



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 870

Column Performance Test Mixture for Liquid Chromatography¹

SRM 870 is a mixture of five organic compounds in methanol intended for use in characterizing general aspects of liquid chromatographic (LC) column performance, including efficiency, void volume, methylene selectivity, retentiveness, and activity toward chelators and organic bases. Other possible uses include (1) column classification to aid column selection during method development, (2) as a control material for monitoring LC column performance over time, and (3) in quality control for column manufacturing. SRM 870 consists of a mixture of the following five organic compounds in methanol: uracil, toluene, ethylbenzene, quinizarin, and amitriptyline (see Figure 1 for structures). The concentrations and relative detection responses of the components are listed in Table 1. A unit of SRM 870 consists of 5 ampoules each containing 11 mL of the mixture. SRMs are also available for the characterization of other chromatographic properties including shape selectivity (i.e., SRM 869a “Column Selectivity Test Mixtures for Liquid Chromatography”) [2] and chiral selectivity (i.e., SRM 877 “Chiral Selectivity Test Mixture for Liquid Chromatography”) [3].

Expiration of Certification: SRM 870 is valid for its intended purpose until **30 September 2010**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. The certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

NOTICE AND WARNINGS TO USERS

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

Preparation and analytical determinations were carried out by L.C. Sander of the NIST Analytical Chemistry Division. The coordination of the technical measurements leading to certification were performed under the direction of L.C. Sander and S.A. Wise of the NIST Analytical Chemistry Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald.

Willie E. May, Chief
Analytical Chemistry Division

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Certificate Issue Date: 30 October 2000

Nancy M. Trahey, Chief
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¹ Certain commercial equipment, instruments, or materials are identified in this certificate in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. Tabulations of commercial LC columns are not intended to be all inclusive.

INSTRUCTIONS FOR USE

Storage: Sealed ampoules, as received, should be stored in the dark at temperatures between 10 °C to 30 °C.

Chromatographic Conditions: This test mixture is intended primarily for the characterization of C₁₈ columns used in reversed-phase liquid chromatography. To compare columns on the same basis, the user should evaluate column performance by separating the mixture isocratically under the following conditions: mobile phase, 80 % methanol and 20 % buffer (v/v), flow rate 2 mL/min, column temperature 23 °C ± 2 °C, injection volume 5 µL. The buffer composition is 5 mmol/L potassium phosphate adjusted to pH 7 (final phosphate concentration in the mixed methanol/buffer mobile phase is 1 mmol/L). This buffer can be prepared by mixing 5 mmol/L monobasic potassium phosphate (KH₂PO₄) and 5 mmol/L dibasic potassium phosphate (K₂HPO₄) solutions to obtain the desired pH 7 solution, as indicated by a pH meter. Because changes in absolute retention, selectivity, and peak shape can occur with changes in temperature and composition, these conditions should be used for all column evaluations to enable comparisons with the results reported in this certificate.

INTERPRETATION OF RESULTS

Separations of the test mixture are illustrated in Figures 2A through 2F for several different C₁₈ columns. These chromatograms are representative examples of possible types of retention behavior. The most typical elution order is shown in Figure 2C. Uracil elutes near the void volume, followed by toluene and ethylbenzene. The elution order for quinizarin and amitriptyline varies with column properties. Quinizarin may elute before, after, or coelute with amitriptyline.

In most instances, peak identification can be made on the basis of elution order (uracil, toluene, ethylbenzene) and detector response (quinizarin, amitriptyline). Relative peak areas are dependent on the detection wavelength (see Table 1). Quinizarin has significant absorbance at 480 nm, and separations of SRM 870 carried out at this wavelength are selective for this single component. Conversely, quinizarin exhibits reduced absorbance at 210 nm, permitting measurement of amitriptyline in the presence of quinizarin. A comparison of separations carried out with detection at 210 nm, 254 nm, and 480 nm is provided in Figure 3. In the event of coelution of quinizarin and amitriptyline, data for each component can be obtained by selective detection at 210 nm and 480 nm (see Table 1). At 210 nm, the area of quinizarin is approximately 2 % of the area of amitriptyline, making the interference to amitriptyline small.

The retention behavior of reversed-phase LC columns often differs in a variety of ways. The components in this test mixture were selected as indicators of several types of chromatographic properties. The determination of peak width (efficiency; theoretical plates), peak asymmetry (A_s), absolute retention (k'), and relative retention (a , i.e., k'_1/k'_2) for these components may provide useful measures of these properties. See Reference [1] for a discussion of the calculation of these parameters.

Uracil: This component is commonly used as an indicator of the void volume (unretained volume) in an LC column. The measurement of void volume is somewhat controversial; however, uracil provides an acceptable approximation of this property.

Toluene/Ethylbenzene: The retention of these compounds can be considered to result primarily from solvophobic interactions. The selectivity coefficient $a_{E/T}$ is the k' ratio of ethylbenzene and toluene, and this value has been used to characterize differences among C₈ and C₈ columns. Absolute retention of a nonpolar component such as ethylbenzene provides a measure of column retentiveness (column strength). Toluene and/or ethylbenzene are also useful markers for calculation of column efficiency (theoretical plates, N).

