Fast and Accurate Identification of Pesticides by Direct Analysis in Real Time (DART) Ionization with Orbitrap Mass Spectrometry

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Key Words

Direct Analysis in Real Time ionization, DART, Exactive, Orbitrap, pesticides, water analysis

Goal

To describe a method incorporating direct analysis in real time (DART) ionization and Thermo Scientific™ Orbitrap™ high-resolution mass spectrometry for rapid analysis and identification of contaminating substances in water.

Introduction

When water or soil is contaminated by chemical substances, quick methods of analysis are required to assess the negative impact on the environment. Accidents that have an impact on drinking water require rapid, real-time diagnosis of the chemical substance involved. The contamination is confirmed in the lab after often tedious extraction and concentration processes and instrumental analysis of the target compounds.

Full-scan mass spectrometry is a powerful compound identification technique. However, conventional quadrupole-type scanning produces low-resolution mass spectra. Most contamination accidents involve concentrations at ng/mL levels; therefore, it is essential that the samples be concentrated prior to instrumental analysis. Care must be taken as to not lose the target compound during the pre-treatment or concentration process. For example, polar substances can be lost during liquid-liquid extraction, and limitations in selectivity of materials used in solid-phase extraction (SPE) can hinder adsorption and concentration of the target compound.

Direct analysis in real time (DART®) has recently been introduced as a desoprtion ionization technology that requires limited or no sample pre-treatment prior to introduction into the mass spectrometer.[1] As a direct spray ionization technique, DART bypasses the conventional high-performance liquid chromatography (HPLC) routinely coupled to MS analysis. It is therefore amenable to high-throughput screening (HTP) and attractive to use in forensics, defense, clinical research, and food applications.[2] Although DART has successfully been coupled to triple quadrupole mass spectrometry [3,4] technology, combining it with high-resolution, accurate mass (HRAM) mass spectrometry [5-7] might lead to higher probability of identifying unknown substances.

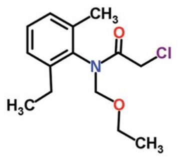
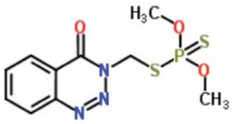
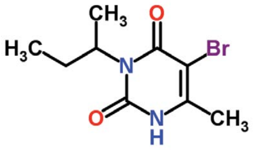
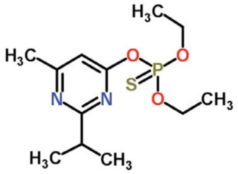
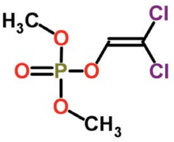
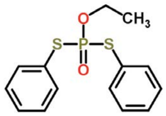
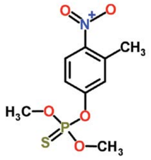
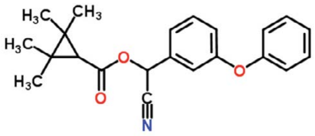
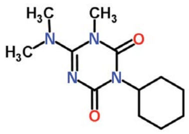
In this study, a method incorporating DART and Orbitrap high-resolution mass spectrometry was developed for rapid analysis and identification of contaminating substances in water. A total of 23 commonly used agricultural pesticide target compounds were analyzed (Table 1). The possibility of screening target compounds at the ng/mL concentration level in water samples, indicative of real case scenarios, was also reviewed.

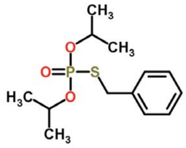
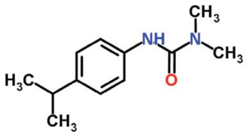
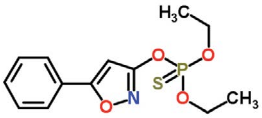
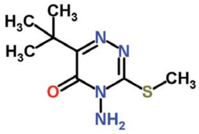
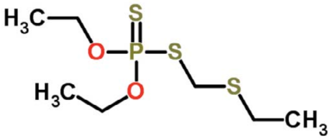
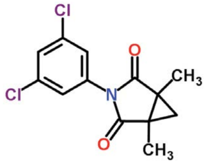
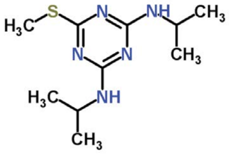
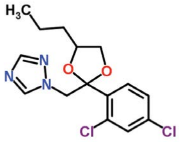
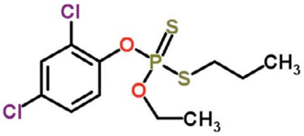
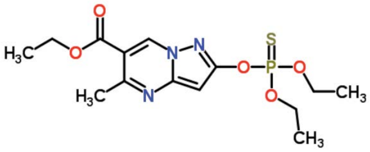
Experimental

