Standard Operating Procedure For The Determination of Diquat By Liquid-Solid Extraction And High Performance Liquid Chromatography

1.0 Purpose

1.1 The purpose of this standard operating procedure (SOP) is to allow for the qualitative and quantitative determination of diquat herbicide that may be present in water.

2.0 Scope

2.1 The diquat determination is achieved by combining solid-phase extraction with High Performance Liquid Chromatography (HPLC). This method is modeled after EPA Method 549.1[1]. Diquat is extracted from the water matrix by isolation on a solid phase cartridge. The diquat is then eluted with a small volume of solvent to concentrate the analyte. It is then injected on the HPLC system and detected by ultra-violet(UV) detection. This SOP is applicable to water samples such as drinking water, ground water, and surface water. The method detection limit is currently 0.4 μg/L. Paraquat herbicide may also be analyzed by this procedure but will not be addressed in this SOP. Therefore, compounds that will be analyzed by this procedure are:

Diquat (Analyte number 30680)

3.0 Safety

3.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Therefore, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. Material safety data sheets (MSDS's) should be on file for all analytes and reagents.

4.0 Interferences

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts of elevated baselines in liquid chromatograms. The method must be demonstrated to be free from interferences by running laboratory reagent blanks with each analysis Several potential sources of interferences are discussed on the next page.

4.1.1 Glassware must be thoroughly cleaned. Wash the glassware with water

and detergent followed by rinsing with distilled water. Allow the glassware to dry and rinse with acetone followed by hexane.

4.1.2 EPA Method 549 recommends the silanization of all glassware which will come in contact with the method analytes to prevent adsorption of the diquat cations onto the glass surfaces. The silanization procedure can be found in Section 13 of this SOP. Plasticware should be substituted whenever possible for glassware when doing this procedure. The diquat will not adsorb to the plastic surfaces. Brief experiments done in our lab indicate that silanization of glassware is not necessary for good method performance. Blank spikes were prepared simultaneously using silanized and non-silanized glassware. Plasticware was used as much as possible. The only places where glassware is required are the final cartridge elution and the autosampler vials. The data to support the use of non-silanized glassware are as follows:

Extraction Date	Silanized Rec(%)	Non-silanized Rec(%)
4-5-93	72	79
4-6-93	51	60
4-12-93	47	55

The data indicate that use of non-silanized glassware does not have a detrimental effect on the diquat analysis.

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.

4.1.5 Interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source.

5.0 Sample Collection, Preservation, and Handling

5.1 Grab samples must be collected in either PVC high density bottles or silanized amber glass bottles. Conventional sampling procedures should be followed[2].

Automatic sampling equipment must be free as possible of adsorption sites that might extract the sample.

5.2 The samples must be iced or refrigerated at 4 degrees C from the time of collection until extraction. Diquat is light sensitive and must be protected from light.

5.3 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 with the addition of sulfuric acid to pH 2 to prevent adsorption of diquat by the humectant material.

5.4 Samples must be extracted within 7 days of collection. Extracts must be analyzed within 21 days of extraction.

6.0 Chemicals, Reagents, and Stock Solutions

6.1 Chemicals

6.1.1 Reagent water - For use in the HPLC mobile phase and in the preparation of other reagents. Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Distilled water purified by a Barnstead Nanopure II system is suitable for this procedure. The water from the Barnstead system must be filtered through 0.45 μM filters using mechanical means to remove microparticulates that are detrimental to the HPLC system.

