

Resonant and Non-Resonant Femtosecond Ionization Mass Spectrometry of Organochlorine Pesticides

Lakshitha, Madunil Siddihalu
Faculty of Design, Kyushu University

Imasaka, Totaro
Division of International Strategy, Center of Future Chemistry, Kyushu University

Imasaka, Tomoko
Faculty of Design, Kyushu University

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ARTICLE

Resonant and non-resonant femtosecond ionization mass spectrometry of organochlorine pesticides†

Siddihalu Lakshitha Madunil,^a Totaro Imasaka,^{b,c} and Tomoko Imasaka^{*a}

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Thirteen organochlorine pesticides in a standard sample mixture were measured by gas chromatography combined with mass spectrometry using an ultraviolet femtosecond laser (267 nm) as the ionization source, and the observed mass spectra were compared with the corresponding spectra obtained using an electron ionization source. When an ultrashort optical pulse was used for ionization, molecular ions were typically produced which was preferential for reliably identifying the analytes. The ionization mechanism was studied based on three models constructed for resonance-enhanced two-photon ionization, non-resonant two-photon ionization, and non-resonant three-photon ionization. The optimal conditions for observing a molecular ion were investigated using data obtained for three pulse widths. The results suggest that two-photon ionization with minimum excess energy would be optimal for observing a molecular ion.

Introduction

Organochlorine pesticides (OCPs) have been extensively used as insecticides for protection against malaria, typhoid fever, cholera, and other insect transmitted diseases. They are also employed in agricultural fields to enhance the productivity of various crops.^{1–4} These compounds have unique chemical structures and contain multiple chlorine atoms in an aliphatic/aromatic molecule, making them semi-volatile and less soluble in water and allowing them to easily bio-accumulate in lipids.^{5,6} It should also be noted that OCPs are persistent, not generally metabolized, and are transported for long distances in the atmosphere.^{7,8} It is now recognized that OCPs are highly-toxic and pose many adverse effects to non-targeting organisms such as marine mammals, birds, and even humans. They induce neurological problems, hyper activity disorders, type II diabetes, changes in gestation age, and cancer for humans.^{9,10} Such health hazards are more serious in developing countries because of improper legislation, a lack of regulation of their use, and ignorance by agricultural workers. OCPs are now banned by law but continue to be utilized in many countries with weak law enforcement.^{2,11} Due to a long history of using these chemicals, they accumulate in agricultural fields.¹²

Many techniques have been developed to analyze OCPs in various matrices such as soil, food, blood, human fat, liver, kidney, etc.^{13–15} In general, analytes in a real sample are measured after a pretreatment to reduce matrix effects.¹⁶ Trace

levels of OCPs are then separated by gas chromatography (GC) or liquid chromatography and are measured using an electron-capture detector or an ultraviolet-visible absorption detector. The former has a high sensitivity and selectivity for OCPs. However, it is not suitable for trace analysis when numerous interfering substances are present in the sample. GC combined with mass spectrometry (MS) is also widely used for the trace analysis of OCPs, in which an electron ionization (EI) source is most frequently used for non-specific hard ionization. In order to minimize matrix effects, the selected-ion-monitoring (SIM) mode is used in MS.¹⁷ However, interference in the matrix, which provides fragment patterns that are similar to the analyte, decreases reliability of this analysis. Because of this, it would be desirable to observe a molecular ion and fragment ions as well for achieving a more selective determination. To accomplish this, it will be necessary to develop a technique for soft ionization to address the problem arising from EI.

Photoionization is a well-known advanced technique for soft ionization which permits a molecular ion to be observed in most cases.^{18,19} This technique can be used for selective ionization by adjusting the laser wavelength at the absorption band of the analyte, as is referred to as resonance-enhanced multiphoton ionization (REMPI). Many organic compounds, including aromatic hydrocarbons, have absorption bands in the ultraviolet (UV) region and can be measured selectively. For OCPs such as hexachlorobenzene (HCB), the molecule consists of many chlorine atoms and has a short excited-state lifetime by intersystem crossing to triplet levels, which decreases the efficiency of ionization when a nanosecond laser is used. A laser with a shorter pulse width has been employed to overcome this problem.¹⁹ For example, many chlorinated aromatic hydrocarbons such as dioxins and polychlorinated biphenyls have been measured, and molecular ions have been observed as major ions.^{20–22} It should be noted that a short optical pulse has been successfully used for the efficient ionization of even non-

