ANALYTICAL SCIENCES JULY 2012, VOL. 28

Deoxidation of Fenthion Sulfoxide, Fenthion Oxon Sulfoxide and Fensulfothion in Gas Chromatograph/Mass Spectrometer, and the Prevention of Sulfoxide Deoxidation by Polyethylene Glycol 300

Kuniyo SUGITATE,*1,*2† Takashi YAMAGAMI,*3,*4 Sadao NAKAMURA,*1 Akira TORIBA,*2 and Kazuichi HAYAKAWA*2

*1 *Agilent Technologies Japan, Ltd., 9-1 Takakura-cho, Hachioji, Tokyo 192–8510, Japan*
*2 *Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920–1192, Japan*
*3 *Nishikawa Keisoku Co., Ltd., YBP West Tower 2F, 134 Godo-cho, Hodogaya, Yokohama, Kanagawa 240-0005, Japan*
*4 *The University of Kitakyushu, 1-1 Hibikino, Wakamatsu, Kitakyushu, Fukuoka 808–0135, Japan*

Fenthion, fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion showed two different mass spectra in GC/MS, depending on their concentrations. The base peaks shifted to lower levels by 1 *m/z* at lower concentration, and no retention time shifts were observed. The "shifted base peaks" were not obtained by a general EI fragmentation. The product ion scan spectra of the "shifted base peaks" were coincident with those of molecular ions of their corresponding sulfides. These phenomena can be ascribed to the conversion of sulfoxide into sulfide by the dominant deoxidation reaction than EI fragmentation in an ion source. Adding polyethylene glycol 300 (PEG300) into a test solution prevented sulfoxide deoxidation.

(Received March 2, 2012; Accepted June 4, 2012; Published July 10, 2012)

Introduction

Fenthion is one of the organophosphorous pesticides, and is widely used as an effective insecticide for rice, fruits, *etc*. On the other hand, fenthion raises serious concern because of its strong toxicity to wild birds.[1] Fenthion is photooxidized to fenthion sulfoxide in the environment,[2] and fenthion sulfoxide shows higher toxicity than fenthion.[3]

In Japan, five derivatives of fenthion (fenthion sulfoxide, fenthion sulfone, fenthion oxon, fenthion oxon sulfoxide and fenthion oxon sulfone) are controlled by the water supply law.[4] We had several chances to determine fenthion and its five derivatives, and noted that two sulfoxides sometimes showed mass spectra different from those in the NIST/EPA/NIH Mass Spectral Database (hereinafter called NIST library), that is, each base peak shifted to a lower level by 1 *m/z*. We also found that fensulfothion, which is not a fenthion derivative, but has a sulfoxide structure, also acted in the same manner. We extensively examined the data on those compounds to find similar analytical situations.

The base peak of fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion in the NIST library, are *m/z* 279, 263 and 293, respectively. In contrast, in the acquired mass spectra, the base peak of fenthion sulfoxide, fenthion oxoxn sulfoxide and fensulfothion were *m/z* 278, 262 and 292, respectively. The base peaks shown in the NIST library are derivered from demethylation, which is very popular for EI fragmentation. On the contrary, the 1 *m/z* lower base peaks are slightly unique because the difference based on molecular weight (mono-isotopic) is an even number for all sulfoxides. In this report, we identify the base peak equal to that of the NIST library as the "usual base peak", and call the 1 *m/z* lower base peak the "shifted base peak". Properly choosing the quantification ion is essential for precise GC-MS quantitative analyses; thus, it is very important to comprehend the cause of producing the shifted base peak result.

GC-MS analyses of sulfoxides were difficult for the following reasons: 1) Sulfoxide is easily produced from sulfide by oxidation, and tends to change into sulfone by additional oxidation. 2) Sulfoxide has a higher polarity than sulfide or sulfone because of its strong polarization between sulfur and oxygen. Fedrak *et al*.[5] reported that methylbenzothiophene sulfoxides were decomposed in a GC injection port. Tanaka *et al*.[6] reported that disulfoton sulfoxide was degraded at a GC injection port, and that a programmed temperature vaporization (PTV) inlet in the pulsed splitless mode reduced the degradation. Ueno *et al*.[7] reported that oxydemeton-methyl (sulfoxide of demeton-*S*-methyl) also decomposed at a GC injection port, and that demeton-*S*-methyl was easily oxidized to oxydemeton-methyl in the sample preparation. They thus added both L-ascorbic acid and butylhydroxytoluene as an antioxidant. Maśtovská *et al*.[8] also reported on the thermodegradation of some sulfoxides. They said that the analyses of sulfoxides were difficult because of their unstable behavior in GC analyses.

