United States Environmental Protection Agency

Office of Water

www.epa.gov August 1993

Method 1660: The Determination of Pyrethrins and Pyrethroids in Municipal and Industrial Wastewater

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of pyrethrins and pyrethroids in wastewater by extraction and high-performance liquid chromatography (HPLC) with an ultra-violet detector (UV). The compounds in Table 1 may be determined by this method.

1.2 This method is designed to meet the monitoring requirements of the U.S. Environmental Protection Agency under the Clean Water Act at 40 *CFR* Part 455. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.

1.3 When this method is applied to analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method lists a second UV wavelength that can be used to confirm measurements made with the primary wavelength.

1.4 This method is specific to the determination of two pyrethrins and seven pyrethroids, but should be applicable to other pyrethroids as well. The quality control requirements in this method give the steps necessary to determine this applicability.

1.5 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 typify the minimum quantity that can be detected with no interferences present.

1.6 This method is for use by or under the supervision of analysts experienced in the use of a high-performance liquid chromatograph and interpretation of liquid chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

2.1 A 750-mL sample is saturated with salt and extracted by stirring with acetonitrile in a 1-L volumetric flask. A small portion of the acetonitrile rises into the neck of the flask.[1] The extract is evaporated to a volume of 7.5 mL.

2.2 A 40-µL aliquot of the extract is injected into the HPLC. Chromatographic conditions are described that permit the separation and measurement of the pyrethrins and pyrethroids by reverse-phase C18 column HPLC with a multiple-wavelength UV detector.

2.3 Identification of compound is performed by comparing the retention time of the compound with that of an authentic standard. Compound identity is confirmed when the retention times agree, and when the response at a second wavelength agrees with the response at the primary wavelength.

2.4 Quantitative analysis is performed using an authentic standard of each compound to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of that compound in the extract. The concentration in the sample is calculated using the sample and extract volumes.

2.5 Quality is assured through reproducible calibration and testing of the extraction and HPLC systems.

3. CONTAMINATION AND INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.4.

3.2 Glassware and, where possible, reagents are cleaned by rinsing with solvent and baking at 450°C for a minimum of 1 hour in a muffle furnace or kiln. Some thermally stable materials may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality acetonitrile may be required.

3.3 Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled.

4. SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. 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 handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 2 through 4.

4.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting requirements of this method is the responsibility of the laboratory.

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottle: Amber glass, 1-L, with screw-cap. If amber bottles are not available, samples shall be protected from light.

5.1.2 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.3 Cleaning.

5.1.3.1 Bottles are detergent water washed, then rinsed with solvent or baked at 450°C for a minimum of 1 hour before use.

5.1.3.2 Liners are detergent-water washed, then reagent water and solvent rinsed, and baked at approximately 200°C for a minimum of 1 hour prior to use.

5.1.4 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for glassware cleaning.

5.2.1 Laboratory sink with overhead fume hood.

5.2.2 Kiln: Capable of reaching 450°C within 2 hours and holding 450°C within ±10°C, with temperature controller and safety switch (Cress Manufacturing Co, Sante Fe Springs, CA, B31H or X31TS, or equivalent).

5.3 Equipment for sample extraction.

5.3.1 Laboratory fume hood.

5.3.2 Stirring plate: Thermolyne Cimarec 2 (Model 546725), or equivalent.

5.3.3 Stirring bar: PTFE coated, approximately 1 cm x 4 cm.

5.3.4 Extraction flask: 1000-mL volumetric flask cleaned by rinsing with solvent or baking at 450°C for a minimum of 1 hour.

5.3.5 pH meter, with combination glass electrode.

5.4 Equipment for sample concentration.

5.4.1 Nitrogen evaporation device: Equipped with heated bath that can be maintained at 35 to 40°C (N-Evap, Organomation Associates, Inc., or equivalent).

