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原　　報

DETERMINATION OF COCAINE IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION: COMPARISON WITH NITROGEN-PHOSPHORUS 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コカインの定量：窒素リン検出との比較

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Summary

Cocaine has been found to be measurerable by gas chromatography (GC) with surface ionization detection (SID), with high sensitivity. The calibration curve showed satisfactory linearity in the range of 0.1−5.0 ng on column; the detection limit of cocaine-HCl was about 20 pg on column (0.5−1.0 ng/ml of a sample). Cocaine could be extracted from human whole blood and urine with Bond Elut Certify columns, with satisfactory recovery and low backgrounds. After establishment of a detailed procedure of extraction of the drug from human samples, capillary gas chromatograms were obtained with both SID and nitrogen-phosphorus detection (NPD). Much more impurity peaks appeared with NPD than with SID for both whole blood and urine; the cocaine peak partially overlapped an impurity peak with NPD, but not with SID.

Key words: Cocaine; Gas chromatography (GC); Capillary GC; Surface ionization detection; Nitrogen-phosphorus detection; Bond Elut Certify columns

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Introduction

Gas chromatography (GC) is the most common tool for quantitative analysis of cocaine because of its relatively high volatility [1−7]; nitrogen-phosphorus detection (NPD) is usually used for sensitive GC analysis [2, 3, 6, 7].

In the present study, we demonstrate that cocaine can be determined by GC- surface ionization detection (SID) with higher sensitivity than by GC-NPD.

Experimental

Materials

Pure powder of cocaine-HCl was purchased from Shionogi & Co., Ltd., Osaka; and Bond Elut Certify columns from Analytichem International, Harbor City, CA, USA. Other common chemicals used were of the highest purity commercially available. Whole blood and urine were obtained from healthy subjects.

Extraction with Bond Elut Certify columns

For pretreatment of a Bond Elut Certify column, 2 ml of methanol and 2 ml of 0.1 M potassium phosphate buffer (pH 6.0) were passed through it by aspiration.

To 1 ml of whole blood in the presence and absence of cocaine, 5 ml of distilled water was add to hemolyze it completely; then 6 ml of 0.1 M potassium phosphate buffer (pH 6.0) was added to it, followed by centrifugation at 3,000 rpm for 5 min. The supernatant fraction was applied to the above pretreated Bond Elut column by aspiration, and washed with 6 ml of distilled water. After being dried by aspiration for 5 min, it was washed again by passing 3 ml of 0.1 M HCl and 9 ml of methanol. Finally, 2 ml of methylen chloride/isopropanol (8 : 2) with 2 % ammonium hydroxide was passed through it elute cocaine. The eluate was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μl of methanol and a 2-μl aliquot of it was injected into the GC port.

In the case of urine, 2 ml volume of it, in the presence and absence of cocaine, was mixed with 6 ml of 0.1 M potassium phosphate buffer (pH 6.0), and directly applied to the Bond Elut column without any centrifugation. The following procedure was exactly the same as that for the whole blood sample.

GC conditions

GC analyses were carried out on a Shimadzu GC-15A instrument equipped with an SID system and on a Hewlett-Packard HP5890A gas chromatograph with NPD. A DB-1 non-polar fused-silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm) (J & W Scientific,

Folsom, CA, USA) and split-splitless injectors were used for both GC instruments. The GC conditions for both instruments were as follows: column temperature, programmed from 100 ℃ (1 min hold for splitless injection) to 300 ℃ at 8 ℃/min; injection temperature, 250 ℃; detector temperature, 280 ℃ for both SID and NPD; and helium flow rate, 3 ml/min. The SID conditions were as described in our previous report [8].

