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Original

SENSITIVE DETERMINATION OF ACETONITRILE AND PROPIONITRILE IN HUMAN WHOLE BLOOD BY HEADSPACE CAPILLARY GAS CHROMATOGRAPHY WITH CRYOGENIC OVEN TRAPPING

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低温オーブントラッピングキャピラリーガスクロマトグラフィーによるヒト全血中アセトニトリル及びプロピオニトリルの高感度分析

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Summary

We have developed a simple and sensitive method for determining acetonitrile and propionitrile in human whole blood using capillary gas chromatography (GC) with cryogenic oven trapping. After heating 0.5 ml of a whole blood sample containing both nitriles and 0.5 ml of distilled water in a 7-ml vial at 75°C for 15 min, 5 ml of the headspace vapor was drawn into a 10-ml syringe and injected to GC with a flame thermionic detector. A sharp peak was obtained for acetonitrile at −10 °C of the initial oven temperature. The extraction efficiencies for acetonitrile and propionitrile were 0.27 and 0.40%, respectively. For quantitation of acetonitrile, propionitrile was used as internal standard, and *vise versa*. The calibration curves for both compounds gave good linearity in the range of 0.2–6 μg/ml whole blood. The detection limits (signal to noise ratio = 3) were 0.05 μg/ml for acetonitrile and 0.01 μg/ml for propionitrile. The coefficients of intra-day variations were 1.0% at 0.4 μg/ml and 1.7% at 2 μg/ml for acetonitrile; 7.1% at 0.4 μg/ml and 1.0 % at 2 μg/ml for propionitrile. This method can be applicable for determining these nitriles in whole blood samples in acute and chronic intoxication cases.

Key words : Acetonitrile; Propionitrile; Gas chromatography; Cryogenic oven trapping; Flame thermionic detector (FTD); NPD

Introduction

Aliphatic nitriles, such as acetonitrile and propionitrile, are highly polar organic solvents being widely used in various chemical industries and laboratories. Acetonitrile is also available to the public in the form of acrylic nail removers. They are readily absorbed into the body through the skin, by inhalation or by ingestion; their acute toxicities appear due to the production of cyanide [1]. Acetone potentiates the acute toxicity of acetonitrile, because its inhibition of the metabolism of acetonitrile to cyanide results in the prolongation of relatively high concentrations of cyanide in blood [2,3]. The poisoning cases of

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nitriles were reported [4-12]; the symptoms caused by their ingestion are vomiting, lethargy, slurred speech, ataxia, stupor, coma and respiratory depression. The oral LD_{50} of acetonitrile in mice, one of most susceptible animals to acetonitrile, is 170–520 mg/kg, and LC_{50} value after one-hour inhalation was about 2,700 ppm [13]. In most reports [4-11], however, only cyanide was measured. A previous method dealing with analysis of acetonitrile by gas chromatography (GC), reported in a fatal poisoning case, gave relatively low sensitivity and poor separation [12].

Microcomputer systems controlling cryogenic oven temperatures below 0°C are widely available for modern types of GC instruments; it was designed for rapid cooling of the oven to reduce analysis time. Our group used it to trap volatile organic compounds (VOCs) in headspace samples, and name it "cryogenic oven trapping (COT)-GC" [14]. This method enabled us to introduce a large volume of headspace vapor (5 ml or more) for cyanide [15], thinner components [16], xylenes [17], ethanol [18], general anaesthetics [19] and other VOCs (see review [20]).

In this paper, we present a simple and sensitive GC method for determining acetonitrile and propionitrile in human whole blood using COT.

Experimental

Materials

Acetonitrile was obtained from Kanto Chemical Co., Inc. (Tokyo); propionitrile from Aldrich (Milwaukee, WI, USA); a Supel-Q PLOT fused-silica capillary column (30 m x 0.32 mm i.d.) from Spelco Inc. (Bellefonte, CA, USA). Other chemicals used were of the analytical grade. Whole blood samples were obtained from healthy volunteers.

Extraction procedure

To 0.5 ml of a whole blood sample containing the nitrile compounds in a 7-ml vial with a silicone septum cap, 0.5 ml distilled water was added. The vial was sealed rapidly, mixed well and then put on an aluminum block heater (Reacti-Therm Heating/Stirring Module, Pierce, Rockford, IL, USA). After heating the sample at 75°C for 15 min, the 5 ml of headspace (HS) vapor was drawn into a 10-ml syringe and injected into the GC instrument.