Here, we found that the problem with the concerned sulfoxides is derived from deoxdation in an EI ion source. With a decrease

† To whom correspondence should be addressed.
E-mail: kuniyo_sugitate@agilent.com

in the concentration, the ratio of the "shifted base peak" became higher. This becomes a problem in the measurement corresponding to the water supply law. To meet the legal regulations, we have to analyze the pesticides at ppb levels, and reproducible experimental data of the mass spectra is necessary.

By the way, a matrix-induced enhancement effect is often observed in the pesticide residue analysis by GC or GC-MS.[9-18] This phenomenon means that the response of a pesticide in the matrix solution is higher than that in the matrix-free standard solution. It was reported that the matrix protected the analytes from adsorption or alternation during transfer from the injector to the column.[9,10,12,13,16] In order to compensate for the matrix-induced enhancement effect, the priming injection technique by real samples,[19] the standard addition technique, the matrix matching technique, or using a pseudo matrix, such as polyethylene glycol 300 (PEG300),[20] or analyte protectants,[21,22] are often used.

In the present work, we examined the effect of PEG300 on the GC-MS behavior of the sulfoxides, and found that PEG300 prevented deoxidation in an EI ion source and a dirty injection port.

Experimental

Reagents and chemicals

The standards for pesticides with a purity of 98% or higher, except for fenthion sulfoxide (94.7%), were obtained from Hayashi Pure Chemical Ind., Ltd. (Osaka, Japan). The chemical structures of sulfide, sulfoxide and sulfone are shown in Fig. 1. Acetone, dichloromethane, *n*-hexane and ethyl acetate were high-purity solvents for pesticide and PCB analysis, obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Methanol, HPLC grade, and polyethylene glycol 300 (PEG300), special grade, were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Bottled water was "Natural water of Minami-alps" from Suntory Holdings., Ltd. (Osaka, Japan).

Apparatus

GC-MS measurements were performed on an Agilent 7890A GC system coupled to a 5975C TAD mass spectrometer (Little Falls, DE, USA). GC-MS/MS analyses were performed on an Agilent 7890A GC system coupled to a 7000B triple quadrupole mass spectrometer (Santa Clara, CA, USA). Both systems were equipped with a 7693 autoinjector (Little Falls, DE, USA). The inlet temperature was 250°C, the total flow was set at 50 mL/min, and a split valve was opened 1.0 min after pulsed splitless injection (25 psi). The injection volume was 2 µL. A fused silica capillary column, HP-5msUI (30 m × 0.25 mm i.d. × 0.25 µm film thickness, Agilent, Folsom, CA, USA) was used. At the beginning of injection, the oven temperature was set at 60°C for 1.0 min, ramped to 310°C at 20°C/min and then held for 3.0 min. The helium carrier gas flow rate was constant at 1.0 mL/min, and the transfer line temperature was set at 280°C. GC-MS was operated in a scan or SIM mode (SIM was for ppb level analyses), and GC-MS/MS was operated in the product ion scan mode. The source temperature was 230°C in both systems. As for the GC-MS/MS, nitrogen gas was used as the collision gas.

All data from Chemstation were converted to MassHunter software.