^a Faculty of Design, Kyushu University, 4-9-1, Shiobaru, Minami-ku, Fukuoka 815-8540; 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

^b Division of International Strategy, Center of Future Chemistry, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

^c Hikari Giken, Co., 2-10-30, Sakurazaka, Chuou-ku, Fukuoka 810-0024, Japan

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aromatic hydrocarbons through non-resonant multiphoton ionization (NRMPI). The ionization efficiency can be improved to a level comparable to REMPI by decreasing the laser pulse width to < 100 fs.²³ This technique has been applied for the measurement of analytically important compounds, e.g., chlorinated aliphatic/aromatic hydrocarbons such as OCPs.²⁴ However, the mechanism responsible for this ionization has not yet been investigated to date.

In this study, we report on the measurement of a sample mixture containing thirteen OCPs, which were not chosen by the authors but, rather, are routinely used as standard chemicals. The analytes were ionized using the third harmonic emission (267 nm) of a femtosecond Ti:sapphire laser to study three types of ionization mechanisms, i.e., resonance-enhanced two-photon ionization (RE2PI), non-resonant two-photon ionization (NR2PI), and non-resonant three-photon ionization (NR3PI) at three laser pulse widths of 160, 47, and 35 fs. A guideline is proposed for identifying optimal conditions for achieving a more sensitive and selective determination in the trace analysis of a real sample containing numerous interfering species.

Experimental

Analytical instrument

Analytes in a sample mixture were separated by GC (6890N, Agilent Technologies, Santa Clara, CA) equipped with an auto sampler (7683B, Agilent Technologies). The GC column used was DB-5ms (capillary length 30 m, inner diameter 0.25 mm, film thickness 0.25 μ m, Agilent Technologies). The temperature program for the GC column was as follows; the temperature of the inlet port of GC was set at 250 °C, the initial temperature of the column was held at 50 °C for 1 min, a ramp of 25 °C/min to 125 °C, then increased to 300 °C by 10 °C/min rate, and finally held for 10 min. The temperature of the transfer line between the GC to the MS was maintained at 250 °C. The elution of analytes from the GC column was measured by time-of-flight MS (HG-1, Hikari GK, Fukuoka, Japan). The signal from a microchannel detector (F4655-11, Hamamatsu Photonics, Shizuoka, Japan) was recorded using a digitizer (Acqiris AP240, Agilent Technologies) and was analyzed using laboratory-made software. The third harmonic emission (267 nm) of a Ti:sapphire laser (800 nm, 100 fs, 1 kHz, 1 mJ, Libra, Coherent Inc., Palo Alto, CA) was used for measuring the limit of detection (LOD) and another Ti:sapphire laser (800 nm, 35 fs, 1 kHz, 6 mJ, Soltice-Ace, Spectra-Physics, Santa Clara, CA) for measuring the two-dimensional display at pulse widths of 35, 47, 160 fs. The temporal duration was stretched by a chirp of the pulse by passing the UV beam (110 mW) through fused silica plates with different thicknesses to change the dispersion.²³ The relationship between the pulse width, Δt_1 , and the path length, l , of the beam in the fused silica can be calculated using the following equation.

$$\Delta t_1 = \Delta t_0 \sqrt{1 + 16 \left(\ln 2 \frac{l \cdot GVD}{\Delta t_0^2} \right)^2} \quad (1)$$

The parameter, Δt_0 , represent the pulse width of the incident beam and GVD is the group velocity dispersion calculated using

the Sellmeier equation. In this study, a transformed-limited pulse was passed through a fused silica plate with a positive GVD and was then down-chirped.

Samples

A sample mixture containing thirteen OCPs, i.e., α -hexachlorocyclohexane (α -HCH), β -hexachlorocyclohexane (β -HCH), γ -hexachlorocyclohexane (γ -HCH), heptachlor, aldrin, *trans*-chlordane, *cis*-chlordane, dieldrin, endrin, α -endosulfan, β -endosulfan, HCB, dichlorodiphenyltrichloroethane (DDT), and acetone used as the solvent (analytical grade) were purchased from Wako Pure Chemicals, Japan.

Computational method

The Gaussian 16 program series package was used for calculating the energies of the neutral and ionic states. The minimum geometries were obtained by the B3LYP method based on the density functional theory (DFT) with a cc-pVDZ basis set.²⁵ The difference between the energies obtained at the level of B3LYP/cc-pVTZ of the ground and ionic states was calculated as a vertical ionization energy. The lowest one-hundred singlet transition energies and the oscillator strengths were calculated using time-dependent DFT (TD-DFT) at the level of B3LYP/cc-pVTZ. The absorption spectra were predicted using the GaussView 5 software program.

