



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

メタデータ	<p>言語: English</p> <p>出版者: 日本法中毒学会</p> <p>公開日: 2013-08-27</p> <p>キーワード (Ja):</p> <p>キーワード (En): Benzamides</p> <p>作成者: Ishii, Akira, Kumazawa, Takeshi, Seno, Hiroshi, Nishikawa, Masanobu, Watanabe, Kanako, Suzuki, Osamu</p> <p>メールアドレス:</p> <p>所属:</p>
URL	<p><a href="http://hdl.handle.net/10271/1702">http://hdl.handle.net/10271/1702</a></p>

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

Akira ISHII\*<sup>a</sup>, Takeshi KUMAZAWA<sup>b</sup>, Hiroshi SENO<sup>a</sup>, Masanobu NISHIKAWA<sup>a</sup>, Kanako WATANABE<sup>a</sup> and Osamu SUZUKI<sup>a</sup>

<sup>a</sup> *Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan*

<sup>b</sup> *Department of Legal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan*

*Received June 30, 1995*

*Accepted July 8, 1995*

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

石井 晃<sup>a</sup>, 熊澤武志<sup>b</sup>, 妹尾 洋<sup>a</sup>, 西川正信<sup>a</sup>, 渡部加奈子<sup>a</sup>, 鈴木 修<sup>a</sup>

<sup>a</sup> 浜松医科大学法医学教室 〒421-31 静岡県浜松市半田町3600番地

<sup>b</sup> 昭和大学医学部法医学教室 〒142 東京都品川区旗の台 1-5-8

### 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<sub>18</sub> 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<sub>18</sub> cartridge

\*Correspondence should be addressed to Akira Ishii.

©1995 Japanese Association of Forensic Toxicology. All rights reserved. 0915-9606/95/\$05.00

## Introduction

Benzamide derivatives 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

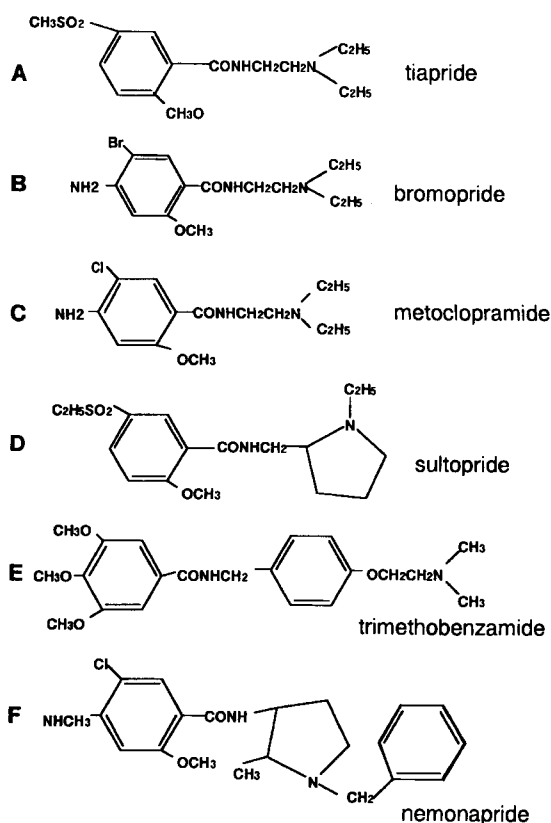


Fig. 1. Structures of six benzamide derivatives used in this study.

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  $\mu$ m) 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<sub>18</sub> 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<sub>3</sub> solution. The sample solutions were loaded on the pretreated Sep-Pak C<sub>18</sub> 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  $\mu$ l of methanol and 2  $\mu$ l 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  $^{\circ}$ C (1 min hold at 150  $^{\circ}$ C, 20  $^{\circ}$ C/min from 150 to 250  $^{\circ}$ C, 10  $^{\circ}$ C/min from 250 to 300  $^{\circ}$ C); injection temperature, 230  $^{\circ}$ C and helium flow rate, 3 ml/min. The SID conditions were: detection temperature, 280  $^{\circ}$ C; heating current through platinum emitter, 2.2 A; emitter temperature, about 600  $^{\circ}$ C; and ring electrode bias voltage, +200 V with respect of the collector electrode. The samples were injected in the splitless mode at a column temperature of 150  $^{\circ}$ C 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