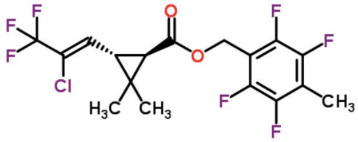
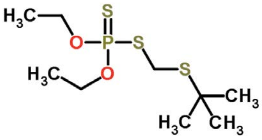
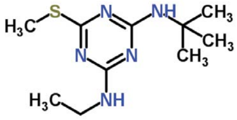
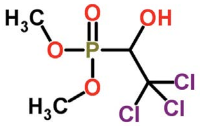
Target Compounds

Table 1 lists the chemical formulas, molecular weights, and structures of the target pesticides.

Table 1. Target pesticides

Compund	CAS Number	Formula	Molecular Weight	Chemical Structure
Acetochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269.7	
Azinphos-methyl	86-50-5	$C_{10}H_{12}N_3O_3PS_2$	317.3	
Bromacil	314-40-9	$C_9H_{13}BrN_2O_2$	261.1	
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.3	
Dichlorovos	95828-55-0	$C_4H_7Cl_2O_4P$	220.9	
Edifenfos	17109-49-8	$C_{14}H_{15}O_2PS_2$	310.3	
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	277.2	
Fenpropathrin	39515-41-8	$C_{22}H_{23}NO_3$	349.4	
Hexazinone	51235-04-2	$C_{12}H_{20}N_4O_2$	252.3	

Compund	CAS Number	Formula	Molecular Weight	Chemical Structure
Iprobenfos	26087-47-8	$C_{13}H_{21}O_3PS$	288.3	
Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206.2	
Isoxathion	18854-01-8	$C_{13}H_{16}NO_4PS$	313.3	
Metribuzin	21087-64-9	$C_8H_{14}N_4OS$	214.2	
Phorate	298-02-2	$C_7H_{17}O_2PS_3$	260.3	
Procymidone	32809-16-8	$C_{13}H_{11}Cl_2NO_2$	284.1	
Prometryn	7287-19-6	$C_{10}H_{19}N_5S$	241.3	
Propiconazole	60207-90-1	$C_{15}H_{17}Cl_2N_3O_2$	342.2	
Prothiofos	34643-46-4	$C_{11}H_{15}Cl_2O_2PS_2$	345.2	
Pyrazophos	13457-18-6	$C_{14}H_{20}N_3O_5PS$	373.3	

Compund	CAS Number	Formula	Molecular Weight	Chemical Structure
Tefluthrin	79538-32-2	$C_{17}H_{14}ClF_7O_2$	418.7	
Terbufos	13071-79-9	$C_9H_{21}O_2PS_3$	288.4	
Terbutryn	886-50-0	$C_{10}H_{19}N_5S$	241.3	
Trichlorfon	66758-31-4	$C_4H_8Cl_3O_4P$	257.4	

Direct Analysis in Real Time (DART)

A DART source with a Standardized Voltage and Pressure (SVP) controller (IonSense™, MA, USA) was used as the ionization source. The ionization mechanism in DART is Penning ionization.[8] It relies upon fundamental principles of atmospheric pressure chemical ionization (APCI). Excited-state helium atoms produce reactive species for analyte ionization.[1] Figure 1 shows a schematic diagram of DART technology.

The operating temperature range of the DART-SVP source is 50–500 °C, and the optimal temperature for the studied compounds was found to be 300 °C. The ionization and instrumental analysis time was set to 30 sec. The DART operating conditions are summarized in Table 2.

Samples were loaded onto a strip that contained 10 spots for sample deposition. Each sample was individually deposited on a metal mesh and allowed to dry before the strip was fitted on the DART source. The strip was then set to run and each sample was presented in front of the mass spectrometer for analysis. Hot helium gas flowed through the sample/mesh, ionizing the sample by an atmospheric pressure chemical ionization (APCI) -like mechanism.

