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# DETERMINATION OF THE α,β-ADRENOCEPTOR BLOCKER YM-09538 IN URINE BY GAS CHROMATOGRAPHY WITH A NITROGEN-SENSITIVE DETECTOR

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## SUMMARY

A gas chromatographic method for the quantitative determination of the α,β-adrenoceptor blocker YM-09538 in urine is described. YM-09538 was extracted from alkalinized urine with ethyl acetate and converted to its cyclic methylboronate derivative. Analysis by gas chromatography using a nitrogen-sensitive detector allowed quantitation of the drug over a concentration range of 0.2—5.0 μg/ml. Urinary excretion of YM-09538 was determined in humans after oral administration of 50 mg.

## INTRODUCTION

A combined α- and β-adrenoceptor blocker, 5-{1-hydroxy-2-[2-(*o*-methoxyphenoxy)ethylamino]ethyl}-2-methylbenzenesulphonamide hydrochloride (YM-09538), has been introduced as a dose-dependent antihypertensive agent [1,2]. Biopharmaceutical studies require estimation of urinary excretion of YM-09538. The concentration of this drug in plasma can be analyzed by high-performance liquid chromatography with fluorescence detection [3]. However, that method is not applicable to determine urinary YM-09538 since endogenous fluorescent materials in the urine interfere with the analysis; a gas chromatographic (GC) method was therefore investigated. YM-09538, being a

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multifunctional amino alcohol, requires derivatization of the reactive groups before it can be analyzed by GC. Aryl- or alkylboronic acids have been used as specific reagents for derivatization of various bifunctional amino alcohols for GC or gas chromatography—mass spectrometry (GC—MS) (for a review see ref. 4). Some attempts have been made to increase the sensitivity of the derivatives. Poole et al. [5,6] introduced electron-capturing groups into benzeneboronic acid and the reagents were used to analyze nanogram levels of β-blocking drugs in biological fluids by GC with electron-capture detection [7,8]. Recently, a series of β-blocking drugs in plasma was determined by GC with a nitrogen-sensitive detector after conversion to their *n*-butyl- or phenylboronic esters [9]. This present report describes a GC method of determining YM-09538 in urine using a nitrogen-sensitive detector and methylboronic acid as a reagent for derivatization.

## EXPERIMENTAL

### *Chemicals and reagents*

YM-09538 and 5-{1-hydroxy-2-[2-(*o*-methoxyphenoxy)ethylamino]ethyl} 2-methoxybenzenesulphonamide hydrochloride, used as the internal standard, were synthesized in our laboratory by the method of Imai et al. [1]. Methylboronic acid was purchased from Applied Science Labs. (State College, PA, U.S.A.) and *n*-butyl- and phenylboronic acid were from Tokyo Kasei (Tokyo, Japan).

The methylboronic acid solution was prepared by dissolving 3 mg of the boronic acid in 10 ml of ethyl acetate.

### *Instrumentation*

A Hewlett-Packard Model 5730A gas chromatograph equipped with a nitrogen-sensitive detector Model 18789A was used. The column was a glass tube (90 cm × 1.8 mm I.D.) packed with 2% OV-7 on Chromosorb W (AW DMCS, 80—100 mesh). The injection port, column oven and detector were maintained at 350°C, 290°C and 300°C, respectively. The helium carrier gas was dried over molecular sieves and passed at a flow-rate of 30 ml/min. Hydrogen and air flow-rates to the nitrogen-sensitive detector were 4 and 100 ml/min, respectively. Mass spectra of cyclic methylboronate esters of YM-09538 and the internal standard were obtained with a JMS D-300 mass spectrometer (JEOL, Tokyo, Japan) which was combined with a Hewlett-Packard Model 5710A gas chromatograph. The operating conditions were: accelerating voltage, 3.0 kV; ionizing potential, 70 eV; total emission current, 300 μA; separator and ion source temperature, 250°C.

