National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 968b

Fat-Soluble Vitamins and Cholesterol in Human Serum

Standard Reference Material (SRM) 968b is intended for use in validating methods for determining fat-soluble vitamins, carotenoids, and cholesterol in human serum and plasma. This SRM can also be used for assigning values to in-house control materials for those constituents. Certified values are provided for retinol, retinyl palmitate, α-tocopherol, *trans*-ß-carotene, total ß-carotene (*trans* plus *cis* isomers), total α-carotene, lutein, and cholesterol in each of the three levels. Noncertified values are also provided for γ-tocopherol (includes ß-tocopherol), δ-tocopherol, zeaxanthin, ß-cryptoxanthin, *trans*-lycopene, *trans*-α-carotene, total lycopene, 9-*cis*-ß-carotene, 13- plus 15-*cis*-ß-carotene, and 15-*cis*-ß-carotene. A unit of SRM 968b consists of three vials of lyophilized human serum, one each of three concentration levels. The lyophilized serum in each vial must be reconstituted with 1.00 mL of HPLC-grade water before use.

Certified Concentration Values: The certified concentration values of retinol, retinyl palmitate, α-tocopherol, cholesterol, and four carotenoids in reconstituted SRM 968b are provided in Table 1. Certified values are based on the results obtained with NIST definitive methods and/or the concordant results from two or more different methods. The certified concentration values for the fat-soluble vitamins and carotenoids were derived from the agreement of results from three different liquid chromatography (LC) procedures developed at NIST and from an interlaboratory comparison exercise among institutions that participate in the NIST/National Cancer Institute (NCI) Micronutrients Measurement Quality Assurance (QA) Program. An alphabetized listing of these institutions and the analytes that they measured is provided in Appendix A.

The certified values for the vitamins and carotenoids listed in Table 1 are the equally weighted means of the combined results from at least three of the analytical methods used to provide measurements for the SRM. The certified values for cholesterol listed in Table 1 were determined from measurements using the NIST definitive method, gas chromatography-isotope dilution mass spectrometry (GC-IDMS). The uncertainties listed are expanded uncertainties [1] at the 95 % level of confidence, and with the certified values, define the ranges in which the true concentrations are expected to fall for each vial. (**Note:** The certified and noncertified concentration values apply to measurements performed after the serum has been reconstituted using the procedure described in "Instructions for Use.")

Noncertified Concentration Values: Values for *trans*-α-carotene, *trans*-lycopene and total lycopene, γ-tocopherol, δ-tocopherol, zeaxanthin, β-cryptoxanthin, 9-*cis*-ß-carotene, 13- plus 15-*cis*-ß-carotene, and 15-*cis*-ß-carotene are provided in Table 2 as noncertified values. These values are provided for information only because of the greater disagreement of the results among methods or because they are derived from a limited number of LC analyses performed by NIST and the collaborating laboratories.

The overall direction and coordination of the preparation and technical measurements leading to the certification of this SRM were performed by J.M. Brown Thomas, S.A. Wise, and W.E. May of the NIST Analytical Chemistry Division.

Preparation of SRM 968b and analytical measurements at NIST were performed by J.M. Brown Thomas, K.E. Sharpless, P.M. Ellerbe, and L.T. Sniegoski of the NIST Analytical Chemistry Division, and M.C. Kline of the NIST Biotechnology Division. Collaborating laboratories that performed analytical measurements are listed in Appendix A.

The technical and support aspects involved with the certification and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

Gaithersburg, MD 20899
August 7, 1995

Thomas E. Gills, Chief
Standard Reference Materials Program

Statistical consultation was provided by S.B. Schiller of the NIST Statistical Engineering Division and D.L. Duewer of the NIST Analytical Chemistry Division.

The certification of this SRM was supported in part by the Division of Cancer Prevention and Control, NCI.

NOTICE AND WARNING TO USERS

Warning: The supplier of this serum has reported that the source materials used to prepare this product were found to be nonreactive when tested for Hepatitis B Surface Antigen (HB_sAg) and for human immunodeficiency virus (HIV) by Food and Drug Administration required tests. However, because no test method can offer complete assurance that HIV, Hepatitis B virus, or other infectious agents are absent, this SRM should be handled at the Biosafety Level 2 for any potentially infectious human serum or blood specimen, as recommended in the Center for Disease Control/National Institutes of Health Manual [2].

