



A case of death after ingestion of an agrochemical spreading agent

Kiyotaka Usui*, Yoshie Hayashizaki, Yoshikazu Okubo, Masaki Hashiyada, Masato Funayama

Division of Forensic Medicine, Department of Public Health and Forensic Medicine, Tohoku University School of Medicine, Sendai 980-8575, Japan

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ABSTRACT

An agrochemical spreading agent was found near the slightly decomposed corpse of a deceased female. The appearance of the stomach contents suggested that ingestion of a surfactant had occurred before death. The spreading agent was found to contain nonionic nonylphenol ethoxylates (NPEO_n) and anionic sulfonated naphthalene-formaldehyde condensates (SNFC_n). A solid phase extraction cartridge containing a mixed reversed phase-weak anion exchange sorbent (Oasis WAX, Waters) was used to successfully extract both NPEO_n and SNFC_n from the blood. The cartridge was preconditioned with methanol and acetic acid (AcOH). After the dilute blood sample was applied to the cartridge, it was washed with AcOH, and then NPEO_n and SNFC_n were eluted with methanol/dichloromethane (7:3, v/v) and 5% NH₃/80% methanol, respectively. The concentrations of NPEO_{n=2-9} and SNFC_{n=0} in the blood sample were 7.7 μg/mL and 1.8 mg/mL, respectively. It is possible that postmortem changes increased the concentration of SNFC_{n=0} monomer by breaking down the polymer. However, the behavior of these compounds in the human body is unclear and further case studies are needed to investigate this result.

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1. Introduction

Pesticides and other agrochemicals are often mixed with an agrochemical spreading agent before use. Surfactants are a major component in these agents, and help to decrease the surface tension in the agrochemical fluid and improve the adhesion to plants and insects. Only two fatal cases of poisoning by spreading agents have been reported in Japan, but in both these cases a toxicological study was not carried out [1]. In addition, while a few quantitative reports have detailed fatal poisoning cases with cationic surfactant antiseptics [2–4], there have been no reports on nonionic and anionic surfactants. In the present case, we investigated a case of death after ingestion of a spreading agent containing nonylphenol ethoxylates (NPEO_n, 15%) (Fig. 1a) and sulfonated naphthalene-formaldehyde condensates (SNFC_n, 4%) (Fig. 1b). We developed a new method for extraction of both surfactants using a solid phase cartridge. A quantitative study of the blood and gastric contents was performed by liquid chromatography tandem mass spectrometry (LC–MS/MS).

2. Case

In late December, a female in her sixties went home after quarreling with a family member. Five days later, she was found

dead and an empty 500 mL bottle of an agrochemical spreading agent was found near the body. The head and neck of the corpse had been attacked by animals, the face was skeletonized, and organs around the neck were missing. At autopsy, the remaining organs were found to be soft and slightly decomposed. There was 110 mL of a foul smelling brown viscous fluid in the stomach. The gastric fluid was placed in a test tube with water and bubbled when shaken by hand. The gastric mucosa appeared to be eroded. There were no significant antemortem injuries or disease. The cause of death appeared to be ingestion of a large amount of the agrochemical spreading agent. For toxicological analysis, blood from the decedent's external iliac vein and the gastric contents were collected and stored at –30 °C until analysis 25 days later. No preservative agent such as NaF was added to these samples.

3. Materials and methods

3.1. Reagents

Sulfonated naphthalene-formaldehyde condensate was purchased from Kao Chemicals (Tokyo, Japan). LC–MS grade acetonitrile and methanol, and analytical grade dichloromethane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ammonium formate was purchased from Kanto Chemical Co. Ltd. (Osaka, Japan). All other chemicals used were of analytical grade.

3.2. Solid phase extraction

To extract both nonionic NPEO_n and anionic SNFC_n from the blood, a mixed reversed phase-weak anion exchange sorbent (Oasis WAX, Waters) was used for solid phase extraction. The reversed phase retained NPEO_n and the weak anion exchange phase

* Corresponding author. Tel.: +81 22 717 8110; fax: +81 22 717 8112.
E-mail address: usui@forensic.med.tohoku.ac.jp (K. Usui).