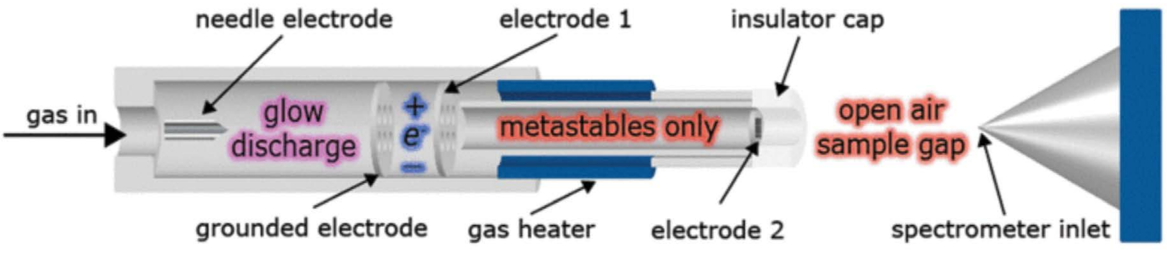


Figure 1. DART technology

Table 2. Operating conditions of desorption ionization probe

Instrument	DART-SVP
Temperature	300 ˚C
Sample loading volume	5 µL
Carrier gas, pressure	Helium, 75 psi

Mass Spectrometry

Due to the absence of separation in the DART source, the whole sample is introduced into the mass spectrometer. This unavoidably leads to a significant number of spectral interferences. To correctly determine the masses of relevant compounds and potential unknowns in the case of fingerprinting analysis, it is essential to separate them from the matrix ions. A mass spectrometer based on Orbitrap™ technology achieves high mass resolving power while maintaining excellent mass accuracy, without the use of internal mass correction.[9] These features make it an ideal tool to complement DART ionization for the analysis of complex samples.

A Thermo Scientific™ Exactive™ Orbitrap high-resolution, accurate-mass mass spectrometer was used in full scan mode. The resolving power was set to 50,000 (FWHM) at *m/z* 200. The detailed conditions for the operation of the mass spectrometer are summarized in Table 3.

Table 3. MS operating conditions

Parameter	Setting
Scan range	*m/z* 100–500
Resolving power	50,000 (FWHM at *m/z* 200)
Polarity	Positive
Run time	0.5 min
Spray voltage	0 kV
Capillary temperature	250 °C
Capillary voltage	25 V
Tube lens voltage	170 V
Skimmer voltage	36 V

Results and Discussion

Mass Spectrum of Quinine and Mass Accuracy

Prior to analyzing the agricultural pesticides under review, quinine ($C_{20}H_{24}N_2O_2$) was selected as a standard compound for preliminary testing. A spectrum of quinine was collected and analyzed using the DART-Exactive MS. Five microliters of 1 ng/µL solution was applied to a metal mesh using a micropipette. The mass spectrum for quinine shown in Figure 2, was acquired under the operating conditions outlined in Table 3. Comparison using the simulated elemental composition feature in Thermo Scientific™ Xcalibur™ software version 2.1 confirmed the results and presence of carbon isotopes in the form of $[M+H]^+$. A mass accuracy 0.632 ppm was measured, so it was possible to confirm the compound within an accuracy of <1ppm.

