USEPA METHOD 1613
HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY DETERMINATION OF TETRA- THROUGH OCTA-CHLORINATED DIBENZO-*P*-DIOXINS AND DIBENZOFURANS BY ISOTOPE DILUTION

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ABSTRACT

The United States Environmental Protection Agency is promulgating a new method for the analysis of PCDDs and PCDFs in waters, wastewaters, and solids. This method, designated Method 1613, is a high resolution gas chromatography and high resolution mass spectrometry method employing isotope dilution for the quantification of fifteen of the seventeen 2,3,7,8-substituted PCDDs and PCDFs. The method contains an extensive QA/QC protocol, and is being required for use in monitoring dioxins and furans in industrial and municipal discharges permitted under the National Pollutant Discharge Elimination System.

INTRODUCTION

In 1976 the U.S. District Court for the District of Columbia issued a consent decree requiring the United States Environmental Protection Agency (USEPA) to measure and limit 65 compounds and classes of compounds in effluents discharged to receiving waters in the United States. The list of 65 was subsequently refined by USEPA to a list of 129 specific analytes termed the "Priority Pollutants" and codified as the Section 307(a) list of "toxic pollutants" in the 1977 Clean Water Act (CWA) amendments. Priority Pollutant number 129 is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), one of the most toxic substances known.

Section 304(h) of the CWA requires the Administrator of USEPA to *"promulgate guidelines establishing test procedures for the analysis of pollutants . . ."*. As a result, all test procedures to be used by the Office of Water or dischargers for monitoring compliance with national environmental regulations must be approved under the guidelines developed for Section 304(h). To date, methods have been promulgated for over 260 different parameters, including the priority pollutants as well as radiological, bacteriological, and physical parameters.

Currently, USEPA Method 613 is the only method for the analysis of 2,3,7,8-TCDD that has been promulgated under CWA Section 304(h). This method, developed in the late 1970's, utilizes gas chromatography and low resolution mass spectrometry, with minimal extract cleanup steps. The detection limit for 2,3,7,8-TCDD in water is 2000 ppq for Method 613. As a result, it does not achieve the target detection limit for 2,3,7,8-TCDD currently required by the Agency for analysis of treated effluents (10 - 25 ppq). Further, it is specifically designed for the analysis of TCDD, and is not directly applicable to the other 2,3,7,8-substituted dioxins or furans.

Method 1613 was developed by the Industrial Technology Division (ITD) of the USEPA Office of Water Regulations and Standards (OWRS) in response to the need for analyses of treated effluents at low levels of 2,3,7,8-TCDD (10 ppq). It is designed for regulatory development purposes and compliance monitoring under the National Pollutant Discharge Elimination

System (NPDES, CWA Section 402). Method 1613 is designed to quantitate 2,3,7,8-TCDD as well as the other sixteen 2,3,7,8-substituted polychlorinated dioxins and furans (PCDDs/PCDFs). It employs high resolution gas chromatography coupled with a state-of-the-art high resolution mass spectrometer for the analysis. Method 1613 incorporates the quality assurance and quality control program from the Agency's 500/600 series methods, including an initial demonstration of proficiency and ongoing demonstrations of laboratory performance. The method employs isotope dilution as a means of quantifying the analytes of interest through the addition of the carbon-labeled analogs of 15 of the 2,3,7,8-substituted PCDDs and PCDFs prior to extraction of the sample. This technique results in a correction of the concentrations of the unlabeled PCDDs/PCDFs for the losses that occur during sample extraction and cleanup.

SYNOPSIS OF THE METHOD

As with the other OWRS 1600 series methods for analysis of organic compounds (Methods 1624 and 1625), isotope dilution is the cornerstone of Method 1613. The use of isotope dilution has been shown to significantly improve the accuracy and reduce the bias of environmental analyses for dioxins and furans as well as for other organic compounds. The ^{13}C-labeled analogs of 15 of the 2,3,7,8-substituted PCDDs and PCDFs are added to each sample prior to extraction. The labeled analogs are used to quantitate their respective unlabeled compounds. Because the unlabeled and labeled compounds behave similarly during extraction and cleanup, losses of the labeled analogs are similar to those of the unlabeled analytes, and the resulting concentration data are recovery corrected. The labeled analog of OCDF is *not* added to the samples, because it produces an ion that interferes with the mass spectrometric determination of the unlabeled OCDD. The labeled analog of 1,2,3,7,8,9-HxCDD is used as an instrument internal standard added immediately prior to injection of the extract into the GC/MS. Because it is not added *prior to* extraction, this labeled analog is not used for quantitation of the unlabeled 1,2,3,7,8,9-HxCDD. The recovery correction of the unlabeled 1,2,3,7,8,9-HxCDD result is based on the other two labeled HxCDDs. For the unlabeled OCDF, the recovery correction is based on the labeled OCDD, but given the low relative toxicity of this unlabeled compound, this shortcoming is minimal.

