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Method 1667, Revision A: Formaldehyde, Isobutyraldehyde, and Furfural by Derivatization Followed by High Performance Liquid Chromatography

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1.0 Scope and Application

1.1 This method is for surveying and monitoring under the Clean Water Act. It is used to determine certain organic pollutants specific to the pharmaceutical manufacturing industry (PMI) that can be derivatized and analyzed by high-performance liquid chromatography (HPLC).

1.2 The chemical compounds listed in Table 1 may be determined in waters, soils, and municipal sludges by this method.

1.3 The detection limits of the method are usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 are the minimum levels that can be reliably quantified by this method with no interferences present.

Furfural (2-furaldehyde) forms two relatively stable geometric isomers upon derivatization with 2,4-dinitrophenylhydrazine (DNPH). The first isomer (probably anti-) elutes after the formaldehyde derivative and before the isobutyraldehyde derivative. The second isomer (probably syn-) elutes after the isobutyraldehyde derivative. Experience with this system has shown that the best quantitative results (lowest detection limits) are obtained using the area from the first eluted peak rather than that from the second peak or the sum of the two areas. This method is for use only by analysts experienced with HPLC or under the close supervision of such qualified persons.

1.4 This method is performance-based. The analyst is permitted to modify the method to overcome interferences or to lower the cost of measurements, provided that all performance criteria in this method are met. The requirements for establishing method equivalency are given in Section 9.1.2.

2.0 Summary of the Method

2.1 For solid wastes or for aqueous wastes containing significant amounts of solid material, the aqueous phase, if any, is separated from the solid phase and stored for later analysis. If necessary, the particle size of the solids in the waste is reduced. The solid phase is extracted with an amount of extraction fluid equal to 20 times the weight of the solid phase. The extraction fluid employed is a function of the alkalinity of the solid phase of the waste. Following extraction, the aqueous extract is separated from the solid phase by filtration employing 0.6 to 0.9-µm glass-fiber filter.

2.2 If compatible (i.e., multiple phases will not form on combination), the initial aqueous phase of the waste is added to the aqueous extract, and these liquids are analyzed together. If

incompatible, the liquids are analyzed separately and the results are mathematically combined to yield a volume-weighted average concentration.

2.3 A measured volume of aqueous sample or an appropriate amount of solids leachate is buffered to pH=5 and derivatized with 2,4-dinitrophenylhydrazine (DNPH), using either the solid-sorbent or methylene chloride derivatization/extraction option. If the solid-sorbent option is used, the derivative is extracted using solid-sorbent cartridges, followed by elution with ethanol. If the methylene chloride option is used, the derivative is extracted with methylene chloride. The methylene chloride extracts are concentrated using the Kuderna-Danish (K-D) procedure and solvent exchanged into methanol prior to HPLC analysis. Liquid chromatographic conditions are described that permit the separation and measurement of formaldehyde, isobutyraldehyde, and furfural derivatives in the extract by absorbance detection at 365 nm.

2.4 The quality of the analysis is assured through reproducible calibration and testing of the derivatization/extraction procedure and the HPLC system.

3.0 Definitions

There are no specific definitions unique to this method.

4.0 Interferences

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in chromatograms. 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 9.3.

4.1.1 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and reagent water. It should then be drained, dried, 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 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 Analysis of formaldehyde is complicated by its ubiquitous occurrence in the environment. Acetic acid, even high-purity acetic acid, is often contaminated with formaldehyde. For this reason, a phthalate buffer is used in this method instead of an acetate buffer. Wherever acetic acid is used, it must be demonstrated to be formaldehyde free.

4.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. If matrix interferences occur, some additional cleanup may be necessary.

4.4 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for resolution of the specific compounds covered by this method, other matrix components may interfere.

5.0 Safety

5.1 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 safety data sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 through 3.

5.2 Formaldehyde has been classified as a potential carcinogen. Primary standards of formaldehyde should be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator should be worn when high concentrations are handled.

