METHOD 549.1

DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Revision 1.0

August 1992

J.W. Hodgeson

W.J. Bashe (Technology Applications Inc.)

James W. Eichelberger

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 549.1

DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

1.0 SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatography (HPLC) method for the determination of diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) and paraquat (1,1'-dimethyl-4,4'- bipyridilium dichloride salt) in drinking water sources and finished drinking water[1,2].

Analyte	Chemical Abstract Services Registry Number
Diquat	85-00-7
Paraquat	1910-42-5

1.2 When this method is used to analyze unfamiliar samples, compound identification should be supported by at least one additional qualitative technique. The use of a photodiode array detector provides ultraviolet spectra that can be used for the qualitative confirmation.

1.3 The method detection limits (MDL, defined in Sect. 13) (3) for diquat and paraquat are listed in Table 1.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.

2. SUMMARY OF METHOD

2.1 A measured volume of liquid sample, approximately 250 mL, is adjusted to pH 10.5. The sample is extracted using a C_8 solid sorbent cartridge or a C_8 disk which has been specially prepared for the reversed-phase, ion-pair mode. The disk or cartridge is eluted with 4.5 mL of an acidic aqueous solvent. After the ion-pair reagent is added to the eluate, the final volume is adjusted to 5.0 mL. Liquid chromatographic conditions are described which permit the separation and measurement of diquat and paraquat in the extract by absorbance detection at 308 nm and 257 nm, respectively. A photodiode array detector is utilized to provide simultaneous detection and confirmation of the method analytes[1,2].

2.2 Analysis of diquat and paraquat is complicated by their ionic nature. Glassware should be deactivated to prevent loss of analytes. The substitution of polyvinylchloride (PVC) for glass is recommended.

3.0 DEFINITIONS

3.1 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 Field Reagent Blank (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.3 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 Stock Standard Solution (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.6 Primary Dilution Standard Solution (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 Quality Control Sample (QCS) -- A solution of method analytes of known concentration which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.9 External Standard (ES) -- A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard(s) is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

4.0 INTERFERENCES

4.1 Method interference may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatogram. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.2.

4.1.1 Glassware must be scrupulously cleaned[4]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and distilled water. It should then be drained dry and heated in a laboratory oven at 130°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 Before the initial use of all glassware, the procedure described in Section 4.1.1 should be followed. Silanization of all glassware which will come in contact with the method analytes is necessary to prevent adsorption of the diquat and paraquat cations onto glass surfaces (Section 7.13).

4.1.3 Plasticware should be washed with detergent and rinsed in tap water and distilled water. It should be drained dry before use.

4.1.4 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source. Because of the selectivity of the detection system used here, no

interferences have been observed in the matrices studied. If interferences occur, some additional cleanup may be necessary.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

6.0 EQUIPMENT AND SUPPLIES

6.1 Sampling Equipment -- Discrete or composite sampling.

6.1.1 Grab sample bottle -- Amber polyvinylchloride (PVC) high density, 1 L, fitted with screw caps. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with deionized water, and dried before use to minimize contamination.

6.2 Glassware

6.2.1 Volumetric flask -- 5 mL, silanized.

6.2.2 Autosampler vials -- 4 mL, silanized.

6.3 Balance -- analytical, capable of accurately weighing 0.0001 g.

6.4 pH Meter -- capable of measuring pH to 0.1 units

6.5 HPLC Apparatus

6.5.1 Isocratic pumping system -- Constant flow (Waters M6000A HPLC pump or equivalent).

6.5.2 Manual injector or automatic injector -- Capable of delivering 200 µL.

6.5.3 Analytical column -- Any column which produces results equal to or better than those listed below may be used.

6.5.3.1 Hamilton PRP-1 -- 5 µm, 150 mm x 4.1 mm, or equivalent.

6.5.3.2 Guard column -- C_8 packing.

6.5.4 Column oven -- Fiatron, Model CH-30 and controller, Model TC-50, or equivalent.

6.5.5 Photodiode array detector -- LKB 2140 Rapid Spectral Detector or equivalent. Any detector which has the capability to switch between 257 nm and 308 nm may be used.

6.5.6 Data system -- Use of a data system to report retention times and peak areas is recommended but not required.

6.6 Extraction Apparatus

6.6.1 Liquid-solid extraction cartridges -- C_8, 500 mg or equivalent.

6.6.2 Liquid-solid extraction system -- Baker, 10 SPE, or equivalent.

6.6.3 Liquid-solid extraction disks -- C-8 Empore, 47 mm, or equivalent.

6.6.4 Liquid-solid extraction system -- Empore, 47 mm, six-position manifold (Varian Associates or equivalent). If a disk extraction manifold is not used, all glassware used instead should be silanized or substituted with polypropylene ware.