Water samples are spiked with the labeled analogs and then filtered. Both the aqueous filtrate and the particulates collected on a 2.7 um glass fiber filter are extracted separately, and the extracts are combined prior to cleanup and analysis.

Solid samples such as soils, sediments, and sludges are spiked with the labeled analogs and extracted using an azeotropic distillation. This distillation/extraction is carried out in a combination of a Soxhlet extractor and a Dean Stark water trap. The combination, developed at Dow Chemical and referred to as a Soxhlet/Dean-Stark, or SDS, has significant advantages over more traditional extraction techniques involving sonication or Soxhlet alone. These latter techniques require that the water in the sample matrix be removed by adsorption on drying agents such as sodium sulfate. Sodium sulfate often contains carbon particles that result from the pretreatment of this reagent in a muffle furnace to remove organic contaminants. These particles may irreversibly adsorb the analytes of interest during the lengthy Soxhlet extraction process. Furthermore, the drying action of the sodium sulfate may actually seal some pores in the sample matrix with hydrated sodium sulfate, thus reducing the exposure of the sample surface to the extraction solvent.

Using toluene as an extraction solvent, the water is removed from the sample matrix by azeotropic distillation, and is collected in the Dean Stark trap, where it may be measured to determine the moisture content of the actual aliquot of sample used for extraction. The particulates filtered from an aqueous sample are extracted by the SDS procedure as well. Given that PCDDs and PCDFs in an aqueous sample are believed to be strongly associated with the particles in the sample, the use of an extraction technique that is more rigorous than simple separatory funnel extraction of unfiltered water improves the accuracy and precision of these measurements.

Prior to further sample handling and cleanup, all extracts are spiked with $^{37}Cl_4$-2,3,7,8-TCDD. This labeled analog is used to monitor the efficiency of the subsequent handling and cleanup steps. In this manner, losses of the analytes during cleanup, particularly TCDD, the most toxic isomer, may be differentiated from any losses that occur during the extraction process itself.

The cleanup procedures employed in Method 1613 include the use of back extraction of extracts with acidic and basic aqueous solutions, acidic or basic alumina chromatography, acidic *and* basic silica gel chromatography, activated carbon chromatography (AX-21/Celite), and gel permeation chromatography (GPC). The method also discusses the use of high performance liquid chromatography (HPLC) as an option for particularly difficult samples.

Two additional labeled analogs, ^{13}C-1,2,3,4-TCDD and ^{13}C-1,2,3,7,8,9-HxCDD, are added to each concentrated extract just prior to injection into a high resolution gas chromatograph coupled to a high resolution mass spectrometer operating in the selected Ion monitoring mode. These last two labeled analogs function as traditional internal standards and are used to quantitate the labeled compounds added prior to extraction and the ^{37}Cl-labeled cleanup standard. Two ions are monitored for each analyte. Identification is based on simultaneous elution of peaks for both ions, retention times of unlabeled compounds relative to the labeled analogs, and ratios of the abundances of both ions. Quantitative results are based on the areas of both ions.

For GC columns that cannot resolve the 2,3,7,8-TCDD and 2,3,7,8-TCDF isomers from all others, a confirmatory analysis is required on a second GC column for all samples in which 2,3,7,8-TCDF is identified. Second column confirmation is also required for any samples in which peaks are present for 2,3,7,8-substituted PCDDs/PCDFs that meet all of the qualitative identification criteria except the ion abundance ratios.

QA/QC PROGRAM

Promulgation of a method under Section 304(h) of the CWA requires that the method contain an extensive quality assurance and quality control program (QA/QC). The 1600 series methods for organic analyses contain QA/QC requirements that involve a variety of checks on method and laboratory performance.

