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原　　報

SENSITIVE DETECTION OF BENZAMIDES BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION AND THEIR RAPID CLEAN-UP FROM BODY FLUIDS

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表面電離ガスクロマトグラフィーによるベンザマイド系薬物の高感度検出と体液からの迅速抽出

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Summary

Six benzamides could be detected by gas chromatography with surface ionization detection (GC-SID) with high sensitivity. They were classified into two groups, a higher response group (Group I: tiapride, bromopride and metoclopramide) and a lower response group (Group II: sultopride, trimethobenzamide and nemonapride). The detection limits were 0.02 to 0.17 pmol on column (1 to 8.5 pmol/ml) in Group I drugs and 0.2 to 0.9 pmol on column (10 to 45 pmol/ml) in Group II drugs. Sep-Pak C_{18} cartridges were used for rapid extraction of benzamides from human whole blood and urine, and their recoveries were above 90 %.

Key words: Benzamides; Tiapride; Bromopride; Metoclopramide; Sultopride; Gas Chromatography (GC); Surface ionization detection; Sep-Pak C_{18} cartridge

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Introduction

Benzamide derivertives are widely used as antipsychotic, antiemetic or antispasmodic drugs. It is thus important to develop simple and sensitive methods for determination of these drugs.

In 1985, Fujii and Arimoto developed surface ionization detection (SID) for gas chromatography (GC) [1, 2], which can detect tertiary amines highly selectively and sensitively [3]. In this report, we demonstrate simple quantitative analysis of six benzamides with high sensitivity by GC-SID.

Experimental

Materials

Bromopride, metoclopramide-HCl and trimethobenzamide were purchased from Sigma Chemical Co. (St Louis, MO, USA). Nemonapride, sultopride-HCl and tiapride-HCl were kindly donated by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo), Mitsui Pharmaceuticals, Inc. (Tokyo) and Fujisawa Pharmaceutical Co., Ltd. (Osaka), respectively. Their chemical

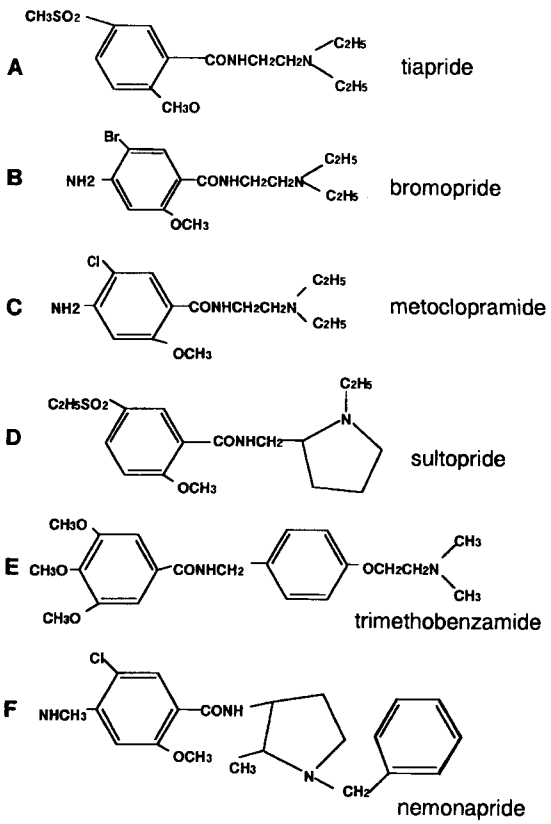

Fig. 1. Structures of six benzamide derivertives used in this study.

structures are shown in Fig. 1. Sep-Pak cartridges were purchased from Waters Associates (Milford, MA, USA) and a DB-1 fused silica capillary column (30 m×0.32 mm i.d., film thickness 0.25 μm) from J & W Scientific (Folsom, CA, USA). Other common chemicals used were of analytical grade. Whole blood and urine were objected from healthy subjects.

Extraction with Sep-Pak C_{18} cartridges

Solid phase extraction of benzamides from body fluids was based on the previous report [4]. Sep-Pak cartridges were pretreated by passing 10 ml of chloroform/methanol (8: 2), 10 ml of methanol and 10 ml of distilled water, and this procedure was repeated more than 3 times.