5.4.2 Concentrator tube: 10- to 15-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified.

5.5 Sample vials: Amber glass, 10- to 15-mL with PTFE-lined screw- or crimp-cap, to fit HPLC autosampler.

5.6 Balance: Analytical, capable of weighing 0.1 mg.

5.7 Miscellaneous glassware.

5.7.1 Pipettes, glass, volumetric, 1.00-, 5.00-, and 10.0-mL.

5.7.2 Pipettes, glass, Pasteur, 150 mm long x 5 mm ID (Fisher Scientific 13-678-6A, or equivalent).

5.7.3 Volumetric flasks, 10.0-, 25.0-, and 50.0-mL.

5.8 High-performance liquid chromatograph (HPLC): Analytical system complete with pumps, sample injector, column oven, and multiple-wavelength ultra-violet (UV) detector.

5.8.1 Pumping system: Capable of isocratic operation and producing a linear gradient from 70% water/30% acetonitrile to 100% acetonitrile in 25 minutes (Waters 600E, or equivalent).

5.8.2 Sample injector: Capable of automated injection of up to 30 samples (Waters 700, or equivalent).

5.8.3 Column oven: Capable of operation at room ambient to 50°C (Waters TCM, or equivalent).

5.8.4 Column: Two 300 Angstrom C18 columns 150 mm long x 4.6 mm ID (Vydac 201 TP5415, or equivalent) connected in series and preceded by a 300 Angstrom C18 guard column 30 mm long x 4.6 mm ID (Vydac 201 GCC54T, or equivalent), operated at the conditions shown in Table 2.

5.8.5 Detector: UV operated at 235 and 245 nm (Waters 490E, or equivalent).

5.9 Data system.

5.9.1 Data acquisition: The data system shall collect and record LC peak areas and retention times on magnetic media.

5.9.2 Calibration: The data system shall be used to calculate and maintain lists of calibration factors (response divided by concentration) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) are used to test calibration linearity.

5.9.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each analysis. Displays of chromatograms are required to verify results.

5.9.4 Statistics on initial (Section 8.2) and ongoing (Section 12.5) performance shall be computed and maintained.

6. REAGENTS AND STANDARDS

6.1 Sample preservation: Sodium thiosulfate (ACS), granular.

6.2 pH adjustment.

6.2.1 Sodium hydroxide (10N): Dissolve 40 g NaOH in 100 mL reagent water.

6.2.2 Sulfuric acid (1+1): Reagent grade, 6N in reagent water. Slowly add 50 mL H_2SO_4 (specific gravity 1.84) to 50 mL reagent water.

6.3 Solvents: Acetonitrile and acetone; pesticide-quality; lot-certified to be free of interferences.

6.4 Reagent water: HPLC grade water in which the compounds of interest and interfering compounds are not detected by this method.

6.5 Salt: Sodium chloride, spread approximately 1 cm deep in a baking dish and baked at 450°C for a minimum of 1 hour, cooled and stored in a precleaned glass bottle with PTFE-lined cap.

6.6 Standard solutions: Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard.

NOTE: The pyrethrins are normally available in a mixed standard consisting of the six naturally occurring compounds (pyrethrin I and II, cinerin I and II, and jasmolin I and II). The concentrations in this standard will be on the order of 10% each of pyrethrin I and II. The concentration in the stock solution prepared from this mixed standard is to be corrected for the exact concentration.

When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use.

6.7 Preparation of stock solutions: Prepare in acetonitrile per the steps below. Observe the safety precautions in Section 4.

6.7.1 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg allethrin in a 10-mL ground-glass stoppered volumetric flask and fill to the mark with acetonitrile. After the allethrin is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.

6.7.2 Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.

6.7.3 Stock solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.

6.8 Secondary mixtures: Using stock solutions (Section 6.7), prepare mixtures for calibration and calibration verification (Sections 7.3 and 12.4), for initial and ongoing precision and recovery (Sections 8.2 and 12.5), and for spiking into the sample matrix (Section 8.3).

6.8.1 Calibration solutions: Prepare two solutions in acetonitrile at the concentrations given in Table 3. The midpoint solution is used for calibration verification (Section 12.4).

6.8.2 Precision and recovery standard and matrix spike solution: Prepare two solutions in acetone at 7.5 times the concentration of the midpoint standard (Table 3).