The first part of this program involves a series of so-called start-up tests, or initial demonstrations of precision and recovery (IPR). Each laboratory using the method must prepare a series of at least four replicates of a blank matrix spiked with all the unlabeled analytes of interest. These replicates are carried through the entire analytical procedure, including extraction and cleanup. The recovery of each analyte from the series of samples is compared to predetermined limits in the method, as is the standard deviation of the recoveries. The laboratory may not proceed with analysis of field samples until it can demonstrate that it can meet the method specifications in this fashion. This test must be repeated for each additional sample matrix to be analyzed (i.e., water, soil, sludge, etc.).

With every group of samples received and analyzed together, an additional spike of a blank matrix must be prepared and analyzed. This is the termed the ongoing precision and recovery (OPR) aliquot. The recovery of the unlabeled analytes must also meet the method criteria for this analysis, or else the entire set of samples must be prepared and analyzed again. The data from the IPR and OPR analyses are maintained by the laboratory in order to provide "statements of data quality" regarding the accuracy of the method in the matrix of interest.

The laboratory must perform a five-point initial calibration of the unlabeled and labeled analytes. This calibration must meet linearity criteria as well as the qualitative identification criteria. The resolution of the GC is verified using standards containing the first and last eluting isomers in each level of chlorination. Mass spectrometer resolution of at least 10,000 is verified once every twelve hours. A calibration verification is performed at the beginning of each twelve-hour period of

analyses, and must be within limits specified in the method.

The 15 labeled analogs are added to every sample prior to extraction. The recoveries of these analogs must be within the range of 25-150% for each analysis. The ^{37}Cl-labeled cleanup standard must meet the same limits. These recoveries are used by the laboratory to monitor the accuracy of the method in a given matrix over time. This information may also be employed by the data user to gauge the precision and bias of the measurements for every analyte in every sample.

A blank must be prepared with each group of samples extracted together, and must meet limits for contamination. The absolute retention times of the two internal standards in each sample must meet a minimum criteria, in order to avoid situations where the GC temperature program is increased beyond the ability of the column to separate the analytes. The retention times of the unlabeled analytes relative to their labeled analogs must also meet method specifications, thus minimizing the chances of misidentifying compounds.

PERFORMANCE DATA

During the course of the development of Method 1613, laboratories under contract to ITD have employed this method in over 500 analyses. These analyses include over 300 field samples representing industrial categories such as petroleum refining, pulp and paper, pesticide manufacturers, hazardous waste treaters, and publicly-owned sewage treatment works. In addition, almost 200 analyses of IPR and OPR aliquots and calibration standards were submitted by these laboratories. These data have been evaluated to provide specifications for the first formal revision of the method, Revision A, released in April 1990.

Based on these data, the revised method contains tighter specifications for initial calibration and calibration verification, and analyte-specific limits for the precision and accuracy of the IPR and OPR aliquots. Because the 15 labeled analogs are added to each sample prior to analysis, the recovery of these compounds can be used to judge the overall performance of the method across a variety of matrices.

The recoveries of the labeled analogs from over 200 field samples demonstrate that the method is both capable and rugged. Considering the results from all matrices together as a measure of method accuracy, the mean recoveries of the 15 labeled analogs plus the ^{37}Cl-labeled cleanup standard ranged from 60% for 2,3,4,7,8-PeCDF to 85% for the 1,2,3,4,7,8-HxCDD. Using the standard deviations of those mean recoveries as a measure of precision, the standard deviations ranged from 13% to 25%. Considering the aqueous and solids matrices separately, and calculating the grand mean recovery of all 16 labeled compounds by matrix, the grand mean recovery of the labeled compounds from 60 aqueous samples was 60.4%, and the grand mean recovery from 166 solid samples was 77.4%. The standard deviation of the grand mean recoveries was used to compare the precision of the method in both matrices. The recoveries from the aqueous samples were more precise than the recoveries from the solids, with a standard deviation of 5.6% for aqueous samples versus 8.5% for the solids

FURTHER DEVELOPMENT

USEPA is currently in the process of further evaluating the use of Method 1613 through a round robin study involving at least 14 laboratories from six countries. The results of that study will be used to refine the method specifications and quality control measures, and to assist the Agency in determining the interlaboratory and intralaboratory components of variability that apply to the method. Other studies are underway or planned in the near future, including: evaluation of the use of reverse phase HPLC as a cleanup technique to be applied to particularly difficult matrices; studies of extraction techniques designed to reduce the volumes of solvents used in the method; and investigations of new extract cleanup techniques that may be more effective for interferences such as alkyl dioxins and furans.