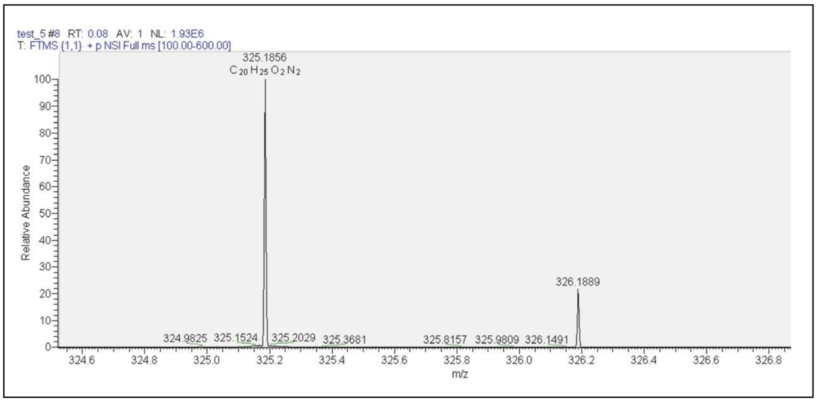


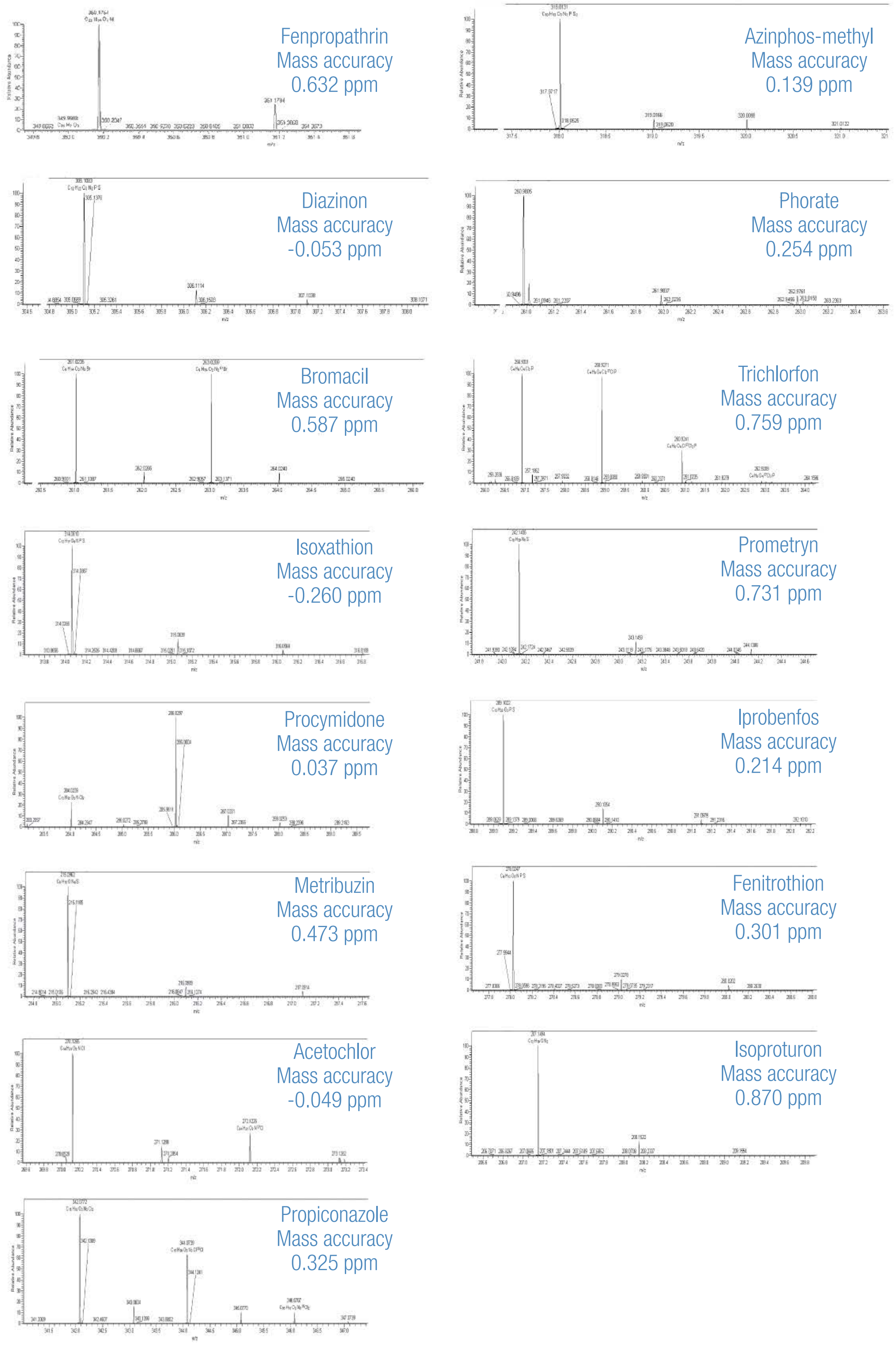
Figure 2. Preliminary expanded ionization spectrum of quinine ($C_{20}H_{25}O_2N_2$)

Mass Spectrum Measurement of Target Compounds

A diluted solution of the 23 standard agricultural pesticides was prepared at a concentration of 500 ng/mL each and was measured three times under the DART-Exactive MS conditions described in the previous section. The mass spectra and corresponding mass accuracies were recorded and confirmed by comparison to the simulated elemental composition. The mass spectra and accuracies of the target compounds are summarized in Table 4 and Figure 3. All agricultural pesticides were detected as $[M+H]^+$, similar to quinine. There were no Na^+ or NH_4^+ adducts detected, confirming the ionization as a Penning-type mechanism. The carbon isotopic distribution was also used to confirm the compounds. Those target compounds with a chlorine atom, such as procymidone, acetochlor, propiconazole, dichlorovos, tefluthrin, and prothiophos, showed isotopic ratios typical of Cl-35 to Cl-37, with its natural abundance ratio of 3:1. Bromacil, with bromine, showed the natural abundance isotopic pattern of Br-79 to Br-81, which is 1:1. Mass accuracy was observed to be in the range of 0.053 to 0.870 ppm, which satisfied the condition of being less than 1 ppm. Thus, DART combined with HRAM mass spectrometry has substantial advantages as an identification analysis method.

