DETERMINATION OF AMINOPYRINE IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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原　　報

DETERMINATION OF AMINOPYRINE IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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表面電離検出ガスクロマトグラフィーによる体液中アミノピリンの定量

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Summary

We could detect aminopyrine, a well known antipyretic, with high sensitivity by gas chromatography with surface ionization detection (GC-SID) using aminopropylon as internal standard. The calibration curve showed excellent linearity in the range of 50 to 800 pg on column. The detection limit was about 30 pg on column (1.5 ng/ml) which is about 70 times lower than those reported by gas chromatography or high-performance liquid chromatography. Solid phase extraction with Sep-Pak C_{18} was useful for extraction of the drugs from body fluids because of its excellent recovery and purification.

Key words: Aminopyrine; Aminopropylon; Gas chromatography (GC); Capillary GC; Surface ionization detection; Sep-Pak C_{18} cartridges

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Introduction

Aminopyrine (aminophenazone), a pyrazolone compound, was widely used as a mild analgesic or antipyretic. Because of its severe side effects, such as agranulocytosis [1], coma and convulsion [2, 3], the drug is not now used in many countries. However, it is available in a number of countries particularly in Asia; and it was reported that drug abusers took herbal preparations containing aminopyrine obtained from an Asian market [4].

In 1985, Fujii and Arimoto developed a new system of surface ionization detection (SID) for gas chromatography, which is said to be very sensitive to tertiary amino compounds [5, 6]. However, the response of SID is quite different in different tertiary amino compounds; thus aminopyrine, containing a tertiary amino group, is worthwhile testing by GC-SID. In this report, we have developed a simple and sensitive method for the detection of aminopyrine from body fluids by GC-SID.

Experimental

Materials

Aminopyrine and aminopropylon (internal standard, IS) were purchased from Sigma (St. Louis, MO, U.S.A.). Their chemical structures were shown in Fig. 1. Sep-Pak C_{18} cartridges were purchased from Waters Associates (Milford, MA, U.S.A.) and a DB-1 column (30 m x 0.32 mm i.d., film thickness 0.25 μm) from J & W Scientific (Folsom, CA, U.S.A.). Other chemicals used were of analytical grade. Whole blood and urine were obtained from healthy subjects.

Extraction of aminopyrine with Sep-Pak C_{18} cartridges

Sep-Pak C_{18} cartridges were pretreated by passing 10 ml of chloroform, 10 ml of methanol and 10 ml of distilled water, and its procedure was repeated more than 5 times.

To 1 ml of whole blood or urine, with or without addition of drugs (40 ng of aminopyrine and 500 ng of aminopropylon), 9 ml of distilled water was added. The sample solution was then loaded on the pretreated Sep-Pak C_{18} cartridge at a flow rate not greater than 5 ml/min. It was washed with 20 ml of distilled water and finally 3 ml of chloroform was passed through it to elute the drugs. The eluate was collected in a vial, and the

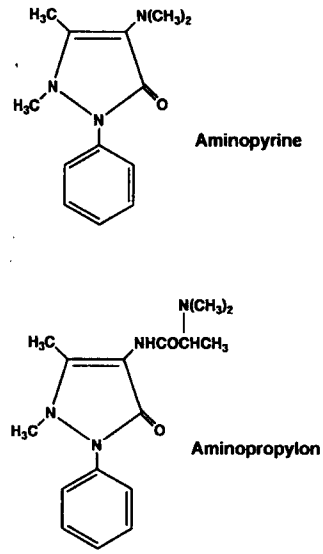

Fig. 1. Chemical structures of aminopyrine and aminopropylon (IS).

aqueous layer was discarded. The organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl of methanol and a 2-μl aliquot of it was subjected to GC analysis.

GC conditions

GC was carried out on a Shimadzu GC-14A gas chromatograph equipped with an SID system (Shimadzu Corp., Kyoto). The GC conditions were: column temperature 150−300℃ (1 min hold at 150℃ , 5℃/min for 16 min and 10℃/min for 13 min); injection temperature 210℃; and helium flow rate, 3 ml/min. The SID conditions were: detector temperature, 280℃; heating current through platinum emitter, 2.2 A; emitter temperature, about 600℃; and ring electrode bias voltage, + 200 V with respect to the collector electrode. The samples were injected in the splitless mode at a column temperature of 150℃ and the splitter was opened after 1 min.

Results and discussion

Figure 2 shows gas chromatograms by GC-SID for 40 ng of aminopyrine and 500 ng of aminopropylon (IS), which were added to 1 ml of whole blood and urine, and extracted with Sep-Pak C_{18} cartridges; as the authentic sample, 800 pg of aminopyrine and 10 ng aminopropylon were applied to the GC-SID. The retention times were 12.6 min for aminopyrine and 22.2 min for aminopropylon. There were some impurity peaks, but none of them overlapped the drug peaks. The recovery of aminopyrine from whole blood and urine was 84.7 and 93.1%, respectively; that of aminopropylon 83.4 and 111%, respectively.

Figure 3 shows a calibration curve for aminopyrine against aminopropylon as IS (10 ng on column). The equation and the regression coefficient for the curve were $y=0.00186x + 0.0235$ and $r=0.997$. The detection limit was about 30 pg on column (1.5 ng/ml). Determinations of aminopyrine by GC with flame ionization detection (FID) and high-performance liquid chromatography (HPLC) were reported [7, 8]; the detection limit of both methods was 100 ng/ml. The detection limit of our method is about 70 times lower than those of the previous methods.

