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Determination of isophorone in food samples by solid-phase microextraction coupled with gas chromatography–mass spectrometry

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Abstract

A simple and sensitive method for the determination of isophorone in food samples was developed by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS). Isophorone was separated within 10 min by GC–MS using a DB-1 capillary column and detected with selective ion monitoring mode. The HS-SPME using a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber provided effective sample enrichment, and was carried out by fiber exposition at 60 °C for 45 min. The extracted isophorone was easily desorbed by fiber exposition in the injection port of a capillary GC–MS system, and carryover was not observed. Using this method, the calibration curve of isophorone was linear in the range 20–1000 pg/mL, with a correlation coefficient 0.9996 ($n = 18$), and the detection limit (S/N = 3) was 0.5 pg/mL. The HS-SPME/GC–MS method showed 25,000-fold higher sensitivity than the direct injection method (1 μL injection). The within-day and between-day precisions (relative standard deviations) at the concentration of 1 ng/mL isophorone were 3.9% and 6.1% ($n = 5$), respectively. This method was successfully applied to the analysis of food samples without interference peaks. The recoveries of isophorone spiked into food sample were above 84% for a 50 or 500 pg/mL spiking concentration. The analytical results of the contents of isophorone in various food samples were presented.

Keywords: Headspace solid-phase microextraction; Isophorone; Gas chromatography–mass spectrometry; Food samples

1. Introduction

Isophorone (3,5,5-trimethyl-2-cyclohexene-1-one) is colorless liquid having peppermint odor, and used widely in industry as a solvent of natural and synthetic resins, wax, oil, pesticides, paints and printing inks [1,2]. Isophorone is detected in environmental waters [1,3], air in working place [1,4], foods [5], food packaging [5] and human urine [6,7]. Allergic contact dermatitis [8,9], respiratory disease [10], liver and renal damage [11], cancer-causing possibility [11,12] by exposition of isophorone have been pointed out in animal experiments, but it is not grasped enough about the reality of exposition for human from foods. Therefore, a sensitive, selective and simple method to determine the presence and contents of isophorone in food samples is required.

The determination of isophorone has been carried out by gas chromatography (GC) [3], GC/mass spectrometry (GC–MS) [4,5] and liquid chromatography/mass spectrometry (LC–MS) [6,7]. Among these methods, GC–MS was used for the analysis of food samples [5]. However, the method requires time-consuming and tremendous sample pretreatment such as steam distillation, solvent extraction and conventional open column chromatography. These techniques require relatively large amounts of food sample and large volumes of organic solvent. More complicated pretreatment may cause error, and the use of large volumes of organic solvent may pose a health hazard to the analyst as well as cause environmental pollution. On the other hand, a solid-phase microextraction (SPME) [13,14] using a fused-silica fiber coated on the outside with a stationary phase is a solvent-free sample preparation technique, and it was applied to the analysis of isophorone in lake water [3]. However, the method coupled with GC does not have enough sensitivity for analysis of real sample, and has not been applied to food samples.

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In this paper, we report a simple and sensitive method for the determination of isophorone by headspace (HS)-SPME coupled with GC–MS. In order to grasp the intake of isophorone from foods, this method was also applied to the analysis of several food samples.

2. Experimental

2.1. Materials

Isophorone purchased from Tokyo Kasei Kogyo (Tokyo, Japan) was dissolved in methanol to make a stock solution at concentration of 1 mg/mL. The solutions were stored at 4 °C and used after dilution with distilled water to the required working concentrations. Distilled water was used after purification with a Model Milli-Q water purifier (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade.

Manual assemblies of SPME with a replaceable and reusable extraction fiber coated with polydimethylsiloxane (PDMS, 100 μm), carboxene/PDMS (CAR/PDMS, 75 μm), StableFlex™ divinylbenzene/CAR/PDMS (DVB/CAR/PDMS, 50/30 μm), StableFlex™ PDMS/DVB (65 μm), polyacrylate (85 μm) and carbowax/DVB (CW/DVB, 65 μm) were purchased from Supelco (Supelco Japan, Tokyo). These fibers were conditioned in a GC injection port at adequate temperature prior to use. One fiber can be used repeatedly at least more than one hundred times.