6.6.5 Vacuum pump -- 100 VAC, capable of maintaining a vacuum of 8-10 mm of Hg.

6.6.6 Membrane filters -- 0.45 µm pore-size, 47 mm diameter, Nylon.

7.0 REAGENTS AND STANDARDS

7.1 Deionized Water -- Water which has been processed through a series of commercially available filters including a particulate filter, carbon bed, ion exchange resin and finally a bacterial filter to produce deionized, reagent grade water. Any other source of reagent water may be used provided the requirements of Section 9.0 are met.

7.2 Methanol -- HPLC grade or higher purity.

7.3 Orthophosphoric Acid -- 85% (w/v), reagent grade.

7.4 Diethylamine -- Reagent grade.

7.5 Concentrated Sulfuric Acid -- ACS reagent grade.

7.6 Sodium Hydroxide -- Reagent grade.

7.7 Concentrated Hydrochloric Acid, 12 N -- Reagent grade.

7.8 Cetyl Trimethyl Ammonium Bromide, 95% -- Aldrich Chemical.

7.9 Sodium Thiosulfate -- Reagent grade.

7.10 1-hexanesulfonic Acid -- Sodium salt, 98%, Aldrich Chemical.

7.11 1-heptanesulfonic Acid -- Sodium salt, 98%, Aldrich Chemical.

7.12 Ammonium Hydroxide -- ACS, concentrated.

7.13 Sylon CT -- Silanization solution, Supelco.

7.14 Reagent Solutions

7.14.1 Conditioning Solution A -- Dissolve 0.500 g of cetyl trimethyl ammonium bromide and 5 mL of concentrated ammonium hydroxide in 500 mL of deionized water and dilute to 1000 mL in volumetric flask.

7.14.2 Conditioning Solution B -- Dissolve 10.0 g of 1-hexanesul-fonic acid, sodium salt and 10 mL of concentrated ammonium hydroxide in 250 mL of deionized water and dilute to 500 mL in volumetric flask.

7.14.3 Sodium hydroxide solution, 10% w/v -- Dissolve 50 g of sodium hydroxide into 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.4 Hydrochloric acid, 10% v/v -- Add 50 mL of concentrated hydrochloric acid to 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.5 Disk or cartridge eluting solution -- Add 13.5 mL of orthophosphoric acid and 10.3 mL of diethylamine to 500 mL of deionized water and dilute to 1000 mL in a volumetric flask.

7.14.6 Ion-pair concentrate -- Dissolve 3.75 g of 1-hexanesul-fonic acid in 15 mL of the disk or cartridge eluting solution and dilute to 25 mL in a volumetric flask with the disk eluting solution.

7.15 Stock Standard Solutions

7.15.1 Diquat dibromide and paraquat dichloride

7.15.2 Stock diquat and paraquat solutions (1000 mg/L) -- Dry diquat and paraquat salts in an oven at 110°C for three hours. Cool in a desiccator. Repeat process to a constant weight. Weigh 0.1968 g of dried diquat salt and 0.1770 g of dried paraquat salt and place into a silanized glass or polypropylene 100 mL volumetric flask. Dissolve with

approximately 50 mL of deionized water. Dilute to the mark with deionized water.

7.15.3 The salts used in preparing the stock standards (Section 7.15.2) were taken to be diquat dibromide monohydrate and paraquat dichloride tetrahydrate[5]. The drying procedure described in Section 7.15.2 will provide these hydration levels.

7.16 Mobile Phase -- Make mobile phase by adding the following to 500 mL of deionized water: 13.5 mL of orthophosphoric acid; 10.3 mL of diethylamine; 3.0 g of 1-hexanesulfonic acid, sodium salt. Mix and dilute with deionized water to a final volume of 1 L.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in either amber PVC high density bottles or silanized amber glass bottles. Conventional sampling procedures should be followed[6]. Automatic sampling equipment must be free as possible of adsorption sites which might extract the sample.

8.2 The samples must be iced or refrigerated at approximately 4°C from the time of collection until extraction. The analytes are light-sensitive, particularly diquat.

