the facile cleavage of the derivative to the fragment $(CH_3)_2\overset{+}{N}{=}CH{-}C_6H_4{-}OCOC_6F_5$. A fragment observed at $m/z$ 195 indicated the loss of a pentafluorobenzoyl moiety. The mass spectrum of the derivatized internal standard was similar.

GLC—The retention time of the derivatized drug and internal standard was 5.8 and 7.6 min, respectively. A representative chromatogram is shown in Fig. 1. No interfering peaks were observed in control (drug free) plasma.

The mean ratio of the peak height for derivatized ciramadol to derivatized internal standard and its coefficient of variation at each concentration of drug added to control plasma (three experiments) is shown in Table I. The coefficient of variation for each mean ratio ranged from 3.8 to 11.1%. Standard curves relating ciramadol concentration to peak height ratio were linear with correlation coefficients ranging from 0.9995 to 1.0000. With a 2-ml plasma sample, the minimum quantifiable concentration was 4 ng/ml (as free base).

## DISCUSSION

The results of the present study demonstrate that the specificity, linearity, precision, and sensitivity of the GLC assay for ciramadol is satisfactory for measuring the drug in plasma in concentrations >4 ng/ml. This method has been applied to samples obtained in studies on ciramadol disposition in rhesus monkeys, dogs, and humans. Urine specimens have also been analyzed by this procedure. An example of the application of the assay, which demonstrates its utility in a multiple dose study in dogs, is presented in Fig. 2. The assay permitted the observation that multiple dosing did not affect the metabolic disposition of ciramadol in dogs. No peaks interfered with the detection of drug or internal standard in samples obtained from other investigated species.

Other methods of analysis have been attempted but were not pursued for various reasons. These included GLC and HPLC analysis of underivatized ciramadol as well as other nonfluorinated derivatives of ciramadol. None proved to be as sensitive as the described procedure.

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# GLC–Mass Fragmentographic Determination of Mannitol and Sorbitol in Plasma


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**Abstract** □ A GLC–mass fragmentographic method was developed for the simultaneous determination of mannitol and sorbitol as their $n$-butyldiboronate derivatives in plasma. The plasma sample was deproteinized, and the subsequent supernatant was concentrated to dryness; the resulting residue was then dissolved in pyridine containing $n$-butylboronic acid to allow derivation. An aliquot of this solution was injected into the gas chromatograph–mass spectrometer and analyzed by a selected-ion monitoring method using galactitol as the internal standard. Detection was limited to 20 ng/0.1 ml of plasma for both mannitol and sorbitol. A rapid, precise, and sensitive assay for the determination of mannitol and sorbitol in plasma was established.



**Keyphrases** □ Mannitol—GLC–mass fragmentographic analysis in plasma □ Sorbitol—GLC–mass fragmentographic analysis in plasma □ GLC–mass fragmentography—analysis, mannitol and sorbitol in plasma □ Hexiol—mannitol and sorbitol in plasma, GLC–mass fragmentographic analysis


Mannitol and sorbitol (glucitol) have been used as artificial sweeteners in pharmaceutical preparations.

Many methods employing GLC with a flame ionization detector have been reported for the determination of common hexitols. Authentic samples of mannitol and sorbitol have been analyzed by this GLC method in studying the acetyl (1–7), trifluoroacetyl (8, 9), trimethylsilyl (10), phenyldiboronate (11), and $n$-butyldiboronate derivatives (12). However, the mannitol and sorbitol contents were determined (13) in pharmaceuticals as their $n$-butyldiboronate derivatives. Mannitol, sorbitol, and other polyols were assayed (14) in human plasma or cerebrospinal fluid as their acetyl derivatives. Furthermore, mannitol was measured (15) in body fluids as an $n$-butyldiboronate derivative.

However, these GLC methods indicate several problems in determining mannitol and sorbitol in plasma samples, such as poor sensitivity or the influence of glucose pooled in plasma. Thus, a GLC–mass fragmentographic determination of mannitol and sorbitol in plasma was examined, and a rapid, precise, and sensitive analytical method for their assay was established. The present method was also found applicable to other human biological fluids.

## EXPERIMENTAL

**Materials**—Mannitol[1], sorbitol[1], galactitol[1] (dulcitol), zinc sulfate[1], barium hydroxide[1], and other chemicals used were obtained commercially. $n$-Butylboronic acid[2] and pyridine[3] were derivating agents for $n$-butyldiboronate formation.