Storage: Until required for use, SRM 968b should be stored in the dark at a temperature between -20 °C and -80 °C. If carotenoids are to be measured, the unit should be stored at or below -70 °C in the dark. Carotenoids appear less stable than the retinoids and the tocopherols at -20 °C [3-6].

Expiration of Certification: When the SRM is stored properly, this certification is valid, within the specified uncertainty limits, for up to two years from the date of shipment from NIST. In the event that the certification becomes invalid prior to that time, users will be notified by NIST. Return of the attached registration form will facilitate notification.

Instructions for Use: SRM 968b is provided as a set of three vials of lyophilized serum, one each of three concentration levels with respect to retinol, α-tocopherol, and β-carotene, and must be reconstituted prior to use. Before reconstitution, the vials should be allowed to stand at room temperature for at least 30 min. At room temperature, the vials appear to be at slight positive pressure, therefore, it is recommended that prior to opening, the vials be vented by insertion of an empty syringe needle. To achieve the certified concentrations, the freeze-dried serum must be reconstituted with 1.00 mL of HPLC-grade water. Dissolution should be facilitated by ultrasonic agitation for 3 min to 5 min or by intermittent swirling for at least 15 min. Vigorous shaking or mechanical swirling should be avoided as it may cause the formation of foam. After reconstitution, the contents should be used immediately or stored at -20 °C for no more than three days with minimal (less than five) freeze/thaw cycles [6]. Precautions should be taken to avoid exposure to strong UV light and direct sunlight.

PREPARATION AND ANALYSIS

Preparation of SRM Serum Pools

Plasma used to prepare the three pools (1.9 L each) of materials comprising SRM 968b was obtained from Interstate Blood Bank Inc. (IBB), Memphis, TN. Several serum subpools were obtained from the blood of donors whose diets had been supplemented with β-carotene. After collection of the plasma at IBB, it was shipped on dry ice to NIST and stored at -80 °C. Aliquots from each of the sub-pools were analyzed by LC to provide values for blending the pools.

Prior to blending, the plasma units were removed from storage at -80 °C, placed in a water bath at about 25 °C until thawed, and filtered through a microfiber filter under vacuum to remove the fibrin. Serum was selected for blending based on the β-carotene level in the pool. Stripped serum (serum that had been sterilized and filtered to remove lipids and fibrin) was obtained from Western States Plasma, Fallbrook, CA, and used as a diluent during the blending process. The desired levels of *trans*-retinol, γ-tocopherol, α-tocopherol, and retinyl palmitate were achieved by supplementing the levels naturally present through the addition of concentrated ethanolic spiking solutions. The concentrations of the spiking solutions were based on target values relative to the physiological levels of the analytes in human serum and were determined by spectrophotometry. The purity of the solutions was determined by LC at the wavelength of maximum absorption.

After spiking, the plasma-converted serum pools were stirred for 30 min and subsequently stored at 4 °C overnight. The serum was removed from storage the next day and stirred again at room temperature for 3 h. The serum pools were filtered once again through a microfiber filter with glass-fiber prefilters. The filtered serum was mixed by swirling and inversion, divided in two 1-liter bottles, and stored at -20 °C until aliquoted for freeze-drying.

Two freeze-drying runs per serum pool were required. Each unit of serum was thawed at 25 °C and filtered as previously described. Aliquots (1 mL each) of serum from each unit were dispensed into 3.5-milliliter serum vials using a calibrated air-displacement pipettor. The vials were then frozen at -35 °C prior to the application of vacuum to start freeze-drying. Each vial was consecutively numbered and color-coded. The average time in the freeze-dryer was 48 h for all samples. Prior to removal from the freeze-drying chamber, the vials were sealed under slight vacuum with argon (at 15 °C). After removal from the freeze-dryer, the vials were crimped, selected for analysis according to a stratified random sampling scheme, and stored at -80 °C.