To 1 ml of whole blood or urine, with or without of the addition of bromopride, metoclopramide and tiapride (100 pmol each) for Group I, or nemonapride, sultopride and trimethobenzamide (1 nmol each) for Group II, were added 8 ml of distilled water and 1 ml of 1 M $NaHCO_3$ solution. The sample solutions were loaded on the pretreated Sep-Pak C_{18} cartridges at a rate not faster than 5 ml/min. Following washing with 20 ml of distilled water, the drugs were eluted with 3 ml of chloroform/methanol (8: 2). The organic layer containing the drugs was evaporated to dryness under the stream of nitrogen. The dried residue was dissolved in 100 μl of methanol and 2 μl of aliquot was subjected to GC-SID.

GC conditions

GC was carried out on a Shimadzu GC-14B gas chromatograph equipped with an SID system (Shimadzu Corp., Kyoto). The GC conditions were: column temperature, 150−300 ℃ (1 min hold at 150 ℃, 20 ℃/min from 150 to 250 ℃, 10 ℃/min from 250 to 300 ℃); injection temperature, 230 ℃ and helium flow rate, 3 ml/min. The SID conditions were: detection temperature, 280 ℃; heating current through platinum emitter, 2.2 A; emitter temperature, about 600 ℃; and ring electrode bias voltage, +200 V with respect ot 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

Table 1 shows retention times and relative intensities (the intensity of bromopride is taken as 100 %). We classified these six drugs into two groups (Group I and II) according to their SID responses.

Gas chromatograms by GC-SID for human urine and whole blood samples, in the presence or absence of six benzamides, are shown in Fig 2. In both Groups I and II, we did not find any impurity peaks which interfered with the drug peaks. Good linearities were obtained in the

Table 1. Retention times and relative intensities of six benzamides measured by GC–SID

Drug	Retention time (min)	Relative intensity[a] (%, mean±SEM, n = 6)
Group I		
A Tiapride	11.4	184±7.2
B Bromopride	12.0	100
C Metoclopramide	12.5	142±2.6
Group II		
D Sultopride	12.4	8.2±0.9
E Trimethobenzamide	13.6	17.3±1.9
F Nemonapride	15.2	6.6±0.7

[a] Bromopride=100%.

Table 2. Percent recoveries of six benzamides from blood or urine

Drug	% for blood (mean±SEM, n = 3)	% for urine (mean±SEM, n = 3)
Group I		
A Tiapride	95.7±7.7	97.3±12
B Bromopride	105±7.1	100±15
C Metoclopramide	102±7.0	101±14
Group II		
D Sultopride	95.3±2.1	100±13
E Trimethobenzamide	99.6±0.9	99.8±18
F Nemonapride	89.0±2.8	108±20

range of 5 to 200 pmol/ml (0.1 to 4 pmol on-column) for Group I, and 20 to 2000 pmol/ml (0.4 to 40 pmol on-column) for Group II. The detection limits ranged from 1 to 8.5 pmol/ml (0.02 to 0.17 pmol on-column) for Group I drugs and from 10 to 45 pmol/ml (0.2 to 0.9 pmol on column) for Group II.

Sep-Pak C_{18} cartridges were found quite useful for rapid extraction of benzamides. Table 2 shows their percent recoveries from blood or urine. They were not lower than 89 % for blood and 97 % for urine.

