
** Obtained by manual integration

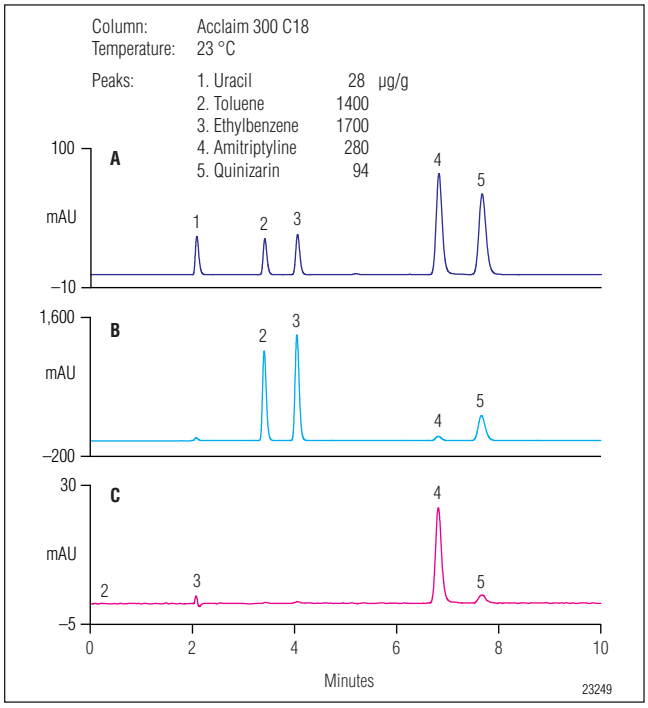

Figure 8. Chromatography of a modified standard with the Acclaim 300 C18 column at a methanol/buffer ratio of 75:25. A) 254 nm, B) 210 nm, C) 480 nm.

of quinizarin and amitriptyline was achieved at 30 ºC. Analysis results at 254 nm on Acclaim 120 C18 column are summarized in Table 3. It was found that on an Acclaim 300 C18 column, quinizarin and amitriptyline could be separated on baseline at the methanol/buffer ratio of 75:25 at 23 ºC. A typical chromatogram is shown in Figure 8.

Although SRM 870 was developed to assist in characterizing, comparing, and monitoring C18 columns, it can also be used to evaluate a number of other phases. This Application Update describes modifications to SRM 870 component concentrations and separation conditions to best test Dionex's Acclaim 120 C18, 120 C8, PA, PA II, and 300 C18 columns. When conditions were adapted, the Acclaim columns demonstrated excellent separation, capacity, and efficiency. The reformulated SRM 870, when used with the conditions described in this Application Update, is a powerful tool for monitoring the performance of these columns and comparing their performance to other columns.

PRECAUTIONS

This test mixture contains small amounts of organic compounds known to be toxic. Care should be exercised during handling and use. Use proper methods for disposal of waste.

The Acclaim 300 C18 column is packed with 3-µm particles, so it has higher backpressure than the others evaluated in this document. Typically, MeCN is the organic solvent in the mobile phase for separations using the 300 C18 columns. If methanol is used in the mobile phase, be careful of higher backpressures, compared to MeCN, when increasing the flow rate.

REFERENCES

1. Certificate of Analysis, Standard Reference Material (SRM) 870, National Institute of Standards and Technology.

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