6.1.2 Methanol - HPLC grade or higher purity

6.1.3 Orthophosphoric acid, 85% (w/v) - reagent grade

6.1.4 Diethylamine (DEA) - reagent grade

6.1.5 Concentrated sulfuric acid - ACS reagent grade

6.1.6 Sodium hydroxide - reagent grade

6.1.7 Concentrated hydrochloric acid - 12 N reagent grade

6.1.8 Cetyl trimethyl ammonium bromide - 95%, available from Aldrich

6.1.9 Sodium thiosulfate - reagent grade

6.1.10 1-Hexanesulfonic acid, sodium salt - 98%, available from Aldrich

6.1.11 1-Heptanesulfonic acid, sodium salt - 98%, available from Aldrich

6.1.12 Ammonium hydroxide - ACS concentrated

6.1.13 Sylon CT - silanization solution, available from Supelco

6.2 Reagent Solutions

6.2.1 Conditioning solution A - Dissolve 0.500 g cetyl trimethyl ammonium bromide and 5 ml concentrated ammonium hydroxide in 500 ml of deionized water and dilute to 1000 ml in a volumetric flask. This solution is used to condition the solid phase extraction cartridges.

6.2.2 Conditioning solution B - Dissolve 10.0 g of 1-hexanesulfonic acid, sodium salt, and 10 ml of concentrated ammonium hydroxide in 250 ml deionized water and dilute to 500 ml in a volumetric flask. This solution is used to condition the solid phase extraction cartridges.

6.2.3 Sodium hydroxide solution, 10% w/v - Dissolve 50 g sodium hydroxide in 400 ml deionized water and dilute to 500 ml in a volumetric flask. This solution is used for the pH adjustment of the samples, if necessary.

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

6.2.5 Cartridge eluting solution - Add 13.5 ml of orthophosphoric acid and 10.3 ml diethylamine to 500 ml deionized water and dilute to 1000 ml in a volumetric flask. This solution is used to elute the analytes from the solid phase cartridges and can be stored for several months at room temperature.

6.2.6 Ion-pair concentrate - Dissolve 3.75 g 1-hexanesulfonic acid in 15 ml of the cartridge eluting solution and dilute to 25 ml with eluting solution. An aliquot of this solution is added to each standard and sample to form the ion-pair that allows for the diquat to be retained on the HPLC column.

6.3 Diquat stock solution

6.3.1 Diquat standards can be prepared from dry, pure powder or can be purchased from several vendors at certified concentrations. It is

recommended to use the certified solutions. Diquat is only available as diquat dibromide. Therefore, extra care must be taken to be sure that the concentration of the standard is in terms of diquat and not diquat dibromide.

6.3.1.1 Preparation from dry powder - According to EPA Method 549, 196.8 mg diquat dibromide monohydrate in 100 ml water is equivalent to 1.0 mg/ml diquat. Therefore, the conversion factor for calculation of the diquat is:

1.0/1.968 = 0.51 mg diquat/mg diquat dibromide

Dry the diquat dibromide in an oven at 110 degrees C for 3 hours. Repeat the process until a constant weight is achieved. Weigh the diquat dibromide into a volumetric flask and dissolve with water. Multiply the weight of the diquat dibromide by the conversion factor to obtain the concentration of diquat in the stock solution.

6.3.1.2 Preparation from commercially prepared solutions - Diquat is available from several commercial sources. Be sure the concentration is in terms of diquat. Prepare dilutions as necessary for the analysis. See Section 9.2.3 for the details on standard preparation.

7.0 Equipment and Apparatus

7.1 Analytical equipment

7.1.1 LDC Analytical Constametric 4100 solvent delivery system with Membrane Degasser or an equivalent system that is capable of delivering an isocratic mobile phase at a constant flow.

7.1.2 TSP SpectraSYSTEM AS3000 autosampler or equivalent automatic sampling system capable of delivering consistent injection volumes ranging from 100 μl to 1000 μl.

7.1.3 LDC Analytical SM5000 or equivalent detection system capable of monitoring ultraviolet(UV) absorption at 308 nm.

7.1.4 Fiatron CH-30 oven and TC-50 temperature controller or equivalent system capable of maintaining a constant elevated temperature for the

HPLC column.

7.2 Extraction apparatus

7.2.1 Solid phase extraction cartridges, C8, 500 mg. Available from Varian.

7.2.2 Solid phase extraction manifold capable of holding up to 24 solid phase extraction cartridges. Available from Supelco.