**GLC–Mass Fragmentographic Conditions**—A mass spectrometer[4] with an electron-impact ion source connected to a gas chromatograph[5] was used.

The coiled glass column (1 m × 2-mm i.d.) of the gas chromatograph was packed with 3% OV-17 on Chromosorb W-AW, 80–100 mesh[6] and conditioned at 280° for 24 hr. The temperatures of the injector, column,

[1] Wako Pure Chemical Co., Osaka, Japan.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] Pierce Chemical Co., Rockford, Ill.
[4] Model JMS-D 300, JEOL, Tokyo, Japan.
[5] Model JGC-20kP, JEOL, Tokyo, Japan.
[6] Gaschro Kogyo Co., Tokyo, Japan.


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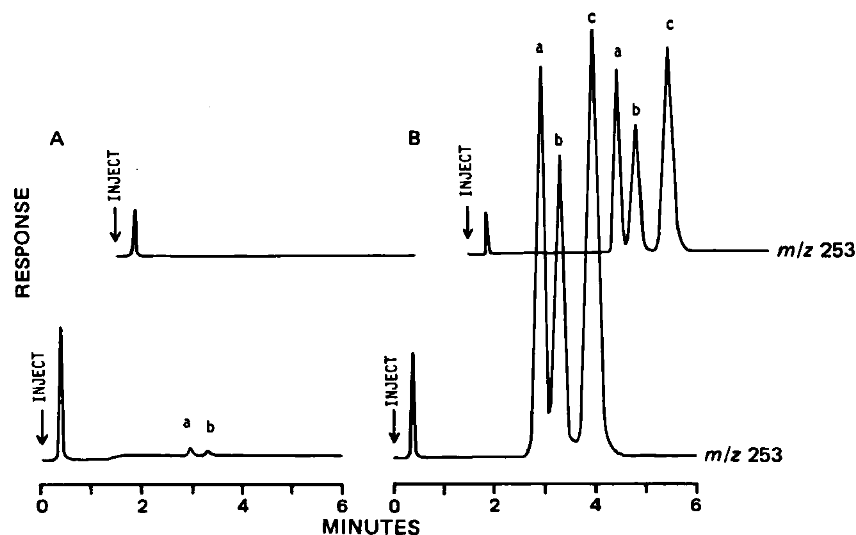


**Figure 1**—*GLC–mass fragmentograms of human control plasma (A) and human plasma prepared following addition of authentic samples at 500 ng/0.1 ml for each compound (B). Results are for the* n-*butyldiboronate derivatives and are recorded under two different attenuators. Key: (a) mannitol; (b) sorbitol; and (c) galactitol (internal standard).*

and ion source were 280, 270, and 250°, respectively. Helium was used as the carrier gas at a flow rate of 30 ml/min.

For mass fragmentography the mass spectrometer was set at the following conditions: ionization energy, 70 eV; ionization current, 300 $\mu$A; accelerating voltage, 3.0 kV; and ion multiplier voltage, 1.2–1.4 kV. The peak of $m/z$ 253 for each $n$-butyldiboronate derivative of mannitol, sorbitol, and the internal standard galactitol was selected for mass fragmentographic analysis. The chromatograms were recorded under four different attenuators (1, 2, 5, and $10 \times 10^2$).

The mass spectra of the $n$-butyldiboronate derivatives of mannitol, sorbitol, and galactitol were measured under the same GLC–MS conditions.

**Analytical Procedure**—Blood samples were collected in heparinized containers and centrifuged to separate the plasma. The plasma then was frozen until analysis.

The plasma (0.1 ml) was diluted with 1.0 ml of 0.13 $M$ $ZnSO_4$ and 0.1 ml of aqueous solution containing 100 ng of galactitol (internal standard) and mixed well. To this suspension 1.0 ml of 0.125 $M$ $Ba(OH)_2$ was then added, mixed again, and centrifuged at 2000$\times g$ for 15 min to remove the precipitates. The supernatant was concentrated to dryness under nitrogen gas at 50° or in a freezing desiccator. The residue was dried thoroughly over phosphorus *pentoxide* under reduced pressure and subjected to $n$-butyldiboronate derivation at room temperature for a few minutes by the addition of ~10–15 mg of $n$-butylboronic acid and 100 $\mu$l of pyridine. Analyses by GLC–mass fragmentography were carried out on 1–3-$\mu$l samples of the resultant solution.

