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ISOMER SPECIFIC SEPARATION AND QUANTITATION OF TETRACHLORO DIBENZO-p-DIOXINS BY HRCG AND HRGC/MS

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SÉPARATION ET QUANTIFICATION DES ISOMÈRES DES TÉTRACHLORODIBENZO-p-DIOXINES PAR CGHR ET CGHR-SM

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MANAGEMENT PERSPECTIVE

This paper entitled "Isomer Specific Separation and Quantitation of Tetrachloro Dibenzo-p-Dioxins by HRGC and HRGC/MS" describes high resolution narrow-bore gas chromatography (HRGC) and HRGC/mass spectrometry (MS) for the determination of all 22 isomers of tetrachloro dibenzo-p-dioxins in various environmental matrices which enables analysts to report sifnificantly more accurate data for all 22 isomers.

It is the first time that relative response factors for all 22 isomers using both the electron capture detector and the electron impact ionization selected ion monitoring has been reported.

PERSPECTIVE DE GESTION

Ce document intitulé " Séparation et quantification des isomères des tétrachlorodibenzo-p-dioxines par CGHR et CGHR-SM" décrit la méthode de chromatographie gazeuse de section étroite à haute résolution (CGHR) et de CGHR-spectrométrie de masse (SM) utilisée pour déterminer chacun des 22 isomères de tétrachlorodibenzo-p-dioxines dans diverses matrices environnementales permettant aux analystes d'enregistrer des données considérablement plus précises sur chacun des 22 isomères.

C'est la première fois que les facteurs de réponse relatifs sont signalés au moyen d'un détecteur à capture d'électrons et du contrôle, pour chaque ion, de l'ionisation par choc électronique.

ISOMER-SPECIFIC SEPARATION AND QUANTITATION OF TETRACHLORO DIBENZO-p-DIOXINS BY HRGC AND HRGC/MS

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SUMMARY

A method is described for the determination of 22 tetrachlorodibenzo-p-dioxin isomers at the low part-per-trillion (ppt) level. High resolution, narrow bore, open tubular columns (OTCs) with 100 um i.d. can achieve better separations than presently used 320 or 250 um i.d. columns in about half the total time.

This paper describes preparation of fused silica OTCs, coated with a cyanopropylpolysiloxane stationary phase. Relative retention times and response factors for all 22 TCDD isomers are presented for the electron capture detection and for the molecular ion mass of individual isomers under electron impact ionization with selected ion monitoring (SIM).

INTRODUCTION

A considerable degree of recent environmental research activity on polychlorinated dibenzo-p-dioxins (PCDDs) has been devoted to developing improved methods for the cleanup and unambiguious identification of 2,3,7,8-tetrachloro dibenzo-p-dioxin (2,3,7,8-TCDD) [1-4]. In addition, increasing attention is also being focused on the improvement of precision and accuracy during quantitative analysis of PCDDs at low picogram levels. The analytical scheme for quantitative analysis, for a given matrix, involves several pretreatment and cleanup steps, and those used depend upon the nature of the sample and the concentration of interfering compounds present in the sample [5]. Beside these methodological problems, the most important requirement is the

performance of the separation column.

It is known that cyanopropyl siloxane phases, such as Silar 10 c can very effectively separate TCDD-isomers [6-8], however, due to its thermal degradation when heated over 240 °C, it does not separate all PCDDs.

In our laboratory, various methods of sample treatment, extraction and cleanup steps were evaluated [9], including preparation of narrow-bore open tubular columns with 100 um i.d., that produce the most acceptable separation tool for TCDD analyses [10]. This paper summarizes the evaluation of various concentration and cleanup methods for a wide range of environmental samples, as well as describing the separation of the components of interest on narrow-bore WCOT columns used with high resolution gas chromatography (HRGC) and HRGC/MS.

EXPERIMENTAL

The extraction techniques vary widely from one laboratory to another [11], and varying recoveries are reported for similar sample types. A wide variety of digestion and/ or extraction techniques are used by laboratories for extraction of PCDDs from fish tissue and sediment samples. It was found that no single set of conditions were optimal for all samples. Matrice variations, such as particle size in sediments and lipid content in fish gave varying extraction efficiency. Figure 1 shows the UV-traces of 10-g sediment samples extracted under different conditions. The samples were Soxhlet extracted for 36 hours and the resultant extracts were passed through high pressure gel permeation chromatography (GPC) using a high capacity Styrogel column with UV-detection. Those samples treated with trisodium phosphate and

sodium sulfate gave the highest extraction efficiency for organics with a molecular weight range between 250 to 1000. A detailed procedure is given in ref.[2].