Quinizarin: Quinizarin (1,4-dihydroxyanthraquinone) is a strong metal chelating reagent (see Figure 1). The retention behavior of this component is expected to be indicative of the presence or absence of metals in the chromatographic system. Columns demonstrate one of two types of retention behavior. Low activity toward chelating reagents is indicated by symmetric peak shape, and high activity toward chelating reagents is indicated by tailing, asymmetric peak shape. Quinizarin typically elutes after ethylbenzene and before amitriptyline. It is interesting to note that for columns known to contain certain embedded polar functional groups, quinizarin elutes last, with good peak symmetry. Peak asymmetry is not strongly correlated with retention for quinizarin.

Amitriptyline: Amitriptyline is a basic ($pK_a = 9.4$) pharmaceutical (antidepressant) commonly used by column manufacturers for column characterization. Elution of organic bases with severe peak tailing is often associated with SRM 870

high silanol activity; however, the elution of such compounds with symmetrical peak shape is considered indicative of column deactivation. Because peak tailing is the most objectionable property associated with silanol activity, A_s is an appropriate measure of this property. Peak asymmetry is not strongly correlated with retention for amitriptyline.

DISCUSSION

Selection of the components in SRM 870 was based on published testing protocols [4,5] and commercial column literature [6]. An effort was made to provide a simple, easy to evaluate test with a limited number of components. Component concentrations were adjusted to facilitate identification. This test is not intended for column classification as “good” or “bad”; however, columns that exhibit certain properties may be more suitable for a given application than others.

Separations of SRM 870 were carried out on a variety of previously unused LC columns (see Appendix A). The selected columns are intended to represent a broad sampling of currently available columns. Retention data for the columns are listed in Table 2.

Test Conditions: The influence of chromatographic conditions on test results was examined for several different parameters. Relative changes in retention have been evaluated in reference [4] for pH, temperature, buffer concentration, and mobile phase composition. Because retention, efficiency, and peak shape are influenced by testing conditions, column evaluation should be carried out under standardized conditions to facilitate column comparisons. The largest changes in retention behavior occur with changes in the mobile phase composition. As specified in the “Instructions for Use” section of this certificate, the composition of the mobile phase is 80 % methanol and 20 % buffer, where the buffer composition is 5 mmol/L potassium phosphate adjusted to pH 7. The retention of quinizarin and amitriptyline is strongly dependent on the pH of the potassium phosphate buffer solution (see Figure 4). The retention of quinizarin is reduced at high pH, whereas the retention of amitriptyline is reduced at low pH. At pH 7, both solutes exhibit significant retention. The ionic strength of the buffer is less significant. Only slight changes in retention, efficiency, and peak asymmetry are measurable with changes on the phosphate buffer concentration at pH 7. The presence of the buffer is essential, however. At levels below 1 mmol/L (buffer concentration before dilution with methanol), A_s and k' increase dramatically for amitriptyline. The absolute retention of the polar and nonpolar components increase with the percentage of buffer in the mobile phase (at pH 7, and constant ionic strength in the mixed solution). A composition of 80 % methanol and 20 % buffer was selected to provide appropriate retention for a broad range of column types.

Injection volume can also significantly influence test results (see Figure 5). Separation efficiency typically decreases with increased injection volume. Injection overload results in degraded peak shape and in some instances, reduced retention. An injection volume of 5 μ L is recommended.

Changes in column temperature strongly influence the absolute retention of the components in SRM 870; however, relatively small effects are observed in the peak shape of quinizarin or amitriptyline. It is recommended that column temperature be controlled to $23\text{ }^\circ\text{C} \pm 2\text{ }^\circ\text{C}$.

Column Comparisons: The data in Table 2 exemplify the range in retention properties that exist among commercial LC columns. The first eight columns exhibit unusual retention behavior (i.e., quinizarin elutes last, see Figure 2A), and these columns are grouped separately from the remaining columns. The data within these groups is listed in order of increasing peak asymmetry for amitriptyline. No two columns exhibit identical retention behavior; however, similarities do exist among several columns. Among columns tested, values of k' for ethylbenzene ranged from 0.2 to 2.8. In contrast, only slight differences were observed for methylene selectivity ($a_{E/T}$; range, 1.26 to 1.45). The retention of quinizarin ranged from $k' = 1$ to $k' = 23.6$. In two instances, no elution of this compound was detected. Peak asymmetry values ranged from $A_s = 1.1$ to $A_s = 5.7$ (peaks were not defined well enough in two instances to permit determination of A_s). Finally, the retention of amitriptyline ranged from $k' = 1.4$ to $k' = 72.9$ ($A_s = 1.0$ to $A_s = 11$).

Figure 2 illustrates typical elution patterns for SRM 870. Five of the columns tested are known to utilize embedded polar functional groups within the stationary phase to improve chromatographic performance toward bases (these columns are listed first in Table 2). The separation of SRM 870 was similar for these columns. In each case, quinizarin eluted last, and both amitriptyline and quinizarin exhibited symmetrical peak shape (e.g., Figure 2A).

Peak asymmetry data for quinizarin and amitriptylin are plotted in Figure 6. The scatter in the data indicates independence of the two terms. Thus, it is possible for a column to exhibit high activity toward chelating agents and low activity toward bases, or other combinations (e.g., Figures 2C through 2F).