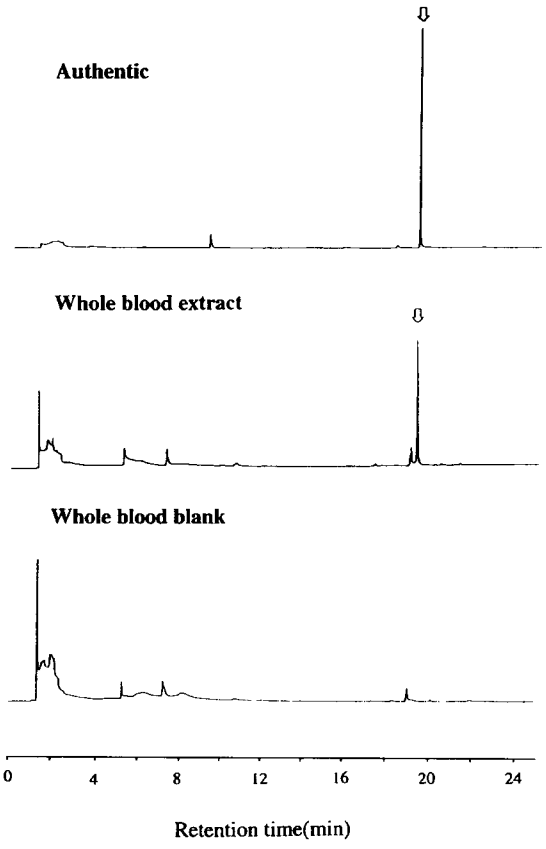

Fig. 1

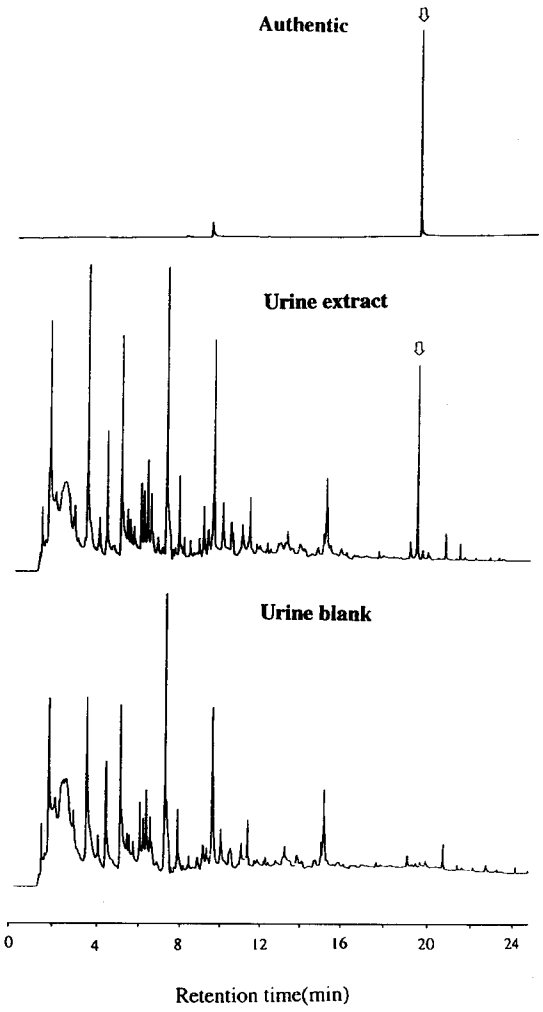

Fig. 2

Fig. 1. Capillary GC-SID for extracts of 1 ml of a human whole blood sample, spiked and not spiked with 100 ng cocaine-HCl. The extraction was made with Bond Elute Certify columns. For GC conditions, see text. The arrows show the peaks of cocaine.

Fig. 2. Capillary GC-SID for extracts of 2 ml of a human urine sample, spiked and not spiked with 100 ng cocaine-HCl. The arrows show the peaks of cocaine.

Results

Figures 1 and 2 show gas chromatograms by GC-SID for whole blood and urine samples, respectively, spiked and not spiked with 100 ng cocaine-HCl. Although the urine extract (Fig. 2) showed many impurity peaks at early stages of the retention time, the whole blood extract showed very clean backgrounds (Fig. 1). The cocaine peaks did not overlapped any impurity peaks. The recovery was 55.8 % for the whole blood sample and 93.9 % for the urine sample.

The calibration curve for cocaine-HCl by GC-SID is shown in Fig. 3. It showed excellent linearity in the range of 100—5000 pg on column. The equation and r value for the curve were: $y = 1.113x + 0.004$, $r = 0.9994$. The detection limit was about 20 pg on column (0.5—1.0 ng/ml of a sample).