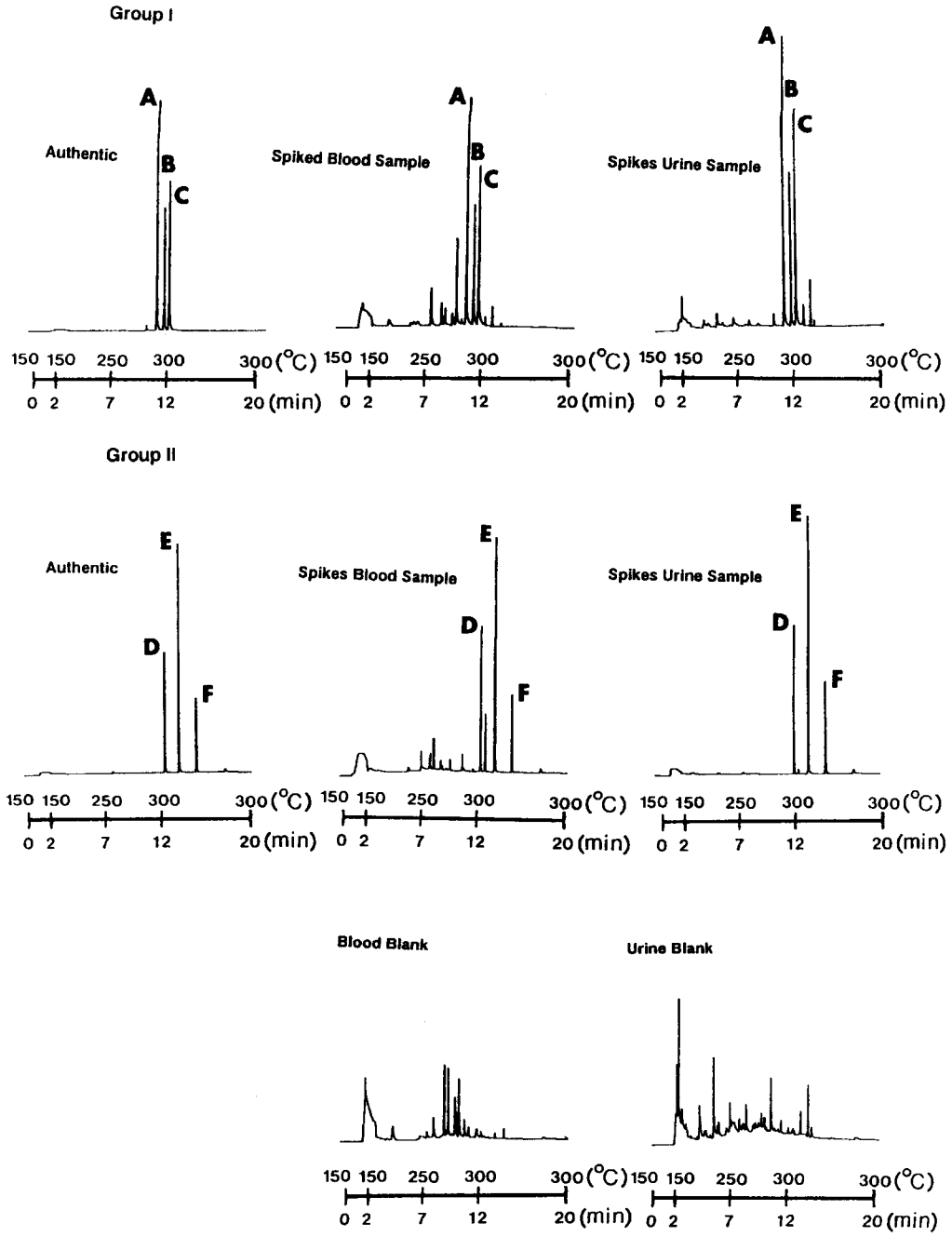

Fig. 2. GC-SID for the extracts of human blood and urine, spiked and not spiked with benzamides with the use Sep-Pak C_{18} cartridges for extraction.; A, tiapride; B, bromopride; C, metoclopramide; D, sultopride; E, trimethobenzamide; and F, nemonapride. The amounts of benzamides spiked to each 1 ml sample were 100 pmol for Group I and 1.0 nmol for Group II.

Discussion

In this study, we have been able to detect six benzamide derivertives with high sensitivity. In the previous reports, high-performance liquid chromatography (HPLC) [5−10], GC-flame ionization detection (FID) [11], GC-electron capture detection (ECD) [12−14] and GC-mass spectrometry (MS) [15] were used for the detection of the benzamides. The detection limits were reported to be 5 to 10 ng/ml for metoclopramide [8, 10], 2 ng/ml for bromopride [6] and 15 ng/ml for sultopride [7] by HPLC. They were around 10 ng/ml for metoclopramide [12, 14] by GC-ECD. The detection limits in this study are much lower than those in the above reports.

GC-SID study had been reported for tiapride and sultopride [16]; our study is the first trial for detection of other four benzamides by GC-SID. Moreover their detection limits for sultopride and tiapride were 4 ng and 200 pg respectively, which were larger than those in our report.

Pharmacokinetic studies of benzamides have been made by many researchers. For example, plasma concentration of bromopride 12 h after oral administration of its 20 mg was about 13 ng/ml [6]. Plasma concentration of sultopride was about 100 ng/ml 24 h after administration of 446 mg (intramuscular) or 376 mg (oral) [7]. Our method, therefore, gives enough sensitivity for such studies, and seems useful in forensic toxicology, clinical toxicology and clinical pharmacology.

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