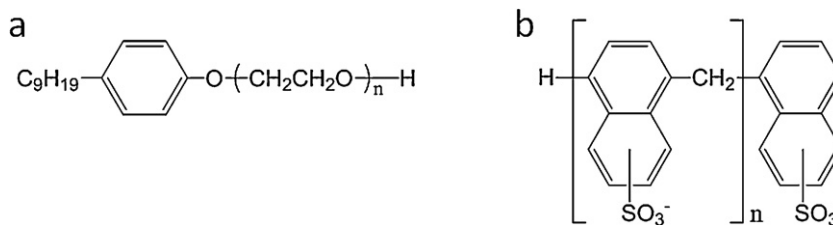


Fig. 1. Structures of nonylphenol ethoxylates (a) and sulfonated naphthalene-formaldehyde condensates (b).

Table 1

List of selected ions for quantitative analysis.

| Compounds | <i>n</i> | Q1 | Q3 |
|--------------------------|--------------------------|-----|-----|
| NPEO _{<i>n</i>} | 2 | 326 | 183 |
| | 3 | 370 | 227 |
| | 4 | 414 | 271 |
| | 5 | 458 | 315 |
| | 6 | 502 | 359 |
| | 7 | 546 | 403 |
| | 8 | 590 | 291 |
| | 9 | 634 | 133 |
| | SNFC _{<i>n</i>} | 0 | 207 |

Q1, precursor ion; Q3, product ion.

retained SNFC_{*n*}. The cartridge was preconditioned with 3 mL of methanol and 3 mL of 10 mmol/L acetic acid (AcOH). The blood sample was diluted 10-fold for NPEO_{*n*} analysis or 1000-fold for SNFC_{*n*} analysis with 1 mL of 10 mmol/L AcOH, and then applied to the cartridge. The cartridge was washed with 3 mL of 10 mmol/L AcOH, and then NPEO_{*n*} and SNFC_{*n*} were eluted with 3 mL of methanol/dichloromethane (7:3, v/v) and 3 mL of 5% NH₃/80% methanol, respectively. The extracts were evaporated to dryness under a stream of nitrogen at 55 °C and reconstituted with 1 mL of MeOH/H₂O (5:5, v/v) for NPEO_{*n*} analysis and distilled water for SNFC_{*n*} analysis. Finally, 10 μL of the reconstituted extract was injected into the LC-MS/MS. The gastric sample was diluted 1:15 000 with distilled water, and injected into the LC-MS/MS.

3.3. LC-MS/MS conditions

LC was performed with a Shimadzu Prominence LC system (Kyoto, Japan). Chromatographic separation was achieved on a Synergi Fusion-RP column (50 mm × 2.0 mm i.d., 2.5 μm, Phenomenex). MS/MS detection was performed with an Applied Biosystems 3200QTRAP MS/MS equipped with an electrospray ionization probe (Foster City, CA, USA). The mobile phase was 95% 10 mmol/L ammonium formate–5% methanol (solvent A) and 5% 10 mmol/L ammonium formate–95% methanol (solvent B). The solvent gradient for elution of NPEO_{*n*} increased linearly from 70 to 100% solvent B in 15 min, and was maintained at this level for 5 min. The solvent gradient for elution of SNFC_{*n*} increased linearly from 30 to 40% solvent B in 16 min, and was maintained at this level for 4 min. The solvent flow rate for each analysis was 0.2 mL/min. The mass spectra of NPEO_{*n*} and SNFC_{*n*} were measured in positive and negative ion modes, respectively. Quantitative analysis was performed in multiple reaction monitoring mode using the ions shown in Table 1. In SNFC analysis, many peaks appeared in the mass chromatogram of SNFC_{*n=1-5*} and it was difficult to integrate all these peaks. Therefore, only the peak of SNFC_{*n=0*} was used to calculate the concentration.

