Investigation of improving innovative dioxins analyis method using Solid phase MicroExtraction (SPME)-HRGC-HRMS

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Introduction

Methodologies for the extraction and cleanup of dioxin isomers have been widely investigated and a reliable, but lengthy analytical method using High-Resolution Gas Chromatography- High-Resolution Mass Spectrometry (HRGC-HRMS) has been established. This method requires an extensive sample cleanup procedure for the purification and isolation of the dioxin isomers from various sample matrices that may contain a wide variety of interferences depending upon the sample type. The current sample preparation steps require many complicated steps, skillful techniques, are lengthy and have a high cost.

A new rapid, simple, inexpensive and accurate dioxin extraction and analysis procedure has been developed using Solid Phase Micro Extraction (SPME) coupled with HRGC-HRMS. This innovative, rapid dioxin analysis method uses carbon coated SPME fibers without the complicated sample cleanup steps that are currently being used for conventional dioxin analytical methods.

During the development of the new SPME method, we noticed a few problems that needed to be addressed. One is the carryover of isomers after analyzing a sample containing a high concentration of dioxin isomers. The second concern are extracts that can not be concentrated to small volumes, for example, 50-100uL due to precipitation because the extract contains a large amount of interfering sample matrices.

This paper describes the research focused on reducing the carryover and the precipitation problems with concentrated samples along with evaluating a new extraction method.

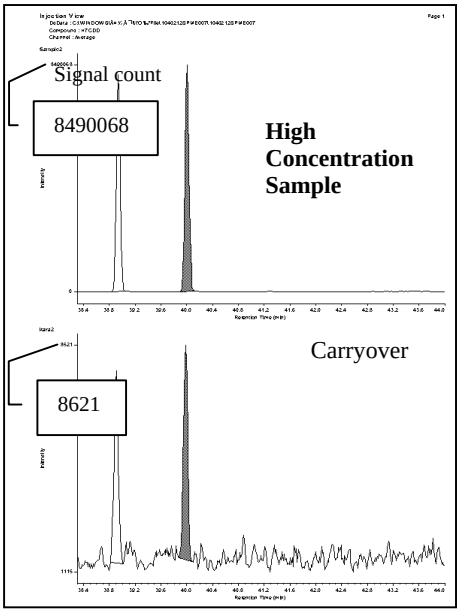


Figure 1. Carryover

Materials & method

1. Reducing carryover

Our current procedure for the analysis of dioxin isomers with SPME uses a 350ºC GC injection port temperature with a desorption time of 15 minutes for both desorption and fiber conditioning prior to extracting the next sample. This condition was satisfactory for most of the samples we evaluated, but a carryover on the SPME fiber was observed after the analysis of high concentration sample of dioxin isomers. Figure 1 demonstrates the carryover using heptachlorodibenzodioxin isomers (HpCDD) as an example. To reduce the