Description of Analytical Measurements Used for Value Assignment

A. Measurement of the Fat-Soluble Vitamins and Carotenoids in SRM 968b

The certified values for selected fat-soluble vitamins and carotenoids in this SRM were derived from the agreement of results from analyses performed by NIST and 40 collaborating institutions (listed in Appendix A). Since the maintenance of pure and stable primary reference compounds for the analytes measured in this SRM is difficult, detector responses were calibrated against solutions whose concentrations were determined by spectrophotometry. NIST analyses were based on the absorptivities provided in Figure 1. Three different LC techniques (chromatograms shown in Figure 2) were used at NIST for the determination of the fat-soluble vitamins and carotenoids in the SRM [7-10]. Details of the three LC methods are provided below. (**Note:** The composition of the solvent mixtures described in these methods is volume fraction expressed in percent. [11])

Reversed-Phase LC using a Polymeric C_{18} Phase

The measurement of retinol, α-tocopherol, γ-tocopherol, *trans*-β-carotene, total α-carotene, and total β-carotene using a 5-micrometer polymeric [12] C_{18} stationary phase column was based on the analysis of one extract from each of six vials per level, injected in duplicate. A binary solvent mixture was used in this method. Solvent A was 60 % methanol/10 % butanol/30 % water. Solvent B was 89.5 % methanol/10 % butanol/0.5 % water. A 40-minute gradient elution program of 75 % solvent A to 90 % solvent B with a 5 min hold of 75 % solvent A was used to sequentially determine these analytes in the SRM. UV/visible detection using a deuterium lamp at the following wavelengths was used: 325 nm for retinol, 292 nm for γ-tocopherol and α-tocopherol, and 452 nm for α-carotene and β-carotene. Retinyl palmitate could not be measured simultaneously using this LC protocol because of co-elution with β-carotene isomers. Therefore, a linear gradient of 25 % of the initial solvent mixture to 100 % of the final solvent mixture in 20 min with UV/visible detection at 325 nm was used for its determination. Tocol (the internal standard) was monitored at 292 nm in both procedures.

Reversed-Phase LC using an Intermediate C_{18} Phase

Six major carotenoids, retinol, retinyl palmitate, δ-tocopherol, γ-tocopherol, and α-tocopherol, as well as total lycopene, total α-carotene, and total β-carotene were measured in two extracts from each of 24 vials of each level of the SRM using an intermediate [12] C_{18} stationary phase column. A ternary solvent method was used to isolate the analytes from the serum extract. Solvent A was acetonitrile, solvent B was methanol containing 0.05 mol/L ammonium acetate, and solvent C was ethyl acetate. Each of the three solvents contained 0.05 % triethylamine (TEA) to enhance carotenoid recovery [8]. The method consisted of two linear gradients and an isocratic component. The first gradient ran from 98 % solvent A/2 % solvent B to 75 % solvent A/18 % solvent B/7 % solvent C in 10 min. A second linear gradient ran from the first gradient composition to 68 % solvent A/25 % solvent B/7 % solvent C in 5 min. The second linear gradient composition was held for 10 min longer, then the system was returned to initial conditions of 98 % solvent A/2 % solvent B over 5 min and re-equilibrated for 10 min. In this method, a programmable UV/visible detector with

a tungsten lamp was used for measurement of the retinoids and the carotenoids at 325 nm and 450 nm, respectively. *Trans*-β-apo-10'-carotenal oxime [13,14] was used as the internal standard for the quantification of the retinoids and carotenoids in this procedure. A fluorescence spectrometer was used to measure the tocopherols and tocol (the internal standard) using an excitation wavelength of 295 nm and an emission wavelength of 335 nm. Signals from both detectors were recorded simultaneously. Retinyl palmitate in the SRM was measured separately using the described chromatographic conditions with absorbance detection at 325 nm.

This method was also used to determine the homogeneity of the SRM material. Statistically significant vial-to-vial variability was detected. The average relative vial-to-vial standard deviations were 3.9 %, 1.4 %, and 1.9 % for the high, middle, and low levels of the SRM, respectively.