### *Procedures*

To 1 ml of urine in a 10-ml glass stoppered centrifuge tube were added 2 ml of an aqueous solution containing 3 or 10 μg of the internal standard and then about 0.5 g of sodium hydrogen carbonate. The mixture was extracted with 4 ml of ethyl acetate and, after centrifugation, the ethyl acetate layer was evaporated under reduced pressure. The residue was dissolved in 100 μl of the methylboronic acid solution and, after 5 min, 6 μl of the solution were in-

jected into the GC column. Ratio of the peak height of YM-09538 to that of the internal standard was used to calculate the amount of YM-09538 by referring to a standard curve. The standard curve was generally prepared by analyzing drug-free control urine spiked with 1—5 μg of YM-09538 using 10 μg of the internal standard. When the concentration of the drug in urine samples was low, we used control urine spiked with 0.2—1.5 μg of YM-09538 and 3 μg of the internal standard.

*Human studies*

Three male volunteers, aged 28—33 years, received 50-mg YM-09538 tablets after overnight fasting. Urine was collected at 2-h intervals up to 12 h and then one fraction between 12 and 24 h. The urine samples were stored frozen until taken for assay.

The excretion rate was calculated by dividing the amount of YM-09538 excreted into urine by the period of collection time. The elimination half-life was demonstrated by least-squares analysis from the linear terminal portion of the curve plotted on semilogarithmic graph paper.

## RESULTS AND DISCUSSION

*Extraction of YM-09538 from urine*

YM-09538 can be considered to dissociate as in Fig. 1. The $pK_1$ value determined by potentiometry and the $pK_2$ value determined by UV spectrophotometry [10] at 25°C were 7.4 and 10.2, respectively. This result is a good explanation of the previous finding [3] that the compound was most efficiently extracted from plasma at pH 7.5—8.5. When control human urine containing 1 μg of YM-09538 was saturated with sodium hydrogen carbonate (pH was about 8.2) and extracted with ethyl acetate, extraction recovery determined by the GC method was 91.7 ± 3.4% ($n$=5).

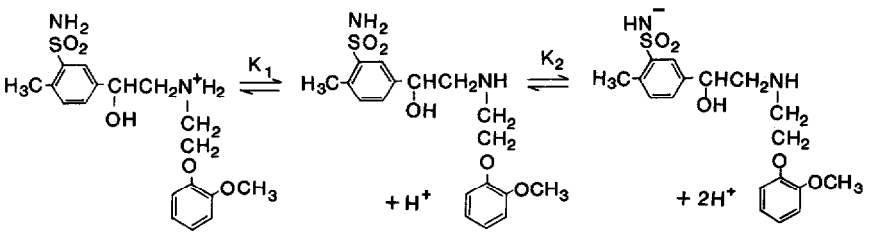


Fig. 1. Dissociation of YM-09538. $K_1$ and $K_2$ are dissociation constants for the secondary amino and sulphonamide groups, respectively.

*Gas chromatography of YM-09538*

YM-09538 has polar multifunctional groups which may render the drug unsatisfactory for GC analysis. Initial attempts to derivatize the compound with various silylating reagents were unsuccessful owing to incomplete silylation of the amino or sulphonamido group. For example, when N,O-bis-(trimethylsilyl)acetamide was used as a silylating reagent, a mixture of a major bis-trimethylsilyl (TMS) derivative with a minor tris-TMS derivative was obtained; the ratio of the tris-TMS derivative to the bis-TMS derivative increased with

the time course. After two days at room temperature, the product had changed to a mixture of tris-TMS and tetrakis-TMS derivatives.

