Synthesis and Use of [$^{13}C_{12}$]-12347-PCDD as an Internal Standard in the Measurement of Chlorinated Dibenzo-p-dioxins and Dibenzofurans

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INTRODUCTION

The use of isotopically labeled compounds in analytical methodology for determining chlorinated dibenzo-p-dioxins and dibenzofurans (CDDs/CDFs) by means of mass spectrometry is of historical precedent.[1] Such constituents are termed *labeled compound spiking standards* and function as reference standards, as *carriers* for related analytes through rigorous cleanup technologies, and they also provide a means for evaluating the recovery of analytes in each sample being examined. The ideal analytical situation involves use of an isotopically labeled analyte for each species being determined; and these components would be added to sample matrices upon commencement of the analysis procedure in a fashion equivalent to that of any native analytes present.

The measurement of CDDs/CDFs by HRGC-HRMS in accordance with EPA methods such as Method 1613 not only requires the use of *labeled compound spiking standards*, but also the incorporation of additional isotopically labeled compounds just prior to HRGC-HRMS examination. These constituents, termed *internal standards*, improve accuracy and reliability of the mass spectrometric measurement processes by permitting evaluation and use of relative response factors (RRFs).

Because of inherent biological response to CDDs/CDFs bearing substituents in each of the 2,3,7,8-positions, it is becoming increasingly common to measure only these seventeen analytes. From this perspective, it is most advantageous to employ *internal standard* isomers that are not fully substituted in the 2,3,7,8-positions which can be readily separated from all native or labeled analytes already present in the sample matrix. Reality emerges to conflict with such optimization in that few isotopically labeled CDD/CDF isomers are commercially available, and of these, most are fully 2,3,7,8-substituted isomers.

In accordance with the current version of EPA Method 1613,[2] two internal standards are specified: [$^{13}C_{12}$]-1234-TCDD and [$^{13}C_{12}$]-123789-HCDD. Two such standards are required because of the wide range of CDD/CDF analytes being measured. Said analyte range necessitates long chromatographic separations be performed during HRGC-HRMS examination procedures and the potential for instrumental variation tends to increase with increasing monitoring periods.

One problem associated with use of these particular internal standards is that [$^{13}C_{12}$]-1234-TCDD is not adequately separated from the [$^{13}C_{12}$]-2378-TCDD spiking standard when using a DB-5 capillary column unless an ~60 m column is employed. Use of this long column results in retention times ≥ 27 minutes for the labeled TCDD isomer standards. Obviously, separation of native analytes would require similar chromatographic conditions, but, in cases where relatively *clean* samples are being examined, or if the analyte concentrations are below a pre-defined limit, then the consequences of incomplete separation of the native isomers could be of less importance and a more rapid chromatographic separation might be applicable. However, because separation of the internal standard from the spiking standard is a critical methodology performance guideline, use of a more rapid separation is thereby precluded.