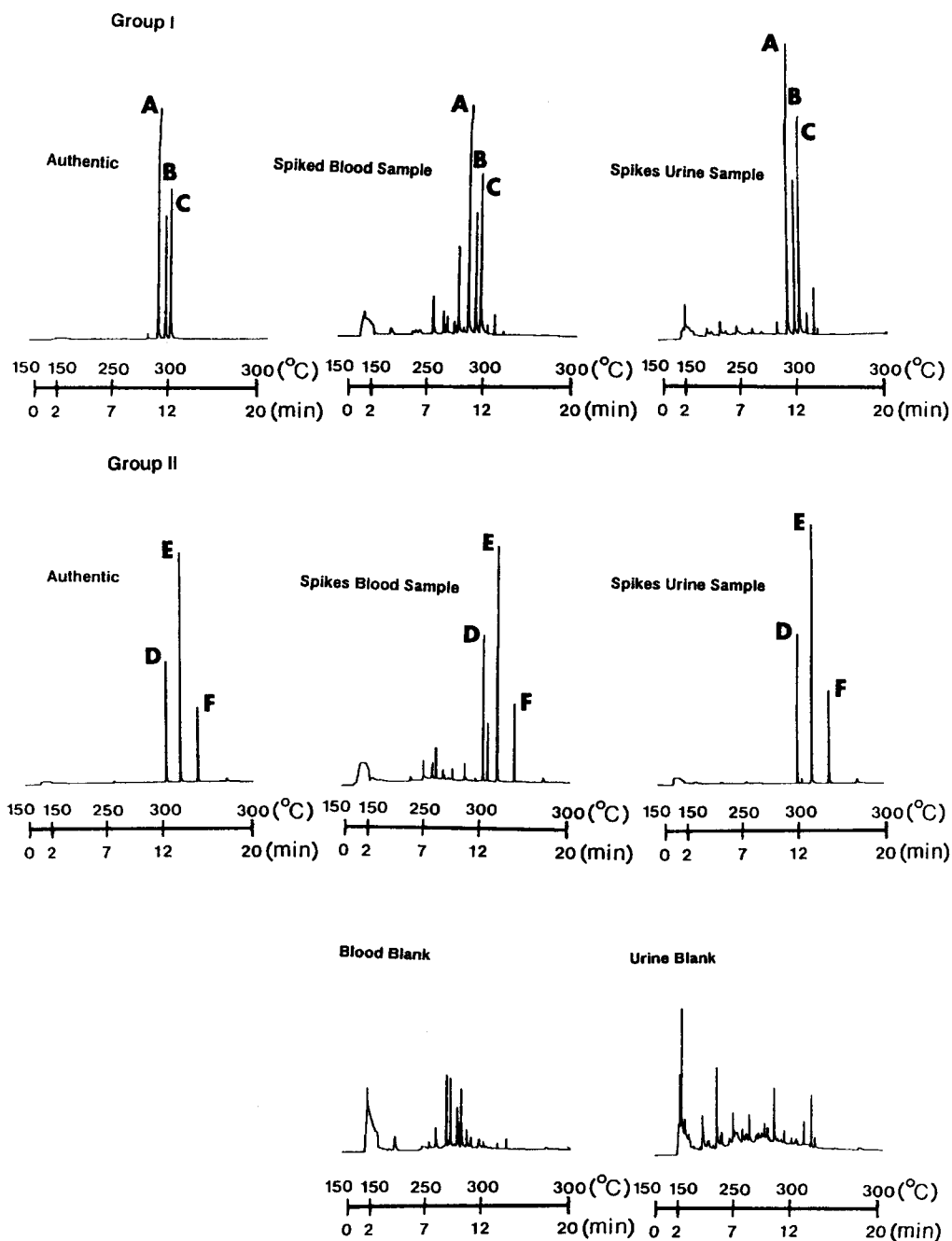
Drug	Retention time (min)	Relative intensity <sup>a</sup> (%, mean $\pm$ SEM, n = 6)
Group I		
A Tiapride	11.4	184 $\pm$ 7.2
B Bromopride	12.0	100
C Metoclopramide	12.5	142 $\pm$ 2.6
Group II		
D Sultopride	12.4	8.2 $\pm$ 0.9
E Trimethobenzamide	13.6	17.3 $\pm$ 1.9
F Nemonapride	15.2	6.6 $\pm$ 0.7

<sup>a</sup>Bromopride = 100%.**Table 2.** Percent recoveries of six benzamides from blood or urine

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

## Acknowledgment

This research was supported by the grant of Yokoyama Foundation.