Reversed-Phase LC using a Polymeric C_{30} Phase

Measurement of lutein, zeaxanthin, β-cryptoxanthin, lycopene, *trans*-α-carotene, *trans*-β-carotene, 9-*cis*-ß-carotene, 13-*cis*-ß-carotene, and 15-*cis*-β-carotene was provided using a NIST-engineered polymeric C_{30} carotenoid column [15,16]. This column was selected for use in this certification exercise because of the resolution of the β-carotene isomers, and to provide values for the xanthophylls, i.e., lutein, zeaxanthin, and β-cryptoxanthin, for comparison with values obtained from the analyses previously described using the intermediate C_{18} column. In this method, one extract from each of eight vials of each level of the SRM was analyzed. The method consisted of two linear gradients and an isocratic component. Solvent A was 8 % water/92 % methanol containing 0.05 mol/L ammonium acetate and 0.05 % TEA; solvent B was methyl *tert*-butyl ether. The initial solvent composition of the first gradient was 83 % solvent A/17 % solvent B and ran to 59 % solvent A/41% solvent B in 29 min. The second linear gradient ran from this composition to 30 % solvent A/70 % solvent B in 5 min. The final composition was held for 4 min longer to allow the elution of lycopene, then the system was returned to initial conditions (83 % solvent A/17 % solvent B) over 5 min and re-equilibrated for 10 min. A programmable UV/visible detector with a tungsten lamp was used for measurement of the carotenoids at 450 nm. *Trans*-β-apo-10'-carotenal oxime was also used as the internal standard for the quantifica tion of the carotenoids in this method [13,14].

Interlaboratory Methods used for the Analysis of SRM 968b

The fat-soluble vitamins and carotenoids in SRM 968b were measured by 40 collaborating institutions that participated in an interlaboratory comparison exercise in which blind samples of the SRM were distributed as part of the NIST/NCI Micronutrients Measurement QA Program. Analyses typically involved precipitation of serum proteins with ethanol followed by extraction of the supernatant with a lipophilic solvent (e.g., hexane or petroleum ether). The extracts were then analyzed by LC using various stationary phase-mobile phase combinations, detectors, and internal standards. Detector responses were calibrated against solutions whose concentrations were determined using spectrophotometry.

B. Measurement of Cholesterol in SRM 968b

Cholesterol concentrations were determined using the NIST GC-IDMS definitive method [17,18]. Two sets of samples, each consisting of three vials from each of the three levels of the SRM, were randomly selected for analysis according to a statistical sampling scheme. An aliquot from each vial was reconstituted with 1.00 mL of HPLC-grade water and prepared using a previously established procedure that employs hydrolysis of cholesterol esters using potassium hydroxide in ethanol, followed by extraction with hexane, and derivatization of cholesterol using *bis*(trimethylsilyl)acetamide [18]. Cholesterol-$^{13}C_3$ was used as the internal standard. Quantitation of cholesterol in each sample was achieved by injecting the sample between measurements of two standards whose unlabeled/labeled ratios bracket that of the sample. The standards were weighed mixtures of SRM 911b, Cholesterol and cholesterol-$^{13}C_3$.

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[19] Certain commercial equipment is identified in this certificate to adequately describe 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 equipment identified is necessarily the best available for this purpose.

Table 1. Certified Concentration Values of Cholesterol, Fat-Soluble Vitamins, and Carotenoids in SRM 968b

The certified values for vitamins and carotenoids are equally weighted means of results from at least three analytical techniques [10]. The certified values for cholesterol are derived from measurements from two sets of samples, each consisting of three vials from each of the three levels of the SRM. Each uncertainty is an expanded uncertainty [1], whose level of confidence is 95 %. It includes random sources of uncertainty within each analytical method as well as vial-to-vial variability.

Analyte	Low Level		Middle Level		High Level	
	μg/mL	*μmol/L*	*μg/mL*	*μmol/L*	*μg/mL*	*μmol/L*
Retinol	0.296 ± 0.013	1.033 ± 0.045	0.514 ± 0.017	1.794 ± 0.059	0.892 ± 0.088	3.11 ± 0.31
Retinyl palmitate[a]	0.099 ± 0.010	0.189 ± 0.019	0.178 ± 0.026	0.339 ± 0.050	0.246 ± 0.034	0.469 ± 0.065
α-Tocopherol	7.07 ± 0.50	16.4 ± 1.2	10.11 ± 0.58	23.5 ± 1.4	17.8 ± 1.3	41.4 ± 3.0
trans-β-Carotene	0.224 ± 0.012	0.417 ± 0.022	0.569 ± 0.029	1.06 ± 0.054	1.13 ± 0.10	2.10 ± 0.19
Total β-Carotene[a,b]	0.253 ± 0.020	0.471 ± 0.039	0.630 ± 0.030	1.17 ± 0.056	1.24 ± 0.11	2.31 ± 0.20
Total α-Carotene[a,c]	0.0220 ± 0.0037	0.0410 ± 0.0069	0.0376 ± 0.0062	0.070 ± 0.012	0.053 ± 0.012	0.099 ± 0.022
Lutein	0.0616 ± 0.0060	0.108 ± 0.011	0.0629 ± 0.0056	0.111 ± 0.010	0.0391 ± 0.0054	0.0687 ± 0.0095
Cholesterol	866.3 ± 8.6	2240 ± 23	1444 ± 12	3735 ± 31	1276 ± 12	3300 ± 31

[a]A systematic component of uncertainty existed between the methods, and was included in the total uncertainty for total α-carotene in all three levels, retinyl palmitate in the middle and high levels, and total β-carotene in the low level.
[b]Includes *cis* and *trans* isomers of β-carotene.
[c]Includes *cis* and *trans* isomers of α-carotene.