Aminopropylon, which has been used as IS in this study, is being actually used for treatment of persistent fever in Japan as injection solution in combination with sulpyrin and diphenylpyraline (Obelon®). Aminopropylon can be measured with aminopyrine as IS conversely, by GC-SID. The sensitivity for aminopropylon was 10 times lower than that of aminopyrine. The drug, however, can be detected by GC-SID with sensitivity better than to that by GC-FID.

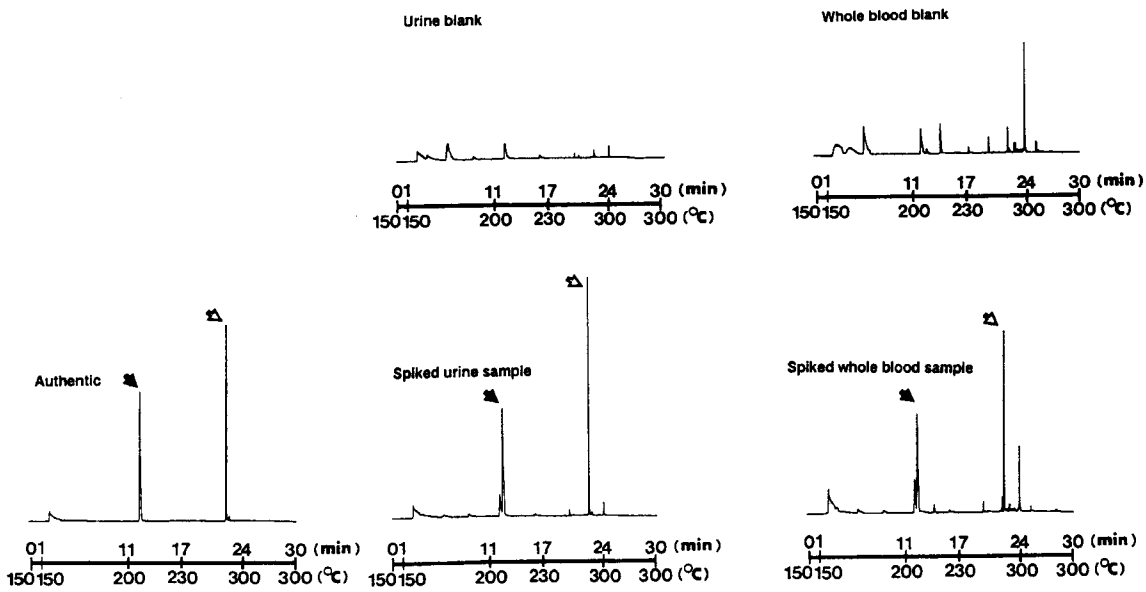

Fig. 2. Chromatograms of capillary GC-SID for aminopyrine (filled arrows) and aminopropylon (open arrows) extracted from whole blood and urine samples. The amounts of aminopyrine and aminopropylon spiked to each 1 ml sample were 40 ng and 500 ng, respectively.

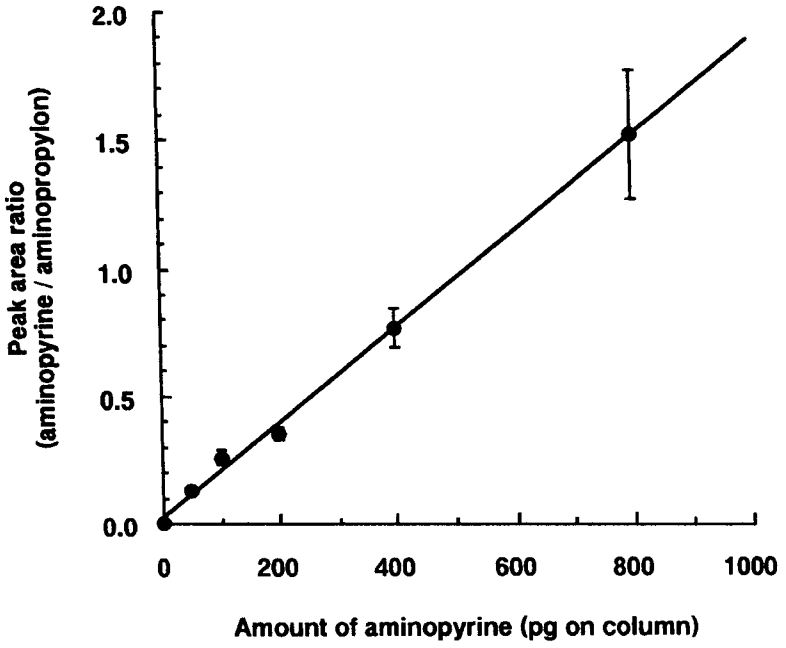

Fig. 3. Calibration curve for aminopyrine. The bars show standard deviations. The amount of the IS was 10 ng on column.

Structures of these two drugs are very analogous (Fig. 1); one of the differences is the existence of an amide bond in the β-position of the teriary amino residue of aminopropylon. A carbonyl group present in the α-position to a tertiary amino group remarkably suppresses the response of SID, but a carbonyl group in the β-position shows no negative effects [9]. In this case, the existence of a secondary amino group in the side chain may cause much lower response of SID for aminopropylon. Further studies on specific structures of a compound which affect response of SID are now under way in our laboratories.

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