6.0 Apparatus and Materials

Disclaimer: The mention of trade names or commercial products in this Method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

6.1 Reaction vessel—250-mL Florence flask.

6.2 Separatory funnel—250-mL, with polytetrafluoroethylene (PTFE) stopcock.

6.3 Kuderna-Danish (K-D) apparatus.

6.3.1 Concentrator tube—10-mL, graduated (Kontes K-570050 or equivalent). A ground-glass stopper is used to prevent evaporation of extracts.

6.3.2 Evaporation flask—500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

6.3.3 Snyder column—Three-ball macro (Kontes K-503000-0121 or equivalent).

6.3.4 Snyder column—Two-ball micro (Kontes K569001-0219 or equivalent).

6.3.5 Springs—½", (Kontes K-662750 or equivalent).

6.4 Vials—10- and 25-mL glass, with PTFE-lined screw-caps or crimp-tops.

6.5 Boiling chips—Solvent-extracted with methylene chloride, approximately 10/40 mesh (silicon carbide or equivalent).

6.6 Balance—Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.7 pH meter—Capable of measuring to the nearest 0.01.

6.8 High-performance liquid chromatograph (modular).

6.8.1 Pumping system—Isocratic, with constant flow control capable of 1.00 mL/min.

6.8.2 High-pressure injection valve with 20-µL loop.

6.8.3 Column—250 mm long × 4.6 mm inside diameter (i.d.), 5-µm particle size, C_{18} (or equivalent).

6.8.4 Absorbance detector—365 nm.

6.8.5 Strip-chart recorder compatible with the detector. Use of a data system is recommended.

6.9 Glass-fiber filter paper, 0.6 to 0.9-µm.

6.10 Solid-sorbent cartridges—Packed with 500 mg C_{18} (Baker or equivalent).

6.11 Vacuum manifold—Capable of simultaneous extraction of up to 12 samples (Supelco or equivalent).

6.12 Sample reservoirs—50-mL capacity (Supelco or equivalent).

6.13 Pipet—Capable of accurately delivering 0.10 mL of solution (Pipetman or equivalent).

6.14 Water bath—Heated, with concentric ring cover, capable of temperature control of ± 2°C at 80-90°C. The bath should be used in a hood.

7.0 Reagents and Standards

7.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may

be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determinations.

7.2 Reagent water—Water in which the compounds of interest and interfering compounds are not detected by this method. It may be generated by any of the methods in this subsection.

7.2.1 Activated carbon—Pass tap water through a carbon bed (Calgon Filtrasorb-300, or equivalent).

7.2.2 Water purifier—Pass tap water through a purifier (Millipore Super Q, or equivalent).

7.2.3 Boil and purge—Heat tap water to 90-100°C and bubble contaminant-free inert gas through it for approximately 1 hour. While still hot, transfer the water to screw-cap bottles and seal with a PTFE-lined cap.

7.3 Methylene chloride—HPLC grade or equivalent.

7.4 Methanol—HPLC grade or equivalent.

7.5 Ethanol (absolute)—HPLC grade or equivalent.

7.6 2,4-Dinitrophenylhydrazine (DNPH, 70% w/w) in reagent water.

7.7 Formalin (37.6% w/w) in reagent water.

7.8 Acetic acid (glacial), demonstrated to be formaldehyde-free.

7.9 Potassium acid phthalate.

7.10 Sodium hydroxide solutions, 1 N, and 5 N.

7.11 Sodium chloride.

7.12 Sodium sulfate solution, 0.1 M.

7.13 Hydrochloric acid, 0.1 N.

7.14 Extraction fluid—Dilute 64.3 mL of 1.0 N sodium hydroxide and 5.7 mL of glacial acetic acid to 900 mL with reagent water. Further dilute to 1 L with reagent water. The pH should be 4.93 ± 0.02. If not, adjust with acid or base.

7.15 Stock standard solutions.

7.15.1 Stock formaldehyde (approximately 1.00 mg/mL)—Prepare by diluting 265 µL formalin to 100 mL with reagent water.

Standardization of formaldehyde stock solution—Transfer a 25-mL aliquot of a 0.1 M sodium sulfite solution to a beaker and record the pH. Add a 25-mL aliquot of the formaldehyde stock solution (Section 7.15.1) and record the pH. Titrate this mixture back to the original pH using 0.1 N hydrochloric acid. The formaldehyde concentration is calculated using the following equation:

$$Concentration\ (mg/mL) = 30.03\ x\ (N\ HCl)\ x\ (mL\ HCl)\ x\ 25$$

where:

$N\ HCl$ = *Normality of the HCl solution*

$mL\ HCl$ = *mL of standardized HCl solution*; *and*

30.03 = *Molecular weight of formaldehyde*.