REFERENCES

- [1] Snyder, L.R. and Kirkland, J.J., "Introduction to Modern Liquid Chromatography," 2nd edition, New York, Wiley-Interscience, (1979).
- [2] Sander, L.C. and Wise, S.A., "SRM 869a Column Selectivity Test Mixture for Liquid Chromatography Polycyclic Aromatic Hydrocarbons," Certificate of Analysis, NIST, Gaithersburg, MD (1998).
- [3] Phinney, K.W. and Sander, L.C., "SRM 877 Chiral Selectivity Test Mixture for Liquid Chromatography," Certificate of Analysis, NIST, Gaithersburg, MD (2000).
- [4] Neue, U.D., Serowik, E., Iraneta, P., Alden, B.A., and Walter, T.H., "Universal Procedure for the Assessment of the Reproducibility and the Classification of Silica-Based Reversed-Phase Packings I. Assessment of the Reproducibility of Reversed-Phase Packings," *J. Chromatogr. A*, **849**, pp. 87-100 (2000).
- [5] Engelhardt, H., Arangio, M., and Lobert, T., "A Chromatographic Test Procedure for Reversed-Phase HPLC Column Evaluation," *LC GC*, **15**, pp. 856-866 (1997).
- [6] Nacalai Tesque, Inc., "Product Catalog," Kyoto, Japan (1998).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet <http://www.nist.gov/srm>

Table 1. Information Value Mass Fractions and Relative Areas for Components in SRM 870

Component	CAS Number ^a	Property Evaluated	Source	Lot	Purity ^{b,c}	Mass Fraction ^b μg/g	Relative Area ^b 254 nm	Relative Area ^b 210 nm	Relative Area ^b 480 nm
Methanol	67-56-1		J. T. Baker	L30330	-				
Uracil	66-22-8	void volume marker	Aldrich	MS15011BS	98	28	0.02	0.00	
Toluene	108-88-3	hydrophobic retention, efficiency	Burdick and Jackson	AH700	-	1400	0.02	0.18	
Ethylbenzene	100-41-4	methylene selectivity, hydrophobic retention, efficiency	Aldrich	PS10785MS	99.8	1700	0.03	0.20	
Quinizarin	81-64-1	activity toward chelators	Aldrich	03116HS	97.9	94	0.10	0.01	1.00
Amitriptyline	549-18-8	activity toward bases	Sigma	48H0468	99.6	2800	0.83	0.61	

^aChemical Abstract Registry Number

^bData are provided for information only as an aid in peak identification, and are not to be used for quantification purposes.

^cPurity data provided by the manufacturer as % mass fraction.

Table 2. Retention, Efficiency, and Peak Asymmetry Data for Selected Commercial C₁₈ Columns

Column	Retention Time, uracil (min)	k' toluene	Theoretical Plates Ethylbenzene ^a	k' ethylbenzene	Asymmetry quinizarin ^b	k' quinizarin	Asymmetry amitriptyline ^b	k' amitriptyline
1 Xterra RP-18	0.86	0.76	3490	0.97	1.12	2.86	1.12	1.68
2 Suplex pKb	1.42	0.60	12800	0.80	1.23	3.67	1.20	1.40
3 Bonus C18	1.32	0.78	10900	1.04	1.63	2.70	1.21	1.97
4 Supelcosil ABZ+plus	1.37	0.74	8320	0.98	2.11	4.28	1.28	1.77
5 OmniSpher 5 C18	1.27	1.77	13200	2.53	2.33	7.16	1.31	5.81
6 Symmetry shield RP18	0.80	1.13	7660	1.49	1.11	5.35	1.56	2.99
7 Symmetry C18	0.75	1.56	7550	2.20	1.31	5.48	2.07	5.19
8 Nucleosil C18 AB	1.23	1.24	7170	1.74	3.21	7.33	4.89	4.18
9 ACE C18	1.41	1.13	12700	1.59	1.07	4.01	1.03	3.93
10 Hypurity Elite C18	1.54	0.78	14800	1.09	1.09	2.70	1.61 ^c	2.63
11 Inertsil ODS-3	1.40	1.92	9660	2.71	1.24	6.35	1.26	6.72
12 Partisil ODS-1	1.74	0.17		0.22	3.61	0.97	1.45	8.91
13 LUNA C18 (2)	1.33	1.44	13800	2.05	1.46	4.17	1.52	5.40
14 Kromasil C18	1.23	1.94	7850	2.81	3.75	7.32	1.59	7.03
15 Discovery C18	1.60	0.73	11700	1.04	1.37	2.50	1.78	2.54
16 Spherisorb ODS-1	1.41	0.63	11200	0.83	2.27	23.6	1.88	34.2
17 Inertsil ODS-2	1.37	1.37	6850	1.92	1.27	5.10	1.93	5.09
18 Nucleosil protect I	1.54	0.46	8450	0.58	2.02	1.55	2.16	1.63
19 Hypersil BDS-C18	1.35	0.94	10800	1.32	1.26	3.28	2.21	3.07
20 Partisil ODS-3	1.52	0.87	1340	1.20	5.68	4.18	2.43	7.61
21 Hydrobond PSC18	1.47	1.29	13800	1.86	1.47	4.19	2.48	5.94
22 Partisil ODS-2	1.42	0.70	8060	0.94	3.07	9.88	2.74	35.8