GC conditions

GC analysis was performed on a Shimadzu (Kyoto) GC-14B instrument equipped with a flame thermionic detector and a COT device. The column temperature was held at –10°C for 1 min and then programmed at 20°C/min up to 200°C. Injection and detection temperatures were set at 230 and 270°C, respectively. The flow rate of helium carrier gas was 3 ml/min. The vapor samples were injected in the splitless mode, and the splitter was opened 1 min after completion of the injection.

Results and discussion

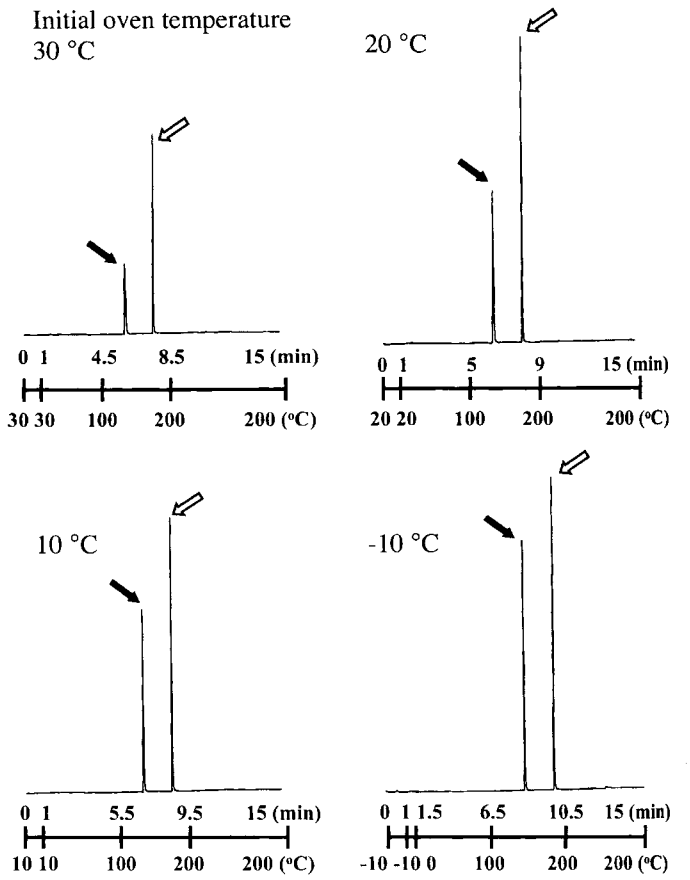

Fig. 1. Headspace COT-GC chromatograms for acetonitrile (filled arrows) and propionitrile (open arrows) in whole blood with various initial oven temperatures. Acetonitrile and propionitrile (1 μg each per vial) were added to 0.5 ml of human whole blood for headspace extraction.

Conditions

Various conditions for the HS extraction of the nitriles from whole blood were tested. When the sample was heated at 45 to 85°C for 5 to 20 min. It was found that the optimal extraction into the HS was obtained at 75°C after 15 min; however, the peak area seemed to be slightly reduced after 20 min at this temperature.

We tested various initial oven temperatures for trapping acetonitrile and propionitrile vapor (Fig.1). At over 20°C, the peak of acetonitrile was broad and became sharper and higher upon lowering the temperature down to -10°C. On the other hand, the initial oven temperatures did not appreciably affect the peak profile of propionitrile. Thus, -10°C was adopted for trapping both compounds in the following analysis.

The addition of salts, such as NaCl, Na_2SO_4 and $CaCl_2$ (0.3 g each) did not improve the extraction efficiencies of the compounds (data not shown).