7.15.2 Stock formaldehyde, isobutyraldehyde, and furfural—Prepare by adding 265 µL of formalin, 0.100 g of isobutyraldehyde, and 0.100 g of furfural to 90 mL of reagent water and dilute to 100 mL. The concentrations of isobutyraldehyde and furfural in this solution are 1.00 mg/mL. Calculate the concentration of formaldehyde in this solution using the results of the assay performed in Section 7.15.1.

7.15.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

7.15.4 Aqueous performance standard—An aqueous performance standard containing formaldehyde (nominally 100 µg/L), isobutyraldehyde at 100 µg/L, and furfural at 100 µg/L shall be prepared daily and analyzed each shift to demonstrate performance (Section 9).

7.15.5 Preparation of calibration standards.

7.15.5.1 Prepare calibration standard solutions of formaldehyde, isobutyraldehyde, and furfural in reagent water from stock standard solution (Section 7.15.2). Prepare these solutions at the following concentrations (in µg/mL) by serial dilution of the stock standard solution: 50, 20, 10. Prepare additional calibration standard solutions at the following concentrations, by dilution of the appropriate 50, 20, or 10 µg/mL standard: 5, 0.5, 2, 0.2, 1, 0.1. Make further dilutions if appropriate.

7.16 Reaction solutions.

7.16.1 DNPH (1.00 mg/mL)—Dissolve 142.9 mg 70% (w/w) reagent in 100 mL of absolute ethanol. Slight heating or sonication may be necessary to effect dissolution.

7.16.2 Phthalate buffer (0.1 N)—Prepare by dissolving 20.42 g of potassium acid phthalate in 1 L of reagent water. Adjust pH to 5 by addition of sodium hydroxide or hydrochloric acid, as necessary.

7.16.3 Sodium chloride solution (saturated)—Prepare by mixing an excess of the reagent-grade solid with reagent water.

8.0 Sample Collection, Preservation, and Storage

8.1 Grab samples are collected in glass containers having a total volume greater than 20 mL. For aqueous samples that pour freely, fill sample bottles so that no air bubbles pass through the sample as the bottle is filled and seal each bottle so that no air bubbles are entrapped. Maintain the hermetic seal on the sample bottle until time of analysis.

8.2 Samples are maintained at 0-4°C from the time of collection until analysis. Samples must be derivatized within five days of collection and analyzed within three days of derivatization.

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 4). The minimum requirements of this program consist of an initial demonstration of laboratory capability and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.1 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 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve separations or lower the costs of measurements. These options include alternative extraction, concentration, cleanup procedures, and changes in columns and detectors. Alternative techniques, such as substitution of immunoassay, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the method detection limit (MDL; 40 CFR Part 136, Appendix B) is lower than

one-third the regulatory compliance level. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information below, at a minimum.

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A list of pollutant(s) measured, including name and CAS Registry Number.

9.1.2.2.3 A narrative stating the reason(s) for the modification.

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method, including:

(a) Calibration (Section 10.1.2)

(b) Calibration verification (Section 10.1.2.2)

(c) Initial precision and accuracy (Section 9.2)

(d) Analysis of blanks (Section 9.3)

(e) Accuracy assessment (Section 9.5)

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

(a) Sample numbers and other identifiers

(b) Extraction dates

(c) Analysis dates and times

(d) Analysis sequence/run chronology

(e) Sample weight or volume (Section 11)

(f) Extract volume prior to each cleaning step (Section 11.1.2)

(g) Final extract volume prior to injection (Section 11.3.4.5 or Section 11.3.5.5)

(h) Injection volume (Section 12)

(I) Dilution data, differentiating between dilution of a sample or an extract

(j) Instrument and operating conditions

(k) Column and operating conditions (nature of column, dimensions, flow rates, solvents, etc.)

(l) Detector operating conditions (wavelength, etc.)