23	Novapak C18	1.12	0.90	5960	1.28	2.42	3.16	3.05	4.43
24	Eclipse XDB-C18	1.29	1.02	11000	1.48	1.07	2.99	3.05	4.48
25	Stablebond C18	1.26	1.14	10200	1.63	1.43	3.11	3.07	6.16
26	Selectapore 90M (Vydac 201sp54)	1.52	0.64	12200	0.87	3.20	1.83	3.10	2.86
27	Nucleosil C18	1.50	1.19	6350	1.64	2.41	5.16	3.19	15.4
28	Selectapore 300M (Vydac 238wp54)	1.50	0.29	9050	0.40	1.34	1.00	3.65	1.77
29	Prontosil 120-5-C18-SH	1.38	1.44	14200	2.03	1.17	5.34	3.81	9.33
30	Capcell Pak SG C18	1.41	1.02	10700	1.42	1.09	2.82	4.51	3.33
31	Lichrosorb RP-18	1.49	1.24	9180	1.72	3.30	7.60	4.89	16.4
32	Hypersil PAH	1.32	0.85	6000	1.15	2.63	7.54	5.22	9.08
33	Lichrospher RP-18	1.34	1.76	10200	2.46	3.66	11.5	5.63	13.3
34	Resolve C18	1.21	1.29	9220	1.83	2.83	9.43	6.32	72.9
35	Selectapore 300P (Vydac 218wp54)	1.42	0.35	8480	0.48	1.44	2.51	6.50	3.12
36	Vydac 201TP54	1.44	0.46	6760	0.63	1.68	3.35	7.58	5.20
37	Hypersil C18	1.35	0.91	10300	1.28		2.57	7.85	4.05
38	Cosmosil C18 AR-II	1.39	1.59	9300	2.20	1.16	7.39	8.39	8.49
39	Spherisorb ODS-2	1.31	1.32	12100	1.85			8.92	15.3
40	μBondapak C18	1.45	0.71	6210	0.97	2.56	2.19	8.99	7.81
41	Zorbax classic ODS	1.21	1.89	11200	2.68			11.0	17.8

^a Column efficiency (theoretical plates) was calculated as $N = 5.54 (t_r / w_{1/2})^2$, where t_r is the retention time and $w_{1/2}$ is the peak width at 50 % of the peak height.

^b Peak asymmetry was calculated using the following equation: $A_s = (w_r + w_l) / (2w_i)$, where w_r and w_l are the right and left peak widths relative to a perpendicular drawn through the peak maximum, determined at 5 % of the peak height.

^c Peak coelution; value estimated.

APPENDIX A. Column Identification

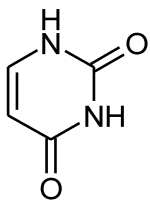
Column	Configuration (i.d. x length; mm)	Particle Size (μm)	Serial Number	Manufacturer	Distributor
1 Xterra RP-18	4.6 x 150	5	M90571D01	Waters Corporation	Waters Corp., Milford, MA
2 Suplex pKb	4.6 x 250	5	18431-03	Supelco	Supelco, Inc., Bellefonte, PA
3 Bonus C18	4.6 x 250	5	AC1208	Mac Mod Analytical, Inc.	Mac Mod Analytical, Inc., Chadds Ford, PA
4 Supelcosil ABZ+plus	4.6 x 250	5	17908-01	Supelco	Supelco, Inc., Bellefonte, PA
5 OmniSpher 5 C18	4.6 x 250	5	839013	Varian: Chrompack International B.V.	Varian: Chrompack International B.V., The Netherlands
6 Symmetry Shield RP18	4.6 x 150	5	T90082	Waters Corporation	Waters Corp., Milford, MA
7 Symmetry C18	4.6 x 150	5	T80781	Waters Corporation	Waters Corp., Milford, MA
8 Nucleosil C18 AB	4.6 x 250	5	9042102	Macherey-Nagel GmbH & Co.	Macherey-Nagel, Inc., Easton, PA
9 ACE C18	4.6 x 250	5	A1352	Advanced Chromatography Technologies	Mac Mod Analytical, Inc., Chadds Ford, PA
10 Hypurity Elite C18	4.6 x 250	5	22105-060	Hypersil	Phenomenex, Torrance, CA
11 Inertsil ODS-3	4.6 x 250	5	5020-01732	GL Sciences, Inc.	Phenomenex, Torrance, CA
12 Partisil ODS-1	4.6 x 250	10	4450191621	Whatman	Waters Corp., Milford, MA
13 LUNA C18 (2)	4.6 x 250	5	326184	Phenomenex	Phenomenex, Torrance, CA
14 Kromasil C18	4.6 x 250	5	99080093	Eka Nobel	Phenomenex, Torrance, CA
15 Discovery C18	4.6 x 250	5	18915-06	Supelco	Supelco, Inc., Bellefonte, PA
16 Spherisorb ODS-1	4.6 x 250	5	105391861	Waters Corporation	Waters Corp., Milford, MA
17 Inertsil ODS-2	4.6 x 250	5	326186	GL Sciences, Inc.	Phenomenex, Torrance, CA
18 Nucleosil protect I	4.6 x 250	5	8061092	Macherey-Nagel GmbH & Co.	Macherey-Nagel, Inc., Easton, PA
19 Hypersil BDS-C18	4.6 x 250	5	99061859	Hypersil	Supelco, Inc., Bellefonte, PA
20 Partisil ODS-3	4.6 x 250	10	2830192301	Whatman	Waters Corp., Milford, MA
21 Hydrobond PSC18	4.6 x 250	5	923692	Mac Mod Analytical, Inc.	Mac Mod Analytical, Inc., Chadds Ford, PA
22 Partisil ODS-2	4.6 x 250	10	5400192521	Whatman	Waters Corp., Milford, MA
23 Novapak C18	3.9 x 300	4	W91791F-14	Waters Corporation	Waters Corp., Milford, MA
24 Eclipse XDB-C18	4.6 x 250	5	NH-1069	Mac Mod Analytical, Inc.	Mac Mod Analytical, Inc., Chadds Ford, PA
25 Stablebond C18	4.6 x 250	5	CL6914	Mac Mod Analytical, Inc.	Mac Mod Analytical, Inc., Chadds Ford, PA

