The Determination of Picloram in Municipal and Industrial Wastewater by HPLC-UV

Instrument: Waters Alliance 2695 HPLC system with Waters 2487 Dual Absorbance UV/Vis Detector

1. SCOPE AND APPLICATION

1.1 This method covers the determination of picloram in municipal and industrial wastewater.

Parameter CAS No.

Picloram 1918-02-1

1.2 The estimated detection limit (EDL) for picloram is listed in Table 1. The EDL was calculated from the minimum detectable response being equal to 5 times the background noise using a 100-µL injection. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.

1.3 This is a high-performance liquid chromatographic (HPLC) method applicable to the determination of picloram in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for picloram, compound identification should be supported by at least one additional qualitative technique.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of liquid chromatograms.

2. SUMMARY OF METHOD

2.1 Picloram is removed from the acidified sample matrix by extraction with methylene chloride. The extract is dried, exchanged to HPLC mobile phase, and analyzed by HPLC with ultraviolet (UV) detection. An alkaline back-extraction is used as necessary to eliminate interferences which may be encountered.

3. INTERFERENCES

3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of liquid chromatograms .All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section

3.1.1 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.

3.1.2 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible 1after 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 reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle

furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

3.2 Matrix interferences may be caused by UV-active contaminants that coelute with picloram. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limit listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. 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.

Additional references to laboratory safety are available and have been identified for the 2-4 information of the analyst.

5. APPARATUS AND EQUIPMENT

5.1 Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and reagent water. Allow the bottles and cap liners to air dry, then muffle at 400°C for 1 hour. After cooling, rinse the bottles and cap liners with hexane, seal the bottles, and store in a dust-free environment.

5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Kuderna-Danish (K-D) glassware.

5.2.1 Synder column: Three-ball macro (Kontes K-503000-0121 or equivalent) and two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-l025 or equivalent) with ground-glass stopper.

5.2.3 Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.3 High-performance liquid chromatography (HPLC) apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detector and strip-chart recorder. A data system is recommended for measuring peak areas.

5.3.1 Isocratic pumping system, constant flow.

5.3.2 Injector valve (Rheodyne 7125 or equivalent) with 100-µL loop.

5.3.3 Column: 250 mm long by 4.6 mm ID, stainless steel, packed with reverse-phase Ultra sphere ODS, 10 µ.

5.3.4 Ultraviolet detector, variable wavelength, capable of monitoring at 225 nm.

5.3.5 Strip-chart recorder compatible with detector, 250 mm. (A data system for measuring peak areas is recommended.)

5.4 Chromatographic column: 300 mm long by 10 mm ID Chromaflex, equipped with coarse-fritted bottom plate and PTFE stopcock. (Kontes K-420540-0213 or equivalent).

5.5 Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.

5.6 Miscellaneous.

5.6.1 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6.2 Separatory funnels: 2-L, 500-mL, and 250-mL, equipped with PTFE stopcocks.

5.6.3 Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride for 2 hours.

5.6.4 Water bath: Heated with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

5.6.5 Pasteur pipettes and bulbs.

5.6.6 Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

6. REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagents.

6.1.1 Acetone, hexane, methanol, and methylene chloride: Demonstrated to be free of analytes and interferences.

6.1.2 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.1.3 Sodium hydroxide (NaOH) solution (0.3N): Dissolve 12 g NaOH in reagent water and dilute to 1000 mL.

6.1.4 Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.

6.1.5 Sodium chloride: ACS, crystals.

6.1.6 Sulfuric acid (H SO) solution (1+1): Add a measured volume of concentrated H_2 SO_4 to an equal volume of reagent water. 2 4

6.1.7 HPLC buffer (pH 2, 0.1M phosphate): Dissolve 5.83 g of KH PO (ACS) and 3.9 mL of 85% phosphoric acid (ACS) in 1-L of reagent water.

6.1.8 HPLC mobile phase: Add 570 mL of HPLC buffer solution to a 1-L volumetric flask and dilute to volume with methanol.

6.2 Standard stock solutions (1.00 µg/µL): These solutions may be purchased as a certified solution or prepared from the pure standard material using the following procedures.

6.2.1 Prepare the stock standard solution by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide-quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.2.2 Transfer the stock standard to a PTFE-sealed screw-cap bottle. Store at 4 C and N protect from light. The stock standard should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from it.

6.2.3 The stock standard must be replaced after 6 months, or when comparison with a quality control check sample indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers.