(m) Chromatograms, printer tapes, and other recording of raw data

(n) Quantitation reports, data system outputs, and other data necessary to link raw data to the results reported

9.1.3 Analyses of blanks are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis (Section 4). The procedures and criteria for analysis of a blank are described in Section 9.3.

9.1.4 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the aqueous performance standard (Section 7.15.4) that the analysis system is in control. This procedure is described in Section 10.

9.1.5 The laboratory shall maintain records to define the quality of data that is generated.

9.2 Initial precision and accuracy—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations for compounds to be calibrated.

9.2.1 Analyze four aliquots of the aqueous performance standard (Section 7.15.4) according to the method beginning in Section 11. Use the solid-sorbent option or the methylene chloride option, whichever will be used routinely.

9.2.2 Using results from Section 9.2.1, compute the average percent recovery (X) and the standard deviation of the recovery (s) for each compound.

9.2.3 For each compound, compare s and X with the corresponding limits for initial precision and recovery found in Table 3. If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any

individual X falls outside the range for accuracy, system performance is unacceptable for that compound. This is an indication that the analytical system is not performing properly for the compound(s) in question. In this event, correct the problem and repeat the entire test (Section 9.2.1).

9.3 Blanks—Reagent water blanks are analyzed to demonstrate freedom from contamination.

With each sample lot (samples analyzed on the same 12-hour shift), a blank shall be analyzed immediately after analysis of the aqueous performance standard (Section 9.1.4) to demonstrate freedom from contamination. If any of the compounds of interest or any potentially interfering compound is found in a blank at greater than 10 µg/L, analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

9.4 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 7.15.5), calibration verification (Section 10.1.2.2) and for initial (Section 9.2) and ongoing (Section 9.1.4) precision and accuracy should be identical, so that the most precise results will be obtained.

9.5 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis.

10.0 Calibration

10.1 Establish liquid chromatographic operating parameters to produce a retention time equivalent to that indicated in Table 2 for formaldehyde derivative. Suggested chromatographic conditions are provided in Section 12.1. Prepare derivatized calibration standards according to the procedure in Section 10.1.1. Calibrate the chromatographic system using the external standard technique (Section 10.1.2).

10.1.1 Process each calibration standard solution through the derivatization option used for sample processing (Section 11.3.4 or 11.3.5).

10.1.2 External standard calibration procedure.

10.1.2.1 Analyze each derivatized calibration standard using the chromatographic conditions specified in Section 12.1, and tabulate peak area against concentration injected. The results may be used to prepare calibration curves for formaldehyde, isobutyraldehyde, and furfural.

10.1.2.2 The working calibration curve must be verified at the beginning of each 12-hour shift or every 20 samples, whichever is more frequent, by the measurement of one or more calibration standards. If the response

for any analyte varies from the previously established responses by more than 10%, the test must be repeated using a fresh calibration standard after it is verified that the analytical system is in control. Alternatively, a new calibration curve may be prepared for that compound. If an autosampler is available, it is convenient to prepare a calibration curve daily by analyzing standards along with test samples.

11.0 Sample Extraction, Cleanup, and Derivatization

11.1 Extraction of solid samples.

11.1.1 All solids must be homogeneous. When the sample is not dry, determine the dry weight of the sample using a representative aliquot.

11.1.1.1 Determination of dry weight—In certain cases, sample results are desired based on a dry weight basis. When such data is desired, a portion of the sample is weighed out at the same time as the portion used for the analytical determination.

Warning: ***The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from drying a heavily contaminated hazardous waste sample.***

11.1.1.2 Immediately after weighing the sample for extraction, weigh 5-10 g of the sample into a tared crucible. Determine the percent dry weight of the sample by drying overnight at 105°C. Allow to cool in a desiccator before weighing.

$$\%\ dry\ weight = \frac{g\ of\ dry\ sample}{g\ of\ sample} \times 100$$

11.1.2 Measure 25 g of solid into a 500-mL bottle with a PTFE-lined screw-cap or crimp-top, and add 500 mL of extraction fluid (Section 6.13). Extract the solid by rotating the bottle at approximately 30 rpm for 18 hours. Filter the extract through glass-fiber filter paper and store in a sealed bottle at 4°C. Each mL of extract represents 0.050 g of solid.

