Method Comparison Table

SM 3500-Cr D	**14552Cr6 - June, 1999**
1.0 Scope and Application This procedure measures only hexavalent chromium (Cr^{6+}). Hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution. Subsequently a red-violet color is produced that is measured spectrophotometrically at 540 nm. The reaction is very sensitive. The absorptivity, based on chromium is approximately 40.000 L g^{-1} cm^{-1}. The standard method provides for a 0.1 – 1.0 mg/L range. This method is applicable for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Clean Water Act.	**1.0 Scope and Application** This test measures hexavalent chromium (Cr^{6+}) present in the sample as chromate or dichromate ions. Diphenylcarbazide dissolved in a weak phosphoric acid mixture is oxidized to diphenylcarbazone by Cr^{6+}. The Chromium III (Cr^{3+}) intermediate, produced during this reaction, reacts with diphenylcarbazone to give a red-violet color complex, which is measured photometrically at or near 543 nm. The reaction producing the red-violet color only occurs in the presence of Cr^{6+}, i.e. chromate or dichromate, as only Cr^{6+} gives rise to reactive, non-hydrolyzed Cr^{3+} intermediary during the reaction. Cr^{3+} in water sample is either hydrolyzed or complex bound, and hence are not available for reaction. Similar to that of the reference method, this reaction is very sensitive, the absorptivity based on chromium is approximately 41.350 L g^{-1} cm^{-1}. The test range of the Spectroquant® Cr^{6+} Cell Tests is 0.05 - 2.00 mg/L. The MDL for the method has been established at 0.02 mg/L. The ML for this method is 0.05 mg/L. This method is applicable for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Clean Water Act.
2.0 Summary of Method A small aliquot of homogenized, acidified sample is reacted with diphenylcarbazide in acid solution. Upon completion of a 5 to 10 minute reaction period full color development occurs. The color intensity can then be compared against color intensity of standards made to the same volume. Measurement is made with a spectrophotometer at 540 nm.	**2.0 Summary of Method** The Spectroquant® Cr^{6+} cell contains pre-measured granules of diphenylcarbazide. A 6 drop aliqout (0.18 ml) of dimethyl sulfoxide–phosphoric acid solution (Spectroquant® Reagent Cr-3K) is added to the cell. The cell is closed tightly and mixed vigorously to soak the solids. Five ml of digested and/or pretreated sample solution is added and mixed. Five minutes are allowed for the reaction to complete. Sample, standard, and reagent blank concentrations are determined photometrically at or near 543 nm. Values for samples are obtained from the Merck Spectroquant®-type system photometers. Direct absorbance

	measurements from other photometric devices are plotted against the calibration curve prepared as stated in Section 12.
3.0 Definitions See Section 18.0–There are no terms, acronyms, or symbols which have been defined in this method.	**3.0 Definitions** See section 18.0 – This method defines, in great detail, the terms, acronyms, and symbols, which appear in the body of the method.
4.0 Interferences For sample which are filtered and acidified and only hexavalent chromium is desired, the analyst can proceed directly to the determinative protocol (color development). The reference method advocates this progress inasmuch as this procedure only measures Cr^{6+}. The reaction of the diphenylcarbazide is nearly specific for Cr^{6+}. Hexavalent molybdenum and mercury salts will react to form color with the reagent, but the intensities are much lower than that for Cr^{6+} at the specified pH. Concentrations as high as 100 mg Mo^{6+}/L and 10 mg Hg^{2+}/L can be tolerated. Vanadium interferes at levels exceeding 1 mg V^{5+}/L, producing false negative results. Concentrations as high as 100 mg V^{5+}/L can be tolerated in blanks. Iron in concentrations greater than 100 mg Fe^{3+}/L interferes. The method also suggests that for turbid samples (after filtration) the absorbance of the sample blank be subtracted from colored sample.	**4.0 Interferences** The reaction of the diphenylcarbazide is nearly specific for Cr^{6+}. Hexavalent molybdenum and mercury salts will react to form color with the reagent, but the intensities are much lower than that for Cr^{6+} at the specified pH. Concentrations as high as 100 mg Mo^{6+}/L and 10 mg Hg^{2+}/L can be tolerated. Vanadium interferes at levels exceeding 1 mg V^{5+}/L, producing false negative results. Concentrations as high as 100 mg V^{5+}/L can be tolerated in blanks (Reference 16.2). Iron in concentrations greater than 100 mg Fe^{3+}/L interferes (Reference 16.2). With logic consistent with the reference methods, two potential inhibitors of accurate quantitation are identified and accommodation provided via the method protocol. These are: a) To compensate for possible slight losses of chromium during analytical operations, standards are treated by the same procedure as the sample. b) If after filtration (for determination of dissolved Cr^{6+}), turbidity persists, take the absorbance reading of the sample prior to its introduction to the diphenylcarbazide reagent. Correct for the absorbance of the sample blank by subtracting from the result obtained when the colored sample is analyzed.
5.0 Safety The reference method does not define potential health risks associated with the use of the chemicals in this method.	**5.0 Safety** This method employs the use of Spectroquant® Cr^{6+} Cell Tests containing pre-measured reagents and prepackaged small quantities of reagents, hence the handling of hazardous chemicals is significantly reduced. As with any analytical procedure, the analyst is cautioned to become familiar with the potential health hazards described in the reference Material Safety Data Sheet (MSDS) records.

