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Determination of chlorhexidine (CHD) and nonylphenolethoxylates (NPEO*n*) using LC-ESI-MS method and application to hemolyzed blood

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Abstract

Rapid and reliable methods for identification of chlorhexidine (CHD) and nonylphenolethoxylates (NPEO*n*) in antiseptic and hemolyzed blood using electrospray ionization mass spectrometry (ESI-MS) were developed. Fragmental analysis provides accurate evidence for the presence of CHD in the samples. For the determination of CHD in hemolyzed blood, the method was also developed using LC-ESI-MS. Linearity of calibration curve was obtained over the concentration range of 0.1–11 μg/mL with residuals from −4.3 to 6.7%. We applied the methods to the case of suicidal injection of antiseptic and successfully detected CHD and NPEO*n* from hemolyzed blood. The CHD concentration was 352 μg/mL.

Keywords: Chlorhexidine; Nonylphenolethoxylates; LC-ESI-MS; Human; Blood; Forensic science; Forensic medicine; LC-ESI; Disinfectant; Toxicology; Suicide; Antiseptic

1. Introduction

A case of accidental intravenous injection to a patient, a fatal case of suicidal injection, and a case of mixing these compounds into beverages have been reported [1–3]. Therefore, antiseptics and surfactants are important compounds from a forensic toxicological point of view.

Chlorhexidine (CHD), 1,1′-hexamethylenebis [5-(4-chlorophenyl) biguanide], is one of the most widely used antiseptics. In addition, it is also used as ingredient of healthcare products such as mouth rinses, contact lens cleaners, burn cream, and cosmetics. Some products of CHD contain surfactants such as nonylphenolethoxylates (NPEO*n*) as additives. So, the detection of these additives supports the identification of CHD containing preparats.

There are many reports about the determination of CHD in biological fluids using high performance liquid chromatography with a UV detector (HPLC-UV)[1,4,6–10] and gas chromatography mass spectrometry (GC-MS) [5].

LC-ESI-MS has higher sensitivity and selectivity than HPLC-UV and gives more information for identification. For the detection of surfactants in samples such as beverages, the methods using liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) and LC-ESI tandem mass spectrometry (LC-ESI-MS/MS) have been reported from standpoint of forensic chemistry [2,3]. But there is no report investigating for CHD and NPEO*n* in human fluids using these methods.

Electrospray ionization is a suitable technique for the detection of the compounds which have the lack of volatility, thermal stability, and the presence of quaternary amine moieties [11–19]. Therefore, it is very suitable to use ESI-MS technique for determination of CHD and NPEO*n*. In an analysis of CHD and NPEO*n* by ESI-MS, specific molecular-related ions would be measured. Furthermore, fragmental analysis would strongly identify these compounds. In this study, appropriate extraction and ESI technique was applied to a surely identification of CHD and NPEO*n* in hemolyzed blood.

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2. Materials and methods

2.1. Chemicals and reagents

CHD gluconate solution (20%) was purchased from Wako pure chemical industries, Ltd. (Osaka, Japan) and stored at 4 °C in the dark. Acetonitrile was of LC-MS grade, and perchloric acid (60%) was of analytical grade from Wako pure chemical industries, Ltd. (Osaka, Japan) ammonium acetate and trifluoroacteic acid were purchased from Kanto chemical co Ltd. (Osaka, Japan). Extrelut® NT3 was purchased from Merck (Darmstadt, Germany). Product of CHD, STERICLON® solution 5 (5% CHD gluconate), was purchased from Kenei pharmaceutical co., Ltd. (Osaka, Japan). Other chemicals used were of analytical grade.

2.2. Sample preparation and extraction procedure

Stock solution of CHD standard was prepared in deionized water (120 μg/mL) and stored in a plastic vessel at 4 °C in the dark, because CHD is known to be absorbed to glassware [4]. To obtain the calibration standards for CHD at the concentration range of 0.11–11.0 μg/mL, the working solution was spiked appropriately into drug-free hemolyzed blood in polypropylene microtubes.