11.2 Cleanup and separation.

11.2.1 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 sample types. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must meet the specifications in Section 9.1.2.

11.2.2 If the sample is not clean, or the complexity is unknown, the entire sample should be centrifuged at 2500 rpm for 10 minutes. Decant the supernatant liquid from the centrifuge bottle and filter through glass-fiber filter paper into a container that can be tightly sealed.

11.3 Derivatization.

11.3.1 For aqueous samples, measure a 50- to 100-mL aliquot of sample. Quantitatively transfer the sample aliquot to the reaction vessel (Section 6.1).

11.3.2 For solid samples, 1-10 mL of leachate (Section 11.1.2 or Section 11.2.2) will usually be required. The amount used for a particular sample must be determined through preliminary experiments.

11.3.3 Derivatization and extraction of the derivative can be accomplished using the solid-sorbent (Section 11.3.4) or methylene chloride option (Section 11.3.5).

11.3.4 Solid Sorbent Option

11.3.4.1 Add 4 mL of phthalate buffer (Section 7.16.2) and adjust the pH to 5.0±0.1 with sodium hydroxide or hydrochloric acid. Add 10 mL of DNPH reagent, adjust the total volume to approximately 100 mL with reagent water, seal the container and place on a wrist-action shaker at room temperature for 1 hour. Samples or standards containing high analyte concentrations may require more DNPH reagent for complete reaction.

11.3.4.2 Assemble the vacuum manifold and connect to a water aspirator or vacuum pump. Assemble solid sorbent cartridges containing a minimum of 1.5 g of C_{18} sorbent, using connectors supplied by the manufacturer, and attach the sorbent train to the vacuum manifold. Condition each cartridge by passing 10 mL dilute phthalate buffer (10 mL 5 N phthalate buffer dissolved in 250 mL of reagent water) through the sorbent cartridge train.

11.3.4.3 Remove the reaction vessel from the shaker and add 10 mL of saturated sodium chloride solution to the vessel.

11.3.4.4 Add the reaction solution to the sorbent train and apply a vacuum so that the solution is drawn through the cartridges at a rate of 3 to 5 mL/min. After the solution has eluted, allow air to be drawn through the cartridge for approximately 2 minutes to remove all traces of solution, then release the vacuum.

11.3.4.5 Elute each cartridge train with approximately 9 mL of absolute ethanol, directly into a 10-mL volumetric flask. Dilute the solution to

volume with absolute ethanol, mix thoroughly, and place in a tightly sealed vial until analyzed.

11.3.5 Methylene chloride option.

11.3.5.1 **Add 5 mL of phthalate buffer (Section 7.16.2) and adjust the pH to 5.0±0.1 with sodium hydroxide or hydrochloric acid. Add 10 mL of DNPH reagent, adjust the volume to approximately 100 mL with reagent water, seal the container, and place on a wrist-action shaker at room temperature for 1 hour. Samples or standards with high analyte concentrations may require more DNPH reagent for complete reaction.**

11.3.5.2 **Extract the solution with three 20-mL portions of methylene chloride, using a 250-mL separatory funnel, and combine the methylene chloride layers. If an emulsion forms upon extraction, remove the entire emulsion and centrifuge at 2000 rpm for 10 minutes. Separate the layers and proceed with the next extraction.**

11.3.5.3 **Assemble a K-D concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporator flask. Wash the K-D apparatus with 25 mL of extraction solvent to complete the quantitative transfer.**

11.3.5.4 **Add one or two clean boiling chips to the evaporation flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80-90 °C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 10-15 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood with condensed solvent. When the apparent volume of the liquid reaches 10 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.**

11.3.5.5 **Prior to liquid chromatographic analysis, the solvent must be exchanged to methanol. The analyst must ensure quantitative transfer of the extract concentrate. The exchange is performed as described below.**

11.4 **After cooling and draining as described in Section 11.3.5.4, momentarily remove the Snyder column and add 5 mL of methanol and a new boiling chip. Attach the micro Snyder column. Concentrate the extract using 1 mL of methanol to prewet the Snyder column. Place the K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required,**

to complete the concentration. When the apparent volume of the liquid reaches less than 5 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 with 1-2 mL of methanol and add to the concentrator tube. A 5-mL syringe is recommended for this operation. Adjust the extract volume to 10-mL with methanol. Stopper the concentrator tube and store refrigerated at 4 °C if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a vial with a PTFE-lined screw-cap or crimp-top. Proceed with the liquid chromatographic analysis if further cleanup is not required.