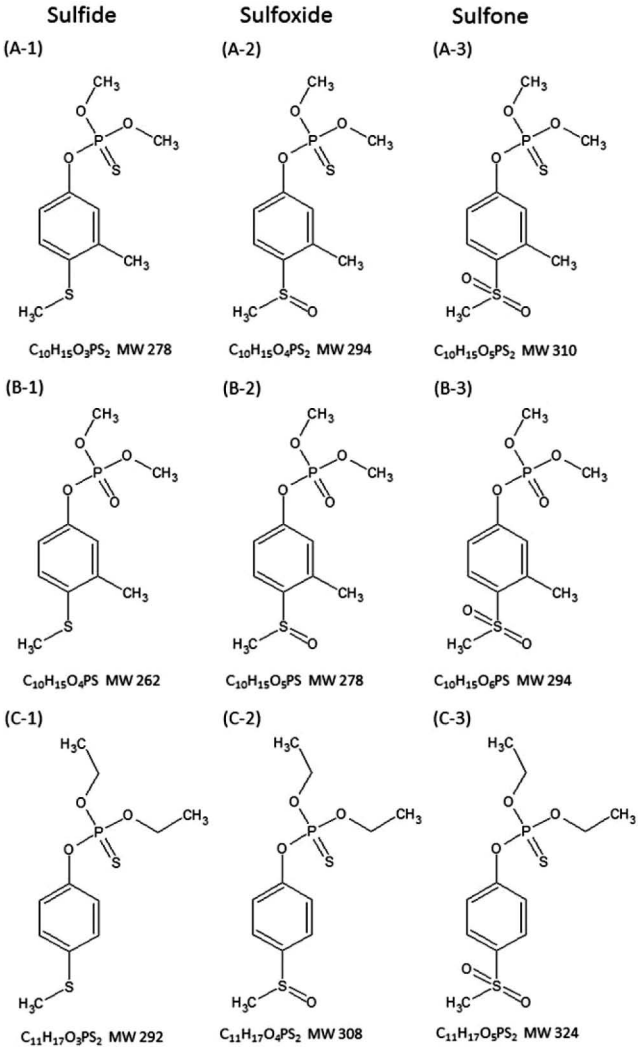

Fig. 1 Chemical structures of sulfide, sulfoxide and sulfone. (A-1) Fenthion, (A-2) fenthion sulfoxide, (A-3) fenthion sulfone, (B-1) fenthion oxon, (B-2) fenthion oxon sulfoxide, (B-3) fenthion oxon sulfone, (C-1) fensulfothion sulfide, (C-2) fensulfothion, and (C-3) fensulfothion sulfone.

Results and Discussion

Concentration and solvent

The mass spectrum of each sulfoxide standard solution at 10 ppm in acetone was almost the same as that in the NIST library. The base peaks for fenthion sulfoxide, fenthion oxon sulfoxide, and fensulfothion were *m/z* 279, 263 and 293, respectively. However, the base peaks of sulfoxide at 1 ppm were shifted to a lower level by 1 *m/z*. The "shifted base peaks" of fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion were *m/z* 278, 262 and 292, respectively (Fig. 2). The concordance of the retention time at both concentrations showed that the change in these spectra occurred neither in the GC injection port nor in the column. Furthermore, we tested various solvents, such as acetone, dichloromethane, *n*-hexane and ethyl acetate which are common in pesticide analyses, but there was no significant difference. This means that the changes in the spectra were occurred in an EI ion source. On the other hand, sulfones were stable, and no base peaks shift was observed.

Ion source temperature

Considering that the spectral change occurred in the ion source, as described above, we observed the spectra at various temperatures of the ion source. The results showed that the