8.3 Samples which are known or suspected to contain residual chlorine must be preserved with sodium thiosulfate (100 mg/L). Samples which are biologically active must be preserved by adding sulfuric acid to pH 2 to prevent adsorption of method analytes by the humectant material.

8.4 Analyte stability over time may depend on the matrix tested. Analyte stability in representative drinking water matrices is listed in Table 3. All samples must be extracted within seven days of collection. Extracts must be analyzed within 21 days of extraction[1].

9.0 QUALITY CONTROL

9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified matrix samples, and laboratory fortified blanks. The laboratory must maintain records to document the quality of the data generated. Additional quality control practices are recommended.

9.2 Laboratory Reagent Blanks (LRB) -- Before processing any samples, the analyst must analyze a LRB to demonstrate that all deactivated glassware or plasticware, and reagent interferences are reasonably free of contamination. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window (Section 11.3.2) of the analyte of interest, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.3 Initial Demonstration of Capability

9.3.1 Prepare laboratory fortified blanks (LFBs) at analyte concentrations of 100 µg/L. With a syringe, add 25 µL of the stock standard (Section 7.14.2) to at least four 250 mL aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.2.

9.3.2 Calculate the recoveries, relative standard deviation (RSD), and the MDL[3]. The recovery (R) values should be within ±30% of the R values listed in Table 2 for at least three of four consecutive samples. The RSD of the mean recovery should be less than 30%. The MDL must be sufficient to meet the requirements of the SDWA regulations. For analytes that fail this critera, initial demonstration procedures should be repeated.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method the quality of the data should improve beyond the requirements stated in Section 9.3.2.

9.4 The analyst is permitted to use other HPLC columns, HPLC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.3.

9.5 Laboratory Fortified Blanks (LFB)

9.5.1 The laboratory must analyze at least one LFB sample per sample set (all samples extracted within a 24-hour period). The fortified concentration of each analyte in the LFB should be 10 times the MDL. If the recovery

of either analyte falls outside the control limits (Section 9.5.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

9.5.2 Until sufficient data become available, usually a minimum of results from 20-30 analyses, the laboratory should assess laboratory performance against the control limits in Section 9.3.2. When sufficient internal performance data become available, develop control limits from the mean percent recovery (R) and standard deviation (S_r) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = $R + 3S_r$
LOWER CONTROL LIMIT = $R - 3S_r$

After each 5-10 new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points.

9.6 Laboratory Fortified Sample Matrix

9.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the laboratory fortified blank (Section 9.5). Over time, samples from all routine samples sources should be fortified.

9.6.2 Calculate the accuracy as percent recovery (R) for each analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Section 9.5.2 from the analyses of LFBs.

9.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 9.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the original sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.7 Quality Control Samples (QCS) -- Each quarter the laboratory should analyze one or more QCS. If criteria provided with the QCS are not met, corrective action should be taken and documented.

9.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the

environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Establish HPLC operating conditions indicated in Table 1. The chromatographic system can be calibrated using the external standard technique.

10.2 In order to closely match calibration standards to samples, process standards by the following method: Using C_8 disks or C_8 cartridges conditioned according to Section 11.2.1, pass 250 mL of reagent water through the disk or cartridge and discard the water. Dry the disk or cartridge by passing 5 mL of methanol through it. Discard the methanol. Pass 4.0 mL of the eluting solution through the disk or cartridge and catch in a 5 mL silanized volumetric flask. Fortify the eluted solution with 100 µL of the ion-pair concentrate and with 500 µL of the stock standard and dilute to the mark with eluting solution. This provides a 10:1 dilution of the stock. Use serial dilution of the calibration standard by the same method to achieve lower concentration standards.

10.3 Analyze a minimum of three calibration standards prepared by the procedure described in Section 10.2 utilizing the HPLC conditions given in Table 1. From full spectral data obtained, extract the 308 nm chromatographic trace for diquat and the 257 nm trace for paraquat. Integrate and record the analyte peak areas. Any mathematical manipulations performed to aid in data reduction must be recorded and performed on all sample chromatograms. Tabulate the peak area against quantity injected. The results may be used to prepare calibration curves for diquat and paraquat.

10.4 The working calibration curve must be verified on each working day by measurement of a calibration check standard, at the beginning of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals. If the response for any analyte varies from the predicted response by more than ±20%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

11.0 PROCEDURE

11.1 Sample Cleanup -- Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various sample types. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of the analytes is within the limits specified by the method.