## References

- 1) Fujii, T. and Arimoto, H.: New sensitive and selective detector for gas chromatography: surface ionization detector with a hot platinum emitter. *Anal Chem*, **57**, 2625–2628 (1985).
- 2) Fujii, T. and Arimoto, H.: The surface ionization detector. In Hill, H. and McMinn, D. G. (eds.), *Detectors for Capillary Chromatography*, Chemical Analysis Series, Vol. 121, John Wiley & Sons, Inc., Chichester, 1992, pp. 169–191.
- 3) Hattori, H., Yamada, T. and Suzuki, O.: Gas chromatography with surface ionization detection in forensic analysis. *J Chromatogr A*, **674**, 15–23 (1994).
- 4) Suzuki, O., Kumazawa, T., Seno, H. and Hattori, H.: Rapid isolation with Sep-Pak C<sub>18</sub> cartridges and wide-bore capillary gas chromatography of some barbiturates. *Med Sci Law*, **29**, 242–248 (1989).
- 5) Nishihara, K., Kohda, Y. and Tamura, Z.: Determination of sultopride in serum and saliva by high-performance liquid chromatography. *Chem Pharm Bull*, **31**, 4144–4146 (1983).

- 6) Brodie, R. R., Chasseaud, L. F. and Rooney, L.: Determination of bromopride in human plasma and urine by high-performance liquid chromatography. *J Chromatogr*, **310**, 353–360 (1984).
- 7) Bressolle, F. and Bres, J.: Dosage du sulpiride et du sultopride par chromatographie liquide à haute performance en vue de leur étude pharmacocinetique. *J Chromatogr*, **341**, 391–399 (1985).
- 8) De Jong, A.P., Wittebrood, A.J., Du Chatinier, W.M. and Bron, J.: Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction. *J Chromatogr*, **419**, 233–242 (1987).
- 9) Suleiman, M. S., Najib, N. M., El-Sayed, Y. M. and Badwan, A.: Stability-indicating high-performance liquid chromatographic assay for the determination of metoclopramide hydrochloride in pharmaceutical dosage forms. *Analyst*, **114**, 365–368 (1989).
- 10) Buss, D. C., Hutchings, A. D., Scott, S. and Routledge, P. A.: A rapid liquid chromatographic method for the determination of metoclopramide in human plasma. *Ther Drug Monit*, **12**, 293–296 (1990).
- 11) Anderson, W. H. and Archuleta, M. M.: The capillary gas chromatographic determination of trazodone in biological specimens. *J Anal Toxicol*, **8**, 217–219 (1984).
- 12) Tam, Y. K., Axelson, J. E. and Ongley, R.: Modification of metoclopramide GLC assay: Application to human biological specimens. *J Pharm Sci*, **68**, 1254–1256 (1979).
- 13) Ross-Lee, L. M., Eadie, M. J., Bochner, F., Hooper, W. D. and Tyrer, J. H.: Electron capture gas chromatographic assay for metoclopramide in plasma. *J Chromatogr*, **183**, 175–184 (1980).
- 14) Riggs, K. W., Axelson, J. E., Rurak, D. W., Hasman, D. A., McErlane, B., Bylsma-Howell, M., McMorland, G. H., Ongley, R. and Price, D. E.: Electron-capture determination of metoclopramide in biological fluids using fused silica capillary columns. Application to placental transport studies in sheep and human. *J Chromatogr*, **276**, 319–328 (1983).
- 15) Bateman, D. N., Kahn, C., Mashiter, K. and Davies, D. S.: Pharmacokinetic and concentration-effect studies with intravenous metoclopramide. *Br J Clin Pharmacol*, **6**, 401–407 (1978).
- 16) Kamizono, A., Inotsume, T., Arimoto, H. and Nakano, M.: Determination of sultopride and tiapride in serum by gas chromatography using a surface ionization detector. *J Chromatogr*, **567**, 113–120 (1991).