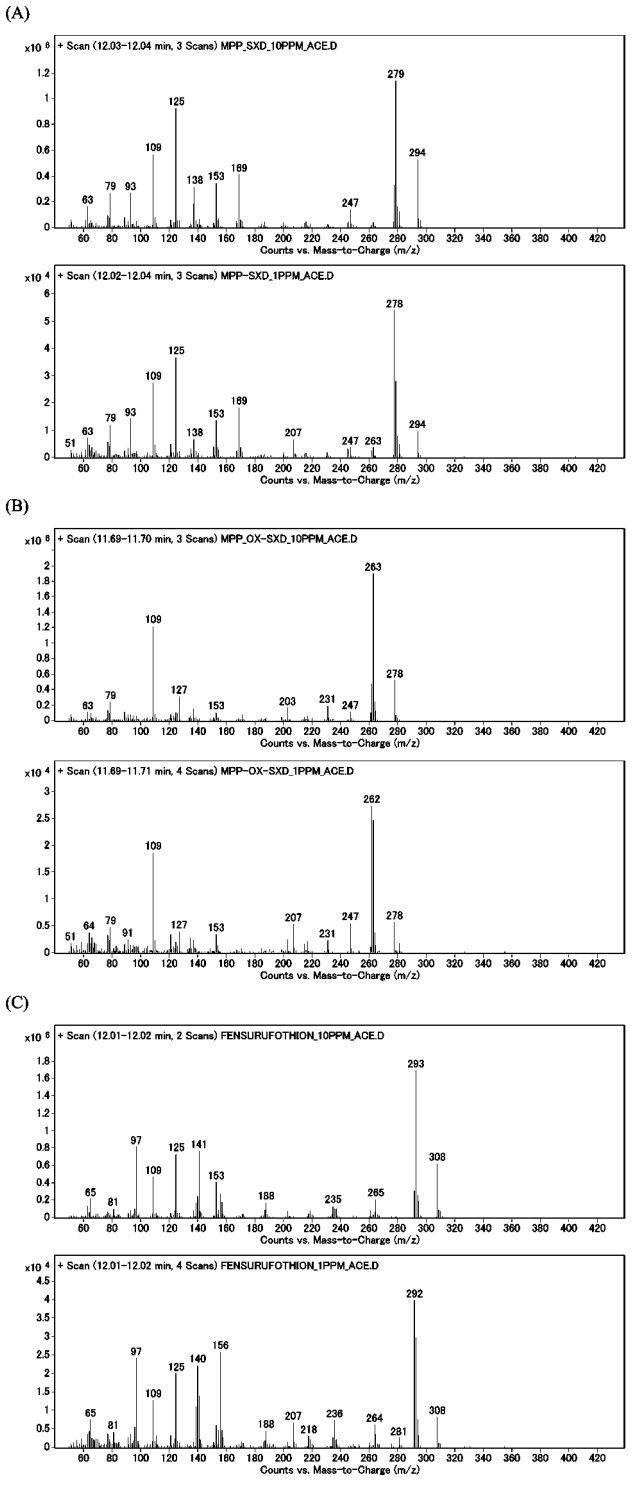

Fig. 2 Spectra of fenthion sulfoxide (A), fenthion oxon sulfoxide (B) and fensulfothion (C). Upper, 10 ppm; lower, 1 ppm. Oven temp., 60°C (1 min)-20°C/min-310°C; inj. temp., 250°C; transferline temp., 280°C; ion source temp., 230°C; injection volume, 2 µL.

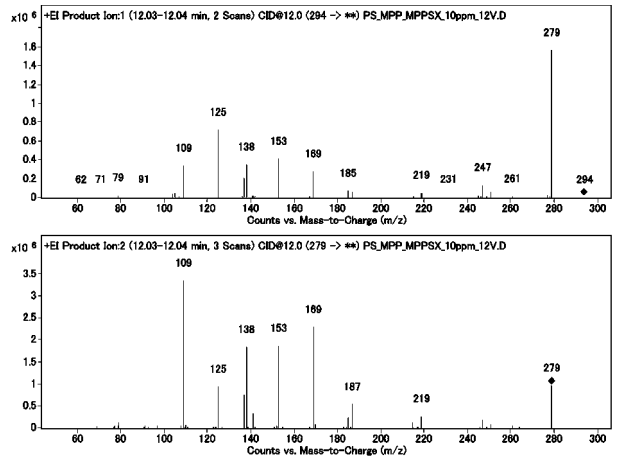

Fig. 3 Product ion scan spectra of fenthion sulfoxide. Upper, precursor ion = molecular ion, *m/z* 294; lower, precursor ion = "usual base peak", *m/z* 279. Other conditions are the same as in Fig. 2.