Figures 4 and 5 show gas chromatograms obtained by GC-NPD from the same vials of the whole blood and urine samples as those used for GC-SID (Figs. 1 and 2). In the chromatograms of the whole blood extract, some large impurity peaks appeared before the cocaine peak; two impurity peaks appeared very closely to the cocaine peak, and one of them overlapped the latter.

In the chromatograms by GC-NPD of the urine extract, very big impurity peaks appeared at 12—15 min of retention time; two impurity peaks also appeared very closely to the cocaine peak.

In comparison of the chromatograms by GC-SID (Figs. 1 and 2) with those by GC-NPD (Figs. 5 and 6), the sensitivity by GC-SID is about 10 times higher than that by GC-NPD.

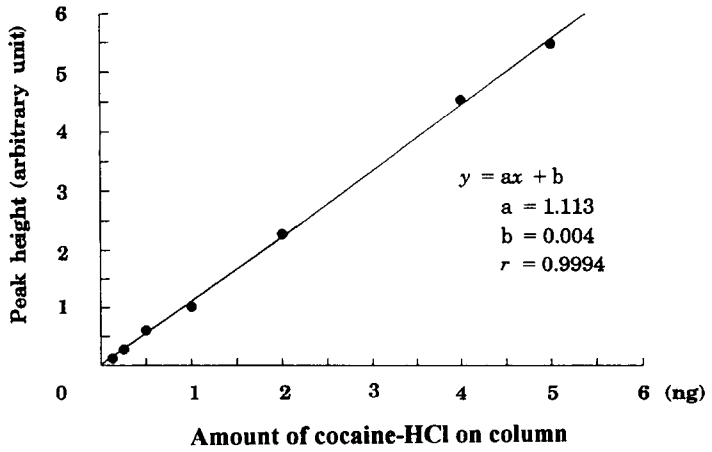

Fig. 3. Calibration curve for cocaine.