11.1.1 If the sample contains particulates, or the complexity is unknown, the entire sample should be passed through a 0.45 µm Nylon membrane filter into a silanized glass or plastic container.

11.1.2 Store all samples at 4°C unless extraction is to be performed immediately.

11.2 Cartridge Extraction

11.2.1 Before sample extraction, the C_{-8} extraction cartridges must be conditioned by the following procedure.

11.2.1.1 Place a C_8 cartridge on the cartridge extraction system manifold.

11.2.1.2 Elute the following solutions through the cartridge in the stated order. Take special care not to let the column go dry. The flow rate through the cartridge should be approximately 10 mL/min.

11.2.1.2.1 Cartridge conditioning sequence

a. Deionized water, 5 mL
b. Methanol, 5 mL
c. Deionized water, 5 mL
d. Conditioning Solution A, 5 mL
e. Deionized water, 5 mL
f. Methanol, 10 mL
g. Deionized water, 5 mL
h. Conditioning Solution B, 10 mL

11.2.1.2.2 Retain conditioning Solution B in the C_8 cartridge to keep it activated.

11.2.2 The C_8 cartridges should not be prepared more than 48 hours prior to use. After conditioning, the cartridge should be capped and stored at 4°C.

11.2.3 Measure a 250 mL aliquot of the sample processed through Section 11.1 in a silanized, volumetric flask.

11.2.4 Immediately before extraction, adjust the pH of sample to 10.5 ±0.2 with 10% w/v NaOH (aq) or 10% v/v HCl (aq).

11.2.5 Place a conditioned C_8 cartridge on the solid phase extraction vacuum manifold. Attach a 60 mL reservoir to the C_8 cartridge with the appropriate adapter. Put a 250 mL beaker inside the extraction manifold to catch waste solutions and sample. Transfer the measured

volume in aliquots to the reservoir. Turn on the vacuum pump or house vacuum and adjust the flow rate to 3-6 mL/min. Filter the sample through the C_8 cartridge, and wash the column with 5 mL of HPLC grade methanol. Continue to draw the vacuum through the cartridge for one additional minute to dry the cartridge. Release the vacuum and discard the sample waste and methanol.

11.2.6 Place a silanized 5 mL volumetric flask beneath the collection stem in the vacuum manifold. Add 4.5 mL of the eluting solution to the sample cartridge. Turn on the vacuum and adjust the flow rate to 1-2 mL/min.

11.2.7 Remove the 5 mL volumetric flask with the extract. Fortify the extract with 100 µL of the ion-pair concentrate. Adjust the volume to the mark with cartridge eluting solution, mix thoroughly, and seal tightly until analyzed.

11.2.8 Analyze sample by HPLC using conditions described in Table 1. Integration and data reduction must be consistent with that performed in Section 10.3. Figure 1 presents a representative, sample chromatogram.

11.3 Disk Extraction -- The top surface of the disk matrix must remain covered with liquid at all times. If the disk is exposed to air at any step in the disk cleanup procedure, the elution procedure should be restarted. Eluants applied to the disk should be allowed to soak into the disk before drawing them through. Vacuum should then be applied to draw most of the eluant through the disk, leaving a thin layer of solution on the top of the disk. Flow rate through the disk is not critical.

11.3.1 Assemble the 47 mm disk in the disk holder or a filter apparatus. Be sure that the surfaces of the holder are either glass or Teflon coated to avoid adsorption or decomposition of the analytes.

11.3.2 Adjust the pH of the sample to 10.5 ±0.2 with 10% w/v aqueous sodium hydroxide or 10% v/v aqueous hydrochloric acid solution. Once the pH has been adjusted, the steps below must be performed immediately.

11.3.3 Apply 10 mL of methanol to the disk. Apply vacuum to begin elution, then immediately vent the vacuum when drops of liquid appear at the drip tip. Allow the methanol to soak into the disk for a minimum of one minute, then reapply the vacuum to bring the methanol to just above the top surface of the disk.

11.3.4 Draw two 10 mL aliquots of reagent water through to just above the top surface of the disk to remove the methanol.

11.3.5 Apply 10 mL of conditioning Solution A to the disk. As with the methanol, draw a few drops through, then allow the disk to soak for at least one minute. Draw the conditioning Solution A through the disk to just above its top surface.

11.3.6 Draw two 10 mL aliquots of reagent water through to just above the top surface of the disk.

11.3.7 Apply 10 mL of conditioning Solution B to the disk. Draw a few drops through using vacuum and allow the disk to soak for at least one minute. Draw the remaining conditioning Solution B through to just above the top surface of the disk.