To obtain a single derivative of YM-09538, aryl- or alkylboronic acids were very useful. Initially, the reaction of YM-09538 with the substituted boronic acids was studied by thin-layer chromatography (TLC) on silica gel 60 $F_{254}$ plates (Merck, Darmstadt, G.F.R.) with the solvent system chloroform—tetrahydrofuran—methanol—28% ammonia solution (20 : 20 : 4 : 0.05). When YM-09538 and the internal standard were admixed with methylboronic acid and examined by TLC, YM-09538 and the internal standard, detectable by short-wave UV light at $R_F$ 0.45 and 0.38, disappeared within 5 min at room temperature and was completely replaced by new compounds which appeared at $R_F$ 0.25 and 0.21, respectively. MS analysis showed these compounds to be cyclic methylboronate esters of YM-09538 and the internal standard (Fig. 2). When the reaction mixture was injected into a GC column (OV-7, 2%, oven temperature 290°C) and monitored by a flame ionization detector (FID), well-shaped single peaks were obtained at retention times of 1.9 and 2.6 min, respectively. However, the large solvent peak disturbed the analysis and the sensitivity was relatively low. In order to increase the sensitivity, we examined selected ion monitoring using the base peaks at *m/z* 281 and *m/z* 297. However, both YM-09538 and the internal standard derivatives gave broad peaks. This was probably due to the adsorption of these derivatives on the separator or ion source. In fact, the shape of the peaks improved when the temperature of the separator and ion source was increased (up to 310°C); it was still insufficient for analytical purposes. The most satisfactory results, in terms of sensitivity and peak shape, were obtained with a nitrogen-sensitive detector. The two compounds gave sharp peaks as with FID and the sensitivity with the nitrogen-sensitive detector was 10—15 times higher than with FID.

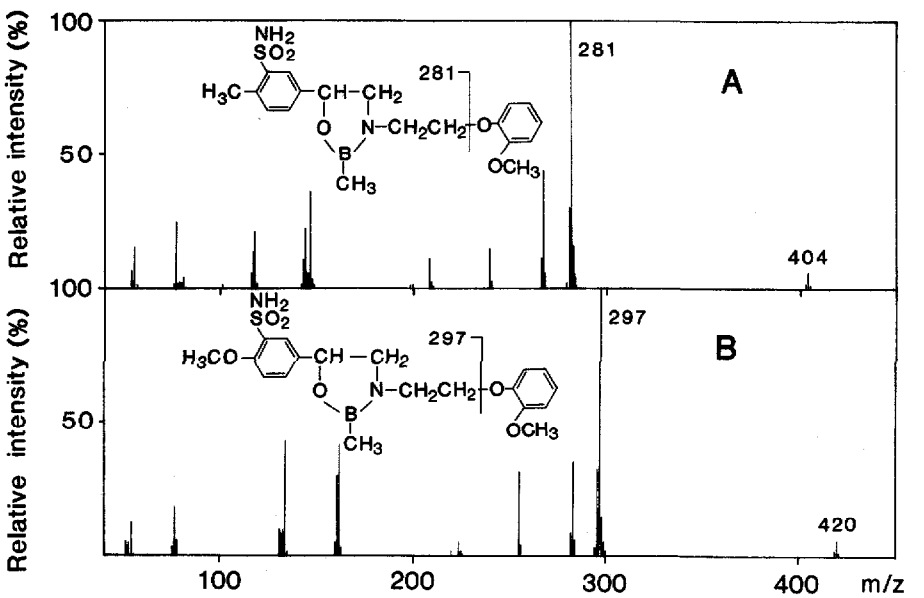


Fig. 2. Mass spectra of YM-09538 (A) and the internal standard (B) cyclic methylboronates.

TABLE I

RETENTION INDICES OF CYCLIC BORONATE ESTERS OF YM-09538 AND THE INTERNAL STANDARD

| Boronate | YM-09538 | The internal standard | $\Delta I$ |
|---|---|---|---|
| Methyl | 3628 | 3798 | 170 |
| Butyl | 3849 | 3993 | 144 |
| Phenyl | 4274 | 4417 | 143 |

Among various boronic acid commercially available, *n*-butylboronic acid has most frequently been used [4]. Brooks and Maclean [11] have reported the relative merits of methyl-, *n*-butyl-, phenyl- and cyclohexylboronate derivatives of mandelic and salicylic acids and noted the advantage of the *n*-butylboronate derivatives regarding their relatively low retention indices and their stability. Table I shows comparative retention indices of methyl-, *n*-butyl- and phenylboronate derivatives of YM-09538 and the internal standard. To determine urinary YM-09538, we selected methylboronic acid because it gave the lowest retention indices and complete separation of the peaks of YM-09538 and the internal standard. Since methylboronic derivatives were reportedly the least stable [11], the stability of the methylboronates of YM-09538 and the internal standard was studied. When the sample analyzed by GC was stored at room temperature, no significant difference in response was found after two days.