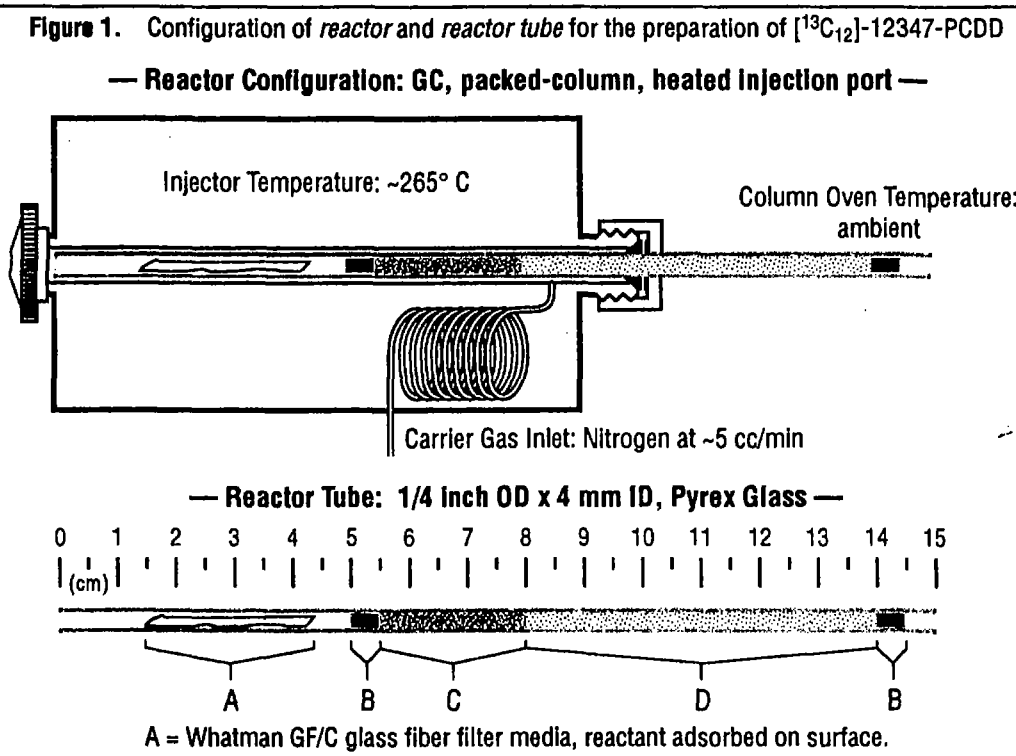
The use of [$^{13}C_{12}$]-123789-HCDD as an internal standard represents another potential problem, namely that it cannot function as a spiking standard for its native analog. Hence, results produced for this native analyte may not be as accurate or reliable as for the other analytes which have corresponding isotopically labeled spiking standards incorporated into the sample matrices during preparation routines.

In response to these problems, we have developed a convenient synthesis of [$^{13}C_{12}$]-12347-PCDD from commercially available [$^{13}C_{12}$]-1234-TCDD. This compound is readily separable from 2,3,7,8-substituted CDD isomers on short DB-5 capillary columns (~20 m) and is near the center of the chromatographic retention window for all 2,3,7,8-substituted CDD/CDF isomers typically measured. Because of these characteristics, [$^{13}C_{12}$]-12347-PCDD is a useful replacement for both of the currently specified internal standards. It provides the potential for faster chromatographic separations, where applicable, while allowing use of [$^{13}C_{12}$]-123789-HCDD as a spiking standard for its native analog.

EXPERIMENTAL & RESULTS

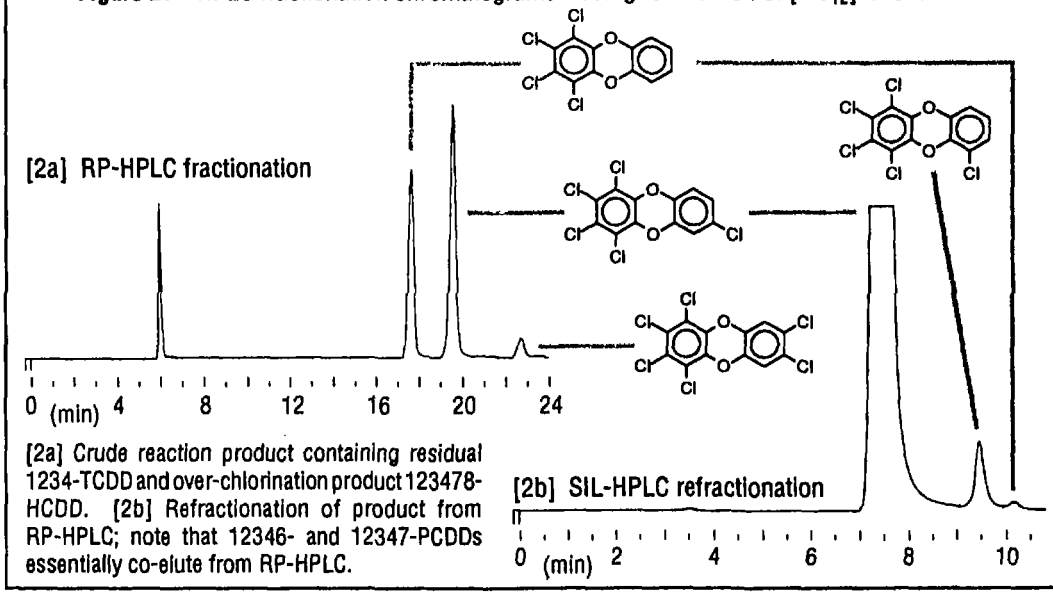
Macro-scale chlorination chemistries for aromatics are well known.[3-6] However, when these techniques have been applied to microgram quantities of CDDs/CDFs, the product yield is often unsatisfactory. Several micro-scale chlorination or bromination reactions have been developed to surmount such difficulties.[7-8] Of these techniques, one permitting convenient and preferential micro-synthesis of 2,3,7,8-substituted brominated dioxins and furans[8] has been modified to accommodate preparation of [$^{13}C_{12}$]-12347-PCDD from 25 µg of [$^{13}C_{12}$]-1234-TCDD precursor at yields of ~42 mole% after recovery and purification. Based upon preliminary experimentation using 25 µg quantities of unlabeled 1234-TCDD, optimum reaction conditions were found