7.2.3 Vacuum system capable of maintaining a vacuum at 8-10 mm Hg. A water aspirator is sufficient to maintain this level of vacuum.

7.2.4 60 ml reservoirs to attach to the solid phase cartridges.

7.2.5 Syringe membrane filters, 0.45 μM pore size, 25 mm diameter, Teflon membrane. Samples must be filtered to remove particulates before analysis.

7.2.6 Disposable syringes to be used when filtering samples.

8.0 Sample Extraction Procedure

8.1 Prior to the sample extraction, the C8 solid phase cartridges must be conditioned as described below.

8.1.1 Attach the C8 cartridges to the extraction manifold.

8.1.2 Elute the following solutions through the cartridge in the order given. The cartridge must not be allowed to go dry. The flow rate should be about 10 ml/min.

8.1.2.1 Deionized water, 5 ml
8.1.2.2 Methanol, 5 ml
8.1.2.3 Deionized water, 5 ml
8.1.2.4 Conditioning solution A, 5 ml
8.1.2.5 Deionized water, 5 ml
8.1.2.6 Methanol, 5 ml
8.1.2.7 Deionized water, 5 ml
8.1.2.8 Conditioning solution B, 10 ml
Retain this solution in the cartridge to keep it activated.

8.1.3 The C8 cartridges may be conditioned up to 48 hours in advance. After

conditioning, they must be capped and stored at 4 degrees C if they are not used immediately.

8.1.4 Blank spike, sample spike, and check sample preparation

8.1.4.1 Prepare blank and matrix spike samples prior to the extraction of the samples. Using a diquat stock solution other than that used for standard preparation, make the appropriate dilutions into 1 L of reagent and matrix water to make a diquat concentration of about 4 μg/L. This is 10 times the method detection limit (MDL) of 0.4 μg/L. These should be prepared in the same PVC bottles that are use for sample collection.

8.1.4.2 Using an old Water Supply check sample of known concentration, prepare a check sample in 1 L of reagent water at about 4 μg/L. This should be prepared in the same PVC bottles that are used for sample collection.

8.1.5 Using plastic graduated cylinders, measure 250 ml aliquot of each sample into separate 400 ml plastic beakers. Also measure 1 blank, blank spike, sample duplicate, and check sample for every 10 samples to be analyzed.

8.1.6 Immediately before extraction, adjust the pH of the sample to 10.5 plus or minus 0.2 with the 10% sodium hydroxide or 10% hydrochloric acid solutions described in Section 6.2.

8.1.7 Place the conditioned C8 cartridges on the extraction manifold. Attach the 60 ml reservoirs to the cartridges with the appropriate adapters. Transfer the measured 250 ml volumes in aliquot from the beakers to the reservoirs. Do this before turning on the vacuum to keep the cartridge wetted. Turn on the vacuum and adjust the flow rate to 3 to 6 ml/min. Allow the waste to be discarded. With an extraction volume of 250 ml and a flow of about 5 ml/min, it should take about 50 minutes to extract the samples. After the sample has been filtered, wash the column with 5 ml methanol. Continue to draw the vacuum through the cartridge for one additional minute to dry the cartridge. Release the vacuum.

8.1.8 Place 5 ml volumetric flasks beneath the collection stems in the vacuum manifold. Add 4.5 ml of the eluting solution (Section 6.2.5) to the sample cartridge. Elute the cartridge at a flow of about 1 to 2 ml/min.

8.1.9 Remove the 5 ml flasks with the extracts. Add 100 μl of the ion-pair

concentrate (Section 6.2.6) to each extract. Dilute to volume with eluting solution, mix well, and transfer the entire solution to plastic scintillation vials until analysis.