Models for multiphoton ionization

Resonance-enhanced two-photon ionization

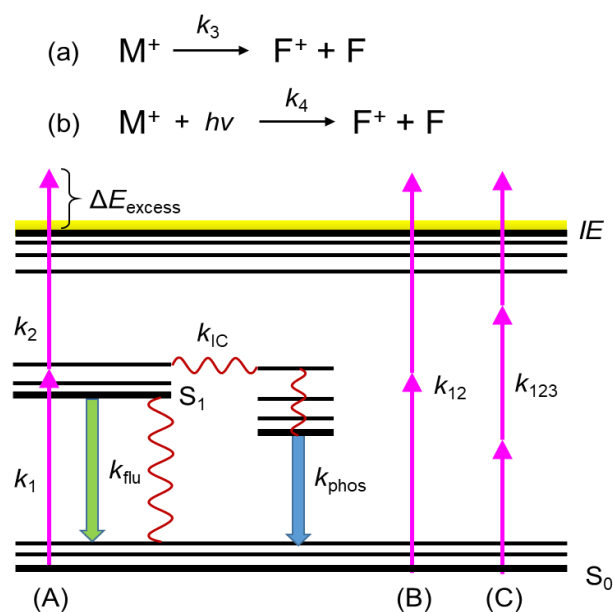


Fig. 1 Scheme showing the MPI processes. (A) RE2PI (B) NR2PI (C) NR3PI. A molecular ion gives rise to fragment ions by auto-dissociation (a) photo-dissociation (b). F and F^+ are the neutral fragment and fragment ion, respectively.

The scheme for RE2PI is shown in Fig. 1 (A). The rates of production of an intermediate (excited) state and a molecular ion can be expressed by Eqs. (2) and (3), respectively.

$$\frac{d[N_1]}{dt} = k_1[N_0]I(t) - k_2[N_1]I(t) - k_{ic}[N_1] - k_{isc}[N_1] - k_{flu}[N_1] \quad (2)$$

$$\frac{d[M^+]}{dt} = k_2[N_1]I(t) - k_3[M^+] - k_4[M^+]I(t) \quad (3)$$

The parameters, $[N_0]$, $[N_1]$, and $[M^+]$, are the population densities of the analyte in the ground, intermediate, and ionized states, respectively, k_1 and k_2 , are the rate constants for transitions from the ground to intermediate states and from the intermediate to the ionized states, respectively, $I(t)$ is the intensity temporal profile of the laser pulse, k_{ic} , k_{isc} , and k_{flu} are the rate constants for internal conversion, intersystem crossing, and fluorescence from the intermediate state, respectively, and k_3 and k_4 are the rate constants for auto-dissociation and photo-dissociation from the molecular ion, respectively. The last three terms in Eq. (2) are negligible, even for highly chlorinated aromatic hydrocarbons when a femtosecond laser is used.²⁰⁻²² Note that this assumption is not valid for compounds that contain heavy halogen atoms such as bromine/iodine or a nitro group, since they have femtosecond lifetimes. The rate of producing an intermediate state is thought to be faster than the rate of ionization due to a larger population in the ground state, i.e., $k_1[N_0] > k_2[N_1]$, $[N_0]$ being constant under non-saturation conditions. When the excess energy remaining in the molecular ion, ΔE_{excess} , is small (< 3 eV), the term, $k_3[M^+]$, can be neglected.²⁶ In addition, the rate of photo-dissociation can be neglected when the molar absorptivity of the ionized state, ϵ_i , is small at the laser wavelength and pulse energy used in the experiment. Consequently, the following equations can be obtained.

$$\frac{d[N_1]}{dt} = k_1[N_0]I(t) \quad (4)$$

$$\frac{d[M^+]}{dt} = k_2[N_1]I(t) \quad (5)$$

The rate of producing $[M^+]$ can then be calculated as follows when a Gaussian optical pulse is used for ionization, where E is the laser pulse energy and τ is the laser pulse width (the duration between the times at which the intensity decreases to $1/e$) which can be converted into the laser pulse width defined as the full width at half maximum, Δt , by multiplying a factor of $\sqrt{\ln 2}$.

$$\begin{aligned} [M^+] &= k_2(k_1[N_0] \int I(t) dt) \int I(t) dt = k_1 k_2 [N_0] (\int I(t) dt)^2 \\ &= k_1 k_2 [N_0] I_0 \tau \frac{\sqrt{\pi}}{2\sqrt{2}} \propto k_1 k_2 [N_0] E^2 \end{aligned} \quad (6)$$

From Eq. (6), the rate of formation of the molecular ion is proportional to the product of the peak intensity, I_0 , and the pulse

width, τ , and then to the square of the pulse energy, E . Thus, the rate is independent of the pulse width.