Figure 1. Configuration of *reactor* and *reactor tube* for the preparation of [$^{13}C_{12}$]-12347-PCDD



A = Whatman GF/C glass fiber filter media, reactant adsorbed on surface.
B = Pyrex glass wool plug serving as a bed support.
C = 2.5% (w/w) Ferric Chloride (hydrated) on 100/200-mesh silica gel.
D = 100/200-mesh silica gel.

Figure 2. HPLC fractionation chromatograms leading to isolation of [$^{13}C_{12}$]-12347-PCDD

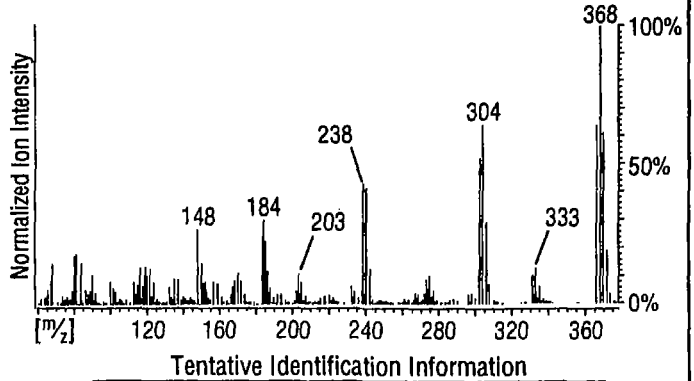


[2a] Crude reaction product containing residual 1234-TCDD and over-chlorination product 123478-HCDD. [2b] Refractionation of product from RP-HPLC; note that 12346- and 12347-PCDDs essentially co-elute from RP-HPLC.

to be as shown in Figure 1 using a reaction time of ~2 minutes. The entire reaction sequence was performed in the injection port of a Varian 1400 gas chromatograph designed to use 1/4 inch OD columns. Preparation of the specified reagents, as well as the reaction sequence, are similar to those previously described.[8]

The crude reaction products were initially subjected to semi-preparative scale reverse phase high performance liquid chromatography (RP-HPLC) to isolate the intended product and any residual precursor as shown in Figure 2a. General operating parameters for RP-HPLC fractionation are: column = 9.4 mm ID x 500 mm ODS/Zorbax; isocratic mobile phase = 3.0 mL/min methanol; column temperature = 50°C. Isolated [$^{13}C_{12}$]-12347-PCDD product was next subjected to HRGC-LRMS examination to confirm product identity and to attempt the determination of any remaining impurities; see Figure 3. Recovered precursor was subjected to the reaction and RP-HPLC fractionation sequence to improve the final product yield.