*Quantitative determination of YM-09538 in urine*

The chromatograms obtained from control human urine and the urine to which 5 μg of YM-09538 and 10 μg of the internal standard had been added are shown in Fig. 3. Drug-free control urine gave no interfering peaks and the sepa-

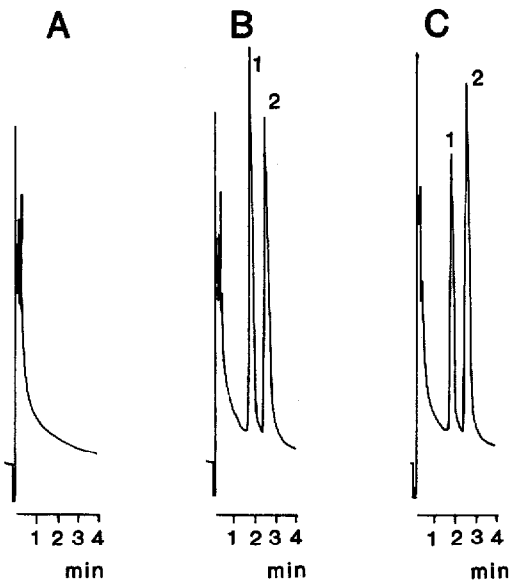


Fig. 3. Chromatograms of control human urine (A), human urine spiked with 5 μg of YM-09538 (B) and 0—2 h urine obtained from a volunteer who took 50 mg of YM-09538 orally (C). Peaks: 1, YM-09538; 2, internal standard.

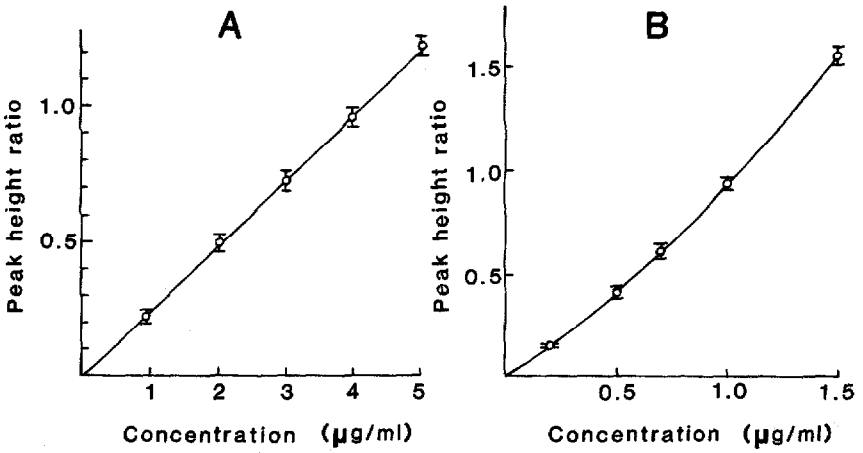


Fig. 4. Standard curves obtained from control human urine spiked with 1—5 μg of YM-09538 and 10 μg of the internal standard (A) and with 0.2—1.5 μg of YM-09538 and 3 μg of the internal standard (B). Each point represents the mean ± S.E.M. from four experiments.

ration of the two compounds added was complete. The standard curve prepared by subjecting control urine spiked with known amounts of YM-09538 (1—5 μg/ml) to the above procedure, after addition of 10 μg of the internal standard, is shown in Fig. 4A. A linear response was obtained for the drug over the concentration range examined; the equation of the line was $Y=0.244x - 0.012$ and the correlation coefficient was 0.984. For the analysis of the urine samples which contained a low concentration of YM-09538, we prepared another standard solution by adding known amounts of the drug (0.2—1.5 μg/ml) to control human urine. Fig. 4B shows the standard curve obtained by subjecting the standard solution to the above procedure using 3 μg of the internal standard. Although the graph did not show a linear response, the reproducibility at each concentration was good. For example, the intra- ($n$=4) and inter-assay ($n$=6) coefficients of variation at 0.5 μg/ml were 5.4% and 6.4%, respectively.