9.0 Analytical Procedure

9.1 Analytical conditions

9.1.1 HPLC column - Hamilton PRP-1, 5μm, 150 X 4.1 mm Part number: 79444

9.1.2 HPLC guard column - Hamilton PRP-1, using the Hamilton cartridge system.

9.1.3 HPLC mobile phase - isocratic - To a 1 L volumetric flask, add the following:

9.1.3.1 13.5 ml orthophosphoric acid, 85%
9.1.3.2 10.15 ml diethylamine
9.1.3.3 3 g heptanesulfonic acid, sodium salt

Depending on the sample load, several liters of mobile phase may be required.

9.1.4 Mobile phase flow rate: 2.0 ml/min

9.1.5 Column temperature: 30 degrees C

9.1.6 Injection volume: 400 μl

9.1.7 Data collection time: 20 minutes

9.1.8 Detector: UV at 308 nm

9.2 Standard Preparation

9.2.1 The commercially available diquat standards are typically at a a concentration of 1000 μg/ml.

9.2.2 Prepare an intermediate standard in reagent water as follows:

<u>Intermediate</u>: 1000 μg/ml X 100 μl/5000 μl = 20 μg/ml

9.2.3 The analytical standards are prepared in the cartridge eluting solution.

<u>Standard 1</u>: 20 μg/ml X 10 μl/10000 μl = 0.02 μg/ml
Add 200 μl ion-pair concentrate
<u>Standard 2:</u> 20 μg/ml X 12.5 μl/5000 μl = 0.05 μg/ml
Add 100 μl ion-pair concentrate
<u>Standard 3:</u> 20 μg/ml X 50 μl/5000 μl = 0.20 μg/ml
Add 100 μl ion-pair concentrate
<u>Standard 4:</u> 20 μg/ml X 100 μl/5000 μl = 0.40 μg/ml
Add 100 μl ion-pair concentrate

9.2.4 Spike and check sample preparation

9.2.4.1 The spike and check samples are prepared when the samples are extracted. The preparation will vary depending on the concentrations of the stock solution used for the preparation. See Section 8.1.4 for the basic preparation procedure.

9.3 HPLC setup and equilibration

9.3.1 Turn on the HPLC system components. Flush the column with a 50/50 mixture of reagent water/acetonitrile. Then flush with a 10/90 mixture of reagent water/acetonitrile. Follow this with a brief 50/50 flush to ease the transition to the next step. Lastly, flush with 90/10 mixture of reagent water/acetonitrile. When the flushing has been completed, begin pumping the mobile phase through the column. It may be convenient to distribute the mobile phase into 2 or more reservoirs. The mobile phase has a high salt content, so the pump should not be allowed to stand without flow for an extended period of time. Salts can deposit and damage pump parts if allowed to stand without flow.

9.4 HPLC analysis

9.4.1 Transfer standards and samples to autosampler vials. Inject the standards at the beginning and end of the run. If the run is long, a third set of standards should be added to the middle of the run. Computer setup and use of software will not be included here. It will be left to the analyst to determine the procedures as the procedures vary from instrument to instrument.

9.5 HPLC shutdown

9.5.1 When the analysis has been completed, several steps are necessary to prepare the system for shutdown. First, flush the column with a 90/10 mixture of reagent water/acetonitrile to remove the mobile phase with it's high salt content. Do this until about 30 to 40 ml of solvent has been flushed. Then flush with a 50/50 mixture of reagent water/acetonitrile. Then flush with a 10/90 mixture of reagent water/acetonitrile. Lastly, flush with a 50/50 mixture of reagent water/acetonitrile. The column is stored in the 50/50 mixture.

10.0 Data Analysis

10.1 Identification of diquat

10.1.1 Identification of diquat is done by comparing retention times of diquat in the samples to diquat in the standard injections. The retention time of diquat under these conditions is typically around 9 minutes. HPLC analytes are subject to retention time shifts. The window of acceptability is usually $\pm$ 20 seconds of the average standard retention time.

10.1.2 Typical chromatograms are included at the end of this SOP.

10.2 Calculations of results

10.2.1 The computer software is typically used to calculate results. The procedures will vary depending upon the system used. It will be left to the analyst to implement the software.