6.9 Stability of solutions: All standard solutions (Sections 6.7 through 6.8) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within ±15% of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

7.1 Configure the HPLC system as given in Sections 5.8 through 5.9 and establish the operating conditions in Table 2.

7.2 Attainment of minimum level: Determine that the minimum levels in Table 2 are met at each wavelength.

7.3 Calibration.

7.3.1 Inject 40 µL of each calibration solution (Table 3) into the HPLC system, beginning with the lowest level mixture and proceeding to the highest. For each compound, compute and store, as a function of the concentration injected, the retention time and the peak area at each wavelength (primary and confirmatory).

7.3.2 Calibration factor (ratio of area to amount injected).

7.3.2.1 Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range for each at each wavelength.

7.3.2.2 Linearity: If the calibration factor is constant (C_v <20%) over the calibration range, an average calibration factor may be used; otherwise, the complete calibration curve (area vs. amount) shall be used.

8. QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality control program.[5] The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If detection limits will be affected by the modification, the analyst is required to repeat demonstration of the detection limit (Section 7.2).

8.1.3 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the precision and recovery standard (Section 6.8.2) that the analysis system is in control. These procedures are described in Sections 12.1, 12.4, and 12.5.

8.1.4 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.3.

8.1.5 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.4.

8.2 Initial precision and recovery: To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

8.2.1 Extract, concentrate, and analyze two sets of four 750-mL aliquots of reagent water spiked with 1.0 mL of each solution of the precision and recovery standard (Section 6.8.2) according to the procedure in Section 10.

8.2.2 Using results of each set of four analyses, compute the average recovery (X) and the standard deviation of recovery (s), in milligrams per liter, for the each compound.

8.2.3 Compare s and X with the corresponding limit for initial precision and recovery in Table 4. If s and X meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or X falls outside the range for accuracy, system performance is unacceptable. In this case, correct the problem and repeat the test.

8.3 Method accuracy: The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.

8.3.1 The concentration of the matrix spike shall be determined as follows.

8.3.1.1 If, as in compliance monitoring, the concentration of allethrin in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration is larger.

8.3.1.2 If the concentration is not being checked against a regulatory limit, the matrix spike shall be at the level of the precision and recovery standard (Section 6.8.2) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.

8.3.1.3 If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, at the level of the precision and recovery standard (Section 6.8.2) or at 1 to 5 times the expected background concentration, whichever is larger.

8.3.2 Analyze one sample aliquot to determine the background concentration (B) of the pyrethrins and pyrethroids. If necessary, prepare a standard solution appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (A) of each analyte. Calculate the percent recovery (P):

Equation 1

$$P = \frac{100\ (A-B)}{T}$$

where
T = True value of the spike

8.3.3 Compare the percent recovery of each compound with the corresponding QC acceptance criteria in Table 4. If any analyte fails the acceptance criteria for recovery, the sample is complex and must be diluted and reanalyzed per Section 15.

8.3.4 As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (water, sludge) in which the recovery test (Section 8.3.3) is passed, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from P-2s_p to P+2s_p for each matrix. For example, if P = 90% and s_p = 10% for five analyses of wastewater, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).

8.4 Blanks: Reagent water blanks are analyzed to demonstrate freedom from contamination.

8.4.1 Extract and concentrate a 750-mL reagent water blank with each sample batch (samples started through the extraction process on the same 8-hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the precision and recovery standard (Section 12.5) to demonstrate freedom from contamination.

8.4.2 If any compound or any potentially interfering compound is found in an aqueous blank at greater than 20 µg/L (assuming the same calibration factor as allethrin for interfering compounds), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

8.5 Other pyrethroids may be determined by this method. To establish a quality control limit for another analyte, determine the precision and accuracy by analyzing four replicates of the analyte along with the precision and recovery standard per the procedure in Section 8.2. If the analyte coelutes with an analyte in the QC standard, prepare a new QC standard without the coeluting component(s). Compute the average percent recovery (A) and the standard deviation of percent recovery (s_n) for the analyte, and measure the recovery and standard deviation of recovery for the other analytes. The data for the new analyte is assumed to be valid if the precision and recovery

specifications for the other analytes are met; otherwise, the analytical problem is corrected and the test is repeated. Establish a preliminary quality control limit of A $\pm 2s_n$ for the new analyte and add the limit to Table 4.

8.6 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 12.4), and for initial (Section 8.2) and ongoing (Section 12.5) precision and recovery should be identical, so that the most precise results will be obtained. The HPLC instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.