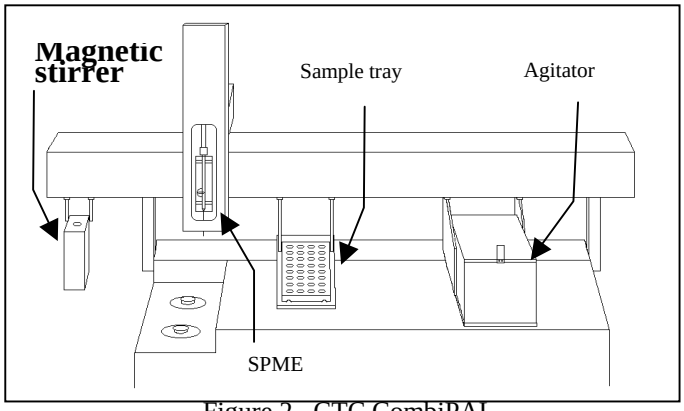


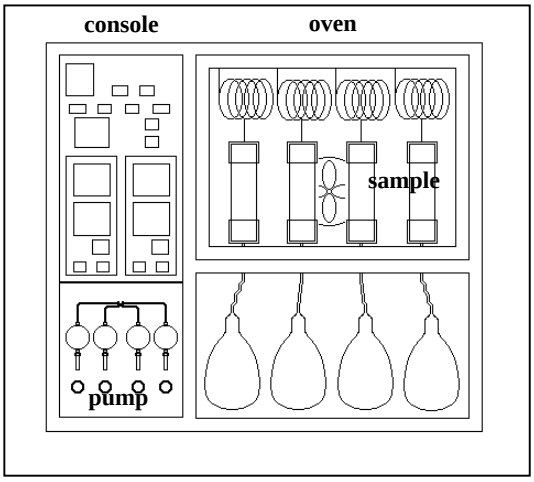
Figure 2 CTC CombiPAL

carryover on the SPME fiber, we tried to use a toluene washing before the next sample extraction. Figure 2 depicts the automated SPME extraction/desorption equipment on the CTC CombiPal autosampler. To aid the extraction, we installed a magnetic stirrer on CTC CombiPAL and added a wash vial containing toluene. One analysis cycle is to immerse SPME fiber into n-nonane or n-hexane sample solution for 45 min, inject on GC and desorb for 5 min. The SPME fiber was then washed with toluene for 5 min. prior to the next extraction.

2. Concentration sample (Sample volume) for SPME extraction

In the SPME method for dioxin analysis, the final sample of n-nonane solution is usually concentrated to 50-100uL before SPME extraction to obtain higher recovery of dioxins. Some samples when processed without using the cleanup procedure are concentrated to small volume less than 1mL, precipitates sometimes occurred in the n-hexane solutions. Due to the precipitation problems, concentration of the final sample volume will be limited. Some samples can be concentrated to 100uL without precipitation but it is dependent on the sample matrix. For samples that precipitated, we must clean up the sample with sulfuric acid before concentrating to 50-100uL.

In this study we examined the possibility extracting dioxin isomers using SPME from large volumes (2-8mL) of an n-hexane solution to avoid precipitation problem which occurred during concentration of the sample. Automated SPME extraction was performed using the CTC CombiPAL auto sampler. We used two types of test solution, a 2mL n-hexane solution containing 500pg dioxin standard in a 2mL vial and an 8mL n-hexane solution containing 1000pg dioxin in transferred into a 10mL vial. With the 2mL volume, we immersed the SPME fiber into sample solution for 45 min and injected/desorbed at the GC injection port as described previously. In the case of 8mL sample, the SPME fiber was immersed into sample and the sample was agitated by the agitator for 45 min prior to injected/ desorbing into the GC.



Figue.3 SE-100 Extraction Instrument

3. Extraction

Conventional dioxin extraction methodology for solid samples is Soxhlet extraction with toluene for 16 hours. In this study, we evaluated a new extraction instrument, SE-100 (Mitsubishi Chemical) to reduce the extraction period. The SE-100 instrument, overviewed in Figure 3, can extract dioxins at high speed under high temperature, with two kind of solvents that can be switched using a solvent switching valve. We extracted a soil sediment and ash samples on this instrument. The ash sample was first treated with HCl, filtered, and then the filtrate was extracted on the SE-100 with dichloromethane as the eluent solvent. The soil or sediment sample was weighed and extracted with SE-100 directly. The sample was first extracted with 130mL ethanol to remove water in the sample, and the ethanol solution was extracted using 150mL toluene. After that the oven was the heated and the sample extracted with 180mL of toluene.