Column	Configuration (i.d. x length; mm)	Particle Size (µm)	Serial Number	Manufacturer	Distributor
26 Selectapore 90M (Vydac 201sp54)	4.6 x 250	5	905550-1-3 #016	The Separations Group	The Separations Group, Hesperia, CA
27 Nucleosil C18	4.6 x 250	5	99050471	Macherey-Nagel GmbH & Co.	Supelco, Inc., Bellefonte, PA
28 Selectapore 300M (Vydac 238wp54)	4.6 x 250	5	E970520-8-5 #014	The Separations Group	The Separations Group, Hesperia, CA
29 ProntoSIL 120-5-C18-SH	4.6 x 250	5	02029D03	Bischoff Chromatography	Mac Mod Analytical, Inc., Chadds Ford, PA
30 Capcell Pak SG C18	4.6 x 250	5	A*AD8670	Shiseido	Phenomenex, Torrance, CA
31 Lichrosorb RP-18	4.6 x 250	5	99040252	EM Separations Technology	Supelco, Inc., Bellefonte, PA
32 Hypersil PAH	4.6 x 250	5	1071132R	Hypersil	Keystone Scientific, Inc., Bellefonte, PA
33 Lichrospher RP-18	4.6 x 250	5	99070182	EM Separations Technology	Supelco, Inc., Bellefonte, PA
34 Resolve C18	3.9 x 300	5	T92001E-03	Waters Corporation	Waters Corp., Milford, MA
35 Selectapore 300P (Vydac 218wp54)	4.6 x 250	5	E970520-9-5 #033	The Separations Group	The Separations Group, Hesperia, CA
36 Vydac 201TP54	4.6 x 250	5	E970225-8-6 #189	The Separations Group	The Separations Group, Hesperia, CA
37 Hypersil C18	4.6 x 250	5	326185	Hypersil	Phenomenex, Torrance, CA
38 Cosmosil C18 AR-II	4.6 x 250	5	KS0686	Nacalai Tesque	Phenomenex, Torrance, CA
39 Spherisorb ODS-2	4.6 x 250	5	123391941	Waters Corporation	Waters Corp., Milford, MA
40 :Bondapak C18	3.9 x 300	10	W91941A-030	Waters Corporation	Waters Corp., Milford, MA
41 Zorbax classic ODS	4.6 x 250	5	F52911	Mac Mod Analytical, Inc.	Mac Mod Analytical, Inc., Chadds Ford, PA

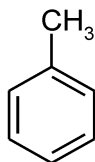
APPENDIX B. Participants

The following individuals and organizations participated in the development and evaluation of SRM 870:

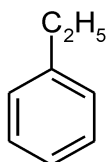
G. Boone, Varian, Inc., Harbor City, CA
J. DeStefano, Hewlett-Packard Co., Newport, DE
K. Harrison, The Separations Group, Inc., Hesperia, CA
R. Henry, Keystone Scientific, Inc., Bellefonte, PA
J. Higgins, Higgins Analytical, Mountain View, CA
B. Hornbake, Macherey-Nagel, Eastin, PA
J. Lamb, Hypersil, Astmoor, Runcorn, England
U. Neue, Waters, Milford, MA
M. Przybyciel, ES Industries, Marlton, NJ
M. Woelk, MetaChem Technologies, Inc., Torrance, CA
V. Yearick, Supelco, Inc., Bellefonte, PA
C. Young, R. Weigand, Alltech Associates, Inc., Deerfield, IL
K. Zimmerman, Mac-Mod Analytical, Inc., Chadds Ford, PA



