APPENDIX D-12
Analytical Procedure for Chelator (EDTA): Method AP-0047

1.0 PURPOSE

This procedure provides instructions to perform (Ethylene dinitrilo)tetraacetic Acid (EDTA) determinations by high performance liquid chromatography (HPLC). See note 9.1.

2.0 SCOPE

This procedure is applicable to aqueous samples or liquid extracts from soil samples.

3.0 SUMMARY

Reagent containing ferric ion (Fe^{3+}) is added to all samples and standards. The EDTA forms a complex with the ferric ion to form a UV-absorbing chromophore. The analysis is accomplished using ion-pair HPLC with a diode array detector.

4.0 REFERENCES

4.1 "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods", SW-846, 3rd Edition, Most Recent Update (July 1992 with proposed methods dated November 1992)

4.1.1 Chapter 1, "Quality Assurance"

4.1.2 Chapter 4, "Organic Analysis"

4.1.3 Method 8000A, "Gas Chromatography"

4.2 "Extraction of EDTA from Soils", AP-0057, Environmental Applications, Tennessee Valley Authority, Muscle Shoals, Alabama

5.0 RESPONSIBILITIES

5.1 The Specialty Laboratory supervisor, or his designee, shall ensure that this procedure is followed during the determination of EDTA.

5.2 The laboratory group leader, or his designee, shall delegate the performance of this procedure to personnel experienced with this procedure. The group leader is responsible for reviewing all data generated. The group leader is responsible for training new personnel on this procedure.

5.3 The chemist or analyst shall follow this procedure, shall ensure the accuracy of all calculations, and shall report any abnormal results or nonconformances to the laboratory group leader.

6.0 **REQUIREMENTS**

6.1 Prerequisites

6.1.1 All soil samples must be extracted by the method: "Extraction of EDTA from Soils" AP-0057 before analysis.

6.2 Limitations and Actions

6.2.1 High levels of EDTA (>500 ppm) affect the response to EDTA in subsequent samples. Samples following those with high levels of EDTA shall be carefully reviewed and reanalyzed as needed.

6.2.2 All samples reading higher than the calibration curve shall be diluted into the range of the calibration curve.

6.3 Apparatus/Equipment

6.3.1 Analytical balance, capable of reading to 0.1 mg.

6.3.2 HPLC system with diode array detector.

6.3.3 HPLC column, Supelco LC-8DB, 5 micron, 15 cm x 4.6 mm.

6.3.4 Guard column, Supelco LC-ABZ.

6.3.5 Sand bath, constant temperature at approximately 90-95 degrees C.

6.3.6 0.2 micron nylon syringe filter.

6.3.7 0.45 micron, type HA Millipore filter.

6.4 Reagents and Standards

6.4.1 Tetrabutylammonium (dihydrogen) Phosphate (TBAP), reagent grade.

6.4.2 Sodium Hydroxide, NaOH, approximately 25% solution, reagent grade.

6.4.3 Sodium phosphate monobasic, monohydrate, reagent grade.

6.4.4 Phosphoric acid, approximately 40 % solution, reagent grade.

6.4.5 Methanol, HPLC grade.

6.4.6 Ethylenediaminetetraacetic acid, disodium salt, dihydrate (EDTA) reagent grade. Formula weight 372.24 g/mole. Correct all weights of the dihydrate to the anhydrous basis by multiplying by the ratio 336.21/372.24 (0.90321).

6.4.7 Water, HPLC grade.

6.4.8 HPLC Mobile Phase

6.4.8.1 To 400 ml of HPLC grade water, add 1.69g tetrabutylammonium phosphate (TBAP).

6.4.8.2 Add 6.9 g of sodium phosphate monobasic, monohydrate. The pH will be approximately 4.5.

6.4.8.3 Add 100 ml HPLC grade methanol. Mix well.

6.4.8.4 Filter solution through a 0.45 micron type HA millipore filter.

6.4.8.5 Dilute to 1 L with HPLC grade water.

6.4.9 Iron Reagent

6.4.9.1 To 40 ml of HPLC grade water, add 1.69 g of tetrabutylammonium phosphate (TBAP).

6.4.9.2 Add 0.69 g sodium phosphate monobasic, monohydrate.

6.4.9.3 Adjust pH to 3.0 with 0.05 M phosphoric acid.

6.4.9.4 Add 0.5 g ferric nitrate.

6.4.9.5 Mix and allow to stand for 1 hour.

6.4.9.6 Centrifuge solution and decant aqueous phase.

6.4.9.7 Filter the solution through a 0.45 micron type HA millipore filter.

6.4.9.8 Dilute to 100 ml with HPLC grade water.

6.4.10 EDTA, disodium salt, 1000 ppm cal stock. Weigh approximately 0.1 g of EDTA (weighed to the nearest 0.1 mg) and dilute to 100 ml with HPLC grade water. J.T. Baker ultrapure bioreagent.

6.4.11 EDTA, disodium salt, calibration standards. Dilute the 1000 ppm stock standard to produce the following calibration standards: 1 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm calibration standards.

6.4.12 EDTA, disodium salt, lab control sample and spiking solution 1000 ppm stock. Weigh approximately 0.1 g of EDTA (weighed to the nearest 0.1 mg) and dilute to 100 ml with HPLC grade water. Reagents, Inc.

6.4.13 EDTA, disodium salt, secondary QC standard. Dilute the 1000 ppm QC stock to produce the following QC standards: 75 ppm spiking solution and 15 ppm QC check standard.