Sample Preparation

Initially, stock solutions of TCDD-isomers were made by dissolving weighed amounts in toluene, and diluted to fixed volume with iso-octane. These stock solutions, of known concentrations in the range 10 to 50 ng/uL, were used in experiments designed to check on the purity of each isomer, and to determine their GC-characteristics. For mas spectrometric studies, mixtures were made up of original stock solutions.

Procedure

All samples were homogenized utilizing a polytron blender and stirrer. The method of extraction was determined by sample type, i.e. fish or egg samples require an initial acid digestion and subsequent blending by a polytron.

Analysis

Detection of TCDDs was accomplished with a Varian Vista 6000 gas chromatograph equipped with an elctron capture detector (ECD) and splitless injection system. A Specta-Physics SP-4200 computing integrator was used to obtain chromatograms and qualitative data for analysis of specific isomers. In these isomer specific analyses hydrogen was used as a carrier gas. Gas chromatographic conditions were selected to minimize analysis time using a 0.2 um narrow-bore (100 um i.d.) WCOT column capable of separating all 22 TCDD isomers. The OTC was 20 m x 100 um i.d. coated with cyanopropyl-tolyl-allyl siloxane as a liquid phase. The hydrogen

carrier gas was run at 350 kPa and 45 cm/s linear velocity. The number of theoretical plates was 82000 TPs at 200 °C.

High Resolution Gas Chromatography-Mass Spectrometry

HRGC/MS analyses were accomplished using a Varian 311 A mass spectrometer in tandem with a Carlo Erba 4160 GC. The WCOT column was the same as stated above. The carrier gas was helium. Temperature programming : the initial temperature was held at 80 °C for 2 minutes, then programmed ballistically to 180 °C and then to 250 °C at 4 °C/min. The rate was later changed to 12 °C/min until the oven reached 280 °C and the final temperature was held for 5 minutes. The electron multiplier voltage was held at 2.4 kV; source temperature at 200 °C for electron impact ionization; and the manifold temperature at 100 °C. The multiple ion detection hardware permitted the monitoring of a maximum of three ions.

Fish Tissue Samples

A 10 to 25 g sample size spiked with ^{13}C-labelled PCDD congeners was digested and/ or treated with 1 N HCl. The resultant mixture was extracted overnight with 100 mL of toluene and centrifuged for 10 to 15 min. to separate solid materials, aqueous phase and toluene. The resultant toluene extract was passed through high capacity GPC prior to being treated with 0.05 M $Na_2PO_4.12\ H_2O$ and 10 N H_2SO_4, alumina cleanup and carbon fibre chromatography before analysis by HRGC or HRGC/MS.

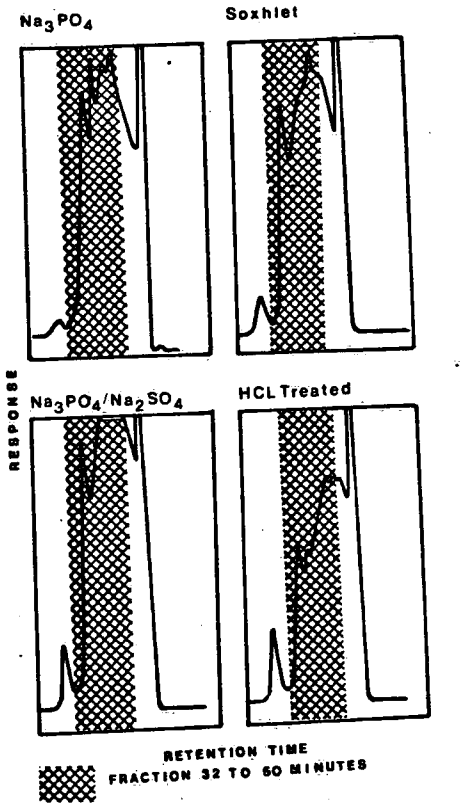

Figure 1. UV-traces of high pressure gel permeation chromatography on sediment sample cleanup under different conditions.