Non-resonant two-photon ionization

A scheme of NR2PI is shown in Fig. 1 (B). The rate of production of the ionic state can be expressed as Eq. (7).

$$\frac{d[M^+]}{dt} = k_{12}[N_0]I(t)^2 - k_3[M^+] - k_4[M^+]I(t) \quad (7)$$

The parameter, k_{12} , represents the rate constant for two-photon ionization. When ΔE_{excess} and ϵ_i are small, the last two terms can be neglected. The rate of producing a molecular ion can then be calculated as follows.

$$\begin{aligned} [M^+] &= \int k_{12}[N_0]\{I_0 e^{(-2t^2/\tau^2)}\}^2 dt \\ &= k_{12}[N_0]I_0^2 \int_0^\infty e^{-4t^2/\tau^2} dt \\ &= k_{12}[N_0]I_0^2 \frac{\tau\sqrt{\pi}}{4} \propto k_{12}[N_0]E^2 \frac{1}{\Delta t} \end{aligned} \quad (8)$$

When the pulse energy is constant, the intensity of the molecular ion is reciprocally proportional to the pulse width of the laser.

Non-resonant three-photon ionization

The scheme for NR3PI is shown in Fig. 1 (C). The rate of production of the ionic state can be expressed as Eq. (9).

$$\frac{d[M^+]}{dt} = k_{123}[N_0]I(t)^3 - k_3[M^+] - k_4[M^+]I(t) \quad (9)$$

The parameter, k_{123} , represents the rate constant for three-photon ionization. The last two terms can be neglected when ΔE_{excess} and ϵ_i are small.

$$\begin{aligned} [M^+] &= \int k_{123}[N_0]\{I_0 e^{(-2t^2/\tau^2)}\}^3 dt \\ &= k_{123}[N_0]I_0^3 \int_0^\infty e^{(-6t^2/\tau^2)} dt \\ &= k_{123}[N_0]I_0^3 \frac{\tau\sqrt{\pi}}{2\sqrt{6}} \propto k_{123}[N_0]E^3 \frac{1}{\Delta t^2} \end{aligned} \quad (10)$$

According, the intensity of the molecular ion is reciprocally proportional to the square of the laser pulse width.

Results and discussion

Comparison with electron ionization

The mass spectrum of aldrin (a typical OCP) measured by femtosecond ionization is shown in Fig. 2. A molecular ion consisting of several isotopomers with different numbers of $^{35}\text{Cl}/^{37}\text{Cl}$ and $^{12}\text{C}/^{13}\text{C}$ atoms could be clearly observed as one of the major ions, in addition to the fragment ions that were mainly

produced by dissociation of the chlorine atoms. Note that broad signal peaks were observed for the fragments, which is due to the release of kinetic energy in a dissociation process of the chlorine atoms (not by mass resolution). This phenomenon can be observed in linear-type time-of-flight MS and is useful for differentiate between molecular and fragment ions. On the other hand, no signal arising from a molecular ion was observed except for an aromatic compound of HCB in EI-MS, as shown in Fig. S-1 in Electronic Supplementary Information (ESI). Thus, MPI-MS is more useful than EI-MS in terms of observing a molecular ion, which is advantageous for the discrimination of the analyte against interfering substances in the matrix because of a large mass/charge (m/z) ratio and for the assignment of the analyte molecule from the intensity distribution of the isotopomers consisting of Cl and C atoms. The mass resolution was 560 in this study, which was sufficient for the measurement of isotopomers. A high-resolution MS is known to be useful for background suppression and then used for trace analysis. The present approach based on photoionization has a benefit for background suppression, due to additional selectivity in the ionization process. Thus, the approach herein would have a potential for use in identification of a potentially unknown pesticide in the environment.