**Calibration Curves**—Calibration curves were prepared by adding known amounts of mannitol and sorbitol to plasma and then assaying the mixture by the same extraction procedure.

The calibration curves for the determination of mannitol and sorbitol by GLC–mass fragmentography were obtained by plotting the ratio of the peak heights of the respective $n$-butyldiboronate derivatives to that of the $n$-butyldiboronate derivatives of the internal standard, galactitol, against the concentration of each compound. These calibration curves were linear.

**Table I—Recoveries of Mannitol and Sorbitol from Human Plasma**

| Drug Added, ng/0.1 ml | Recovery from Plasma[a], % Mannitol | Sorbitol |
|---|---|---|
| 50,000 | 93.4 | 93.0 |
| 10,000 | 92.8 | 93.1 |
| 5,000 | 93.4 | 92.9 |
| 1,000 | 92.7 | 93.4 |
| 500 | 93.4 | 93.2 |
| 100 | 93.5 | 93.1 |
| 50 | 92.9 | 92.9 |
| Mean ± *SD* | 93.1 ± 0.3 | 93.1 ± 0.2 |

[a] Each value is the mean of three determinations.

## RESULTS AND DISCUSSION

To obtain suitable derivatives of mannitol and sorbitol for GLC separation with a flame ionization detector, various derivation procedures were examined on the basis of previous reports (1–15). The silylation procedure enabled rapid progress, but this method did not provide a good chromatographic separation. Although the acetylation procedures gave a suitable chromatographic separation, it was somewhat time consuming. The $n$-butyldiboronate derivation procedure, however, was completed almost immediately, gave excellent chromatographic separation, and was capable of providing quantitative results as reported previously (12, 13, 15). Consequently, the derivation procedure of $n$-butyldiboronate was used in the present experiments.

Since the $n$-butyldiboronate derivatives of mannitol and sorbitol are poorly separated from those of glucose and fructose on a gas-liquid chromatograph, two methods for removing glucose and fructose from plasma were examined (12, 15). One method involved a complicated procedure by transforming the glucose and fructose to their phosphate derivatives (14); however, the excess phosphoric acid hindered the formation of the $n$-butyldiboronate derivative of mannitol and sorbitol. Another method using hydrogenation of glucose and fructose with sodium borohydride (15) or sodium amalgam converted glucose to sorbitol, while fructose produced equal amounts of mannitol and sorbitol. As these methods were not suitable to remove the glucose and fructose, these two sugars were not removed from the plasma samples.

Plasma samples containing glucose and fructose were deproteinized with zinc sulfate followed by the addition of barium hydroxide to remove the surplus zinc sulfate. The supernatant obtained by centrifugation without removing glucose and fructose was dried and subjected to the $n$-butyldiboronate derivation with $n$-butylboronic acid and pyridine. An aliquot of the resultant solution was then injected into the gas chromatograph–mass spectrometer.

Galactitol, a hexitol, was chosen as the internal standard for the simultaneous determination of mannitol and sorbitol by a selected-ion detection technique. This internal standard can also form an $n$-butyl-

**Table II—Changes in the Concentrations of the $n$-Butyldiboronate Derivatives of Mannitol and Sorbitol Prepared from Plasma at Various Conditions**

| Conditions | Concentration Changed[a], $\mu$g/ml Mannitol | Sorbitol |
|---|---|---|
| Control | 5.031 | 5.101 |
| Room temperature, 12 hr | 5.032 | 5.098 |
| Room temperature, 24 hr | 5.001 | 5.073 |
| Room temperature, 3 days | 4.980 | 5.050 |
| Room temperature, 7 days | 4.955 | 5.025 |
| 5°, 12 hr | 5.033 | 5.110 |
| 5°, 24 hr | 5.005 | 5.095 |
| 5°, 3 day | 5.000 | 5.060 |
| 5°, 7 day | 4.998 | 5.056 |

[a] Average of three determinations.

