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Note

Gas chromatographic separation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from polychlorinated biphenyls and tetrachlorodibenzo-*p*-dioxin isomers using a polymeric liquid crystal capillary column

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The extraordinary toxicities of the polychlorinated dibenzo-*p*-dioxins (PCDD) have been demonstrated by animal tests and, to some extent, by accidental exposure of human to these compounds. This has prompted extensive efforts to separate and identify these compounds in various chemical, biological and environmental media[1]. The various experiments have indicated that there is a pronounced difference in toxic and biological effects among different PCDD isomers. Based on the results of whole animal studies and *in vitro* assays, the symmetrical substituted 2,3,7,8-tetrachloridibenzo-*p*-dioxin (2,3,7,8-tetra-CDD) isomer appears to be the most toxic. A factor of 1000 to 10 000 difference in toxicity can be found for the closely related 2,3,7,8- and 1,2,3,8-tetra-CDD isomers[2,3].

The isomer specific separation and isolation of 2,3,7,8-tetra-CDD using reversed-phase and normal-phase high-performance liquid chromatography has been reported previously. The method appears to be too labor-intensive for use as a routine analytical technique; moreover further analysis is needed by ultra-sensitive gas chromatography–mass spectrometry (GC–MS)[4]. The high-resolution GC–MS has shown high potential for the separation and quantitative identification of 2,3,7,8-tetra-CDD in very complex samples. The U.S. Environmental Protection Agency (EPA) has developed a method for the separation and quantification of 2,3,7,8-tetra-CDD where the application of very long capillary columns (60 m) is recommended[5]. The success of the separation and quantification of PCDD depends on the concentration of the interfering compounds; the separation and positive identification of 2,3,7,8-tetra-CDD in sample matrices that contain million fold higher levels of other naturally occurring compounds or other chlorinated industrial pollutants has presented a challenging task for analytical chemists. It is especially difficult to analyse for the 2,3,7,8-tetra-CDD in presence of polychlorinated biphenyls (PCBs) because of interferences in GC and MS. The complex sample clean-up procedures used for PCB separation prior to analysis of 2,3,7,8-tetra-CDD and other PCDD in samples from fires in transformers filled with PCB are partially effective[5].

Liquid crystals have been used as selective stationary phases in GC for the separation of close boiling isomeric organic compounds (6–8). Recently polymeric liquid crystals have been developed and used in capillary column GC (9–14) for separation of polyaromatic compounds. But to date, polymeric liquid crystal columns

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have not been used for the separation of PCDD. In this paper we report, for the first time, the application of polymeric liquid crystals to complete separation of the most toxic 2,3,7,8-CDD from all tetra-CDD isomers present in flyash extrct, and the separation of this isomer from all PCB isomers.

EXPERIMENTAL

The gas chromatograph used was a Hewlett-Packard 5880 equipped with an electron-capture detector and a cool on-column injector. The GC–MS system used was a Hewlett-Packard 5987A with an HP 1000 data system. The Aroclors (1216, 1242, 1248, 1254) were purchased from Ultra Scientific (RI, U.S.A.). The labelled [$^{13}C_{12}$]-2,3,7,8-tetra-CDD was obtained from Cambridge Isotope Labs. (Cambridge, MA, U.S.A.). A fused-silica capillary column coated with polymeric liquid crystal and reported previously for the separation of polyaromatic compounds was used[14]. The Ontario flyash was extracted as reported previously[15]. The extract was spiked with labelled [$^{13}C_{12}$]-2,3,7,8-tetra-CDD and was analysed by GC–MS using the selected ion monitoring mode. The tetra-CDD isomers were confirmed by proper intensities of M, M + 2, M + 4 (320, 322, 324, respectively) ions. In addition 2,3,7,8-tetra-CDD was confirmed by retention time of standard labelled [$^{13}C_{12}$]-2,3,7,8-tetra-CDD.