Table 2. Noncertified Concentration Values of Fat-Soluble Vitamins and Carotenoids in SRM 968b

These values are not certified because either they are based on results from a limited number of analyses or the disagreement among methods is greater than expected for certified values [10]. NIST provides this data for information only.

Analyte	Low Level		Middle Level		High Level	
	μg/mL	*μmol/L*	*μg/mL*	*μmol/L*	*μg/mL*	*μmol/L*
γ-Tocopherol[a]	1.7	4.0	2.3	5.5	3.6	8.6
δ-Tocopherol	0.09	0.2	0.1	0.3	0.2	0.5
Zeaxanthin	0.02	0.03	0.02	0.04	0.01	0.02
β-Cryptoxanthin	0.02	0.04	0.03	0.05	0.03	0.05
trans-Lycopene	0.09	0.2	0.2	0.3	0.2	0.3
trans-α-Carotene	0.02	0.03	0.03	0.05	0.04	0.07
Total Lycopene[b]	0.2	0.3	0.3	0.5	0.3	0.6
9-*cis*-β-Carotene	0.008	0.01	0.02	0.03	0.03	0.05
13- + 15-*cis*-β-Carotene	0.01	0.02	0.03	0.06	0.06	0.1
15-*cis*-β-Carotene[c]			0.03	0.05	0.04	0.08

[a]Includes β-tocopherol.
[b]Includes *cis* and *trans* isomers of lycopene.
[c]Analyte is below the limit of quantitation for the measurement method used.

Appendix A

The analysts, their institutions, and the specific analyte measurements they performed in support of the SRM 968b certification are listed below.

Name	Analyte(s) Measured
Julius Agharanya Liberty Testing Labs Brooklyn, NY	Retinol, α-Tocopherol, β-Carotene
David S. Alberts Arizona Cancer Center Tucson, AZ	Retinol, Retinyl palmitate, γ-Tocopherol, α-Tocopherol
Nancy W. Alcock University of Texas Medical Branch Galveston, TX	Retinol, α-Tocopherol, β-Carotene
Gary R. Beecher U.S. Department of Agriculture Beltsville, MD	Retinol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin
Phyllis Bowen University of Illinois Chicago, IL	Retinol, Retinyl palmitate, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin
Mai H. Bui Institut Suisse des Vitamines Lausanne, Switzerland	Retinol, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein
Ron Bush Central Laboratory Services, Inc. Pittsburgh, PA	Retinol, α-Tocopherol, β-Carotene
Peter P. Chou American Medical Laboratories Chantilly, VA	Retinol, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene
Demetra Collier-Harris Children's Hospital National Medical Center Washington, DC	Retinol, α-Tocopherol
Joanne Curran-Celentano University of New Hampshire Durham, NH	Retinol, γ-Tocopherol, α-Tocopherol, β-Carotene
Ramon Deulofeu Hospital Clinic Servei de Bioquimica Barcelona, Spain	Retinol, γ-Tocopherol, α-Tocopherol

Nikolay Dimitrov Michigan State University East Lansing, MI	γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein
Show-Hong Duh University of Maryland Medical System Baltimore, MD	α-Tocopherol
Henri Faure Hôpital A. Michallon La Tronche, France	Retinol, γ-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin
Adrian Franke University of Hawaii at Manoa Honolulu, HI	Retinol, Retinyl palmitate, γ-Tocopherol, δ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin
Herbert A. Fritsche M.D. Anderson Cancer Center Houston, TX	Retinol, Retinyl palmitate, α-Tocopherol, β-Carotene
Yu-Tang Gao Shanghai Cancer Institute Shanghai, The Peoples Republic of China	Retinol, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin
Carl-Gustaf Gref National Public Health Institute Helsinki, Finland	Retinol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin
George Haenen TNO Nutrition and Food Research Zeist, The Netherlands	Retinol, Retinyl palmitate, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, β-Cryptoxanthin
Mei-Jyn Kang Institute of Biomedical Sciences Taipei, Taiwan	Retinol, α-Tocopherol, β-Carotene
Alda Laschi-Loquerie Institut Pasteur de Lyon Lyon Cedex, France	Retinol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin
Donald LeGatt University of Alberta Hospitals Alberta, Canada	Retinol, α-Tocopherol
Giuseppe Maiani National Institute of Nutrition Rome, Italy	Retinol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, β-Cryptoxanthin
Robert Mansourian Nestle Research Centre Lausanne, Switzerland	Retinol, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, β-Cryptoxanthin