11.3.8 Measure 250 mL of the pH adjusted sample using a polypropylene graduated cylinder. Pour the sample aliquot into the filtration apparatus reservoir and apply vacuum to draw the sample through the disk. Pass the entire sample through the disk, leaving no liquid on the top of the disk, then vent the vacuum.

11.3.9 Assemble a graduated collection tube under the drip tip with the tip descending into the tube slightly to prevent losses of eluants. Be sure the tube will hold at least 10 mL of eluate.

11.3.10 With the vacuum vented, drip enough methanol onto the disk to cover it completely (0.5-1.0 mL). Allow the methanol to soak into the disk for one minute. Add more methanol as needed to keep the disk covered as it soaks.

11.3.11 Pipet 4 mL of disk eluting solvent onto the disk. Apply vacuum until drops appear at the drip tip. Vent the vacuum and allow the disk to soak for one minute.

11.3.12 Draw the disk eluting solution through to just above the top surface of the disk. Add 4 mL of disk eluting solution and draw it completely through the disk. Tap the disk holder assembly gently to loosen adhering drops into the collection tube.

11.3.13 Vent the vacuum, disassemble the disk extraction device, and remove the collection tube. Add disk elution solution to the tube to a known volume.

11.3.14 Analyze samples by HPLC. Some suggested conditions, which were used in developing this method, are listed in Table 1. This table includes the retention times and MDLs that were obtained using the suggested conditions. Figure 1 displays a representative sample chromatogram.

11.4 Identification of Analytes

11.4.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits (Section 11.4.2), to the retention time of a standard compound, then identification is considered positive.

11.4.2 The width of the retention time window used to make identification should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.3 Identification requires expert judgment when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak in a chromatogram, a confirmatory technique must be employed. Through the use of the photodiode array detector, full spectra of the analyte peaks are obtained (Figure 2). When a peak of an unknown sample falls within the retention time windows of method analytes, confirm the peak identification by spectral comparison with analyte standards.

If additional confirmation is required, replace the 1-hexanesulfonic acid salt with 1-heptanesulfonic acid, sodium salt in the mobile phase and reanalyze the samples. Comparison of the ratio of retention times in the samples by the two mobile phases with that of the standards will provide additional confirmation.

11.4.4 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used. Alternatively, the final solution may be diluted with mobile phase and reanalyzed.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Determine the concentration of the analytes in the sample.

12.1.1 Calculate the concentration of each analyte injected from the peak area using the calibration curves in Section 10.3 and the following equation.

$$\text{Concentration, } \mu g/L = \frac{(A) \times (VF)}{(VS)}$$

where: A = Peak area of analyte in sample extract.
VF = Final volume of sample extract, in mL.
VS = Sample volume, in mL.

12.2 Report results as micrograms per liter without correction for recovery data. When duplicate and fortified samples are analyzed, report all data obtained with sample results.

13.0 METHOD PERFORMANCE

13.1 Method Detection Limits (MDL) -- The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level[3]. The MDL data listed in Table 1 were obtained using both cartridges and disks with reagent water as the matrix.

13.2 This method has been tested for linearity of recovery from fortified reagent water and has been demonstrated to be applicable over the range from 4 x MDL to 1000 x MDL.

13.3 Single-laboratory precision and accuracy results at several concentration levels in drinking water matrices using cartridges are presented in Table 2A. Single laboratory accuracy and precision data at a low, a medium, and a fairly high concentration of each compound in several matrices are listed in Table 2B.

14.0 POLLUTION PREVENTION

14.1 Only an extremely small volume of an organic solvent is used in this method. A maximum of 15 mL of methanol is used per sample to condition each cartridge or disk. Methanol is not considered to be a toxic or hazardous solvent. All other chemicals used in this method are not considered toxic when used in the prescribed amounts.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 WASTE MANAGEMENT

15.1 There are generally no waste management problems involved with discarding spent or left over samples in this method since most often the sample matrix is drinking water. If a sample is analyzed which appears to be highly contaminated with chemicals, analyses should be carried out to assess the type and degree of contamination so that the samples may be discarded properly. The Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16.0 REFERENCES

1. Bashe, W.J. "Determination of Diquat and Paraquat in Drinking Waters by High Performance Liquid Chromatography with Ultraviolet Detection," final report, U.S. Environmental Protection Agency, March 1989.