8.7 Depending on specific program requirements, field replicates and field spikes may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Collect samples in glass containers following conventional sampling practices,[6] except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment.

9.2 Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of 5.0 to 7.0 using sodium hydroxide or hydrochloric acid solution. Record the volume used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine.[7]

9.3 Begin sample extraction within 7 days of collection, and analyze all extracts within 40 days of extraction.

10. SAMPLE EXTRACTION

10.1 Preparation of sample and QC aliquots.

10.1.1 Mix sample thoroughly.

10.1.2 Pour 750 mL of sample into a clean 1000-mL volumetric flask. If a matrix spike is to be prepared, pour two 750-mL aliquots into clean flasks.

10.1.3 For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place three 750-mL aliquots of reagent water (Section 6.4) in clean 1000-mL volumetric flasks. One reagent water aliquot serves as the blank.

10.1.4 Spike 1.0 mL of each precision and recovery standard (Section 6.8.2) into the remaining reagent water aliquots.

10.1.5 Spike the samples designated as the matrix spike at the level directed in Section 8.3.

10.1.6 Extract the sample and QC aliquots per Section 10.2.

10.2 Extraction.

10.2.1 Place each sample or QC aliquot on a stirring plate and add a clean PTFE-coated stirring bar.

10.2.2 Add 230 g of clean NaCl (Section 6.5) to each sample and QC aliquot and stir 5 to 10 minutes to dissolve.

10.2.3 Extraction with acetonitrile.

10.2.3.1 Add 160 mL of acetonitrile to each sample and QC aliquot.

10.2.3.2 Begin stirring. Increase the rate of stirring until the vortex is drawn approximately one-half the depth of the water. Stir for 3 to 5 minutes.

10.2.3.3 Stop stirring and invert each flask a minimum of three times will holding the stopper. Return the flask to the stirring plate.

10.2.3.4 Repeat steps 10.2.3.2 through 10.2.3.3 twice.

10.2.4 Allow the solutions to stand for approximately 5 minutes for the phases to separate. If an acetonitrile layer does not appear, add acetonitrile in 5-mL increments, stirring and settling between increments, until a 2- to 5-mL layer appears. If the acetonitrile layer is more than 5 mL, add reagent water, stir, and settle until the acetonitrile volume is reduced to 2- to 5-mL.

10.2.5 Using a Pasteur pipette, transfer the organic phase to a clean K-D concentrator tube (Section 5.4.2).

10.2.6 Add 5 mL of acetonitrile to the extraction flasks, stir, and allow to settle. Transfer the organic phase to the respective concentrator tubes. Repeat the extraction a third time. If all of the extract will not fit into the concentrator tube, evaporate some of the acetonitrile (Section 10.3), then add the remaining extract.

10.3 Concentration of extracts.

10.3.1 Place the concentrator tubes in the evaporation device (Section 5.4.1). Adjust the height of the blow-down tubes to 1 to 3 cm above the surface of the liquid and gently evaporate the acetonitrile until a volume of approximately 5 mL is reached.

10.3.2 Adjust the final extract volume to 7.5 mL and transfer to an HPLC autosampler vial.

11. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Table 2 summarizes the recommended operating conditions for the HPLC system. Included in this table are the retention times for the pyrethrins and pyrethroids achieved under these conditions. An example of the separation achieved by the column system is shown in Figure 1. Pyrethrin I and II are the major peaks in the naturally occurring pyrethrin standard. Pyrethrin II elutes prior to pyrethrin I. Jasmolin II and I will normally coelute with pyrethrin II and I, respectively. Most HPLC columns will resolve cinerin II and I, which are small peaks that elute after the respective pyrethrins. Some HPLC columns may resolve all six of the naturally occurring pyrethrins.

11.1 Calibrate the system as described in Section 7.

11.2 Set the injection volume on the autosampler to inject 40 µL of all standards and extracts of blanks and samples.

11.3 Set the data system or HPLC control to start the gradient upon sample injection, and begin data collection after 10 minutes. Set the data system or HPLC control to stop data collection after the last analyte is expected to elute and to return the gradient to the initial setting.