RESULTS AND DISCUSSION

The complete GC separation of 2,3,7,8-tetra-CDD from PCB isomers using fused-silica capillary column coated with polymeric liquid crystal is shown in Fig. 1. The PCB mixture containing many different isomers was prepared by mixing the commercial Aroclors (1216, 1242, 1248, 1254). Because of the high toxicity of PCBs as well as of 2,3,7,8-tetra-CDD, a very small sample size and the very high sensitive ECD were used in the separation studies. For the positive identification of PCDDs in complex samples by GC–MS in the selected ion monitoring mode various criteria have to be satisfied. It is possible to identify and quantify the PCDDs if the concentrations of the interfering compounds are low. However, in samples such as those obtained from PCB fire, the interfering compounds are million times more concentrated than the PCDDs and it is therefore extremely difficult to satisfy all the criteria for positive identification. The pentachlorobiphenyls have a molecular ion (M = 324) equal to the molecular isotope ion (M + 4) of tetra-CDD, hence the use of criteria such as correct isotopic abundances is very difficult or impossible. It could be possible to identify selectively the 2,3,7,8-tetra-CDD if it could be separated from interfering compounds. With capillary columns such as those coated with SE-30 or DB-5 such separation cannot be obtained presumably because of close volatilities of pentachlorobiphenyls and tetra-CDDs. Liquid crystals are selective stationary phases used in GC, where separation is based on the structural differences of the solutes along with their volatilities. Based on the separation mechanism of liquid crystal stationary phases, for the compounds with equal volatility the linear and symmetrical molecules will be retained longer than the bulkier molecules because of their favorable geometry in retention to that of liquid crystal matrix. Fig. 2 shows that the PCB isomers are flexibil because of the single bond connecting two benzene rings while

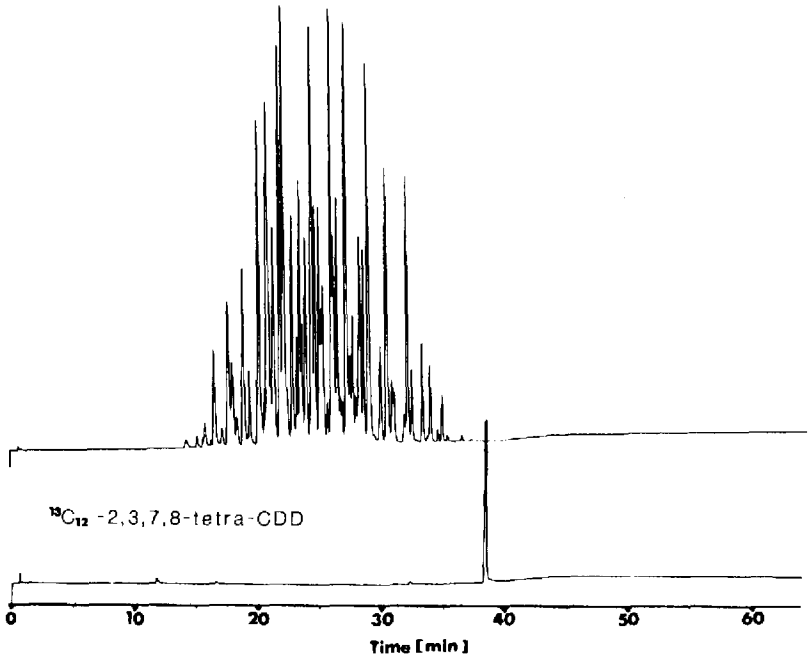

Fig. 1. Gas chromatogram of PCBs (Aroclor mixture) and 2,3,7,8-tetra-CDD. Chromatographic conditions: 20 m × 0.25 mm I.D. liquid crystalline polysiloxane coated fused-silica column; temperature at 100°C for 1 min, programmed to 270°C at 3°C/min.

the PCDD structure is rigid. This structural difference results in longer retention times for PCDDs than for PCBs. In particular the 2,3,7,8-tetra-CDD isomer is the most liner and symmetrical hence it was eluted after all PCB isomers (Fig. 1).

Considering the structural differences of the tetra-CDD isomers it can be seen that 2,3,7,8-tetra-CDD is the most symmetrical isomer. The elution order and separation of 2,3,7,8-tetra-CDD as compared to all other tetra-CDD isomers present in an extract of organic compounds obtained from municipal incinerator flyash was confirmed by GC–MS analysis of a flyash extract spiked by standard [$^{13}C_{12}$]-2,3,7,8-tetra-CDD. There is a very small difference of about two scans in GC–MS analysis of labelled [$^{13}C_{12}$]-2,3,7,8-tetra-CDD and unlabelled 2,3,7,8-tetra-CDD isomers[16]. Hence, the 2,3,7,8-tetra-CDD isomer in flyash extract was considered to be eluted at the same time as labelled 2,3,7,8-tetra-CDD was eluted (Fig. 3). The GC–

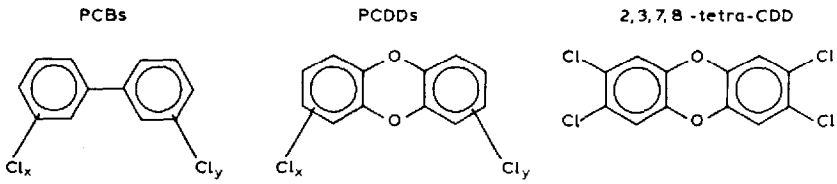

Fig. 2. Structures of PCBs, PCDDs and 2,3,7,8-tetra-CDD.

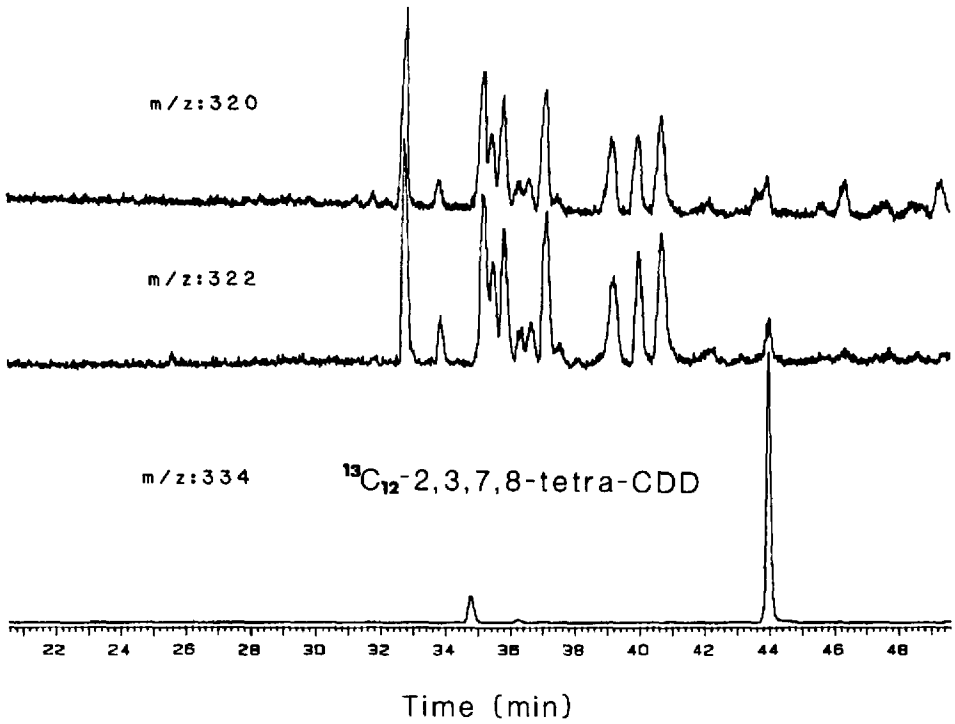

Fig. 3. Separation of 2,3,7,8-tetra-CDD from other tetra-CDD isomers in flyash extract, mass chromatogram of m/z = 320 (M), m/z = 322 (M + 2) (for tetra-CDD isomers) and m/z = 334 (M + 2) (for [$^{13}C_{12}$]-2,3,7,8-tetra-CDD) isomer. Chromatographic conditions: temperature program rate 4°C/min, all other conditions as in Fig. 1.

MS system, was operated in the electron-impact selected ion monitoring mode where m/z = 320, 322, 324 (M, M + 2, M + 4) ions for tetra-CDD and m/z = 332, 334, 336 for labelled 2,3,7,8-CDD were monitored. Flyash extract used in this study was not obtained for the quantation purpose. The concentration of 2,3,7,8-tetra-CDD can be estimated roughly to be 2–3 ng per gram of flyash. In Fig. 3 all peaks present both in M and M + 2 (m/z = 320, 322) ions are the tetra-CDD isomers according to the criteria for positive identification of chlorinated dioxins. The small peak present in M + 2 ion trace of [$^{13}C_{12}$]-2,3,7,8-tetra-CDD isomer is from an unknown compound present in flyash extract. This peak was not observed when only [$^{13}C_{12}$]-2,3,7,8-tetra-CDD was injected. There is a correlation between the chemical structures and the selectivity of the liquid crystal stationary phases[17]. It should be possible to synthesize specific liquid crystalline polymer stationary phases which can separate all PCDDs from PCBs.

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