Conventional sampling practices should be followed, except that the bottle must not be 5 prewashed with sample before collection.

7.2 The samples must be iced or refrigerated at 4°C from the time of collection until analysis.

Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be analyzed within 48 hours of collection, the sample should be adjusted to a pH range of 1.0 to 3.0 with sodium hydroxide or sulfuric acid.

7.3 All samples must be extracted within 7 days of collection and analyzed within 30 days of extraction.6

8. CALIBRATION

8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.

8.2 Prepare calibration standards at a minimum of three concentration levels of picloram by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase. One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

8.3 Using injections of 100 µL of each calibration standard, tabulate peak height or area response against the mass injected. The results are used to prepare a calibration curve for picloram. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

8.4 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for picloram varies from the predicted response by ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.

9. QUALITY CONTROL

9.1 Monitoring for interferences.

9.1.1 Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of picloram, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

9.2.1 After every 10 samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

9.2.1.1 Laboratory control standard concentrate: From the stock standard prepared as described in Section 6.3, prepare a laboratory control standard concentrate that contains picloram at a concentration of 2 µg/mL in methanol or other suitable solvent.7

9.2.1.2 Laboratory control standard: Using a pipette add 1.0 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.

9.2.1.3 Analyze the laboratory control standard as described in Section 10.

Calculate the percent recovery (Pi) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

Si = Analytical results from the laboratory control standard, in µg/L

Ti = Known concentration of the spike, in µg/L

9.2.2 At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.7

9.3 Assessing precision

9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both sample bottles for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of picloram.

9.3.2 Calculate the relative range (RR) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

Ri = Absolute difference between the duplicate measurements X_1 and X_2, in µg/L

Xi = Average concentration found $\left(\frac{X_1 + X_2}{2}\right)$, in µg/L

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 1.5 to 2.5 with sulfuric acid. Add 200 g of sodium chloride and mix to dissolve.

10.1.2 Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for aminimum of 10 minutes. If the emulsion interface between layers is more thanone-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.

10.1.3 Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

10.1.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate. If the extract requires cleanup, collect the extract in a 500-mL separatory funnel and proceed to Section 10.2 (cleanup and separation). If the extract does not require cleanup, collect the extract in a 500-L K-D flask equipped with a 10-mL concentrator tube and proceed with Sections 10.1.5 and 10.1.6.Method

10.1.5 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Pre-wet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Add 50 mL of methanol and a clean boiling chip to the flask and repeat the concentration as described above. When the apparent volume of the liquid reaches 1 mL, remove the K-D apparatus and

allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methanol. A 5-mL syringe is recommended for this operation.

10.1.6 Add a clean boiling chip to the concentrator tube. Attach a two-ball micro-Snyder column. Pre-wet the micro-Snyder column by adding about 0.5 mL of methanol to the top. Place the micro K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methanol. Quantitatively transfer the extract to a 25-mL volumetric flask by means of a Pasteur pipette or other suitable device. Rinse the concentrator tube with about 0.5 mL of methanol and add to the volumetric flask. Adjust the final volume to 25 mL or to a volume suitable for liquid chromatography with HPLC mobile phase. Store refrigerated if further processing will not be performed immediately. Proceed with liquid chromatographic analysis.

10.1.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

10.2.1 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 clean waters and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that recorded in Table 2.

10.2.2 Collect the dried extracts from Section 10.1.4 in a 500-mL separatory funnel. Add in 10 mL of 0.3N NaOH and extract by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow a 10-minute separation time. Drain the methylene chloride and discard. Allow 2 minutes for the aqueous layer to drain from the walls, and collect it in a 25-mL volumetric flask.

10.2.3 Adjust the pH of the aqueous extract to 1.5 to 2.5 with sulfuric acid solution, and dilute to volume with HPLC mobile phase.

10.2.4 Proceed with liquid chromatography as described in Section 10.3.

10.3 Liquid chromatography analysis.

10.3.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and estimated detection limit that can be

achieved by this method. An example of the separation achieved by this column is shown in Figure 1. Figure 2 is a chromatogram of picloram in a POTW wastewater sample. Other columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 is achieved.

10.3.2 Calibrate the system daily as described in Section 8.

10.3.3 Inject 100 µL of the sample extract. Monitor the column eluent at 225 nm.

Record the resulting peak size in area or peak height units.

10.3.4 The retention-time window used to make identifications 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 for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.3.5 If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.

10.3.6 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

References

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