Detection of acetonitrile and propionitrile from human whole blood

Figure 2(a) shows a GC chromatogram for the authentic acetonitrile and propionitrile (10 ng each on-column) dissolved in methanol. The retention times of acetonitrile and propionitrile were 7.9 and 9.6 min, respectively. The whole blood blank gave

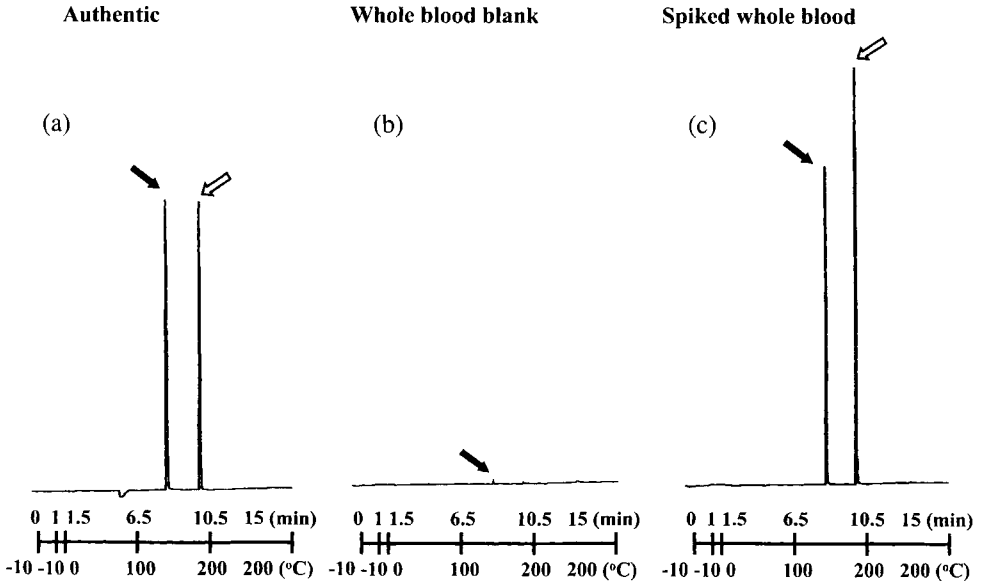

Fig. 2. Headspace COT-GC chromatograms for acetonitrile and propionitrile being set at -10°C of oven temperature. (a): 10 ng each on-column of the authentic acetonitrile (filled arrows) and IS (open arrows) dissolved in methanol with direct injection; (b): whole blood blank with no compounds added; (c): whole blood (0.5 ml) spiked with 1 μg each of the compounds.

a very small interfering peak at the same retention time as that of acetonitrile (Fig. 2(b)). The peak is supposed to be contaminating acetonitrile in the environmental air, but the area seemed to be negligible for quantitating it at concentrations above 0.1 μg/ml. Figure 2(c) shows the chromatogram for the HS vapor for whole blood spiked with 2 μg/ml each of acetonitrile and propionitrile.

Reliability of the method

The extraction effiencies at 2 μg/ml each were 0.27% for acetonitrile and 0.40% for propinitrile (n = 4). The calibration curve for acetonitrile was drawn by plotting six different concentrations in the range of 0.2 to 6 μg/ml using 2 μg/ml propionitrile as IS. For quantitating propionitrile, acetonitrile was used as IS conversely.

The calibration curves gave good linearity in the range of 0.2 to 6.0 μg/ml. Their equations and r^2 values were $y = 0.338x + 0.0392$ and $r^2 = 0.998$ for acetonitrile, and $y = 0.573x + 0.0475$ and $r^2 = 0.999$ for propionitrile. The detection limits of acetonitrile and propionitrile were estimated to be 0.05 and 0.01 μg/ml, respectively. As mentioned in the Introduction section, several cases of acetonitrile or propionitrile poisoning were reported. However, only cyanide was measured in most reports; because it seems difficult to determine these nitriles. In the report by Michaelis *et al.* [12], the lower detection limit of acetonitrile in plasma samples was estimated to be 1 μg/ml by GC-FID, which is much higher than that in our report. Very recently, Shibata *et al.* [21] have reported simultaneous determination of cyanide and aliphatic nitriles using HS-GC. They have claimed that the detection limits are 1.5 ng/ml for acetonitrile and 1.7 ng/ml for propionitrile; they are lower than those by our method. We believe that the use of COT much improves detection limits for VOCs; the discrepancy between the data of the two groups remains to be clarified.

The coefficients of variation for intra-day measurements (n = 5) were 1.68% at 2 μg/ml and 1.04% at 0.4 μg/ml for acetonitrile; 0.986% at 2 μg/ml and 7.09% at 0.4 μg/ml for propionitrile.

To our knowledge, this is the first report to measure acetonitrile and propionitrile by COT-GC. The present method is recommendable for use in forensic and environmental toxicology; because it is simple, needs no special GC apparatus and gives very low background peaks.

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