Dana Marchand Maryland Medical MetPath Baltimore, MD	Retinol, α-Tocopherol
Jukka Marniemi Social Insurance Institution Research Centre Turku, Finland	Retinol, α-Tocopherol, β-Carotene
Israel Mendelson United Health Laboratories, Inc. Woodside, NY	Retinol, α-Tocopherol, β-Carotene
Judy Miller Medical Research Laboratories Highland Heights, KY	Retinol, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene
Sohrab Mobarhan Loyola University Medical Center Maywood, IL	Retinol, γ-Tocopherol, α-Tocopherol, β-Carotene, Lycopene, Lutein
John W. Moore Imperial Cancer Research Fund Oxford, England	Retinol, α-Tocopherol, β-Carotene
Begoña Olmedilla Clinica Puerta de Hierro Madrid, Spain	Retinol, Retinyl palmitate, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, Zeaxanthin, β-Cryptoxanthin, 9- and 13-*cis*-β-Carotene
Iris Osberg University of Colorado Health Sciences Center Denver, CO	Retinol, Retinyl palmitate, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene
Paula Radmacher University of Louisville Louisville, KY	Retinol, α-Tocopherol
Eugene J. Rogers University of Massachusetts-Lowell Lowell, MA	Retinol, γ-Tocopherol, α-Tocopherol, β-Carotene, Lycopene
Willy Schüep F. Hoffmann-La Roche Ltd. Basel, Switzerland	Retinol, Retinyl palmitate, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Lutein, β-Cryptoxanthin
Morton K. Schwartz Memorial Sloan-Kettering Cancer Center New York, NY	Retinol, α-Tocopherol
Anne Sowell Centers for Disease Control Atlanta, GA	Retinol, Retinyl palmitate, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Zeaxanthin, Lutein, β-Cryptoxanthin

Marian Swendseid UCLA School of Public Health Los Angeles, CA	α-Carotene, β-Carotene, Lycopene, Lutein, β-Cryptoxanthin, Zeaxanthin
David I. Thurnham University of Ulster Londonderry, Northern Ireland	Retinol, γ-Tocopherol, α-Tocopherol, α-Carotene, β-Carotene, Lycopene, Zeaxanthin, Lutein, β-Cryptoxanthin
Megan Veldee University of Washington Seattle, WA	Retinol, α-Tocopherol