The sulphonamide function of YM-09538 and the internal standard has not been modified during the analysis. This polar function, which might cause adsorption of the derivative, was probably responsible for the standard curve being concave at low concentration as well as for extreme tailing of the peaks when the derivatives were analyzed by selected ion monitoring. If these problems are severe, further modification of the derivative, trimethylsilylation after reaction with alkylboronic acid [12], or some other derivatizations of sulphonamide [13] is necessary. However, the present method has good reproducibility as described above and offers high sensitivity obtained by introducing the nitrogen-sensitive detector. Because of its simplicity, this method is well suited for the routine analysis of a large number of samples.

*Application of the method*

Fig. 5 shows the excretion rate—time curve of YM-09538. The elimination half-life calculated from the data was 3.5 h and it agreed with the half-life of 3.5 h obtained from plasma concentration gained after oral administration of 50 mg of YM-09538 to humans [3].

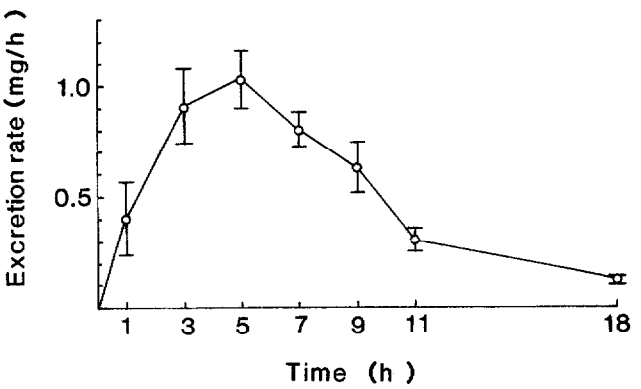


Fig. 5. Urinary excretion rate—time curve of the unchanged drug after oral administration of 50 mg of YM-09538 to humans. Each point represents the mean ± S.E.M. from three experiments.

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REFERENCES

1 K. Imai, K. Niigata, T. Fujikura, S. Hashimoto and T. Takenaka, Ger. Pat. Offen., 2,843,016; C.A., 91 (1979) 157445.
2 T. Takenaka, I. Miyazaki, K. Shiono and H. Maeno, 7th Scientific Meeting of the International Society of Hypertension, New Orleans, LA, U.S.A., May 11—14, 1980.
3 H. Kamimura, H. Sasaki and S. Kawamura, J. Chromatogr., 225 (1981) 115.
4 J.D. Nicholson, Analyst (London), 103 (1978) 1.
5 C.F. Poole, S. Singhawangcha and A. Zlatkis, Chromatographia, 11 (1978) 347.
6 C.F. Poole, S. Singhawangcha and A. Zlatkis, J. Chromatogr., 158 (1978) 33.
7 C.F. Poole, L. Johansson and J. Vessman, J. Chromatogr., 194 (1980) 365.
8 E. Sherwood, C.F. Poole, W.-F. Sye and A. Zlatkis, J. Chromatogr., 205 (1981) 297.
9 T. Yamaguchi, Y. Morimoto, Y. Sekine and M. Hashimoto, J. Chromatogr., 239 (1982) 609.
10 A. Albert and E.P. Serjeant, Ionization Constants of Acids and Bases, Methuen, London, 1962.
11 C.J.W. Brooks and I. Maclean, J. Chromatogr. Sci., 9 (1971) 18.
12 K.-J. Hoffmann, I. Skånberg and K.O. Borg, Xenobiotica, 9 (1979) 79.
13 O. Gyllenhaal and H. Ehrsson, J. Chromatogr., 107 (1975) 327.