2. Lagman, L.H. and Hale, J.R. "Analytical Method for the Determination of Diquat in Aquatic Weed Infested Lakes and Rivers in South Carolina," Technology Conference Proceedings, WQTC-15, American Water Works Association, November 15-20, 1987.

3. Glaser, J.A., Foerst, D.L., McKee, G.M., Quave, S.A., and Budde, W.L. "Trace Analyses for Wastewaters," Environ. Sci. Technol., 15, 1426, 1981.

4. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Container and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

5. Worobey, B.L. "Analytical Method for the Simultaneous Determination of Diquat and Paraquat Residues in Potatoes by High Pressure Liquid Chromatography," Pestic. Sci 18(4), 245, 1987.

6. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

7. "Handbook of Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Analyte	Retention Time (min)	Method Detection Limits[a] (µg/L) (cartridges)	Method Detection Limits[b] (µg/L) (disks)
Diquat	2.1	0.44	0.51
Paraquat	2.3	0.80	0.59

HPLC Conditions:

Column:	Hamilton PRP-1, 5 µ, 4.1 mm x 150 mm
Column Temperature:	35.0°C
Flow Rate:	2.0 mL/min., Ion-Pair Mobile Phase (Section 7.16)
Injection Volume:	200 µL

Photodiode Array Detector Settings:

Wavelength Range:	210-370 nm
Sample Rate:	One scan/sec.
Wavelength Step:	1 nm
Integration Time:	One second
Run Time:	5.0 minutes

Quantitation:

Wavelengths:	Diquat - 308 nm Paraquat - 257 nm

[a]MDL data were obtained from six samples fortified at 2 µg/L diquat and 2.3 µg/L paraquat.
[b]MDL data were obtained from eight samples fortified at 1 µg/L diquat and 1 µg/L paraquat.

TABLE 2A. SINGLE OPERATOR ACCURACY AND PRECISION USING CARTRIDGES

Analyte	Matrix Type	Number of Analytes	Fortified Concentration (µg/L)	Relative Accuracy (Recovery) (%)	Relative Standard Deviation (%)
Diquat	Reagent Water	6	2.0	85.6	5.1
		6	10	2.1	7.3
		7	100	96.2	5.6
		7	1000	90.0	9.8
	Ground Water	6	100	102	3.7
	Tap[a] Water	6	100	91.3	4.7
Paraquat	Reagent Water	6	2.3	87.6	9.1
		7	11	99.7	6.9
		7	113	94.4	12
	Ground Water	6	113	92.1	3.4
	Tap[a] Water	6	113	74.2	1.8

[a]Dechlorinated with $Na_2S_2O_3$ (100 mg/L).

TABLE 2B. SINGLE OPERATOR ACCURACY AND PRECISION USING DISK (N=5 FOR EACH TYPE OF WATER)

DIQUAT

	Fortified 2.62 µg/L		Fortified 10.5 µg/L		Fortified 52.5 µg/L	
Type of Water	**Mean % Rec.**	**% RSD**	**Mean % Rec.**	**% RSD**	**Mean % Rec.**	**% RSD**
RW	99.2	7.0	88.0	2.5	83.8	5.9
DW	93.5	5.4	82.2	4.1	84.1	2.2
GW	87.4	5.8	90.3	4.6	90.5	1.4
SW	86.5	4.2	82.4	5.6	87.4	6.5
WW	90.5	7.1	78.0	5.2	86.1	3.4

PARAQUAT

	Fortified 2.47 µg/L		Fortified 9.9 µg/L		Fortified 49.5 µg/L	
Type of Water	**Mean % Rec.**	**% RSD**	**Mean % Rec.**	**% RSD**	**Mean % Rec.**	**% RSD**
RW	94.7	9.9	92.6	4.7	90.1	1.4
DW	100.1	6.1	85.2	4.1	85.7	1.2
GW	86.4	6.4	81.4	3.2	86.1	4.0
SW	77.4	8.9	71.8	5.3	81.0	2.6
WW	85.9	6.4	81.6	6.1	87.2	2.3

TABLE 3. 14-DAY SAMPLE HOLDING/PRESERVATION DATA[a]