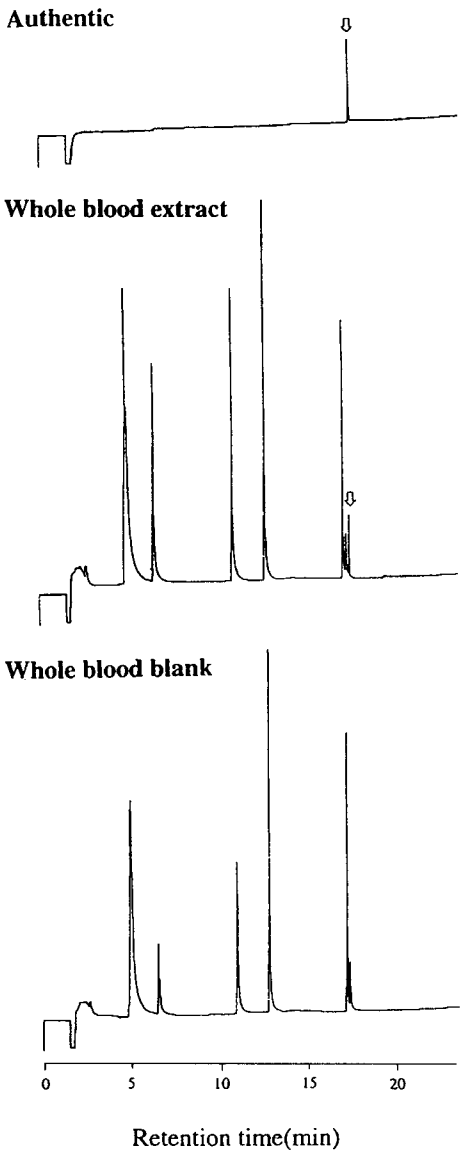

Fig. 4

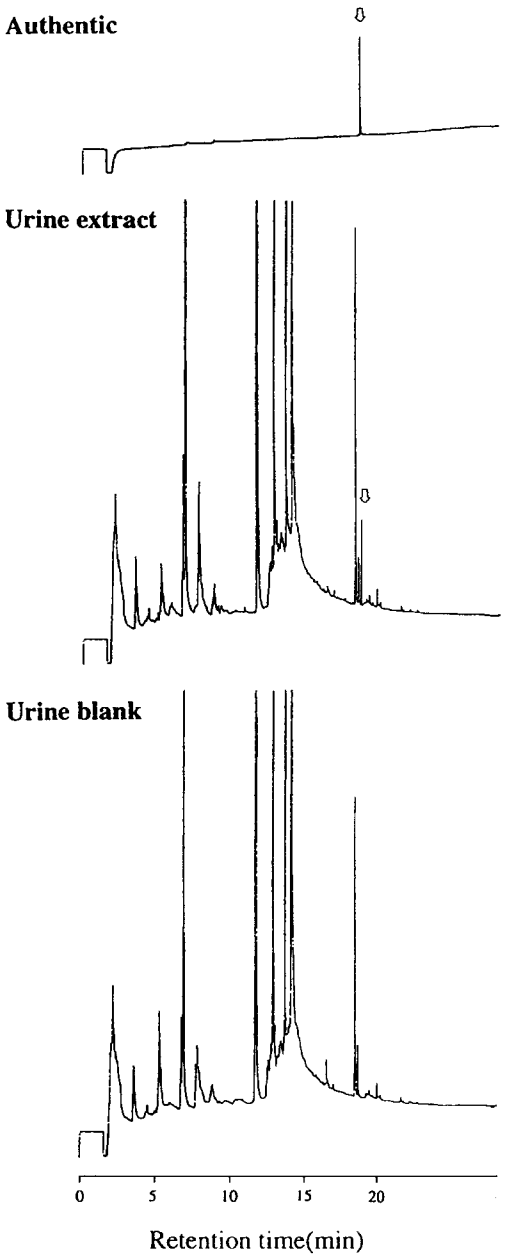

Fig. 5

Fig. 4. Capillary GC-NPD for extracts of 1 ml of a human whole blood sample, spiked and not spiked with 100 ng cocaine-HCl. The extraction was made with Bond Elut Certify columns. For GC conditions, see text. The arrows show the peaks of cocaine.

Fig. 5. Capillary GC-NPD for extracts of 2 ml of a human urine sample, spiked and not spiked with 100 ng cocaine-HCl. The arrows show the peaks of cocaine.

Discussion

This is the first report dealing with detection of cocaine by GC-SID. The high sensitivity obtained is probably due to the methyl amino group positioned outside the polygonal ring structure of cocaine as in the cases of dextromethorphan and dimemorphan [9].

We have used Bond Elut Certify columns for extraction of cocaine from whole blood and urine. There are three reports describing extraction of cocaine with Bond Elut Certify columns [6, 10, 11]. We have compared the present Bond Elut Certify column method with the Sep-Pak C_{18} cartridge method [8]. The Sep-Pak C_{18} could be also used for cocaine extraction because of satisfactory recovery of the drug, but backgrounds were cleaner with Bond Elut Certify than with Sep-Pak C_{18} (unpublished observation).

We have carefully compared gas chromatograms with SID (Figs. 1 and 2) with those with NPD (Figs. 4 and 5) by use of the same sample vials, and have found that the sensitivity by GC-SID is about 10 times higher than that by GC-NPD. The detection limit of the present GC-SID method was about 20 pg on column (0.5−1.0 ng/ml of a sample); the limits of the GC-NPD method reported in the literature were 20−100 ng/ml of a sample. Javaid et al. [4] reported that cocaine as low as 16 ng/ml plasma could be measured by GC-electron capture detection after derivatization with pentafluoropropionic anhydride.

The concentrations of unchanged cocaine in blood and urine of cocaine abusers are generally very low; peak concentrations of cocaine in human plasma after its intravenous, intranasal and oral administration were several ten to several hundred ng/ml [12−14]. Our method with detection limit of 0.5−1.0 ng/ml of a sample can sufficiently meet practical demand for analyses of cocaine in samples of abusers.

Only less than 10 % of total cocaine administrated is excreted into urine; the majority excreted into urine is benzoylecgonine and ecgonine methyl ester [15]. Trials to measure these cocaine metabolites by GC-SID are now in progress in our laboratories.

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