The extraction of CHD from hemolyzed blood was performed according to the method of Kudo et al. [1] with minor modification. Briefly, 200 μL of hemolyzed blood was mixed with equal volume of deionized water in a polypropylene microtube. One hundred microliters of 10% perchloric acid was added to the mixture and mixed vigorously for 30 s. Then the mixture was centrifuged at 1630 × *g* for 5 min and the supernatants were transferred to another microtube. Potassium carbonate (50 μL) was added to neutralize and remove the excess of perchloric acid from the supernatant. An aliquot of 10 μL of the supernatant was injected onto the column.

The extraction of NPEO*n* from hemolyzed blood was performed on Extrelut® NT3 cartridge. Hemolyzed blood (200 μL) was mixed with 2.5 mL of deionized water microtube and the mixture was applied to the Extrelut NT3 cartridge. After wards, the cartridge was held for 5 min. Then NPEO*n* was eluted with 5 mL × 3 of chloroform. After total evaporation of chloroform under a nitrogen stream at 40 °C, the residue was reconstituted with 200 μL of deionized water. An aliquot was directly analyzed by infusion analysis (direct ESI-MS in positive mode).

2.3. Infusion analysis (direct ESI-MS)

Infusion analysis was used for identification of CHD and NPEO*n* in the infused liquid and of NPEO*n* in the blood extracts. The infused liquid and a standard compound in antiseptic were diluted thousand fold with deionized water, and this diluent was directly introduced to a mass spectrometer. A syringe pump used was a Model 11 single syringe (Harvard Apparatus, Inc. USA). Flow rate was set at 10 μL/min. The syringe was connected with a fused silica capillary to a ZMD 4000 mass spectrometer (Micromass, UK) with electrospray ionization probe. The capillary and cone voltage were set at 3.00 kV and ± 20 V, respectively. The source block and the desolvation temperatures were set at 100 and 150 °C, respectively. The nebulizing gas flow rate was set at 200 L/h. MS data were collected as total ion current (TIC).

2.4. LC-MS condition

For LC-MS system, the following instrumentation was used: HPLC system used was a NANOSPACE SI-II (Shiseido, Japan). Reversed-phase chromatography was performed on a TSKgel ODS-100 V (TOSOH, Japan 2.0 mm × 50 mm, 5 μm) with a guard cartridge CAPCELL PAK MG II (Shiseido, Japan; 2.0 mm × 10 mm, 3 μm) using isocratic elution with acetonitrile/water/trifluoroacetic acid (65/35/0.1, v/v/v) at a flow rate of 200 μL/min. The column temperature was set maintained at 22 °C. The injection volume was 10 μL. This HPLC system was

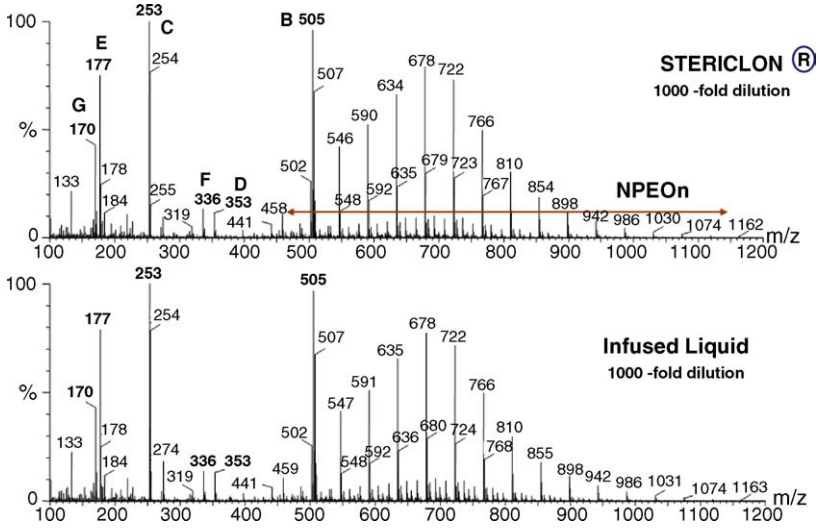

Fig. 1. Comparison of mass spectra (infusion analysis) obtained for antiseptic (upper) and infused liquid (bottom) in positive ion mode. A series of the peaks different by 44 mass units is typical of polyoxyethylene-related surfactant.