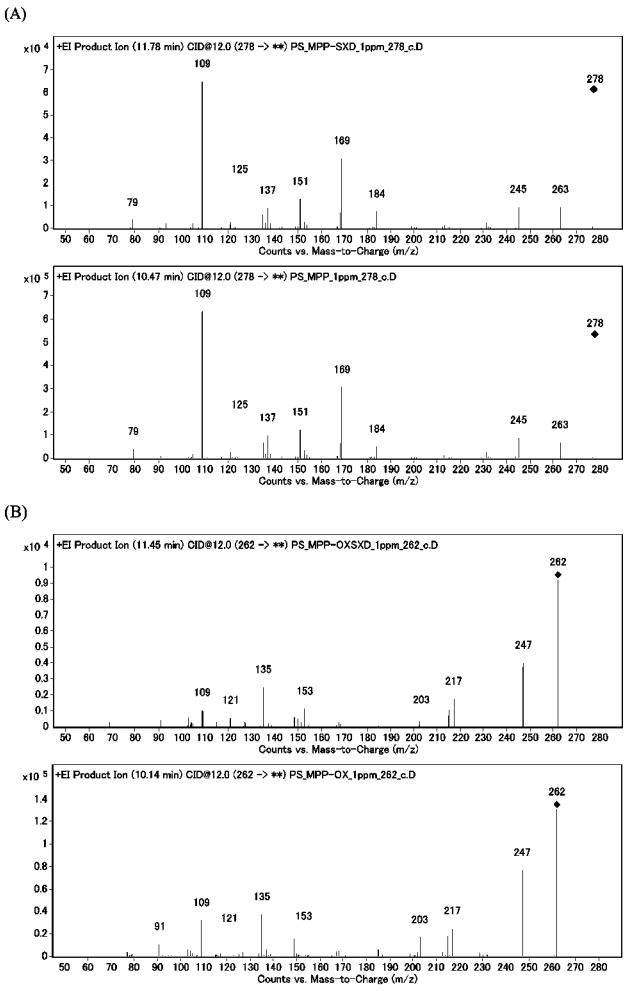

Fig. 4 Product ion scan spectra of the "shifted base peaks" of sulfoxide and those of molecular ions of their sulfides. (A) Upper, fenthion sulfoxide (precursor ion = "shifted base peak" *m/z* 278); lower, fenthion (precursor ion = molecular ion *m/z* 278), (B) upper, fenthion oxon sulfoxide (precursor ion = "shifted base peak" *m/z* 262); lower, fenthion oxon (precursor ion = molecular ion *m/z* 262). Other conditions are the same as in Fig. 2.

lower was the ion source temperature, the higher was the ratio of the "usual base peak". Nevertheless, the spectra were different from those in the NIST library even at low temperature. In addition, the shape of the "shifted base peak" at 150°C showed asymmetry, and the sensitivity was much lower than that at 230°C. Based on these results, the spectra change was caused by the interaction between sulfoxides and the EI ion

source. However, over a 200°C ion source temperature is practical for environmental and food safety analyses in order to prevent any loss of sensitivity due to accumulated contamination from the sample matrix.

The cause of the "shifted base peak"

Demethylation is one of the most basic EI fragmentations, and it has high probability based on the chemical structure of the target sulfoxides. Therefore, the "usual base peak" ($[M–15]^+$) was reasonable, and the result of the product ion scan of the molecular ion supported this (Fig. 3, upper; example, fenthion sulfoxide). On the other hand, it is thought that the "shifted base peak" ($[M–16]^+$) was not formed by the common EI fragmentation. The result that the "shifted base peak" ($[M–16]^+$) was detected neither from the product ion scan of the molecular ion nor from that of the "usual base peak" ion supported, too. Although EI fragmentation and collision induced dissociation (CID) are different mechanisms, both are derive from the structure or functional group of the compounds. The example result of fenthion sulfoxide is shown in Fig. 3. After performing a product ion scan for the "shifted base peak", the spectra were equal to the product ion scan of their sulfides (Fig. 4). However, we could not compare the product ion scan spectrum of fensulfothion with that of fensulfothion sulfide, because we could not obtain the fensulfothion sulfide. In consideration of these results and the similarity of the basic framework of sulfoxide and sulfide, we concluded that the "shifted base peak" was formed from the sulfide, which was produced from the sulfoxide through deoxidation.