11.0 Quality Control

11.1 Quality control consists of the analysis of laboratory reagent blanks, laboratory fortified blanks, and laboratory fortified samples.

11.1.1 Laboratory reagent blanks - A laboratory reagent blank (lab blank) must be analyzed with each set of samples. If within the retention time window of diquat the lab blank produces a peak that would prevent the determination of that analyte, determine the source of the contamination and eliminate the interference before processing samples.

11.1.2 Laboratory fortified blanks - The analyst must analyze at least one laboratory fortified blank (blank spike) with every 10 samples or one per sample set, whichever is greater. The spiking level should be 10 times the method detection limit, which is currently 0.4 μg/L. The spikes should be

prepared along with the sample extractions. See Section 8.1.4 for the basic spike preparation procedure.

11.1.2.1 Control limits - Control limits are determined calculating upper and lower limits from the mean percent recovery (X) and the standard deviation (S) of the percent recovery. The equations for determining the limits are:

Upper limit = X + 3S
Lower limit = X - 3S

After each ten new recovery measurements, new control limits should be calculated using the most recent 30 data points.

11.1.2.2 Acceptability of results - Any diquat blank spike result whose percent recovery for that set of samples is outside the control limits cannot be reported. The set must be reanalyzed with a new spike or not reported.

11.1.3 Laboratory fortified samples - The analyst must analyze at least one laboratory fortified sample (sample spike) with every 10 samples or one per sample set, whichever is greater. The spiking level should be 10 times the method detection limit, which is currently 0.4 μg/L. The spikes should be prepared along with the sample extractions. See Section 8.1.4 for the spike preparation procedure.

11.1.3.1 Control limits - Control limits are determined by calculating upper and lower limits from the mean percent recovery (X) and the standard deviation (S) of the percent recovery. The equation for determining the limits are:

Upper limit = X + 3S
Lower limit = X - 3S

After each ten new recovery measurements, new control limits should be calculated using the most recent 30 data points.

11.1.3.2 Acceptability of results - Any diquat results whose percent recovery in the sample spike for that set of samples is outside the control limits cannot be reported. The set must be reanalyzed with a new spike or not reported.

11.2 System suitability - No formal system suitability is done on a daily basis. The standards are evaluated as they become available. Generally, if the diquat retention time is similar to that of the previous analysis, peak shape is good, and there is resolution between diquat and the minor peak that elutes shortly after diquat, the system is considered suitable for analysis. HPLC system pressures are recorded at the beginning of each analysis and compared to previous readings to detect changes that indicate system problems.

12.0 Documentation

12.1 Results are typically recorded in a notebook.

12.2 Compare the quality control samples to their control limits. Record the spike results and note if they pass or fail to indicate result acceptability.

12.3 Computer files - The computer files should be transferred to diskettes and stored for future reference. After the files have been transferred, they can be deleted from the computer hard drive.

12.4 Hard copies of the reports and chromatograms should be filed.

13.0 Silanization Procedure - Following is the procedure to be followed if the analyst desires to use silanized glassware. Sylon CT Silanizing Reagent is used for this procedure.

13.1 Sylon CT silanizing reagent is a highly reactive material consisting of 5% dimethyldichlorosilane in toluene (w/v). The reagent is stable for up to 2 years when stored in a cool dry place.

13.2 Several precautions must be observed.

13.2.1 Wear protective glasses and gloves when handling this reagent.

13.2.2 Use the reagent only in well ventilated areas.

13.2.3 Keep the reagent away from open flames.

13.3 Procedure[3]

13.3.1 Coat the glass surface by rinsing with the reagent for 10 to 15 seconds, then discard the reagent.

13.3.2 Rinse the surface two times with toluene.

13.3.3 Rinse the surface three times (or until the rinse is neutral) with methanol.

13.3.4 Dry the surface, using nitrogen.

14.0 References

14.1 EPA 549.1 Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and HPLC with Ultraviolet Detection, Revision 1.0 (1992)

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

14.3 Supelco Data Sheet, DS13383A. Data sheet was supplied with the product.