12. SYSTEM AND LABORATORY PERFORMANCE

12.1 At the beginning of each 8-hour shift during which analyses are performed, HPLC system performance and calibration are verified at both wavelengths. For these tests, analysis of the calibration verification standard (Section 6.8.1) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.

12.2 Retention times.

12.2.1 The absolute retention time of sumithrin shall be no earlier than 23 minutes.

12.2.2 The absolute retention time of the peak maxima shall be within ±15 seconds of the average retention times in the initial calibration (Section 7.3.1).

12.3 GC resolution: Resolution is acceptable if the height of the valley between tetramethrin and allethrin is less than 20% of the taller of the two peaks when chromatograms of the two calibration verification solutions (Section 6.8.1) are superimposed.

12.4 Calibration verification.

12.4.1 Inject the two calibration verification standards (Section 6.8.1).

12.4.2 Compute the concentration of the pyrethrins and pyrethroids based on the calibration factor or calibration curve (Section 7.3).

12.4.3 Compare this concentration with the limits for calibration verification in Table 4. If calibration is verified, system performance is acceptable and analysis of blanks and samples may begin. If, however, the recovery falls outside the calibration verification range, system performance is unacceptable. In this case, correct the problem and repeat the test, or recalibrate (Section 7).

12.5 Ongoing precision and recovery.

12.5.1 Analyze the extract of the two precision and recovery standards extracted with each sample batch (Section 10.1.3).

12.5.2 Compute the recovery of the compounds of interest in milligrams per liter.

12.5.3 Compare the recovery with the limits for ongoing recovery in Table 4. If the recovery meets the acceptance criteria, the extraction and concentration processes are in control and analysis of blanks and samples may proceed. If, however, the recovery falls outside the acceptable range, these processes are not in control. In this event, correct the problem, re-extract the sample batch, and repeat the ongoing precision and recovery test.

12.5.4 Add results which pass the specifications in Section 12.5.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r. Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if R=95% and s_r = 5%, the accuracy is 85-105%.

13. QUALITATIVE DETERMINATION

13.1 Qualititative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 12.1), and with data stored in the retention-time and calibration libraries (Section 7.3.1). Identification is confirmed when retention time and amounts agree per the criteria below.

13.2 Establish a retention-time window of ±20 seconds on either side of the mean retention-time in the calibration data (Section 7.3.1).

13.3 If a peak from the analysis of a sample or blank is within a window (as defined in Section 13.2) at the primary wavelength (235 nm), it is considered tentatively identified. A tentatively identified compound is confirmed is confirmed when (1) the retention time of the peak maximum at the confirmatory wavelength (245 nm) is within plus or minus two seconds of the retention-time of the peak maximum at the primary wavelength, and (2) the computed amounts (Section 14) on each system (primary and confirmatory) agree within a factor of 2.

14. QUANTITATIVE DETERMINATION

14.1 Using the HPLC data system, compute the concentration of the analyte detected in the extract (in micrograms per milliliter) using the calibration factor or calibration curve (Section 7.3.2).

14.2 Compute the concentration in the sample using the following equation:

Equation 2

$$C_s = \frac{(V_e)\ (C_{ex})}{V_s}$$

where

C_s = *Concentration in the sample, in µg/L*
V_e = *Extract total volume, in mL (nominally 7.5)*
C_{ex} = *Concentration in the extract, in µg/mL*
V_s = *Volume of sample extracted, in L (nominally 0.75)*

14.3 If the concentration of any analyte exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 40-µL aliquot of the diluted extract is analyzed.

14.4 Report results for pyrethrins and pyrethroids found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

15. ANALYSIS OF COMPLEX SAMPLES

15.1 Some samples may contain high levels (>1000 ng/L) of the pyrethrins and pyrethroids or of interfering compounds and/or polymeric materials. Some samples may form emulsions when extracted (Section 10.2); others may overload the HPLC column and/or detector. In these instances, the sample is diluted by a factor of 10 and re-extracted (Section 10), or the extract is diluted by a factor of 10 and reanalyzed (Section 14.3).

15.2 Recovery of matrix spikes: In most samples, matrix spike recoveries will be similar to those from reagent water. If the matrix spike recovery is outside the range specified in Table 4, the sample is diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method does not work on the sample being analyzed and the result may not be reported for regulatory compliance purposes.