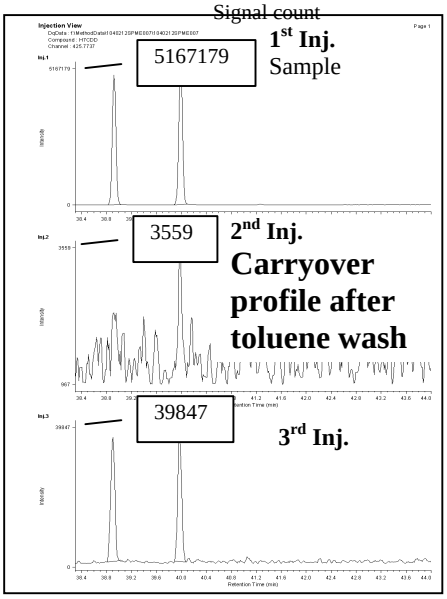


Figure.4 Reducing carryover by toluene wash

After extraction, the toluene extract was concentrated with rotary evaporation, followed by the SPME-HRGC/HRMS analysis. The SPME extraction was performed from a 10mL vial with stirring on the agitator. In the cases of 100uL and 2mL vial, usual extraction with SPME was performed. Extraction and desorption times were 45 min, 15 min respectively.

An Agilent HP6890 GC, JEOL High Resolution Mass Spectrometer JMS-700, and 60 meter x 0.25mm ID RH-12ms capillary column (INVENTX) are used for the separation of dioxins.

The SPME extraction/analysis procedure was also compared to the current method using sample cleanup with a mutli-layer silica gel column, reversible carbon column and HRGC/HRMS. In this analysis, a 60-meter x 0.25mm ID x 0.20µm df SP-2331 capillary column (Supelco) was used for the 4-6 chloro DDs/DFs and the RH-12ms for the 7 and 8 chloro substituted isomers.

Results

1. Reducing carryover

Figure 4 shows that toluene washing significantly reduced the carryover. To confirm the effect of the toluene wash, SPME fiber was first used to extract dioxin isomers from a sample solution and injected/desorbed on GC (1st injection). The fiber was then injected/desorbed on the GC a second time (2nd injection). The response units (signal count) of 1st and 2rd injections are 5167179, 3559, respectively. The carryover is reduced to 0.069%. We could reduce carryover to the level that does not affect the actual measurement. Another experiment was carried out to confirm the effect of toluene. The SPME fiber was immersed into a new dioxin sample solution, followed by washing it with toluene, and then injected into GC (3rd.injection). The response (signal count) of 1st injection and 3rd injection is 5167179, 39847, respectively. The carryover is reduced to 1/100 by toluene washing.

2. Concentration sample (Sample volume) for SPME extraction

Figure 5 shows the chromatogram of $^{13}C_{12}$-PeCB extracted with the SPME fiber from sample volumes of 100uL n-nonane, 2mL n-hexane and 8mL n-hexane. Although the response of $^{13}C_{12}$-PeCB from 2mL sample is about 1/25 of the response from 100uL sample, the response of PeCB was large enough to be detected. However, $^{13}C_{12}$-PeCB could not detected from 8mL sample, even though the PCDDs/DFs could be detected. The response of dioxins from the 8mL volume is 1/25-1/50 of the response to 100uL volume. We found that dioxin isomers can be quantitated from a final volume of 2mL n-hexane solution and this is applicable to the samples that are difficult to concentrate to 100uL due to precipitation. To address the problem for samples with large interferences which cannot be concentrated to 2mL, we increased the sample volume to 8mL for samples that cannot be concentrated to 2mL. PCDD/DF can be measured but PCB could not detected from the 8mL samples. Therefore, for the samples where the PCB contribution to TEQ is very small, these can be measured from 8mL n-

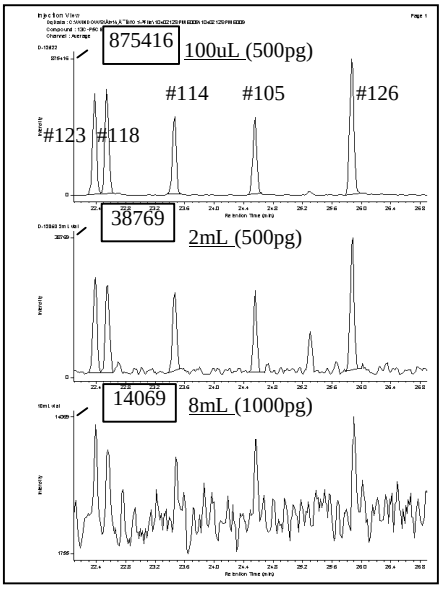


Figure 5. Sample volume

hexane solution. For samples where the PCB contribution to the TEQ is large, sample cleanup using a sulfuric wash or multi-layer silica gel column is inevitable in order to concentrate the volume to less than 2mL.