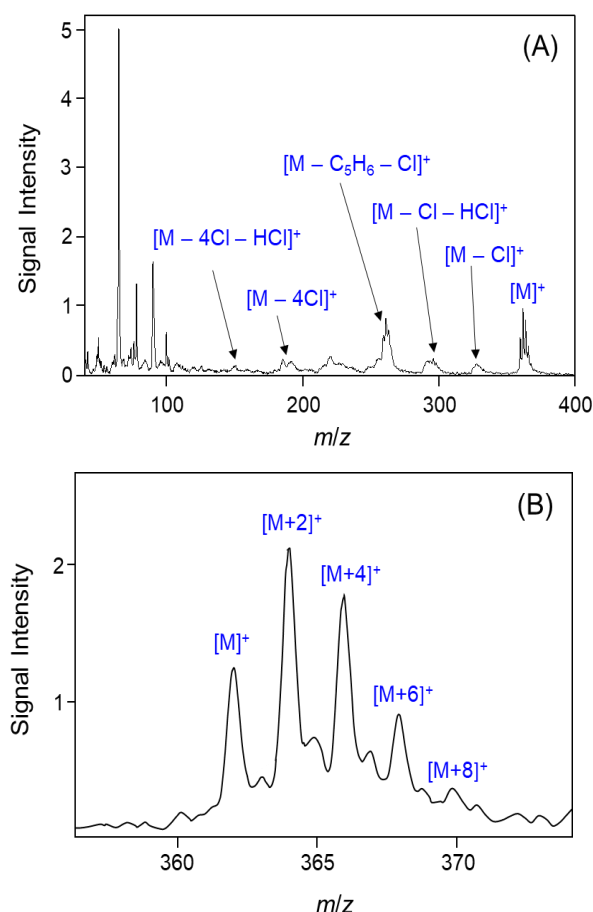


Fig. 2 (A) mass spectrum of aldrin (B) expanded view of (A). Isotopomers consisting of ^{35}Cl and ^{37}Cl atoms are assigned and indicated in the figure.

Limit of detection

A mixture sample containing thirteen OCPs was measured by GC-MS, and the limits of detection (LODs) that were obtained using the molecular ion signal are listed in Table 1. The chemical formula and the spectral data obtained by quantum chemical calculations are summarized in Fig. S-1 in the ESI, which were used to categorize the ionization mechanism shown in the third column of Table 1. A group of compounds categorized as RE2PI provided relatively low LODs. However, DDT provided a smaller signal intensity than HCB, although DDT has a larger molar absorptivity (ca. $6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$) than HCB (ca. $10^3 \text{ M}^{-1}\text{cm}^{-1}$). For compounds categorized as NR2PI, the LODs are higher than those categorized as RE2PI, suggesting lower ionization efficiency, as expected. Note that the LOD for dieldrin was lower, which can be attributed to the fact that its spectral properties were similar to those for HCB (nearly RE2PI). On the other hand, no molecular ions were observed for α -, β -, and γ -HCHs, which can be categorized as NR3PI. It was difficult to separate α -endosulfan from *cis*-chlordane by GC, as shown in the data sheet available from the manufacturer of the sample. The EI-MS data obtained for *cis*- and *trans*-chlordane were very similar to each other, and the situation was identical for α - and β -endosulfan.²⁷ Note that the spectra of *cis*- and *trans*-chlordane are very different from those of α - and β -endosulfan. Since the mass spectrum observed at this retention time, where α -endosulfan appears, was very similar to that observed for *trans*-chlordane, the intensity of the signal arising from α -endosulfan is considered to be very small or negligible, in comparison with the signal for β -endosulfan. This unexpected result can be attributed to the twisted structure of α -endosulfan,^{28,29} resulting in efficient dissociation, e.g., to neutral species, which was not taken into account in the model in the previous section.

Table 1. Limit of detection (LOD) for OCPs

Compound	LOD as molecular ion (ng/ μL)	Ionization mechanism	Excess energy (eV)	Slope
HCB	0.3	RE2PI	0.23	-0.18
DDT	0.5	RE2PI	0.99	+0.38
dieldrin	1	NR2PI	0.36	-1.1
heptachlor	20	NR2PI	0.16	-0.81
aldrin	20	NR2PI	0.60	-0.32
endrin	20	NR2PI	0.51	-0.56
α -endosulfan	-	NR2PI	0.26	-
β -endosulfan	20	NR2PI	0.38	-0.40
<i>trans</i> -chlordane	20	NR2PI	0.04 (4.7)	-4.5
<i>cis</i> -chlordane	20	NR2PI	0.06 (4.7)	-2.0
α -HCH	ND	NR3PI	3.9	ND
β -HCH	ND	NR3PI	3.9	ND
γ -HCH	ND	NR3PI	4.0	-1.7