Addition of polyethylene glycol 300 (PEG300)

We used polyethylene glycol 300 (PEG300), which has been commonly used to compensate for the matrix-induced enhancement effect. The average molecular weight of PEG300 is 300 with a range of approximately 285 to 315, which covered the molecular weight and retention times of these three target sulfoxides. The added amount of PEG300 was 250 ppm, and there was no memory of PEG300 to the system (ion source, column and injection port) from its concentration and molecular weight. Since the ion source was temporarily coated, or PEG300 was preferentially ionized, sulfoxides were avoided to direct contact with the metal surface. As a result, the formation of sulfide from sulfoxide was controlled and provided reproducible experimental data of mass spectra, even at low concentration (Fig. 5).

Possibility of deoxidation at the injection port

We used the plural GC-MS and GC-MS/MS for this test, and found that the deoxidation of sulfoxides normally occured at the ion source. However, we found that deoxidation could occur at the GC injection port, especially when the metal part at the bottom of the injection port was dirty. This phenomenon was observed after one hundred injections of derivatizing reagents (methoxyamine hydrochloride in pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane for another test) in the split injection mode. We renewed the liner and the column after the derivatized compound analysis, but did not exchange the metal part. When deoxidation occurred at the injection port, two different peaks appeared. As for fenthion sulfoxide, one eluted at the fenthion sulfoxide retention time, and the other eluted at the fenthion retention time (Fig. 6). Fenthion oxon sulfoxide also showed two different peaks: one was fenthion oxon sulfoxide and the other was the deoxidized compound, fention oxon. By adding PEG300, the sulfide peaks disappeared and only sulfoxide peaks were obtained. This result indicates that PEG300 prevented the deoxidation of sulfoxides at the injection port when using an extremely dirty metal part.