12.0 High-Performance Liquid Chromatography

12.1 Chromatographic conditions.

Column: C_{18}, 250 mm long × 4.6 mm i.d., 5-µm particle size (or equivalent).

Mobile Phase: Methanol/water, 75:25 (v/v), isocratic at 30°C.

Flow Rate: 1.0 mL/min.

UV Detector: 365 nm.

Injection Vol.: 20 µL.

12.2 Analysis.

12.2.1 Analyze samples by HPLC using conditions described in Section 12.1. Table 2 lists the retention times and MLs that were obtained under these conditions. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 9 are met.

12.2.2 The width of 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 the 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.

12.2.3 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 ethanol or methanol, as appropriate, and reanalyzed.

12.2.4 If the peak area measurement is prevented by the presence of observed interferences, further cleanup may be required.

12.3 Calculations.

12.3.1 Calculate the calibration factor (CF) at each concentration and the mean calibration factor (CF_m) as follows (mean value based on 5 points):

$$CF = \frac{area\ response\ (A_s)}{concentration\ (C_s)}$$

$$mean\ CF = CF_m = \frac{\sum_{i=1}^{5} CF}{5}$$

12.3.2 Aqueous samples—Calculate the concentration of each analyte as follows:

$$g/L = \frac{A_s * V_e * DF}{CF_m * V_s}$$

where:

RF_m is the mean response factor
A_s is the area signal from the analyte
V_e is the extract volume
DF is the dilution factor; e.g. 10, if the sample is diluted by a factor of 10
V_s is the sample volume

12.3.3 Solid samples—Calculate the concentration of each analyte using the equation below. A factor must be included in the equation to account for the weight of the sample used and, if desired, to correct for dry weight.

$$mg/kg = \frac{A_s * V_e * DF}{CF_m * W_s * (1 - \frac{\%m}{100})}$$

where:

W_s is the sample weight
%m is the percent moisture of the sample
the other symbols are the same as in Section 12.3.2

13.0 Method Performance

13.1 The MDLs listed in Table 2 were obtained using reagent water and methylene chloride extraction. Similar results can be obtained using the solid-sorbent method.

13.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the range from the ML to 50 times the ML.

13.3 A representative chromatogram is presented as Figure 1.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be reduced feasibly at the source, the Agency recommends recycling as the next best option. The acids used in this Method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this Method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

15.2 Samples containing acids at a pH of less than 2 are hazardous and must be neutralized before being poured down a drain or must be handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction,* both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

1. "Working with Carcinogens," DHEW, PHS, NIOSH, Publication 77-206 (1977).

2. "OSHA Safety and Health Standards, General Industry," 29 CFR 1910, OSHA 2206, (1976).

3. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety (1979).

4. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," U.S. EPA, EMSL Cincinnati, OH 45268, EPA-600/4-79-019 (March 1979).

17.0 Tables

Table 1. PMI Analytes to Which This Method Applies

PMI Analyte	CASRN[1]
Formaldehyde	50-00-0
Furfural	98-01-1
Isobutyraldehyde	78-84-2

[1] Chemical Abstracts Service Registry Number.

Table 2. Retention Times and Minimum Levels (MLs) for PMI Analytes

PMI Analyte	Retention Time[1] (seconds)	ML[2] (µg/L)
Formaldehyde	326	50
Furfural	495	50
Isobutyraldehyde	714	50

[1] Retention times are for the DNPH derivative.

[2] This is the minimum level at which the entire analytical system shall give a recognizable signal and an acceptable calibration point, taking into account method-specific sample and injection volumes.