Sediment Samples

An appropriate size sample (2-15 g) spiked with ^{13}C-labelled PCDD congeners was treated with 100 mL 1 N HCl and allowed to stand for 1 hr. The mixture was then filtered and washed with 100 mL of distilled deionized water. The filtrate was extracted 3 times with 50 mL of toluene. The solids were mixed with Na_2SO_4 to obtain a free flowing mixture and soxhlet extracted for 6 hrs with 300 mL of toluene. The toluene extracts were used for futher cleanup.

Preparation of Open Tubular Columns

Fused silica tubing (Polymicro Technology, Pheonix, AZ) was leached with an etching solution consisting of 2.3 % HF and 2.2 % HNO_3 at 30 °C for a max. 60 minutes, followed by a rinse with 0.1 % HCl and flushed with methanol. With the capillary tubing connected to the oven, the capillary was heated under nitrogen to 250 °C for 12 hrs. The deactivation reagent was bis-(cyanopropylmethylhydro) polysiloxane dissolved in toluene as a 5 % solution, was obtained from Dr. K. Markides [12]. The deactivation reagent was dynamically coated at 2 cm/s using a high pressure apparatus as described by Schjutes et al. [13], modified as shown in Fig. 2. A tail column connected to the fused silica via a restrictor located at the end of the FS-capillary was employed according to Pretorius [14]. After total solvent evaporation the column was sealed in a flame at atmospheric pressure. The deactivation reaction was performed by a temperature program from 50 °C at 4 °C/min to 250 °C. This temperature was maintained for 10 hrs. The column was cooled and opened under methylene chloride. An excess of the deactivation reagent and

oligomers were rinsed out with methylene chloride. A cyanopropyl siloxane containing 87 % cyanopropyl-, 10 % tolyl- and 3 % allyl-substituents, kindly provided by Prof. M.L. Lee, was statically coated on the columns from a solution in dichloromethane [15]. After conditioning at 230 °C for 12 hrs a dioxin standard was injected into the column. The 2,3,7,8 TCDD peak showed almost a perfect shape and no tailing. However, the efficiency calculated for the 2,3,7,8 TCDD indicated 4100 TP/m at k' 10.3, which was a relatively low value and a coating efficiency of 68 %.

High Pressure Coating Apparatus

A modified version of the coating solution introduction device, according to Schjutes[13] has been used for the filling of narrow-bore OTCs, with coating solutions for both static and dynamic coating techniques. The modified device was shown in Fig. 2. It consists of two cylindrical parts that resemble a piston or a plunger moving inside the barrel of a syringe. The seal was accomplished with a Viton O-ring located on the inner piston. The movement of the barrel can be easily achieved by the lever. The capillary tubing was connected to the upper part of the device using a graphite ferrule. It was possible to de-gas the solution by vacuum. The lower part was provided with a ball-valve and the housing for a 2 mL glass vial (ReactiVial, Pierce Chem.Co., Rockford, IL,USA) containing the coating solution.

RESULTS AND DISCUSSION

a) Gas-chromatographic Retention Pattern

Under the GC-programming conditions described above the data was expressed as relative retention times (RRT), where retention times for 2,3,7,8 TCDD was defined as 1.000. Under the conditions

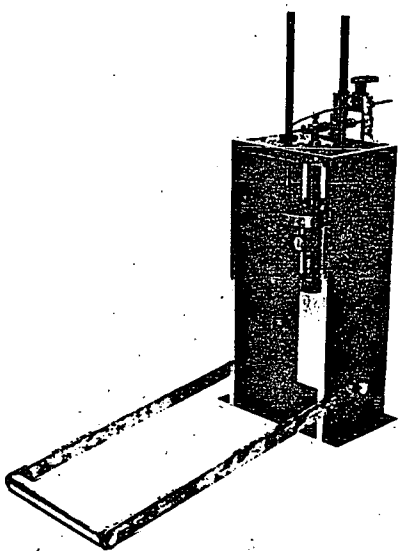

Figure 2. High pressure coating apparatus.