	Spectroquant® Cr^{6+} Cell Tests, 14552, are also clearly labeled as to the analytical parameter, contents, and any potential health hazards. This increases likelihood of user safety.
6.0 Equipment and Supplies This method employs standard laboratory glassware for sampling (1L glass or plastic bottle). Standard glassware for sample handling include pipettes, beakers, volumetric flasks, filtration apparatus, 0.45 µm membrane filters, 10-mm photometric cuvettes. A spectrophotometer is used at a wavelength of 540 nm for measurement.	**6.0 Equipment and Supplies** This method employs all supplies, which are specified in SM 3500-Cr D. Pretreatment supplies are included through reference to the SM. The photometric determination is accomplished using a filter photometer or other standard photometer. ***Merck Spectroquant® -Type System Photometers*** *Chromate cell identification* – The Merck Spectroquant® type system photometers are equipped with a sample identification system. Each Spectroquant® Cr^{6+} Cell Test is bar coded, and when placed correctly in the cell compartment, the instruments recognize the cell, and sets the instrument to the proper measuring parameters (i.e. item number, test range, cell format, wavelength and calibration data). Merck Spectroquant®-type system photometers can also store the sample information within its data files, for printing, downloading to alternate data storage location, or for easy retrieval. *Calibration* - Merck Spectroquant®-type system photometers have been factory calibrated, and the readings obtained from the sample cells are automatically expressed as the Cr^{6+} concentration (mg/L). The calibration curve can be verified, and the data from this verification can be stored, modified or re-entered at any time. The user cannot change the factory program setting. Instead, when the manufacturer generates new calibration data, they supply a new memo chip (transponder) to the user for updating the system. ***Direct Absorbance Measurement*** The use of absorbance mode of the Merck Spectroquant® -type system photometers, or other photometric equipment, is consistent with the reference method specifications.
7.0 Reagents and Standard This method incorporates the following chemicals which are purchased and/or prepared by the laboratory analyst: 7.1 Stock Chromium Solution: dissolve 141.4 mg	**7.0 Reagents and Standards** This method combines, in proportions consistent with SM 3500-Cr D, all of the reaction chemicals, which are specified in the reference method.

$K_2Cr_2O_7$ in water and dilute to 100 ml; 1.00ml = 500 µg Cr. 7.2 Standard Chromium Solution: Dilute 1.0 ml stock chromium solution to 100 ml: 1.00 ml = 5.00 µg Cr. 7.3 Nitric Acid, HNO_3, concentrated. 7.4 Sulfuric Acid, H_2SO_4, concentrated, 7.5 Sulfuric Acid, H_2SO_4, 1 + 1, 7.6 Sulfuric Acid, H_2SO_4, 6 N. 7.7 Sulfuric Acid, H_2SO_4, 0.2 N, Dilute 17 ml of 6 N H_2SO_4 to 500 ml with water. 7.8 Phosphoric Acid, H_3PO_4, concentrated. 7.9 Diphenylcarbazide solution; Dissolve 250 mg 1,5-diphenylcarbazide (1,5-diphenylcarbohydrazide) in 50 ml acetone.	The pre-measured and pre-mixed reagent reduce the risk of error in preparation of the chemical reagents. The cell test contains a premeasured mass of 1,5-diphenylcarbazide for color complexing. Spectroquant® Reagent Cr-3K contains dimethylfulfoxide and phosphoric acid .
8.0 Sample Collection, Preservation, and Storage The sampling is performed in accordance with Standard Methods 1060. The sample is collected in a glass or plastic bottle of 300 ml capacity or greater. When only the dissolved Cr^{6+} content is desired, filter sample through a 0.45 µm membrane filter at the time of collection. After filtration acidify filtrate with concentrated nitric acid to a pH <2. Samples for Cr^{6+} only should be analyzed immediately or within 24 hours. Total chromium samples shall be preserved with HNO_3 to pH <2 and may be held for 6 months.	**8.0 Sample Collection, Preservation, and Storage** The sampling is performed in accordance with Standard Methods 1060. There are no differences in the way the samples are collected. If analysis is not performed immediately, preservation and storage of samples in this method is identical to these procedures in the reference method.
9.0 Quality Control There are no quality control guidelines incorporated into the body of this method.	**9.0 Quality Control** This method includes guidelines for initial demonstration of laboratory capability, quality control and quality assurance measurements. Initial demonstration of performance of the method is required. After initial performance has been established, the analyst is required to provide proof of continued performance through the analysis of ongoing precision and recovery standards. These are tested in conjunction with the entire analytical quality control batch (for up to 20 samples), which include: (1) laboratory blank, (1) laboratory control sample (OPR), (1) field duplicate (DUP).
10.0 Calibration and Standardization The spectrophotometer is calibrated using a blank and at least five (5) Cr standards covering the concentration range of samples to be tested. The absorbance of the standards is	**10.0 Calibration and Standardization** ***Merck Spectroquant®-Type System Photometers*** The Merck Spectroquant®-type system phometers are factory calibrated with standard reference material, and the