ND, not detected

Two-dimensional display of GC-MS

In order to clarify the mechanism for femtosecond ionization of OCPs, two-dimensional displays were measured by changing the laser pulse width at the same pulse energy. The data obtained at 160 fs are shown in Fig. 3. A molecular ion was observed for seven of the OCPs that were examined. The signal intensities of HCB and DDT were larger than those for the others such as dieldrin, heptachlor, aldrin, endrin, and α -/ β -endosulfan, which can be explained by efficient RE2PI for these two compounds. No signal ion was observed for *trans*- and *cis*-chlordane and HCH isomers.

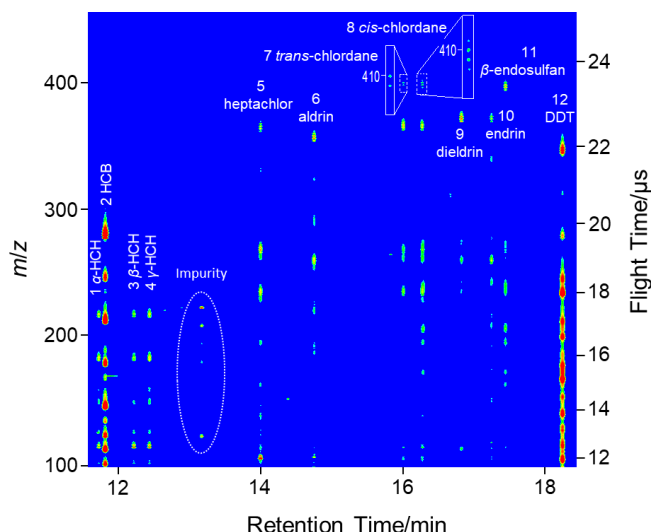


Fig. 4 Two-dimensional display of GC-MS observed at 47 fs

As shown in Fig. 4, when the pulse width was decreased to 47 fs, twelve OCPs were observed. It should be noted that a molecular ion as well as fragment ions were observed for *cis*- and *trans*-chlordane, in addition to the fragment ions from the HCH isomers.

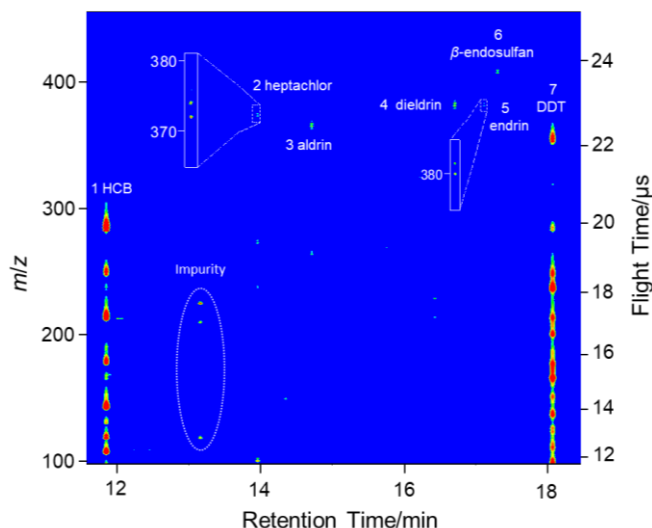


Fig. 3 Two-dimensional display of GC-MS observed at 160 fs

As shown in Fig. 5, a molecular ion was clearly observed at $m/z = 288$ for γ -HCH at 35 fs (not for the other isomers), which can

be explained by a larger enthalpy change by dissociating HCl and HCl₂ for α - and β -HCHs than γ -HCH, suggesting more stable thermodynamic property for γ -HCH.³⁰