2.2. Gas chromatography–mass spectrometry

GC–MS analysis was carried out with a Shimadzu Model QP-2010 gas chromatograph–mass spectrometer in conjunction with a GCMS solution Ver. 2 workstation. A fused-silica capillary column of cross-linked DB-1 (J&W, Folsom, CA, USA: 60 m × 0.25 mm i.d., 1.0 μm film thickness) was used. The GC operating conditions were as follows: injection and detector temperatures, 260 °C; column temperature, held at 120 °C for 2 min, increased to 220 °C at 10 °C/min; inlet helium carrier gas flow rate, 1.5 mL/min maintained by an electronic pressure controller; split ratio, 10:1. The EI-MS conditions were as follows: ion-source temperature, 200 °C; ionizing voltage, 70 eV. The full scan mass spectra were obtained at an m/z range of 50–150 amu. SIM mode detection for isophorone was selected $m/z = 82$ (base ion peak) and $m/z = 132$ (molecular ion peak), and the total ion of each peak was detected. The peak height was measured to construct calibration curve and to determine isophorone concentration in samples.

2.3. Headspace solid-phase microextraction

A 2-mL volume of sample, 0.75 g of sodium chloride and a PTFE-coated magnetic stir bar (2 × 7 mm) were placed in a 4-mL screw-cap vial with a PTFE septum, and set on the SPME sampling stand (Supelco). The solution was stirred with a magnetic hot stirrer (60 °C) at 1000 rpm for 5 min. The SPME needle pierced the septum of sample vial and the fiber was exposed in the headspace above sample for 45 min. After extraction, the fiber was retracted into the needle, the needle was removed from the septum and then inserted directly into the GC-injection port of the GC–MS instrument. Immediately after exposition of fiber, GC–MS temperature programming was started, the fiber was held in the GC-injection port for 10 min. Then the fiber was retracted into the needle, the needle was removed from the GC-injection port and used for the HS-SPME of next sample.

2.4. Preparation of food samples

Food samples were purchased from a local supermarket. All samples were stored in their original packaging under recommended conditions (either refrigerated or at room temperature) until use. Liquid samples were directly used for the analysis. Semi-solid and solid samples were homogenized with a blender, and used as suspension with distilled water. An aliquot of each sample (0.05–0.4 mL for liquid samples or 20–100 mg for semi-solid and solid samples) was weighed into a 4-mL Pyrex glass tube with a PTFE-lined screw-cap, and was made up to total volume 2 mL with distilled water. The mixture was saturated with solid sodium chloride, and then used for HS-SPME/GC–MS analysis.

3. Results and discussion

3.1. Gas chromatography–mass spectrometry of isophorone

In order to select the monitoring ion for isophorone, the full scan mass spectrum was measured at an m/z range of 50–150 amu. As shown in Fig. 1(A), a molecular ion peak ($M^+ = 138$) and main fragment ion peak at $m/z = 82$ [M^+-$(CH_3)_2CCH_2$] were observed on the spectrum and these ions were selected for SIM mode detection. A typical total ion chromatogram of isophorone is shown in Fig. 2. Isophorone eluted as a single and symmetrical peak within 10 min.

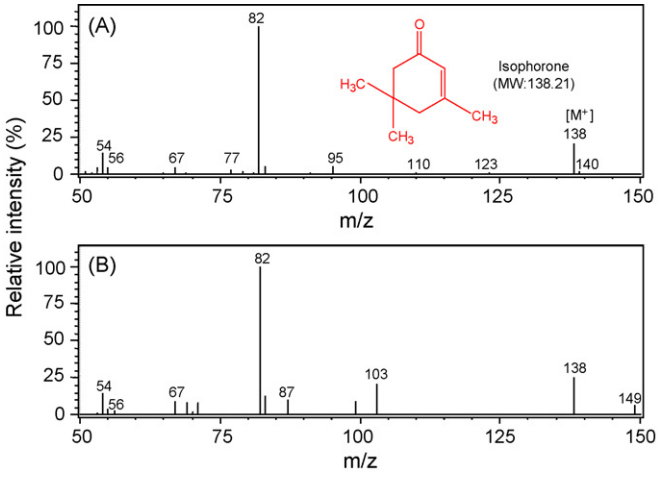

Fig. 1. Mass spectra of isophorone obtained from (A) standard solution and (B) soy sauce.