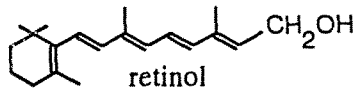
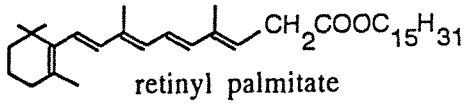
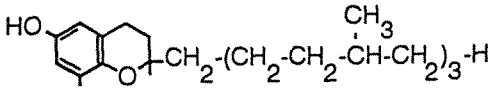
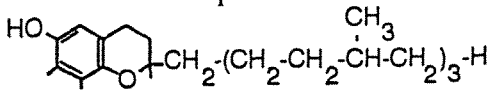
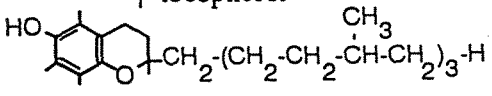
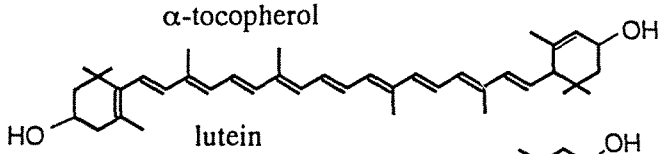
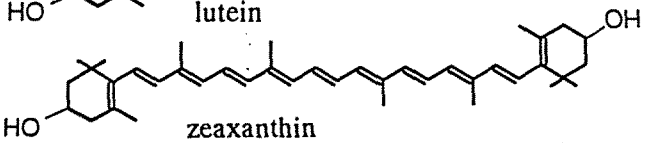
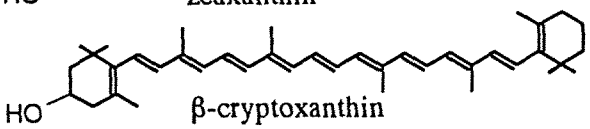
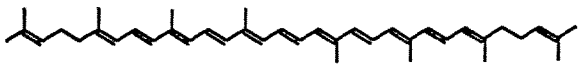
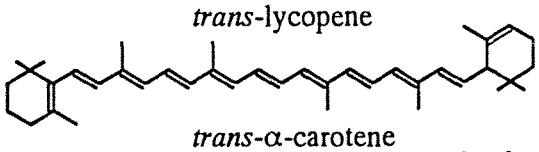
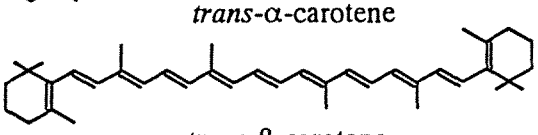
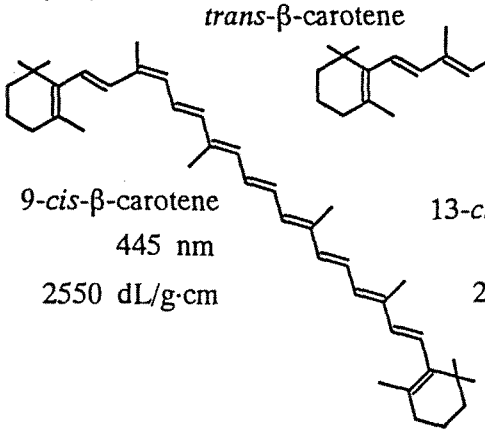
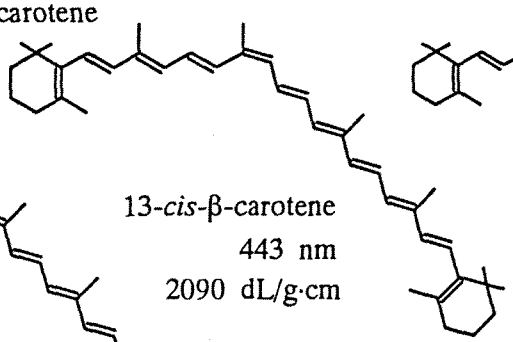
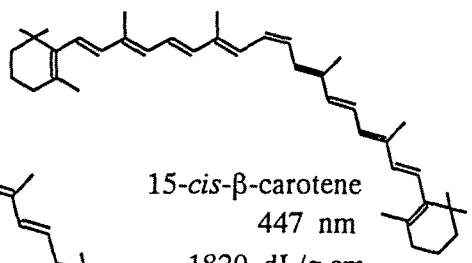
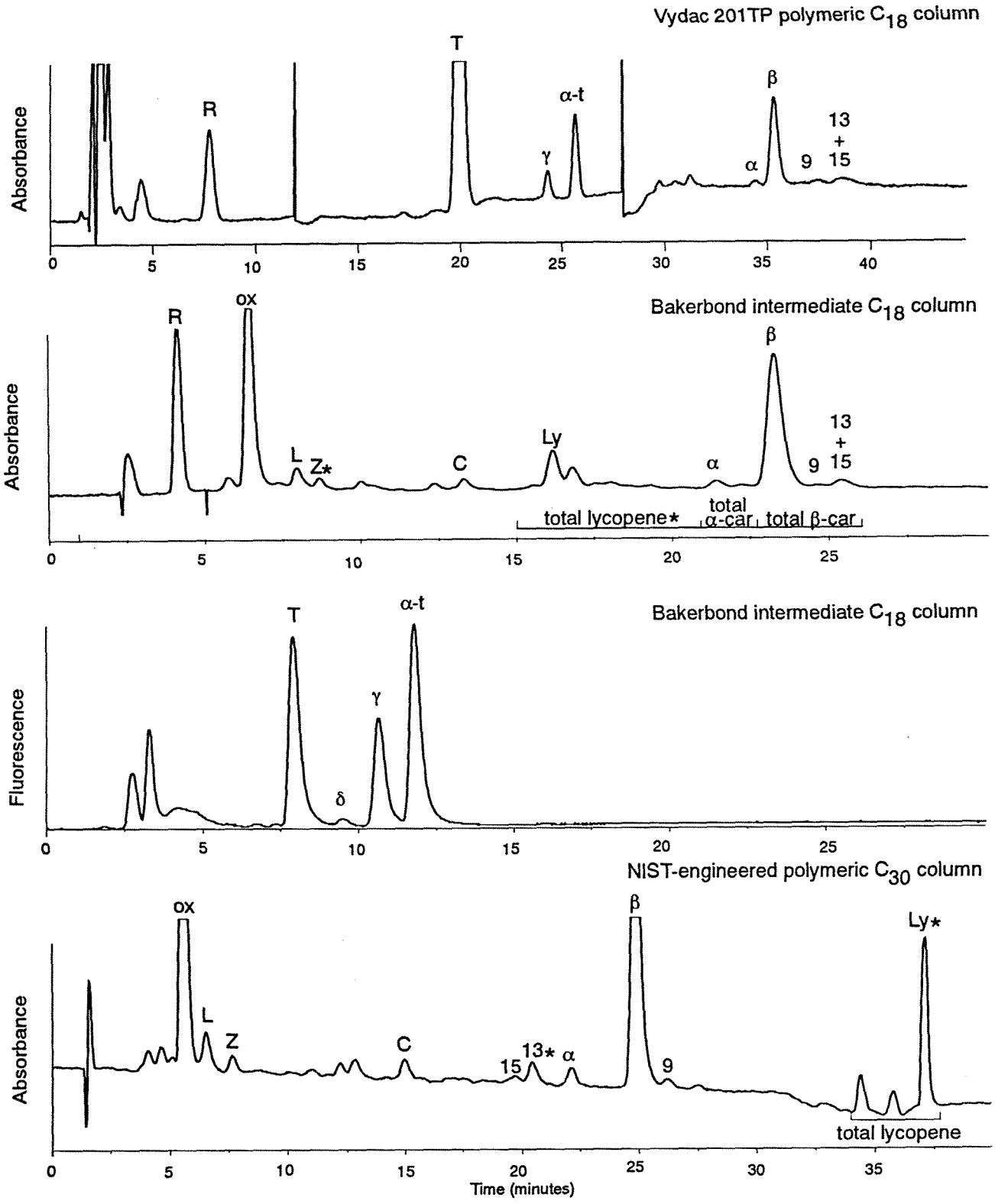
COMPOUND	λ_{max}	ABSORPTIVITY
 retinol	325 nm	1850 dL/g·cm
 retinyl palmitate	325 nm	975 dL/g·cm
 δ-tocopherol	297 nm	91.2 dL/g·cm
 γ-tocopherol	298 nm	91.4 dL/g·cm
 α-tocopherol	292 nm	75.8 dL/g·cm
 lutein	445 nm	2765 dL/g·cm
 zeaxanthin	452 nm	2416 dL/g·cm
 β-cryptoxanthin	452 nm	2486 dL/g·cm
 *trans*-lycopene	472 nm	3450 dL/g·cm
 *trans*-α-carotene	444 nm	2800 dL/g·cm
 *trans*-β-carotene	452 nm	2592 dL/g·cm
 9-*cis*-β-carotene 445 nm 2550 dL/g·cm		
 13-*cis*-β-carotene 443 nm 2090 dL/g·cm		
 15-*cis*-β-carotene 447 nm 1820 dL/g·cm		

Figure 1. Absorptivities and wavelength maxima used for calibration.



R = retinol
T = tocol
δ = δ-tocopherol
γ = γ-tocopherol
α-T = α-tocopherol

L = lutein
Z = zeaxanthin
C = β-cryptoxanthin
Ly = *trans*-lycopene
α = *trans*-α-carotene
β = *trans*-β-carotene

9 = 9-*cis*-β-carotene
13 = 13-*cis*-β-carotene
15 = 15-*cis*-β-carotene
ox = *trans*-β-apo-10'-carotenal oxime
* = includes unknown(s) or co-eluting peak(s)

Figure 2. Chromatogram from the analyses of SRM 968b using three different LC methods developed at NIST [10,19]. Chromatographic conditions are described in the text.