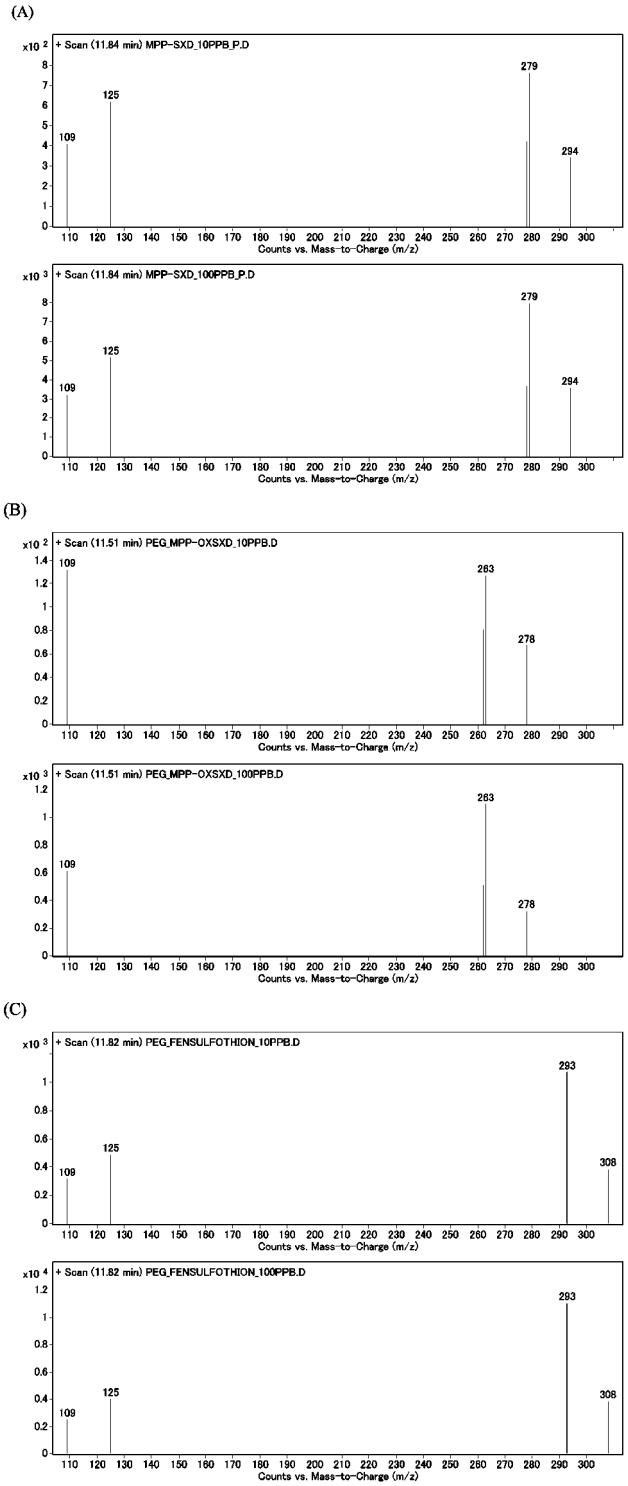

Fig. 5 SIM spectra of fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion at low levels with PEG300 in SIM mode. (A) Fenthion sulfoxide, (B) fenthion oxon sulfoxide, (C) fensulfothion. Upper, 10 ppb; lower, 100 ppb. Other conditions are the same as in Fig. 2.

Additional effect

Since PEG300 has been used to compensate for the

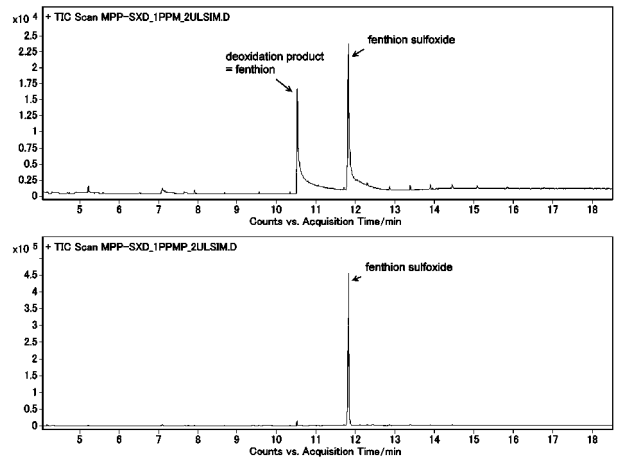

Fig. 6 Chromatogram of fenthion sulfoxide using an extremely dirty metal part at the bottom of the GC injection port. Upper, without PEG300; lower, with PEG 300. Other conditions are the same as in Fig. 2.

Table 1 Matrix-induced enhancement effect value of each pesticide with and without PEG300

Compound	Monitor ion (*m/z*)	Relative response, %[a]	
		Without PEG300	With PEG300
Fenthion	278	141	110
Fenthion oxon	262	161	107
Fenthion sulfone	310	170	117
Fenthion oxon sulfone	294	396	118
Fenthion sulfoxide	279	517	112
Fenthion oxon sulfoxide	263	544	112
Fensulfothion	293	187	113
Fenthion sulfoxide[b]	*278*	*199*	*111*
Fenthion oxon sulfoxide[b]	*262*	*703*	*84*
Fensulfothion[b]	*292*	*167*	*99*

a. Relative response of the analyte in the sample solution to that of the matrix-free standard solution.
b. Deoxidated ion (= "shifted base peak", in Italics).

matrix-induced enhancement effect, we tested PEG300 for other fenthion derivatives including fenthion. The target compounds were fenthion, fenthion oxon, fenthion sulfone, fenthion oxon sulfone, fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion. According to sample preparation reported by the Japanese Ministry of Health, Labour and Welfare,[4] bottled water was extracted by a solid-phase extraction (SPE) column packed with polystyrene divinylbenzene (PS-DVB, 500 mg). The PS-DVB column was conditioned with dichloromethane, methanol and water in succession. Five hundred milliliters of water were loaded onto the PS-DVB column and eluted with 3 ml of dichloromethane. The eluted solution was concentrated with a stream of nitrogen gas, and the volume was adjusted to 1 ml. Fenthion and related compounds were fortified at 100 ppb level into the test solution. As a result, the matrix-induced enhancement effect values of these 7 pesticides in the eluted solution were between 141 and 703% without PEG300, while, those were between 107 – 118% with PEG300 (Table 1). PEG300 not only prevented the sulfoxides deoxidation and provided reproducible experimental data of mass spectra, but also compensated for the matrix-induced enhancement effect.