used, the 20 m x 100 um i.d. and 55 m x 250 um i.d. FS-OTCs permitted reasonably short elution times (~ 20 to 25 min) for the entire mixture of TCDDs. The narrow-bore columns could resolve almost all 22 TCDD-isomers, although as the columns deteriorated with age (2 to 3 weeks or 100 injections) such performance became increasingly difficult to maintain. As it can be seen, from Table 1, even at optimum performance, the column could not resolve three pairs of isomers. The temperature program used in experimental runs, involved an extended maintenance of the polar OTC at the upper temperature limit of 280 °C, which undoubtedly contributed to its deterioration. However, these conditions were found to be suitable for elution of all congeners of PCDDs and thus represents a realistic compromise for analysis of fly ash samples. Elution times for OCDD was shorter than 40 minutes. The reproducibility of

b) Mass Spectrometric Relative Response Factors

Since PCDDs are most often observed as trace impurities at nanogram or even picogram per kilogram, it was usually not feasible to characterize them in such amounts via their complete mass spectra. For this reason, an effort was made to establish relative response factors, under defined conditions of electron impact ionization. The values listed in Table 1 were obtained using an electron energy of 70 eV, and a source tempertaure of 220 °C. The reproducibility of GC peak areas was within ± 8 %. The degree of precision was determined to be ±14.5 %. These experiments were conducted using two mixtures of 2:2 and 3:1 substitution including 1,2,3,4 TCDD.

We do not advise that these results can be directly transferred to other mass spectrometers without re-checking at least some available isomers. It can be justified on the basis of similar data obtained on different types of sector mass spectrometers in different laboratories [16]. Until more work was conducted in this area, it is recommended to calibrate the instrument using as many purified standards as available.

the observed retention times was within 2 seconds.

It should be noted that the elution order of the 22 TCDD isomers from this column was not identical in all cases to that found previously on Silar 10 c. These differences are due to the different polarity between Silar 10 c and the experimental stationary phase.

The results in Fig.3 obtained by HRGC-ECD on two different columns show superior separation of individual TCDD isomers on the narrow-bore column.

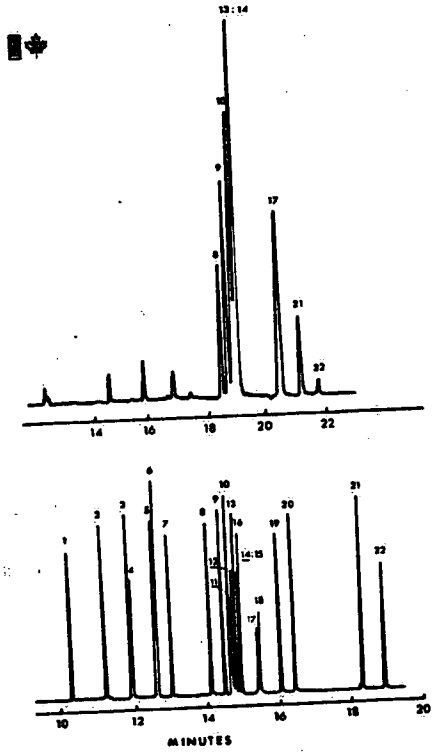

Figure 3. HRGC-ECD chromatograms of 22 TCDD isomers on two WCOT columns of a different internal diameter.

Table 1. Elution Order of Individual TCDD Isomers, their Relative Retention Times and Response Factors on a 20 m x 100 um WCOT Column at 200 °C

No.	Isomer	RT (min)	RRT	RRF-GC	RRF-MS
1	1368	10.39	0.725	1.531	1.250
2	1379	11.34	0.791	1.137	1.055
3	1378	12.01	0.838	1.356	1.120
4	1369	12.04	0.840	1.749	0.650
5	1247	12.76	0.890	1.553	1.210
6	1248	12.76	0.892	1.531	0.975
7	1268	13.10	0.914	1.203	0.885
8	1478	14.17	0.988	0.536	1.220
9	2378	14.34	1.000	1.000	1.000
10	1234	14.56	1.015	1.357	1.100
11	1246	14.70	1.025	0.919	0.980
12	1249	14.74	1.028	NA	1.306
13	1237	14.76	1.028	0.918	0.718
14	1238	14.79	1.029	0.962	1.008
15	1279	14.81	1.033	NA	1.200
16	1236	14.84	1.035	NA	0.955
17	1278	15.46	1.088	0.962	0.805
18	1469	15.49	1.090	NA	1.700
19	1239	16.06	1.120	1.224	1.150
20	1269	16.45	1.149	NA	1.250
21	1267	18.38	1.282	1.530	1.050
22	1289	18.90	1.318	1.138	0.905
	^{37}Cl-2378	m/z 328/322			2.221
	^{13}C-2378	m/z 334/322			1.200

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