**Table III—Mannitol and Sorbitol Pooled in the Plasma of Healthy Males**

| Subject | Concentration in Plasma: Mannitol, μg/ml (nmole/ml) | Sorbitol, μg/ml (nmole/ml) |
|---|---|---|
| A | 1.385 (7.60) | 1.231 (6.76) |
| B | 1.096 (6.02) | 1.030 (5.65) |
| C | 0.741 (4.07) | 0.814 (4.47) |
| D | 0.872 (4.79) | 0.902 (4.95) |
| E | 0.793 (4.35) | 0.961 (5.27) |
| F | 0.720 (3.95) | 0.788 (4.33) |
| G | 1.055 (5.79) | 1.019 (5.59) |
| H | 1.098 (6.02) | 1.159 (6.36) |
| I | 0.546 (3.00) | 0.729 (4.00) |
| J | 0.614 (3.37) | 0.596 (3.27) |
| Mean ± *SD* | 0.892 ± 0.248 (4.90 ± 1.61) | 0.923 ± 0.186 (5.06 ± 1.01) |

diboronate derivative in the same way as mannitol and sorbitol (12), and the mass spectrum of this derivative is similar to that of the mannitol or sorbitol derivative. The mass fragment ion detected for GLC–mass fragmentography was the $m/z$ 253 ion $[C_4H_5O_4B_2(C_4H_9)_2]^+$, since no influence of glucose, fructose, or other plasma constituents could be observed. This $m/z$ 253 ion is a characteristic fragment ion formed from the molecular ion, $m/z$ 380 $[C_6H_8O_6B_3(C_4H_9)_3]^+$, of each compound by loss of $C_2H_3O_2BC_4H_9$ and is not observed in the mass spectra of glucose and fructose. The GLC–mass fragmentography monitoring molecular ions or base peaks, $m/z$ 127 $[C_2H_3O_2BC_4H_9]^+$, showed poor sensitivity and was mainly affected by glucose in certain samples.

To overcome the difficulties in the determination of samples containing high concentrations of mannitol and sorbitol, the ion multiplier voltage of the mass spectrometer was run at ranges from 1.2 to 1.4 kV, or the reactive solution was diluted. On the other hand, the method of simultaneous detection by a combination of GLC–mass fragmentography and GLC–mass spectrometry for total ion monitoring could not be adopted, since the latter method provided poor sensitivity, as was also the case for the GLC method with a flame ionization detector.

Known amounts of mannitol and sorbitol were added to human plasma, and the samples were analyzed. The chromatogram by GLC–mass fragmentography of the *n*-butyldiboronate derivatives of mannitol, sorbitol, and the internal standard, galactitol, prepared from human plasma following addition of authentic samples at 500 ng/0.1 ml for each compound and the chromatogram of the extract of human plasma control are shown in Fig. 1. The retention times of mannitol, sorbitol, and galactitol as their *n*-butyldiboronate derivatives were 3.0, 3.3, and 4.0 min, respectively, and the time required for the assay was ~5.0 min. As summarized in Table I, the recoveries from plasma were 93.1 ± 1.3% for mannitol and 93.2 ± 1.4% for sorbitol.

The detection limit for both mannitol and sorbitol using this GLC–mass fragmentographic method was 20 ng/0.1 ml for plasma. The reproducibility of this method was ±1.7–2.3%.

In addition, the following experiment was conducted. The *n*-butyldiboronate derivatives of mannitol, sorbitol, and galactitol prepared from human plasma were examined at room temperature and 5° under nitrogen gas for stability. As shown in Table II, it was found that no significant difference between the concentrations of the hexitol derivatives in the control and test samples could be observed over a period of several days.

The present method was also applied to other human biological fluids (*e.g.*, urine, cerebrospinal fluid, ascites fluid, bile, or lymph). The results obtained for the GLC–mass fragmentographic separations, recoveries, and sensitivities were in good agreement with those obtained with plasma. This method may also find application in the biological fluids of animals.

Table III shows the results obtained by the present GLC–mass fragmentography for the concentrations of mannitol and sorbitol present in the plasma of 10 healthy males. The quantitative results are the scope of this study.

The present method utilizing GLC–mass fragmentography for the simultaneous determination of mannitol and sorbitol in plasma is rapid and precise and has higher sensitivity than the GLC method with a flame ionization detector (1–15). Furthermore, small amounts of samples (0.1 ml) can be assayed.

Thus, this method should be useful or helpful for clinical profiling of various patients (*e.g.*, uremic, diabetic, or neurological) and for clinicopharmacological studies on therapy with mannitol, sorbitol, or mannitol plus fructose, which is an osmotherapeutic agent for treating increased intracranial pressure (16, 17) and urinary disorders (18).

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