6.5 Quality Control Sample Requirements

6.5.1 Each batch of samples must have the following quality control samples: One spiked sample, one duplicate spike sample, one sample duplicate, one laboratory control sample and one method blank.

6.5.2 The accuracy of the calibration curve is checked on a daily basis with a midpoint check standard analyzed once per every 10 samples analyzed and at the end of the analysis. Recalibration is not required with subsequent analysis unless the midpoint check falls outside the 85 to 115 percent range.

7.0 **PROCEDURE**

7.1 Calibration

7.1.1 Calibrate the instrument with the following standards: 1 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm.

7.1.2 Pipette 1 ml of each known standard into an HPLC sample vial.

7.1.3 Add 0.1 ml of the iron reagent.

7.1.4 Mix thoroughly.

7.1.5 Analyze standards with parameters as in 7.2.3. Utilize vendor-supplied chromatography workstation software to fit the calibration data. Inspect the curve for goodness of fit of 0.99 or better.

7.2 Procedure Instructions

7.2.1 Sample Preparation

7.2.1.1 Filter the aqueous sample through a 0.2 micron nylon syringe filter.

7.2.1.2 Pipette 1 ml of the sample into an HPLC vial.

7.2.1.3 Add 0.1 ml of the iron reagent.

7.2.1.4 Mix thoroughly by shaking.

7.2.3 Instrument Parameters

7.2.3.1 Detector: Photodiode array.

7.2.3.2 Wavelength: 254 nm.

7.2.3.3 Column: Supelcosil LC-8DB; 15 cm x 4.6 mm with guard, LC-ABZ, 2 cm.

7.2.3.4 Flow rate: 1.5 ml/min.

7.2.3.5 Analysis time: 10 minutes.

7.2.3.6 Injection volume: 50 microliters

7.2.4 HPLC Sample Analysis

7.2.4.1 Turn the detector on, allow approximately 1 hour for lamp to warm up.

7.2.4.2 Turn the pump on; 60/40 methanol/water and allow the system to stabilize. NOTE: Prime the pump before operation.

7.2.4.3 Change the composition of the pump to 100% water and allow the system to stabilize.

7.2.4.4 Change the mobile phase of the system to 100% iron reagent mobile phase and allow the system to stabilize.

7.2.4.5 Place the samples on the autosampler and create a sample list. Activate the newly created sample list.

7.2.4.6 Activate the analysis.

7.2.5 Cleaning Column After Analysis

7.2.5.1 Change the mobile phase of the system to 100% water and allow the system to stabilize after the analysis is complete.

7.2.5.2 Change the mobile phase of the system to 60/40 methanol/water and allow the system to stabilize.

7.3 Calculations and Recording Data

7.3.1 The percent recovery for spikes are to be calculated as follows:

$$\% \text{ SPREC} = \frac{\text{SP - SAMP}}{\text{SP1}} \times 100\%$$

where:

SPREC = Percent spike recovery
SP = Actual spike read
SAMP = Spike's corresponding sample read
SP1 = Theoretical value of spike

7.3.2 The percent recovery for control samples and checks are to be calculated as follows:

$$\% CK = \frac{C1}{C2} \times 100$$

where:

CK = Percent recovery for control sample or check standard.
C1 = Actual known value reading
C2 = Theoretical value of known

7.3.3 Utilize commercial chromatography workstation software or a suitable spreadsheet to apply calibration curve factors to peak heights to calculate concentration in samples

Example: When a calibration curve has been fit to the equation $C = A + Bx$ (where x is observed peak height), the concentration would be calculated as:

Conc = (A + Bx) * Volume / Weight * DF

For a soil sample:

A, B = fit parameters of calibration curve
x = observed peak height
Volume = final extraction volume
Weight = weight of soil extracted, corrected for moisture
DF = dilution factor (when sample was diluted) or 1.000

Reporting units would be mg Disodium EDTA/kg soil

(However, see Note 9.2)

For a liquid sample (direct injection):

A, B = fit parameters of calibration curve
x = observed peak height
Volume = 1.000
Weight = 1.000
DF = dilution factor (when sample was diluted) or 1.000

Reporting units would be mg Disodium EDTA/Liter

7.3.4 File all original data, preparation worksheets, chromatograms, calculations, quality control summary sheets, and printouts with the workorder as quality assurance records.

8.0 **SAFETY**

8.1 Read Material Safety Data Sheets (MSDS).

8.2 Wear gloves when handling chemicals. Avoid inhalation of dust.

8.3 Wear lab coat and safety glasses while performing this procedure.

8.4 Material Safety Data Sheets (MSDS) are available for tetrabutyl ammonium phosphate, methanol, sodium hydroxide, EDTA, ferric nitrate and sodium phosphate monobasic, monohydrate.

9.0 **NOTES**

9.1 The chemical names Ethylenediamine tetraacetic acid and (Ethylenedinitrilo)tetraacetic acid are synonyms.

9.2 For the Lead Phytoremediation project, report values as milligrams Disodium EDTA per liter in the extract. Also report sample weight and percent moisture separately.

In this case: Conc = (A + Bx) * Volume / Weight * DF

Where

A, B = fit parameters of calibration curve
x = observed peak height
Volume = final extraction volume
Weight = 1.000
DF = dilution factor (when sample was diluted) or 1.000

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure