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Internal standards for use in the comprehensive analysis of polychlorinated aromatic hydrocarbons using gas chromatography combined with multiphoton ionization mass spectrometry

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ABSTRACT

To decrease health-risks to humans, non-toxic compounds were evaluated for use as internal standards for calibrating data obtained by gas chromatography/multiphoton ionization mass spectrometry (GC-MPI-MS) using an ultraviolet femtosecond laser as the ionization source. The retention time in the mass chromatogram was calibrated using a retention index, in which a series of *n*-alkanes was employed as internal standards for evaluating the retention times for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). To compensate for changes in signal intensity in MPI-MS, the dependence of signal intensity on the laser pulse energy was investigated for the dioxin-like compounds, in addition to five non-toxic aromatic hydrocarbons, that were used as internal standards. Based on their similar behavior,the non-toxic PCDD/PCDF, its ^{13}C-isotope, and pentachlorobenzene behave similarly, we conclude that they can be used for calibrating the signal intensities in MPI-MS.

1. Introduction

Halogenated aromatic hydrocarbons, such as polychlorinated dibenzo- *p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are emitted into the atmosphere as undesirable by-products that are produced in incinerators. In addition, they are produced as by-products during the synthesis of pesticides and other useful organic compounds and are discharged into the environment. Due to their high toxicity, a sensitive and selective analytical method would be desirable for identifying and quantifying such compounds. Currently, gas chromatography combined with high-resolution mass spectrometry (GC-HRMS) based on electron impact ionization (EI) is the standard method for the trace analysis of these compounds [1–3]. This traditional technique, based on EI, is useful for "hard" ionization, and permits compounds to be assigned by comparing observed data with a database. However, the extensive fragmentation by EI sometimes makes quantification, or sometimes even the identification, of such analytes a difficult task, especially when the sample is a complex mixture.

The technique of "soft" ionization, e.g., photoionization, was developed to solve this problem and has been employed for the trace analysis of a wide variety of organic compounds [4–7]. Multiphoton ionization (MPI) provides us with an advanced tool for sensitive and selective analysis; spectral selectivity can be achieved by optimizing the laser wavelength for resonance ionization (see Fig. 1) that suppresses the production of background signals [6–9]. A number of studies based on MPI-MS in which different types of laser sources have been used have appeared [10–12]. This technique has been combined with GC and has proven to be useful for the analysis of environmental samples [13–16]. Femtogram detection limits have been reported for dioxin-like compounds and subfemtogram detection limits for polycyclic aromatic hydrocarbons when this approach is used [17]. The sensitivity can be further improved 2.5-fold by modifying the sample introduction port [18]. The excellent sensitivity provided by MPI-MS allows the trace analysis of toxic compounds in foods and soils. However, special care needs to be taken, since toxic internal standards, e.g., ^{13}C-isotopes of analytes, are typically added to the sample to permit the assignment of the components and also to calibrate signal intensities in a mass chromatogram for quantitative analysis. It should be noted that the toxicity associated with dioxin analysis primarily arises,

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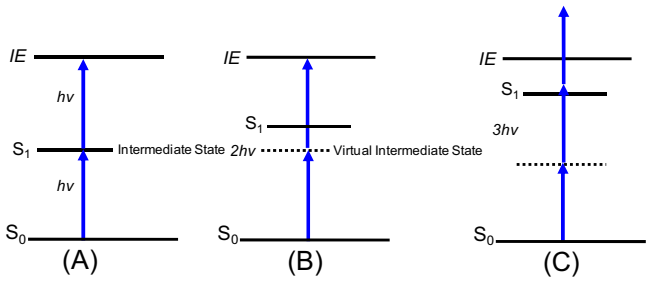

Fig. 1. Ionization mechanisms. (A) resonant two-photon ionization (B) non-resonant two-photon ionization (C) non-resonant three-photon ionization. S_0, ground state; S_1, electronic excited state; *IE*, ionization energy.

in most cases, not from the sample in the environment (e.g., soils) but from the internal standards that are added to the sample. For example, the concentrations of toxic ^{13}C-labeled dioxins used as internal standards are at levels of 50–250 pg/μL in the EPA standard method 8290, the concentration of which are much higher than those of native dioxins in real samples. It is well known that GC retention times can change, depending on the conditions used for separating the analytes in the capillary column and that the ionization efficiency, i.e. signal intensity, depends on the parameters of the laser being used in the experiment, e.g., pulse energy, laser beam focusing conditions, etc. In order to avoid such problems, non-toxic internal standards could be used for calibrating the signals in GC-MPI-MS. Such an approach, i.e., the use of a sensitive analytical instrument and non-toxic internal standards, would allow analyses to be carried out in an analytical laboratory that is not specifically designed for dioxin analysis, thus greatly reducing the capital and operation and maintenance costs for such analyses.

In this study, we report the use of retention indices for calibrating the retention times of dioxin-like compounds, which include seventeen highly-toxic PCDDs, PCDFs, and PCBs, by using a series of *n*-alkanes. We investigated the dependence of signal intensity on laser pulse energy for several non-toxic aromatic compounds in calibrating signals measured by GC-MPI-MS. The suitability of these compounds for use as internal standards in the trace analyses of dioxin-like compounds were compared and the results are discussed.

2. Experimental

2.1. Apparatus

The experimental setup for the mass spectrometer has been reported in detail elsewhere [6]. Briefly, the third harmonic emission (267 nm, 100 μJ) of a Ti:sapphire laser (800 nm, 1 kHz, 100 fs, Libra, Coherent Inc., CA) was utilized as an ionization source. The ions induced by MPI were detected by an assembly of microchannel plates (MCP, F4655-11, Hamamatsu, Shizuoka, Japan). The mass spectrum was recorded using a digitizer (AP240, 1 GHz, 1 GS/s, Acqiris, Agilent Technologies, Santa Clara, CA, USA). A 1-μL sample solution was injected into a GC system (6890N, Agilent Technologies) using an auto sampler (7683B, Agilent Technologies) and the components were separated using a capillary column (DB–5 ms, 30 m, 0.25 mm id., 0.25 μm film thickness, Agilent Technologies). For the analysis of PCDDs/PCDFs, the temperature of the column was programmed to increase from 50 °C (1 min hold) to 180 °C at a rate of 25 °C/min and then to 300 °C at a rate of 5 °C/min, with a 10 min hold. For a PCB analysis, the temperature was programmed to increase from 70 °C (2 min hold) to 300 °C at a rate of 8 °C/min and then a 10 min hold. The temperatures of the injection port and the transfer line were maintained at 300 °C. Analytes eluting from the GC were introduced into a linear-type time-of-flight

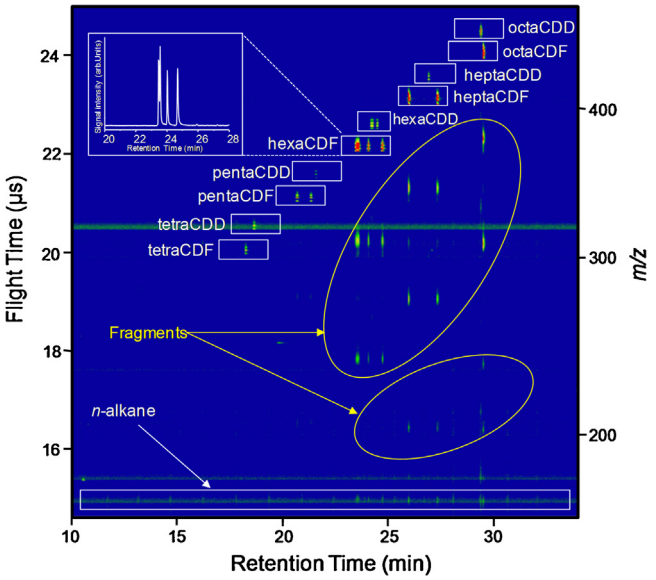

Fig. 2. Two-dimension display of a standard mixture of PCDDs/PCDFs (NK-ST-B2) containing *n*-alkanes measured using GC-MPI-TOFMS. Laser pulse energy, 95 μJ; sample concentration, 50 pg/μL for tetraCDDs/CDFs and pentaCDDs/CDFs, 100 pg/μL for hexaCDDs/CDFs and heptaCDDs/CDFs, 200 pg/μL for octaCDD/CDF. and 50–100 ng/μL for *n*-alkane (C_7—C_{33}). The insert shows the mass chromatogram where the hexaCDFs appear. A line appearing at ca. 20.5 μs can be assigned to a contaminant molecule remaining in the MS.

(TOF) mass spectrometer MS with a mass resolution of 1000. The voltages applied to the electrodes in the MS and the laser beam focusing conditions were optimized by monitoring the mass spectrum of pentachlorobenzene that was directly introduced into the TOFMS as a molecular beam from a reservoir maintained at room temperature. The data were analyzed using the LabVIEW software. The signal intensity was calculated from the data in the mass chromatogram using the software programmed by LabVIEW. Due to the high toxicity of dioxin-like compounds, special care needs to be taken in handling the chemicals. In our laboratory, the maximum amount of dioxins that can be used in one day is restricted to the amount specified as the tolerable daily intake of PCDDs/PCDFs/PCBs by World Health Organization.

2.2. Reagents and samples

A standard sample of NK-ST-B2 containing seventeen toxic PCDDs/PCDFs was purchased from Wellington Laboratories (Guelph, ON, Canada). The three compounds used as internal standards were 2-chlorodibenzofuran, 2,6-dichlorodibenzofuran, and 2,3,7-trichlorodibenzofuran and were also supplied by Wellington Laboratories (Guelph) and pyrene-d_{10} and dibenzofuran were obtained from Supelco (Bellefonte, PA, USA). A sample of KC-mix, a mixture of KC-300, KC-400, KC- 500, and KC-600mainly containing PCBs from diCBs to octaCBs (GL Science, Tokyo, Japan) was utilized as a standard sample mixture of PCBs. A sample containing *n*-alkanes, a mixture of aliphatic hydrocarbons ranging from C_7 to C_{33}, was purchased from Hayashi Pure Chemical Ind., Co., Ltd. (Osaka, Japan). All of these reagents were diluted with nonane supplied for use in dioxin analysis.

2.3. Calculation of retention index

The retention index (*RI*) can be calculated using Eq. (1).

$$RI = 100 \times [\mathrm{n} \frac{t_r(\text{analyte})^{-t_{r(\mathrm{n})}}}{t_r(\mathrm{n}+1)^{-t_{r(\mathrm{n})}}} \quad (1)$$

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