plotted against the concentration. Absorbance readings from samples are plotted against this curve to obtain a concentration value. The calibration curve should be re-run if the linearity of the curve varies more than 5%, or with each new lot of reagents.	products are shipped with Lot Certificates for calibration. ***<u>Other Photometric Devices</u>*** Calibration of these instruments or another photometric device for absorbance measurements is performed as described in the reference method.
11.0 Procedure For this method, only hexavalent chromium is being determined. If only the dissolved Cr^{6+} content is desired, filter sample through a 0.45 µm membrane filter at the time of sample collection: a) acidify filtrate with concentrated nitric acid (HNO_3) to pH <2 if analysis cannot be immediately effected. Alternately, b) use a pH meter and adjust solution to pH 1.0 +/- 0.3 with 0.2 N H_2SO_4. Transfer a portion of this solution to a 100-ml volumetric flask. Dilute to 100 ml with distilled water. Add 2.0 ml diphenylcarbazide solution. Mix. Let stand 5 to 10 minutes for full color development. Transfer an appropriate portion to a 1-cm absorption cell and measure its absorbance at 540 nm.	**11.0 Procedure** This method does not differ from the reference method in reagent addition, sequence of addition, nor in "reagent to sample volume" ratio. As the reagent formulations are pre-measured and prepackaged, the manufacturer's references to reagents is effected with use of test kit product code identifiers (e.g. Spectroquant® Reagent Cr –3K). These formulations are consistent with the reagents necessitated by, and identified in, the reference method. Use prior knowledge of the particular waste stream, when applicable, to dilute sample within the concentration range of this method. Refer to Section 17, Table 1. The following preparation techniques are referenced in the method from SM 4500-Cr D. If only dissolved Cr^{6+} content is desired, filter sample through a 0.45 µm membrane filter at the time of sample collection. If total chromium concentration is desired, digest the sample as outlined in the reference method. *<u>Procedure</u>* Samples should be analyzed immediately for Cr^{6+}. However, if this is not possible, the sample should be preserved with HNO_3 to pH <2. The Spectroquant® Cr^{6+} cell contains pre-measured granules of diphenylcarbazide. A 6 drop aliquot (0.18 ml) of dimethyl sulfoxide - phosphoric acid solution (Spectroquant® Reagent Cr-3K) is added to the cell. The cell is closed tightly and mixed vigorously to soak the solids. After one minute, five ml of digested or pretreated sample

	solution is added to the cell. Wait a five minute reaction period for the reaction to complete. Place the cell in cell compartment of Merck Spectroquant® -type photometer. Read the concentration in mg/L, directly from the instrument display. Record the absorbance (if measuring in this mode) and plot readings against a standard curve, prepared as stated in Section 17, Table 2. The photometric measurements of samples, standards, and blank(s) are conducted at or near 543-nm, measure and record the result**.**
12.0 Data Analysis and Calculations The reference method defines the calculation for samples which have been digested, and where less than the 100 ml original sample was used for color development. The result is calculated as follows: mg Cr/L = $\frac{\mu g \text{ Cr (in 102 ml final volume)}}{A \times B} \times 100$ Where: A = ml original sample, and B = ml portion from 100 ml digested sample. The Minimum Level (ML) is implied from the concentration range tested 0.1 – 1.0 mg/L, as 0.1 mg/L.	**12.0 Data Analysis and Calculations** ***Merck Spectroquant® -Type System Photometers*** Results with these instrument are displayed as Cr concentration (mg/L). ***Other Photometers*** Measurement of samples with equivalent photometric equipment allows for values to be plotted from calibration curves. This method defines the calculation for dilution factor correction in event that samples read outside the calibration range of the test. The Minimum Level (ML) for this method is 0.05 mg/L. If a result is obtained which is lower than this ML, report the result as less than the ML (<0.05 mg/L). Report results to two significant digits.
13.0 Method Performance Referenced in the method are precision and bias data obtained from thirty-one laboratories analyzing one synthetic sample.	**13.0 Method Performance** As in the SM 3500 Cr D reference method, this method incorporates the same chemical components, in the same proportions, with the same reaction time, and determined similarly via photometric measurement. The precision and bias stated in the reference method are achievable by this method.
14.0 Pollution Prevention The reference method does not discuss pollution prevention.	**14.0 Pollution Prevention** Packaging and use of pre-measured, reagents for Spectroquant® Cr^{6+} Cell Tests are designed to minimize risks of spillage, and to reduce the amounts of the