Analyte	Matrix	Percent Recovery					
		Day 0		Day 7		Day 14	
		R	S_R	R	S_R	R	S_R
Diquat	RW[b]	98.8	± 8.6	93.2	± 1.4	102.	± 2.9
	Tw[c]	84.1	± 1.0	94.1	± 5.8	94.4	± 12.0
	Gw[d]	84.9	± 6.6	87.5	± 3.1	72.4	± 4.5
Paraquat	RW	90.8	± 4.4	86.8	± 4.4	89.2	± 3.9
	TW	72.1	± 0.8	86.7	± 4.7	84.7	± 2.9
	GW	98.1	± 1.4	72.5	± 4.8	66.4	± 7.9

[a]Average of four samples for each matrix. All matrices were preserved with H_2SO_4 (pH = 2). Concentration of each analyte was 100 µg/L.
[b]RW = Reagent water.
[c]TW = Tap water -- Dechlorinated with $Na_2S_2O_3$ (100 mg/L).
[d]GW = Groundwater.

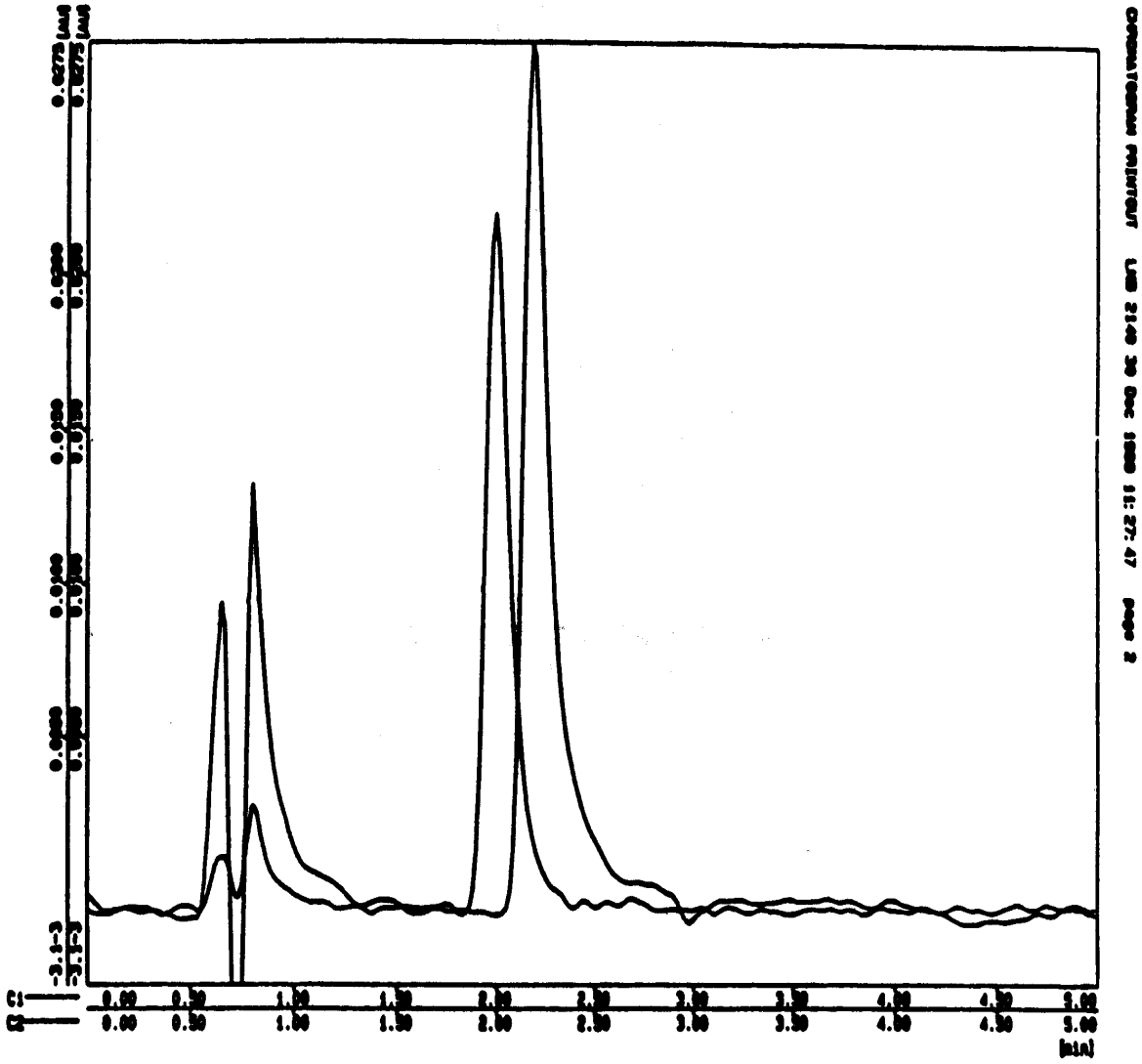

Figure 1. HPLC sample chromatograms of diquat (λ= 308 nm) and paraquat (λ= 257 nm). Retention time of diquat (C = 10 ug/L) is 2.03 min.; retention time of paraquat (C = 11 ug/L) is 2.25 min.