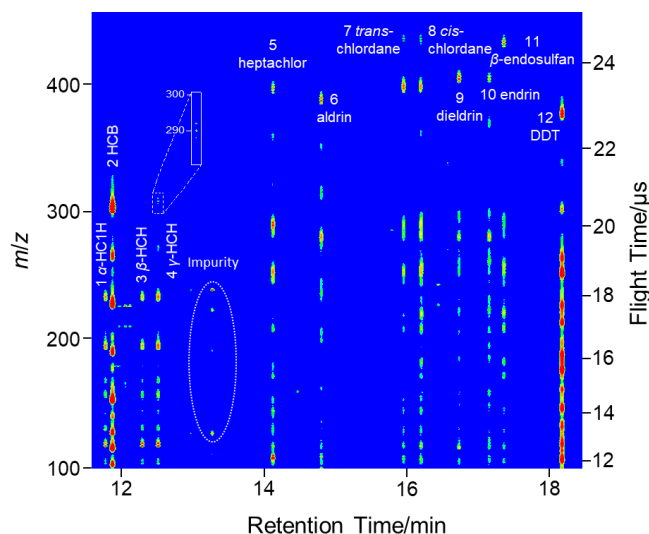


Fig. 5 Two-dimensional display of GC-MS observed at 35 fs

Ionization mechanisms

A slope in the logarithmic plot of the signal intensity and the laser pulse width provides information concerning the ionization mechanism. The values obtained from the data shown in Fig. S-2 are summarized in the last column of Table 1.

RE2PI. Two compounds of HCB and DDT have aromatic rings, and the absorption bands are located at 278 nm (oscillator strength, 0.0000) and 266 nm (0.11), respectively (cf. Fig. S-1). Note that HCB has a symmetry of D_{6h} and the transition to the lowest excited state is electronically forbidden. Actually, the transition would be partially allowed by a vibronic interaction like a benzene molecule. Then, these molecules can be categorized as RE2PI. The slopes observed for HCB and DDT were -0.18 and +0.38, respectively, which are close to the expected value of zero. The slightly smaller value than zero for HCB can be attributed to its low molar absorptivity at 267 nm, suggesting that NR2PI makes a non-negligible contribution. However, DDT was more dissociative (cf. the MS data in Fig. S-1) and the LOD was higher than that for HCB, although the molar absorptivity is larger and is considered to be more preferential for RE2PI. The parameters for ΔE_{excess} were 0.23 and 0.99 eV for HCB and DDT, respectively, as shown in the second last column of Table 1, and these values were much less than 3 eV. Therefore, auto-dissociation would be inefficient. It has been reported that fragmentation is accelerated in MPI-MS when using a near-infrared (NIR) laser when a molecular ion has an absorption band and absorbs an NIR photon.³¹⁻³³ However, the molar absorptivity of the molecular ion for DDT ($3 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$) was similar to or slightly smaller than that for HCB ($7 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$) at 267 nm (cf. Fig. S-1). This makes it difficult to explain efficient decomposition of DDT by photo-dissociation. It has been reported that a bulky side chain, i.e., a low frequency mode in a molecule accelerate fragmentation.²⁴ As can be seen from the chemical formula of DDT (Fig. S-1), it contains two bulky groups and HCB has a rigid

structure, which explains the more dissociative nature of DDT compared to HCB.

NR2PI. From their absorption spectra, dieldrin, heptachlor, aldrin, endrin, α -endosulfan, β -endosulfan, *trans*-chlordane, and *cis*-chlordane are tentatively categorized as NR2PI. The slope for a logarithmic plot of the signal intensity and the laser pulse width would be predicted to be -1 from Eq. (8). The value for dieldrin was reasonable (-1.10). The slopes for heptachlor, aldrin, endrin, and β -endosulfan were in the range from -0.32 to -0.81, slightly larger than the expected value of -1. This result is probably due to the contribution of RE2PI, since the laser wavelength (267 nm) is located at nearly the edge of the absorption band. Prominent molecular ions were observed for these compounds (Fig. S-1), since photo-dissociation from the molecular ion would be negligible because of the low molar absorptivity ($< 3 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$). However, the slopes for *trans*-chlordane and *cis*-chlordane were -4.5 and -2.0, respectively, which are smaller than the expected value of -1. The excess energies calculated for these compounds were 0.04 and 0.06 eV, respectively. Due to unavoidable errors in quantum chemical calculations, it is plausible that they are ionized through NR3PI, leading to a slope of -2, as suggested in Eq. (10). The excess energy calculated under NR3PI were 4.7 and 4.7 eV, respectively, which would be sufficient for auto-dissociation to accelerate the fragmentation.