	chemicals used. The laboratory is reminded to properly manage these reagents in the laboratory to reduce any threat to the environment. General practices, such as ordering of supplies, can seriously impact the amount of materials which require disposal in the laboratory. It is suggested that the laboratory only order supplies as demand dictates, to minimize expired materials requiring disposal.
15.0 Waste Management The reference method does not discuss waste management.	**15.0 Waste Management** Referenced in section 15.3 are two waste management documents for further information on this subject. In using this method, the laboratory must comply with all federal, state, and local regulations governing waste management.
16.0 References Rowland, G.P., Jr. 1939, Photoelectric colorimetry-Optical study of permanganate ion and of chromium-diphenylcarbazide system. Anal. Chem. 11:442 Saltzman, B.E. 1952. Microdetermination of chromium with diphenylcarbazide by permanganate oxidation. Anal. Chem. 24:1016. Urone, P.F. 1955. Stability of colorimetric reagent for chromium, 5-diphenylcarbizide, in various solvents. Anal. Chem. 27:1354. Allen, T.L. 1958, Microdetermination of chromium with 1,5-diphenylcarbohydrazide, Anal. Chem.30:447. Sandell, E.B., 1959, Colorimetric Determination of Traces of Metals, 3rd. ed. Interscience Publishers, New York, N.Y.	**16.0 References** STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 18th Edition, American Public Health Association, 1015 Fifteenth Street, N.W., Washington, D.C. 20005, Methods 3500-Cr D. OSHA Safety and Health Standards, General Industry, (29CFR 1910), Occupational Safety and Health Administration, OSHA 2206, revised January 1976 Safety in Academic Chemistry Laboratories, American Chemical Society Publications, Committee on Chemical Safety, 3rd Edition, 1979 STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 18th Edition, American Public Health Association, 1015 Fifteenth Street, N.W., Washington, D.C. 20005, Methods 1060. Handbook of Analytical Quality Control in Water and Wastewater Laboratories, USEPA, EMSL-Ci, Cincinnati, OH 45268, EPA German Standard Methods for the Examination of Water, and Wastewater and Sludge, Deutsches Institut Für Normung e.V., D-10772, Berlin, DIN Method 38402 Part 51, May 1986. Spectroquant® NOVA 60 Manual, Merck KGaA,

	Frankfurter Strasse 250, 64271Darmstadt, Germany, Release July 1998. Spectroquant® VEGA 400 Manual, Merck KGaA, Frankfurter Strasse 250, 64271Darmstadt, Germany, Release July 1998. Spectroquant® SQ 118 Manual, Merck KGaA, Frankfurter Strasse 250, 64271Darmstadt, Germany, Release July 1998. Spectroquant® Cr^{6+} Product 14552 Insert, Merck KGaA, Frankfurter Strasse 250, 64271Darmstadt, Germany, Release March 1994. Protocol for EPA Approval of Alternate Test Procedures for Organic and Inorganic Analytes in Wastewater and Drinking Water-Draft, March 1998, Environmental Protection Agency, Office of Water (4303) Washington, DC 20460.
17.0 Tables There are no tables, diagrams, flowcharts or validation data reports included in this method.	**17.0 Tables** Three tables are included in this method. ***Table 1*** This table summarizes the Spectroquant® Cr^{6+} Cell Test item 14552 concentration ranges, which can be achieved by this method. The table lists the item number, concentration range, cell size, Merck Spectroquant® -type system photmeter test code, and sample volume. ***Table 2*** This table outlines the procedure for preparing calibration curve solutions for the ranges tested using Spectroquant® Cr^{6+} Cell Test item 14552, when measuring in the absorbance mode. ***Table 3*** This table outlines the performance acceptance criteria for methods cited in 40 CFR Part 136, Table IB. These criteria must be achieved when the analyst initiates the method, attempts to prove initial demonstration of performance, and also ongoing performance of the method.
18.0 Glossary The reference method does not have a glossary.	**18.0 Glossary** See Section 3.0 The glossary defines terminology used in the body of method. Much of the terms defined are specific to the quality control section of the method.