学位論文概要

New application to public health and environmental analysis using photoionization and high-resolution mass spectrometry

質量分析における光イオン化、精密質量測定などの新技術の保健衛生・環境分野への展開

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質量分析は市民生活の安全・安心を守る上で必須の分析手法となっている。また、近年の質量分析に関連する技術の進歩は目覚ましく、特に生命科学等の分野で著しく発展している。本研究はこれら先進の技術を保健衛生・環境分野において展開し、新たに得られた知見を示したものである。

I Introduction

Photoionization has been applied in the analytical chemistry in the 1970s as a detection principle after chromatographic separation. As an atmospheric pressure ionization, Robb et al introduced atmospheric pressure photo-ionization (APPI) to ionization technique of liquid chromatograph/mass spectrometer (LC/MS) in 2000 for the first time. As of now, APPI source becomes commercially available from a number of mass spectrometer manufacturers as well as electrospray (ESI) and atmospheric pressure chemical ionization (APCI) sources. APPI is one of relatively new techniques and it has been considered a major advantage that its application range includes non-polar compounds such as aromatic hydrocarbons that did not ionized by ESI and APCI. In analyses of public health and environmental sectors, the development of rapid and easy analytical methods is essential. Particularly, the sectors have a problem that the number of analytes increases rapidly with an increase in social problems such as crime and accidents by illicit drugs, severe food poisoning by residual pesticides, and environmental pollution by illegal dumping. Because physical properties of the analytes vary greatly, it is urgent to establish robust analytical methods that can be applied to various analytes. In addition, it often happens that the identification of unknown offending substances needs to be made. Recently, the development of analytical instruments in mass spectrometry significantly advances. A mass spectrometer with a mass resolving power of 10,000,000 is already launched. In the identification of unknown offending substances, such ultra-high resolution mass spectrometer is quite useful and is highly anticipated to be applied to the determination of molecular structures by analyses of ions generated using accurate masses.

Past environmental problems were usually caused by pollution that has an obvious source origin as in the case of heavy metal and polychlorinated biphenyl, for example. Recent environmental problems are, however, caused by more familiar substances included in medicines and cosmetics. In addition, emerging substituents that associate with the regulations also draw attention. As well as toxic chemical substances, provision for infection diseases is one of the most urgent problems in the fields of public health and environment. The thesis is a compilation of new knowledge about the development of analytical method, procedure of structural identification, and mechanisms of ionization and fragmentation.

II Steroid Hormone Profiles of Urban and Tidal Rivers Using LC/MS/MS Equipped with ESI and APPI Sources

A highly sensitive method of analyzing steroidal hormones in aquatic samples was developed using an LC/MS/MS equipped with ESI and APPI. Steroidal hormones examined included 10 unconjugated (active) hormones and 7 conjugates. APPI demonstrated greater sensitivity than did ESI for most of the unconjugated hormones. For conjugated hormones, in contrast, ESI was more effective. The method developed was applied to the determination of hormones in the aquatic environment of the Osaka City. The hormones detected were affected by the effluent

from municipal wastewater treatment plants (WWTPs), and hormone concentration values were comparable to those reported in previous studies of WWTP effluent. Because of the two-way flow and stagnancy of streams and watercourses, continuous input of steroidal hormones from WWTPs seems to bring about local accumulation. Levels of androgen were 1 order of magnitude lower than those of estrogen.

III Pesticide analysis by LC/APPI-MS

To evaluate the risk from residual pesticides, the demand for simultaneous analysis of both precursor and metabolite is often issued. The simultaneous analysis is difficult because the physical property of the metabolite is sometimes different from the precursor pesticides. An analytical method of major organochlorine pesticide, chlorothalonil, and its metabolite, 4OH-TPN, was presented. The method was successfully applied to the determination of chlorothalonil in aqueous environment and food samples. Appearance of emerging pesticides is another problem in pesticide analysis. Neonicotinoid is the leading one of the emerging pesticides. An analytical method was developed for 6 neonicotinoids. Dinotefuran was the most frequent and highest among the neonicotinoids examined in the aquatic environment in the Osaka City. Given the toxicity of neonicotinoids for aquatic creatures, the concentrations observed here were substantially low. The change in concentrations was temporally coincident with the period of the neonicotinoid application.