NR3PI. The efficiency of NR3PI strongly depends on pulse width, providing a slope of -2 in the logarithmic plot, as suggested in Eq. (10). No signal was observed for any of the HCH isomers at 160 fs. An energy of a photon at 267 nm is insufficient for NR2PI and, as a result, these compounds are categorized as NR3PI. In fact, the slope observed for γ -HCH (-1.7) is reasonable for MR3PI. The excess energy calculated for α -, β -, and γ -HCH were 3.9, 3.9, and 4.0 eV, respectively, suggesting that the molecular ion undergoes efficient auto-dissociation. Actually, large fragment ions were observed even at shorter pulse widths. The data obtained by quantum chemical calculations indicate that the molar absorptivities for the molecular ions at 267 nm were ca. $10^3 \text{ M}^{-1}\text{cm}^{-1}$ or less. Accordingly, photo-dissociation would be negligible and auto-dissociation would be a major process in fragmentation at 267 nm. This suggests that the use of a deep-ultraviolet (DUV) laser would result in more efficient NR2PI. In fact, molecular ions were clearly observed for all of the HCH isomers at 200 nm.³⁰ However, it should be noted that the background signal from interference also increases when a laser emitting at shorter wavelengths is used.^{34,35} Therefore, further optimization will be needed for this to be used in actual trace analysis.

Guideline for optimization

Sensitivity as well as selectivity can be improved in MPI-MS by optimizing the ionization conditions, which is particularly important in terms of reducing undesirable interference in an authentic sample. A molecular ion that can be observed in MPI-MS, is useful not only for determining the molecular weight of the analyte but also for differentiating fragment ions with low m/z values arising from interferences. The model and the experimental data in this study provide a guideline for observing molecular ions as follows. First, the laser wavelength should be

optimized for 2PI using spectral data obtained by quantum chemical calculations. The ionization efficiency can be improved by RE2PI, in which the signal intensity is independent of the laser pulse width in the femtosecond region. However, when the excess energy is larger than 3 eV, the molecular ion undergoes decomposition by auto-dissociation. On the other hand, photo-dissociation is efficient for a molecule that provides a molecular ion with a large molar absorptivity at the laser wavelength. In NR2PI, the ionization efficiency is inversely proportional to the laser pulse width, and the efficiency increases to a level comparable to that of RE2PI when the pulse width is decreased to $< 100 \text{ fs}$.²³ Note that fragmentation has been suppressed for a molecule (not for an atom) by using a negatively-chirped pulse for MPI in the NIR region (807 nm), which is explained by a ladder-climbing model.^{36,37} However, this model can be applied only for MPI using a NIR (or IR) laser (not for 2PI or 3PI using a UV laser). Molecules with large ionization energies such as HCH isomers are more difficult to be ionized at 267 nm, because the more inefficient NR3PI must be used. Since the ionization efficiency is inversely proportional to the square of the laser pulse, a shorter optical pulse is preferred for ionization. However, ionization efficiency is generally low for NR3PI, and the large excess energy remaining in the molecular ion accelerates fragmentation by auto-dissociation. Accordingly, a laser emitting at shorter wavelengths, e.g., 200 nm for RE2PI or 240 nm for NR2PI, would be preferred for reducing the excess energy. Another approach would be 3PI at longer wavelengths using a near-ultraviolet (NUV) laser, e.g., at 360 nm. This approach would be useful for a molecule with a relatively high ionization energy. In fact, the NUV laser has been successfully used for determination of amino- and nitro-aromatic hydrocarbons based on resonance-enhanced three-photon ionization (RE3PI) due to the absorption bands that are located in the NUV region, although a more complicated laser system using an optical parametric amplifier equipped with a UV option or a different type of femtosecond laser would be needed as an ionization source.^{34,35} It is reported that the difference in ionization efficiency is very small for enantiomers (a few percent or less) even when a circularly-polarized beam is used for ionization (the difference is zero for a linearly-polarized beam).³⁸ Then, a chiral GC column is essential for separation and to quantify the enantiomers of the pesticide.³⁰

Conclusion

The efficiency of NR2PI is lower than RE2PI. However, the efficiency can be improved by decreasing the laser pulse width. Fragmentation is mainly determined by auto-dissociation at an excess energy of $> 3 \text{ eV}$, which can be decreased by optimizing the laser wavelength. A bulky side chain accelerates fragmentation and the laser wavelength would need to be more carefully optimized for reducing the excess energy. The use of an ultrashort NUV pulse for RE3PI or NR3PI is another approach useful for a molecular with a high ionization energy, since the background signal arising from matrix interference could be significantly suppressed in practical trace analysis.

Conflicts of interest

There are no conflicts to declare.

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