Table 3. Quality Control Acceptance Criteria for Initial Precision and Recovery

PMI Analyte	Spike Level (µg/L)	Average Percent Recovery (X)	Standard Deviation (s)
Formaldehyde	50	25-187	81
Furfural	100	70-102	16
Isobutyraldehyde	50	45-121	38

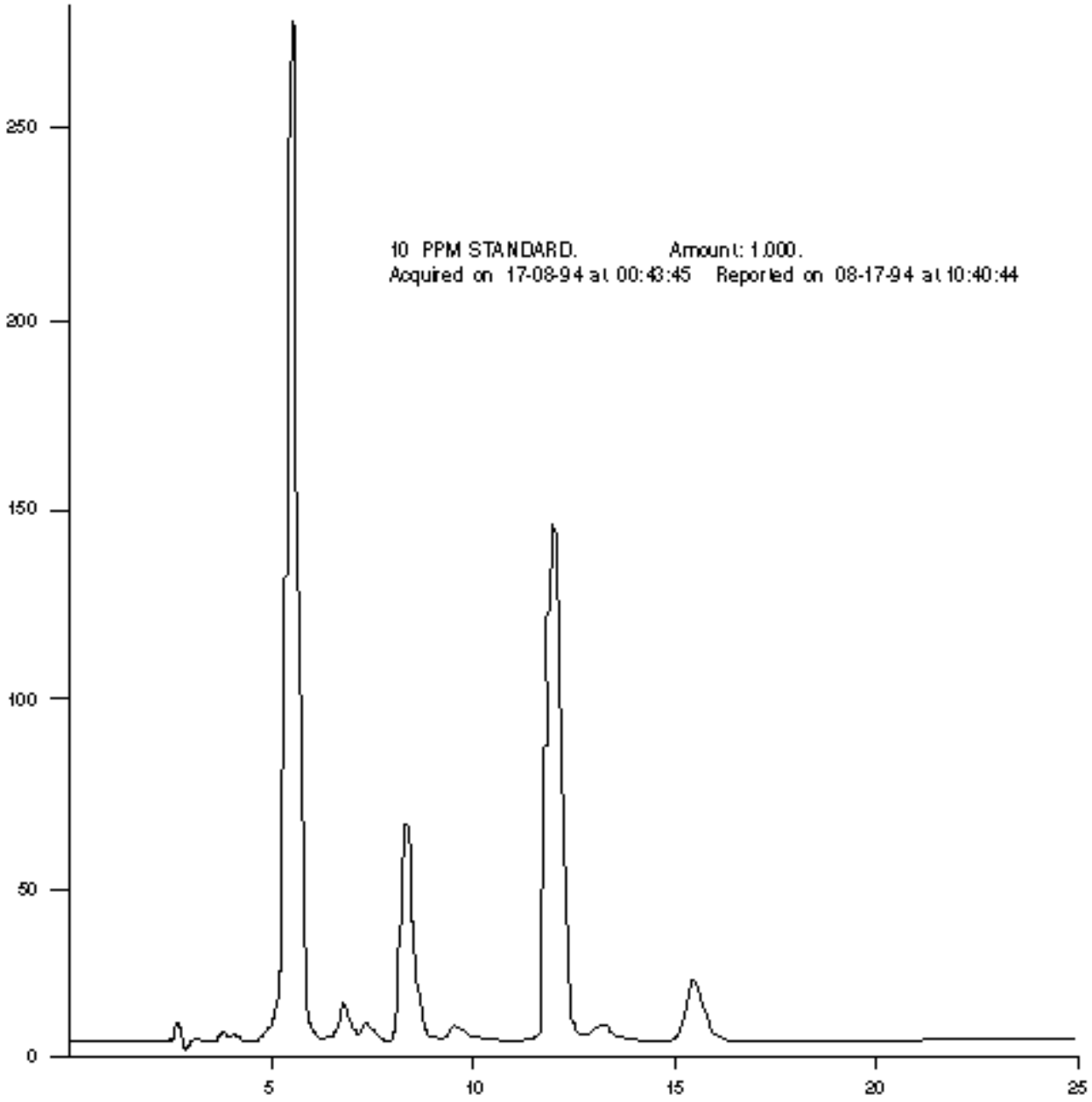

Figure 1. Chromatogram of the 2,4-DNPH Derivative of Formaldehyde, Furfural, and Isobutyraldehyde