16. METHOD PERFORMANCE

16.1 Development of this method is detailed in Reference 8.

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Table 1. Pyrethrins and Pyrethroids Determined by High-Performance Liquid Chromatography with Ultra-Violet Absorption Detector

Compound	***CAS Registry***
Allethrin (Pynamin)	583-79-1
Cyfluthrin (Baythroid)	68359-37-5
Fenvalerate (Pydrin)	51630-58-1
Cis-permethrin	61949-76-6
Trans-permethrin	61949-77-7
Pyrethrin I	121-21-1
Pyrethrin II	121-29-9
Resmethrin	10453-86-8
Sumithrin (phenothrin)	26002-80-2
Tetramethrin	7696-12-0

Table 2. High-Performance Liquid Chromatography of Pyrethrins and Pyrethroids

Compound	***Retention Time (min)***	***Minimum Level*** [1] ***(µg/L)***	***Estimated MDL*** [2] ***(µg/L)***
Pyrethrin II	17.48	3.3	1
Tetramethrin	18.98	5.0	2
Allethrin	19.27	5.0	2
Pyrethrin I	20.89	3.1	1
Cyfluthrin	21.84	5.0	2
Resmethrin	22.07	5.0	2
Fenvalerate	22.68	2.5	2
C/T-permethrin[3]	22.98	5.0	2
Sumithrin	23.47	5.0	1
C/T-permethrin[3]	23.56	5.0	2

1. This is a minimum level at which the analytical system shall give recognizable signals and acceptable calibration points.

2. 40 *CFR* Part 136, Appendix B. Column system and conditions: Two 300 Angstrom C18 columns 150 mm long x 4.6 mm ID 300 Angstrom C18 connected in series preceded by a 300 Angstrom C18 guard column 30 mm long x 4.6 mm ID. Column temperature 30°C. Solvent flow rate 1.5 mL/min. Gradient: linear from 70% water/30% acetonitrile at injection to 100% acetonitrile in 25 minutes.

3. Elution order of cis/trans isomers not known.

Table 3. Concentration of Calibration Solutions

	Solution Concentration (µg/mL)		
Compound	***Low***	***Median***	***High***
Calibration Solution 1			
Cyfluthrin	0.50	4.00	40.0
Fenvalerate	0.25	2.00	20.0
Pyrethrin I	0.31	2.50	25.0
Pyrethrin II	0.33	2.65	26.5
Sumithrin	0.50	4.00	40.0
Tetramethrin	0.50	4.00	40.0
Calibration Solution 2			
Allethrin	0.50	4.00	40.0
Resmethrin	0.50	4.00	40.0
C/T-permethrin*	0.50	4.00	40.0
C/T-permethrin*	0.50	4.00	40.0

Table 4. Acceptance Criteria for Performance Tests for Pyrethrins and Pyrethroids

		Acceptance Criteria			
		Initial Precision and Accuracy (µg/L)			
Compound	***Spike Level (µg/L)***	***s***	***X***	***Calibration Verification[1] (µg/L)***	***Recovery/ Ongoing Accuracy R (µg/L)***
Allethrin	40.0	9.0	16.0–52.0	3.5–4.6	15.0–53.0
Cyfluthrin	40.0	12.5	11.0–61.0	3.0–5.2	9.4–63.0
Fenvalerate	20.0	3.5	12.0–26.0	1.6–2.4	6.2–32.0
C/T-permethrin[2]	40.0	7.5	23.0–53.0	3.0–4.6	22.0–54.0
C/T-permethrin[3]	40.0	7.5	23.0–53.0	3.0–4.6	21.0–54.0
Pyrethrin I	26.5	7.0	8.6–32.0	2.2–2.8	7.7–33.0
Pyrethrin II	25.0	6.0	11.0–33.0	2.0–3.5	10.0–34.0
Resmethrin	40.0	12.5	4.3–51.0	2.3–5.2	2.5–52.0
Sumithrin	40.0	14.0	4.6–57.0	3.5–4.7	2.5–59.0
Tetramethrin	40.0	9.0	17.0–53.0	1.5–6.1	15.0–55.0

1. Verified at the level of the median standard in Table 3.
2. First of two permethrin peaks.
3. Second of two permethrin peaks.