Figure 3. Mass spectrum of crude product [$^{13}C_{12}$]-12347-PCDD



Tentative Identification Information

Molecular ion of m/z 366 with 5Cl pattern
M^+-Cl at m/z 331
M^+-64 (^{13}COCl) with 4 Cl pattern at m/z 302
M^+-128 (2[^{13}COCl]) with 3 Cl pattern at m/z 238
M^+-163 (2[^{13}COCl]-Cl) with 2 Cl pattern at m/z 203
M^{2+} with 5 Cl pattern at m/z 183

Initial HRGC-LRMS analysis of the product following RP-HPLC purification indicated it to be contaminated with ~1.5% of another PCDD isomer presumed to be

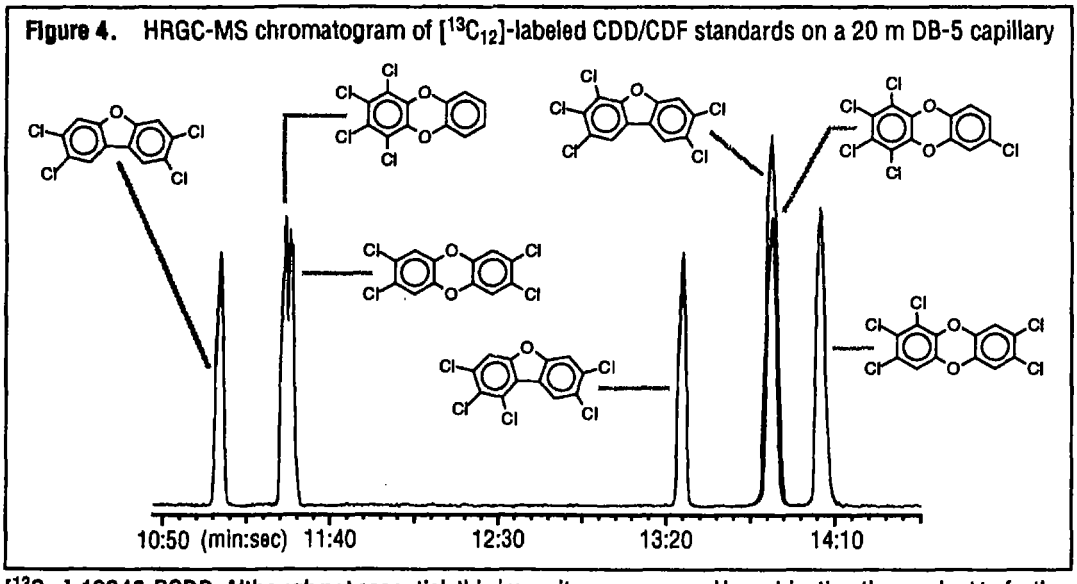


Figure 4. HRGC-MS chromatogram of [$^{13}C_{12}$]-labeled CDD/CDF standards on a 20 m DB-5 capillary

[$^{13}C_{12}$]-12346-PCDD. Although not essential, this impurity was removed by subjecting the product to further normal phase silica adsorption HPLC (SIL-HPLC) refractionation as shown in Figure 2b. General operating parameters for SIL-HPLC refractionation are: column = 6.8 mm ID x 250 mm Ro-Sil (5 μm); isocratic mobile phase = 2.0 mL/min UV-hexane; column temperature = ambient. Subsequent HRGC-LRMS examination of the isolated final product was performed under Selected Ion Monitoring (SIM) conditions so as to permit determination of any native CDDs present. Such measurement of native content is of utmost importance because the concentration of these species determines the maximum amount of labeled standard that may be added to a given sample matrix without incurring a false positive signal for native analytes. Based upon these SIM data, purity of the final product was found to be: native 1234-TCDD = ND (0.05%); native 12347-PCDD = ND (0.09%); [$^{13}C_{12}$]-1234-TCDD = ND (0.05%); [$^{13}C_{12}$]-12346-PCDD = < 0.5%. The HRGC-MS separation of relevant [$^{13}C_{12}$]-labeled CDD/CDF standards on a 20 m DB-5 capillary is shown in Figure 4.

In conclusion, [$^{13}C_{12}$]-12347-PCDD produced as described can be used as an internal standard for the measurement of 2,3,7,8-substituted CDDs/CDFs if the level of standard added to samples is ≤ 500x the instrumental limit of detection for native PCDDs.

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