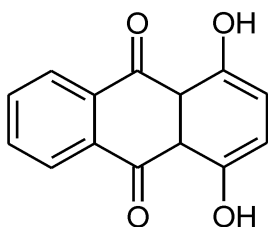
uracil - void volume marker



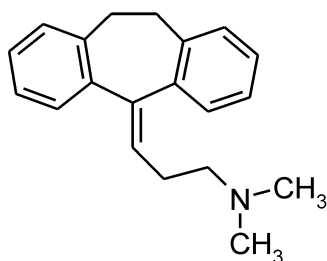
toluene - hydrophobic retention



ethylbenzene - methylene selectivity



quinizarin - activity towards chelating reagents



amitriptyline - activity towards bases

Figure 1. Structures and properties evaluated for components in SRM 870

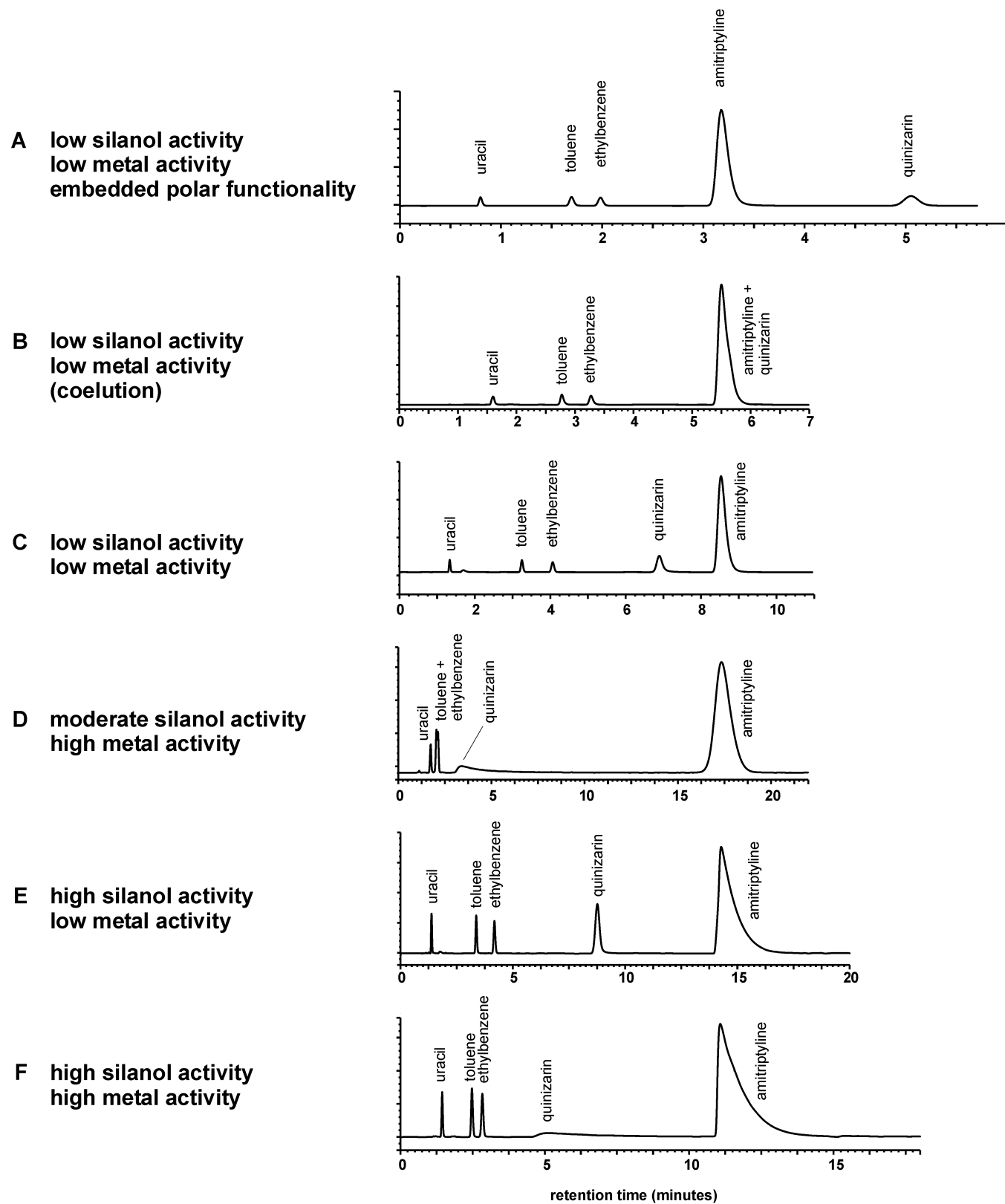


Figure 2. Examples of separations of SRM 870 on commercial C₁₈ columns

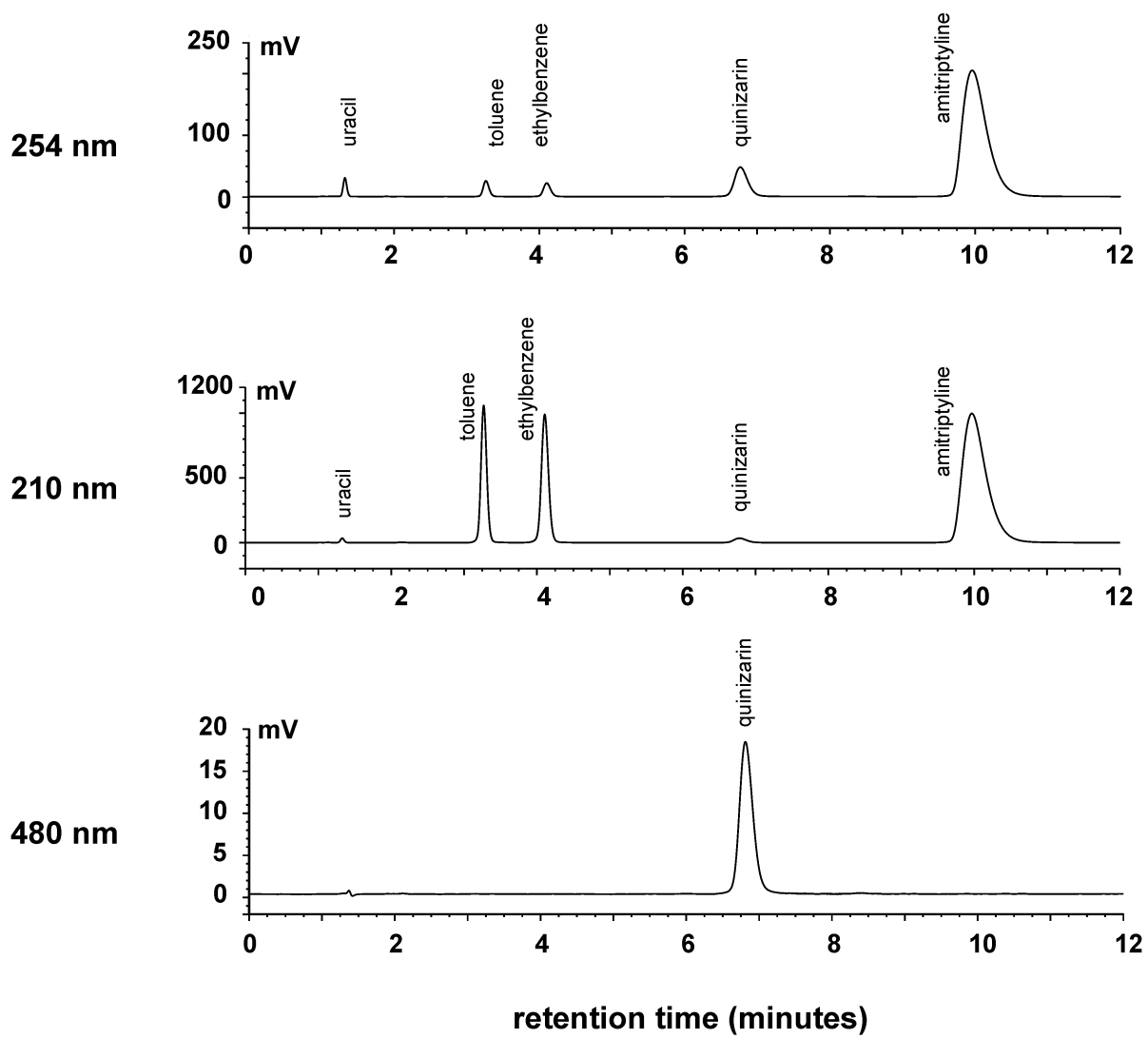


Figure 3. Separations of SRM 870 with detection at 254 nm, 210 nm, and 480 nm

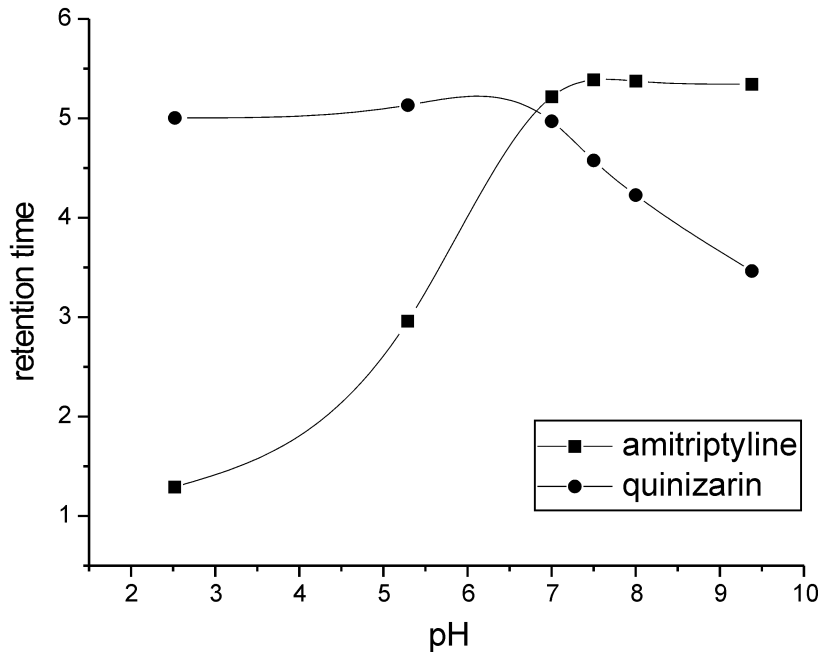