Table 4. Protonated or molecular and isotopes for identification within 5 ppm mass accuracy

Compound	[M+H]$^+$	Qual Ion 1	Qual Ion 2	Qual Ion 3	Qual Ion 4
Acetochlor	270.1255	271.1288	272.1226	273.1262	
Azinphos-methyl	318.0131	319.0166	320.0088	321.0211	322.7027
Bromacil	261.0235	262.0266	263.0209	264.0240	
Diazinon	305.1083	306.1114	307.1038	308.1071	309.2028
Dichlorovos	222.9497	221.9448	224.9465		
Edifenfos	311.0326	312.0306	313.0282		
Fenitrothion	278.0247	279.0278	280.0202	281.0232	282.0270
Fenpropathrin	350.1754	351.1784	352.1815	353.1843	
Hexazinone	253.1660	254.1668	255.1725		
Iprobenfos	289.1022	290.1054	291.0978	292.1010	
Isoproturon	207.1494	208.1522	209.1554		
Isoxathion	314.0610	315.0639	316.0564	317.0595	318.0609
Metribuzin	215.0962	216.0989	217.0914	218.0946	
Phorate	260.9805	261.9837	262.9761		
Procymidone	286.0297	287.0275	288.0253		
Prometryn	242.1436	243.1459	244.1386	245.1417	
Propiconazole	342.0772	344.0739	346.0708		
Prothiofos	346.9667	348.9633	350.1746		
Pyrazophos	374.0932	375.0101	376.3505		
Tefluthrin	419.0645	420.0664	421.0615		
Terbufos	289.0515	290.0550	291.0572		
Terbutryn	242.1435	243.1462	244.1387	245.1420	
Trichlorfon	256.9301	258.9271	260.9241	262.9208	



Fenpropathrin
Mass accuracy
0.632 ppm

Azinphos-methyl
Mass accuracy
0.139 ppm

Diazinon
Mass accuracy
-0.053 ppm

Phorate
Mass accuracy
0.254 ppm

Bromacil
Mass accuracy
0.587 ppm

Trichlorfon
Mass accuracy
0.759 ppm

Isoxathion
Mass accuracy
-0.260 ppm

Prometryn
Mass accuracy
0.731 ppm

Procymidone
Mass accuracy
0.037 ppm

Iprobenfos
Mass accuracy
0.214 ppm

Metribuzin
Mass accuracy
0.473 ppm

Fenitrothion
Mass accuracy
0.301 ppm

Acetochlor
Mass accuracy
-0.049 ppm

Isoproturon
Mass accuracy
0.870 ppm

Propiconazole
Mass accuracy
0.325 ppm

Figure 3. Molecular ions and isotopes in expanded spectra of target pesticides

Low-Concentration Test Considering Water Contamination

The analysis method reviewed in this study enables accurate quantitation analysis within a matter of minutes and is expected to be of significant value in cause identification and result notification, allowing rapid response in the field. However, the majority of water contamination by chemical substances occurs in concentration levels of ng/mL, as observed in dioxane contamination, oil spills, and agricultural pesticide sprays, among others. Thus, there is a need to perform quantitation analysis for low-concentration samples. To review the possibility of detecting trace amounts of the target compounds in low-concentration samples, acetochlor ($C_{14}H_{20}ClNO_2$), one of the pesticides outlined in the previous section, was selected for analysis. The compound was serially diluted using tap water from the lab to 100, 50, 20, 10, 5, and 1 ng/mL solutions, and 10 µL of each of the diluted solutions was applied to the surface of a metal mesh. The mass spectrum for each of the concentrations is shown in Figure 4. The monoisotopic mass of acetochlor is 269.271 amu, with chlorine isotopes at $[M+H]^+$ 270.1258 amu and 272.1231 amu, respectively. These were observed at a ratio of 3:1 at the minimum concentration of 1 ng/mL. We can thus conclude that rapid and accurate quantitation using DART-Exactive MS presents a promising possibility in the analysis of trace amounts of target compounds, the common case in water contamination.