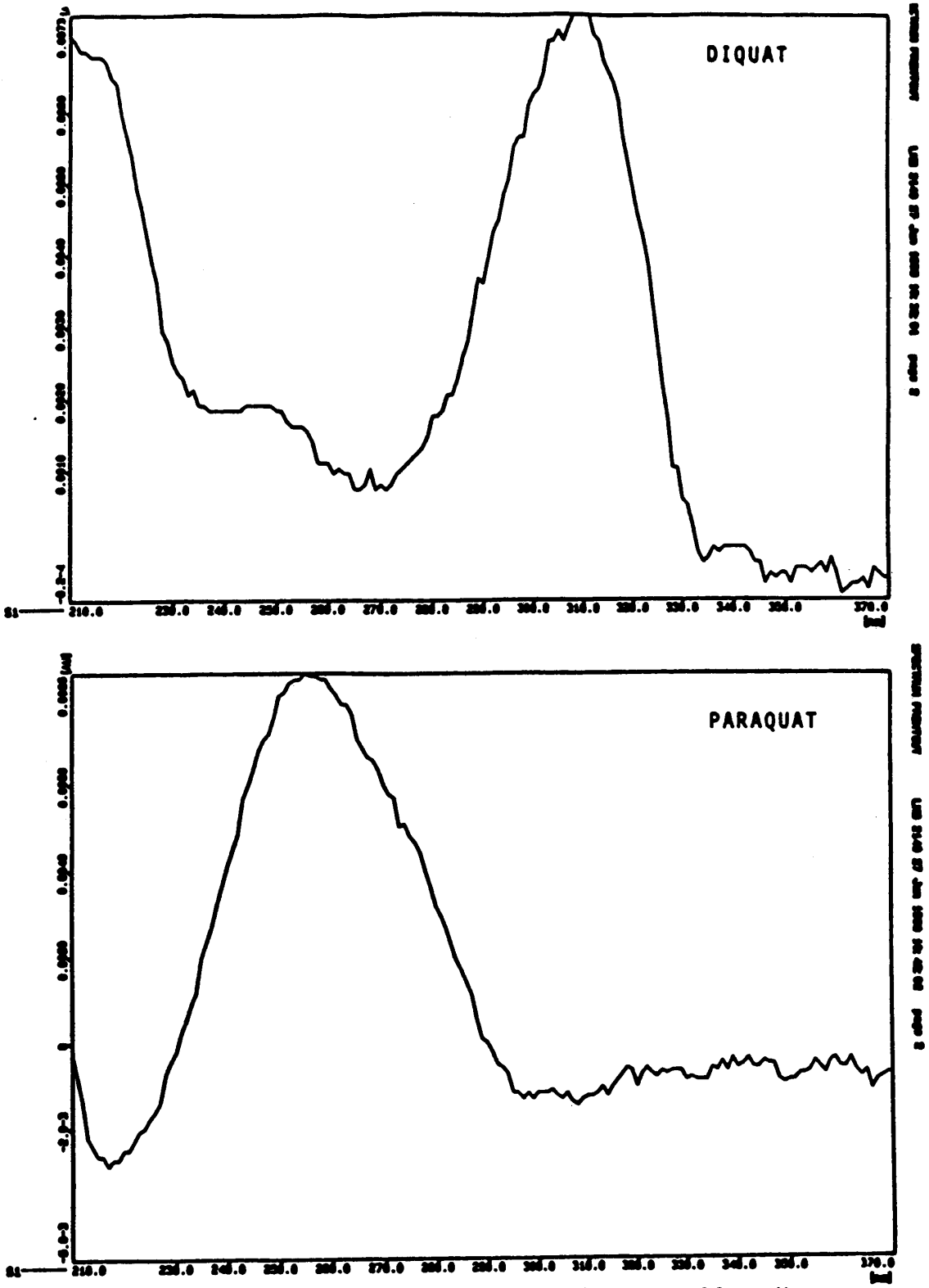

Figure 2. UV spectra of diquat at 10 ug/L and paraquat at 11 ug/L.