Figure 4. Plot of retention vs. pH for amitriptyline and quinizarin

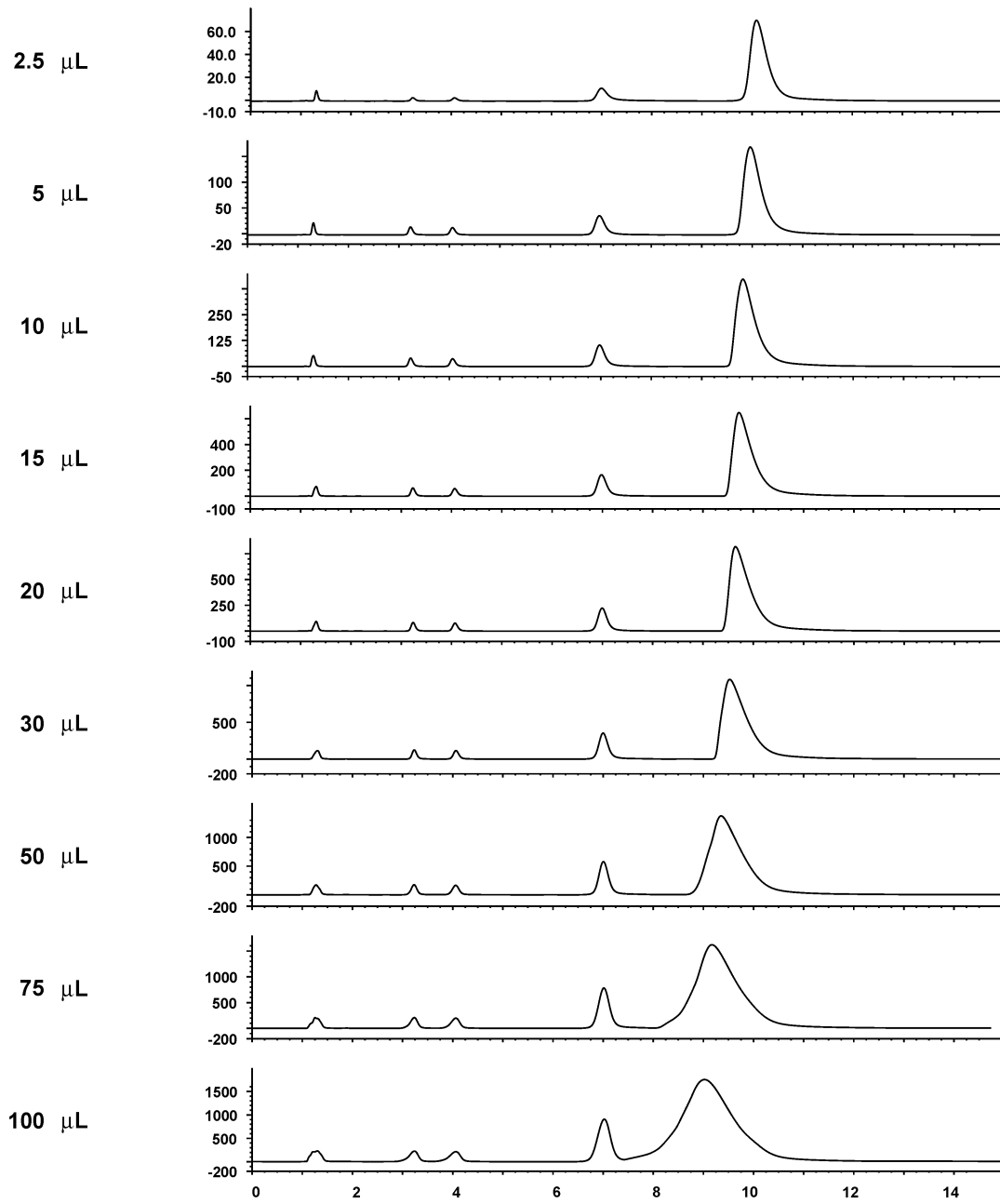


Figure 5. Separations of SRM 870 for different injection volumes

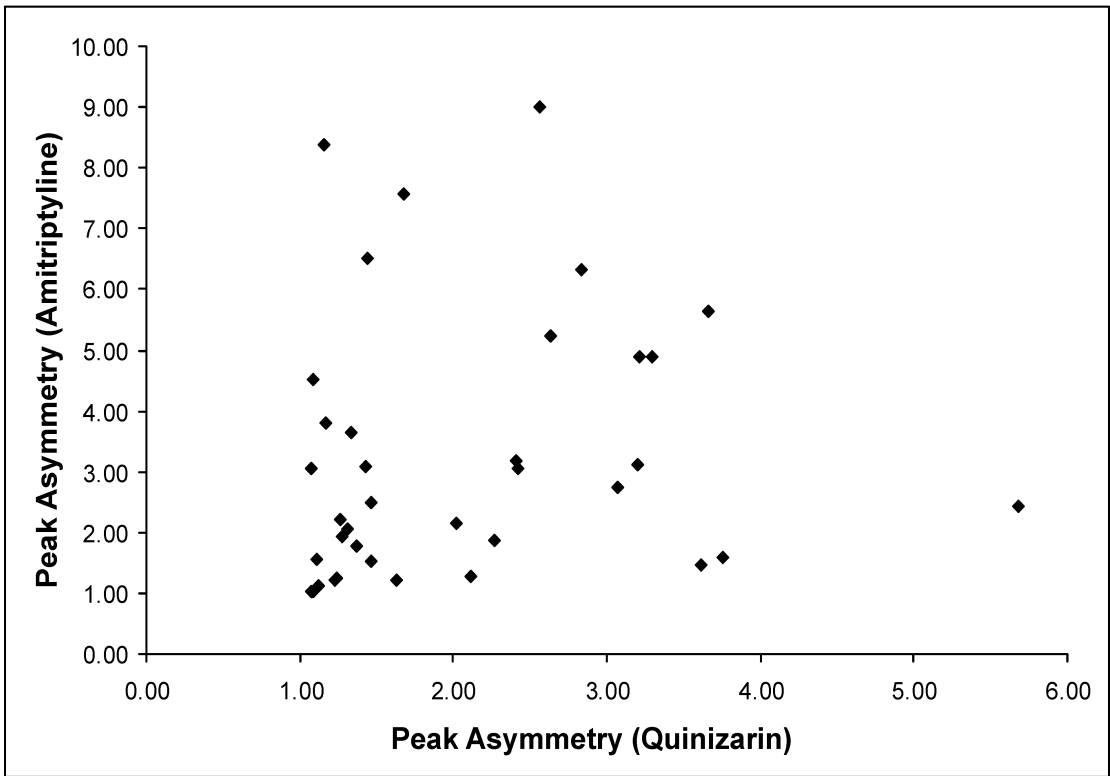


Figure 6. Plot of peak asymmetry for amitriptyline vs. peak asymmetry for quinizarin for the C₁₈ columns listed in Table 2