IV Occurrence of fluoroquinolones and fluoroquinolone-resistance genes in the aquatic environment

Fluoroquinolones (FQs) have been detected in aquatic environments in several countries. Long-term exposure to low levels of antimicrobial agents provides selective pressure, which might alter the sensitivity of bacteria to antimicrobial agents in the environment. The resistance of *Escherichia coli* (*E. coli*) to FQs were examined by phenotyping and genotyping. In the aquatic environment in Osaka, ciprofloxacin, enoxacin, enfloxacin, lomefloxacin, norfloxacin, and ofloxacin were detected. FQ-resistant *E. coli* were also found. Although no obvious correlation was detected between the concentration of FQs and the presence of FQ-resistant *E. coli*, FQ-resistant *E. coli* were detected in samples along with FQs, particularly ciprofloxacin and ofloxacin. Most FQ-resistant *E. coli* carried mutations in *gyrA*, *parC*, and *parE* in quinolone resistance-determining regions. Six strains carried the plasmid-mediated quinolone resistance determinant *qnrS1*. Because plasmids are responsible for much of the horizontal gene transfer, these genes may transfer and spread in the environment.

V Molecular characterization of mycolic acid from *Mycobacterium bovis* BCG substrains by LC/APPI-MS

Two kinds of APPI sources were examined in the application to molecular characterization of mycolic acids. Cell wall structure of microorganisms has a close relation with its phenotype. Mycolic acids are one of the primary constituents in cell wall of *Mycobacterium* spp. Especially, mycolic acids of *Mycobacterium tuberculosis*, an etiological agent of tuberculosis, have high molar masses (approx. 1200-1300) and highly non-polar. In the ionization of mycolic acid methyl ester, APPI was superior to other conventional ionization, such as ESI and APCI. Both APPI sources could sufficiently ionize mycolic methyl ester. The ion species generated in APPI were identified by accurate mass observations. In-source fragmentation that involved dehydration occurred and was accelerated with increasing ion source temperature. In the mycolic acid subclasses examined in the present study, methoxymycolic acid methyl ester was more likely to provoke the fragment reaction than other subclasses. Because the reaction of fragmentation was stationary in each subclass, the composition of mycolic acids could be accurately estimated. APPI is a promising ionization for the analysis of mycolic acids and is expected to be used for further studies to understand cell wall structures.

VII Structural Identification of Chemical Components and Biodegradation Products of Highly Fluorinated Products Using 2D-LC/MS

Structural identification of perfluoroalkyl and polyfluoroalkyl substances found in end-user products and their biodegradation products was performed using ultra-high resolution MS. Little

attention has so far been paid to the environmental burden of perfluorooctane sulfonate and perfluorooctanoic acid generated from compounds with a molar mass of ~2,000. Analysis of end-user waterproofing and stain repellent products revealed the presence of numerous ions with molecular masses ranging from 1,000 to 2,000 and complex mass spectra. Ultra-high resolution MS determined the accurate mass of the observed ions, allowing the cleavage position and fragment structure to be determined. The precursor structures were determined based on reconstitution of the retrieved fragments. Biodegradation tests using activated sludge revealed that biodegradation products consistent with those reported previously were generated even from complex end-user products. The results of the present study suggest that the environmental burden of these compounds should be reevaluated.

VIII General Overview

In the thesis, the advantages of application of APPI and accurate mass measurements to solve various problems of the public health and the environment were demonstrated. APPI was a dominant tool for the analyses of organo-chlorines and lipids with low polarity, and neonicotinoids with high polarity. The APPI process sometimes included various side reactions such as ion-molecule reaction during the ionization and generated inconceivable ions considering the analyte structures. Accurate mass measurement by high-resolution MS could correctly determine the ion species generated in the ionization process. This is great advantage in the elucidation of the reactions that take place in APPI. In the future, data of accurate masses will be accumulated and unknown compounds can be identified only by the knowledge obtained from accurate mass measurements. This strategy has great positive impact on the public health and the environment.

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