4. Results

4.1. NPEO_{*n*}

The full scan chromatogram of NPEO_{*n*} showed one peak for NPEO_{*n=4-10*} (Fig. 2a). Series of ammonium adducts ([M + NH₄]⁺) and protonated molecules ([M + H]⁺) were observed in the mass spectra of NPEO_{*n*} (Fig. 2b). The observed *m/z* of [M + NH₄]⁺ were 458 (*n* = 5), 502 (*n* = 6), 546 (*n* = 7), 590 (*n* = 8), 634 (*n* = 9), and 678 (*n* = 10), and those of [M + H]⁺ were 397 (*n* = 4), 441 (*n* = 5), 485 (*n* = 6), 529 (*n* = 7), 573 (*n* = 8). The 44 u difference between the peaks in each series corresponds to an ethoxylate (–CH₂CH₂O–) unit. We also observed fragment ions corresponding to the loss of a hydroxy group, a nonyl group, and nonylphenol. Fig. 3 shows the proposed fragmentation pathway of NPEO_{*n*}, which was predicted from the MS/MS analysis using [M + NH₄]⁺ of NPEO_{*n=2-11*} as precursor ions.

4.2. SNFC_{*n*}

Fig. 4a shows the SNFC_{*n*} mass chromatograms obtained from the gastric content sample. Many peaks were observed in the mass chromatograms of SNFC_{*n=1-5*} because SNFC_{*n*} is a complex mixture of isomers that have a sulfo group bound at different sites. More peaks were observed in the mass spectra as the degree of condensation (*n*) increased (Fig. 4b). Table 2 shows the expected *m/z* values of protonated molecular ions of SNFC_{*n=0-5*}. The fragmentation patterns of each SNFC_{*n*} oligomer detected by MS/MS were formed by rearrangement of aromatic sulfonate anions [5] and loss of SO₃ from the precursor ions. In addition, a peak at *m/z* 80 was observed because of formation of the SO₃ radical ion. Figs. 5 and 6 show the product ion spectrum and expected fragmentation pathway of SNFC_{*n=1*} from the precursor ions *m/z* 213 and *m/z* 427, respectively. Only SNFC_{*n=0-3*} were detected in the blood sample.

4.3. Method validation

The validation parameters of the developed procedure for blood analysis are summarized in Tables 3 and 4. The calibration curves were established over the concentration ranges of 0.12–12 μg/mL

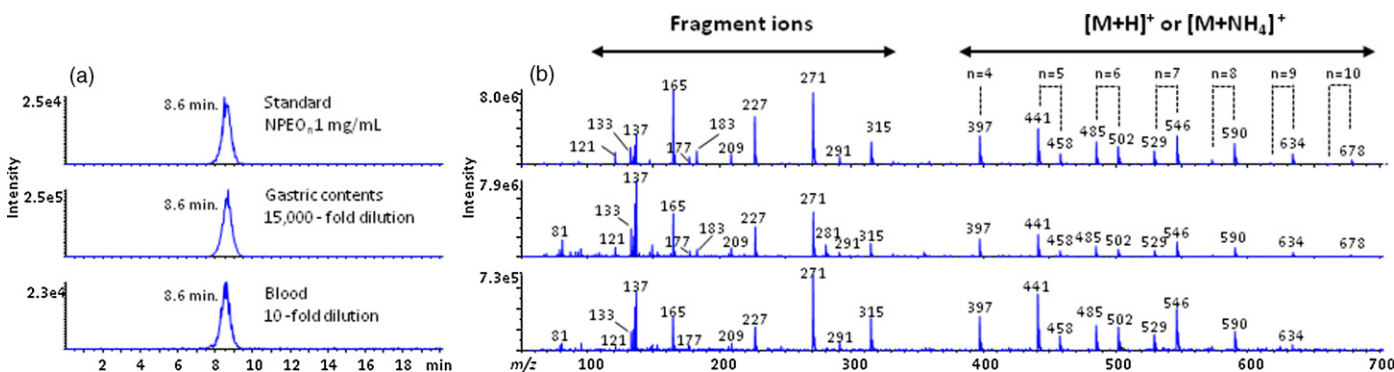


Fig. 2. Full scan chromatograms (a) and mass spectra (b) of NPEO_{*n*} obtained from standard solution and forensic samples.

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