Conclusions

Fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion were deoxidated at the EI ion source in the GC/MS system. The degrees of deoxidation were different depending on their concentrations, and had an influence on quantitative analysis at the ppb level. We found that adding PEG300 prevented sulfoxide deoxidation. Also we observed the deoxidated peaks at their sulfides retention time when sulfoxides were injected into the injection port, which the metal part at the bottom of the injector port was extremely dirty. The addition of PEG300 was also effective in this case. Moreover, PEG300 compensated for the matrix-induced enhancement effect for other fenthion derivatives, such as fenthion oxon, and fenthion sulfone.

References

1. Fact Sheets from United States Environmental Protection Agency (EPA), http://www.epa.gov/oppsrrd1/REDs/factsheets/0290fact.pdf.
2. Y. Hirahara, Y. Sayato, and K. Nakamuro, *Jpn. J. Toxicol. Environ. Health*., **1998**, *44(6)*, 451.
3. W. J. Hayes Jr. and E. R. Laws Jr, "*Handbook of Pesticide Toxicology*", **1991**, Academic Press, California, 1022.
4. Analytical Method for Water Quality Management, Compilation prepared by Japanese Ministry of Health, Labour and Welfare (MHLW), http://www.mhlw.go.jp/topics/bukyoku/kenkou/suido/kijun/dl/kabetten4.pdf.
5. P. M. Fedrac and J. T. Anderson, *J. Chromatogr*., **1992**, *591*, 362.
6. Y. Tanaka, K. Takahashi, T. Kirigaya, S. Hosoi, T. Hidaka, and H. Nakazawa, *J. Food Hyg. Soc*., **2006**, *47(3)*, 105.
7. E. Ueno, Y. Kabashima, H. Oshima, T. Ohno, S. Nemoto, and T. Mitani, *Anal. J. Food Hyg. Soc*., **2009**, *50(2)*, 64.
8. K. Maśtovská and S. J. Lehotay, *J. Chromatogr., A*, **2004**, *10401*, 2592.
9. D. R. Erney, A. M. Gillespie, D. M. Gilvydis, and C. F. Poole, *J. Chromatogr*., **1993**, *638*, 57.
10. D. R. Erney and C. F. Poole, *J. High Resolut. Chromatogr*., **1993**, *16*, 501.
11. J. L. Bernal, M. J. del Nozal, J. J. Jiménez, and J. M. Rivera, *J. Chromatogr., A*, **1997**, *778*, 111.
12. D. R. Erney, T. M. Pawlowski, and C. F. Poole, *J. High Resolut. Chromatogr*., **1997**, *20*, 375.
13. M. Godula, J. Hajšlová, and K. Alterová, *J. High Resolut. Chromatgr*., **1999**, *22*, 395.
14. J. Hajšlová, K. Holadová, V. Kocourek, J. Poustka, M. Godula, P. Cuhra, and M. Kempný, *J. Chromatogr., A*, **1998**, *800*, 283.
15. F. J. Schenc and S. J. Lehotay, *J. Chromatogr., A*, **2000**, *868*, 51.
16. J. Zristlíková, J. Hajšlová, M. Godula, and K. Maśtovská, *J. Chromatogr., A*, **2001**, *937*, 73.
17. J. Hajšlová and J. Zristlíková, *J. Chromatogr., A*, **2003**, *1000*, 181.
18. C. F. Poole, *J. Chromatogr., A*, **2007**, *1158*, 241.
19. S. Nemoto, K. Sasaki, and M. Toyota, in Proceedings of the 35th Symposium on Hygiene Chemistry, **1998**, 56 – 57.
20. T. Okumura, *J. Environ. Chem*., **1995**, *5*, 575.
21. M. Anastassiades, K. Maśtovská, and S. J. Lehotay, *J. Chromatogr., A*, **2003**, *1015*, 163.
22. K. Maśtovská, S. J. Lehotay, and M. Anastassiades, *Anal. Chem*., **2005**, *77*, 8129.