3. Extraction method

The sample extracted with SE-100 was analyzed with SPME-HRGC/HRMS. The same sample was also extracted with current extraction method using toluene/Soxhlet extraction, followed by purification with multi-layer silica gel column/carbon column, HRGC/HRMS (Japanese Industrial Standard method). Both results show good correlation over a wide concentration range as shown in Figure 6.

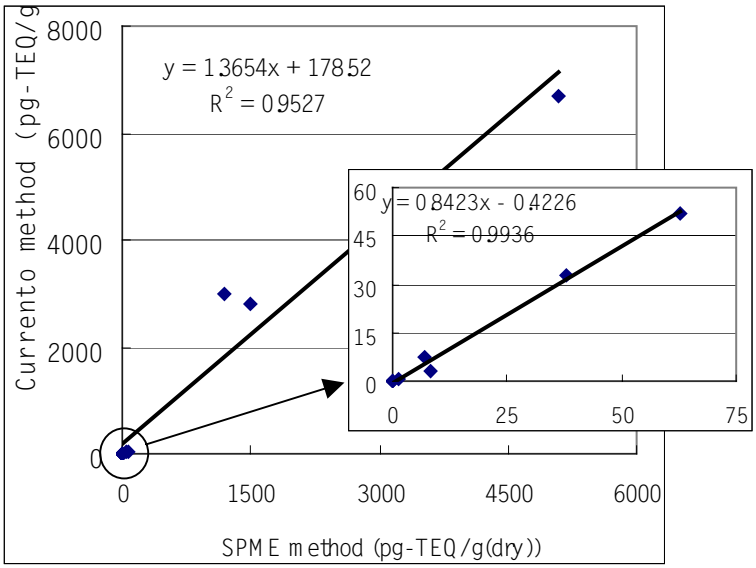


Figure 6. Relationship between SE-100 and Soxhlet extraction method

The SE-100 instrument can extract 4 samples simultaneously in about 2 hours. By using this instrument, total analysis time can be significantly reduced and the combination of SE-100 and SPME was found very useful as a rapid and easy handling analytical method.

Discussion

Toluene washing can reduce carryover on SPME fiber. Longer conditioning at 350°C may also reduce the carryover but it may cause a shorter lifetime for the SPME fiber. Toluene washing is therefore more useful than longer conditioning at 350°C. We also confirmed that dioxin isomers in a 2mL or 8mL n-hexane can be extracted with SPME and quantitated with HRGC/HRMS. This shows that a sample that is difficult to concentrate to 100uL due to precipitation can be analyzed at 2-8mL volume concentration without sample cleanup. In the case of a high concentration dioxin sample, a final volume of 2mL is adequate to measure dioxins. The large volume sample like 2mL can also reduce carryover, and

furthermore combining with a toluene washing it makes SPME method more reliable.

The SE-100 instrument can extract dioxins under atmospheric pressure, by flowing hot toluene (70ºC) at constant flow rate within about 2 hours with same efficiency as toluene/Soxhlet extraction apparatus. By combining this high flow extraction instrument with SPME/HRGC-HRMS, dioxin analyses can be done within one day. This method is only applicable to solid samples but it is very useful for fast analysis.

Through our investigation, we found our SPME method can be applied to various samples without sample cleanup. We will focus on the expansion of this method to more sample matrices other than environmental samples.

References

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