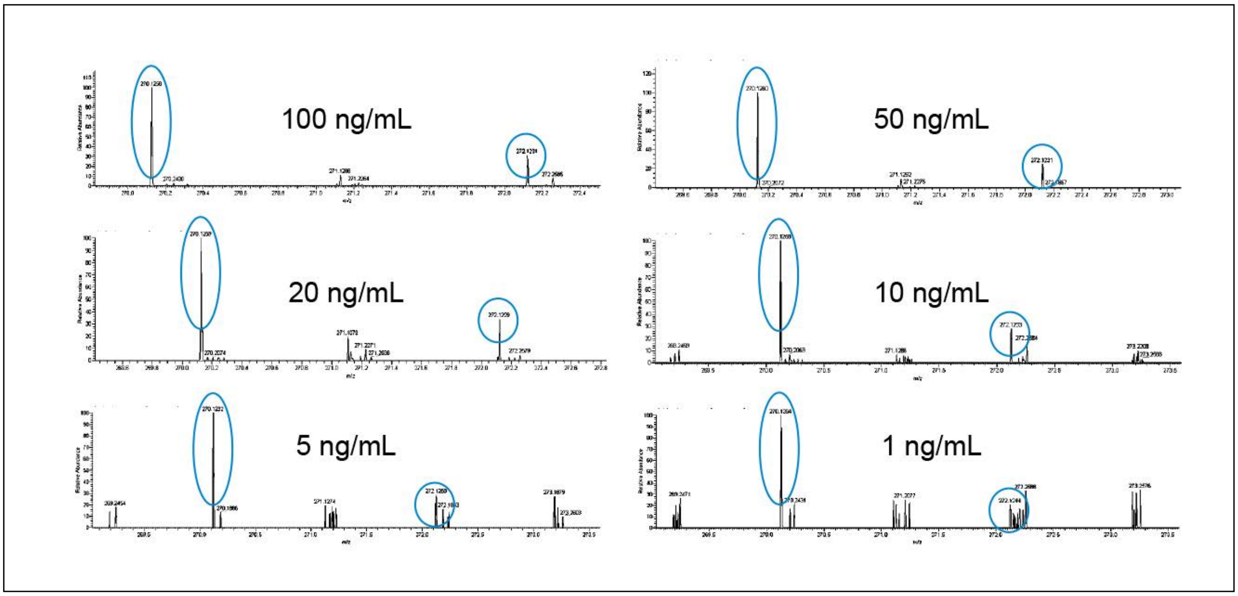


Figure 4. Sensitivity test of acetochlor spiked in tap water

Productivity and Utilization of DART

Chemical terrorism involving contamination of drinking water and/or food targeting a non-specific group creates the need to develop appropriate countermeasures. To confirm the contaminating compound using the conventional microanalysis method on five samples, for example, would require approximately 0.5 to 1 L of sample and take 2.5 to 3 hours for filtration and liquid-liquid extraction (LLE) or solid-phase extraction (SPE) and 1.2 hours for instrumental analysis. On the other hand, the method described here would require only 5 to 10 µL of sample and only 0.5 minutes of analysis time. Thus, DART provides speed in comparison to the conventional method.

Although it was not reviewed in this study, quantitation review cases on the application of the DART-high-resolution mass spectrometry (HRMS) method have been reported.[10,11] If the injection method was to be automated, this method could be especially useful in the fields of water, food, and soil quality control, as it could be used to identify the contaminating compound and confirm its concentration at the same time. Also, desorption ionization methods including DART have a simple ionization mechanism. This reduces the time necessary for optimization and the cost related to the solvent, column, and condition establishment time, etc., necessary when using HPLC. As such, this method is expected to have diverse applicability in environmental analysis including quantitation.

Conclusion

In this study, DART, a direct analysis technique that has been introduced for rapid response to water contamination accidents, was combined with Exactive Orbitrap HRAM MS. Its performance as a microanalysis method for trace amounts of contaminants in water was reviewed. Based on the results, the following conclusions were reached:

- An analysis of agricultural pesticides using DART-Orbitrap MS showed that it was possible to produce accurate identification with a mass accuracy within 1 ppm in a very short period of time without any sample pre-treatment.
- This method demonstrated a detection limit of 1 ng/mL in a sensitivity test using acetochlor, without prior extraction or sample concentration, showing the possibility of using it as a method to detect trace amounts of target compounds.
- The DART method was observed to significantly reduce the analysis time and labor necessary. The speed of the method could also be an advantage if an urgent analysis is needed in the event of an accident that could potentially have a negative impact on the environment. It is also a simple, environmentally-conscious analysis technique, as it does not require large amounts of solvent.

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