# Short Communication

# Determination of methylboronic acid in teboroxime by capillary gas chromatography

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## ABSTRACT

A capillary gas chromatographic method has been developed to quantitate the methylboronic acid in a lyophilized formulation. The formulation is first dissolved in 1 $M$ hydrochloric acid. Next a tetrahydrofuran solution of the internal standard, 1-butaneboronic acid, and a methylene chloride solution of the derivatizing reagent, pinacol (2,3-dimethyl-2,3-butanediol), are added. The two-phase mixture is shaken for 3 h at 60°C during which time the methylboronic acid is extracted into the methylene chloride phase and reacted with pinacol to form the cyclic pinacol-boronate ester. The product formed in methylene chloride is injected in the split mode onto a capillary gas chromatographic system equipped with a flame ionization detector. Quantitation is achieved by an external standard method.

## INTRODUCTION

Methylboronic acid is an essential ingredient in [99mTc] teboroxime (CardioTec), a new technetium-99m based imaging product developed by Bristol-Myers Squibb for non-invasive diagnosis of myocardial infarction [1–3]. The imaging product is prepared *in situ* from a lyophilized formulation by the addition of a solution of pertechnetate containing 10 to 100 mCi of technetium-99m followed by heating at 100°C for 15 min. CardioTec is the methylboronic acid adduct of chloro, triscyclohexyl dioxime technetium and is formed from its member parts of methylboronic acid, cyclohexanedione dioxime, sodium chloride and technetium. In addition to these ingredients, the lyophilized formulation contains pentetic acid, $\gamma$-cyclodextrin, citric acid and stannous chloride. The methylboronic acid content in the formulation is 2.0 mg/vial. The specification allows the methylboronic content to vary between 1.7 and 2.3 mg/vial. The total content of all ingredients in the formulation is 165 mg/vial; thus, the methylboronic acid content is only 1.2% of the total formulation.

A capillary gas chromatographic (GC) method has been developed to quantitate the methylboronic acid in the lyophilized formulation. The sample preparation part of the method involves dissolving the formulation in 1 $M$ hydrochloric acid, adding a tetrahydrofuran solution of the internal standard, 1-butaneboronic acid, adding a methylene chloride solution of the derivatizing reagent, pinacol (2,3-dimethyl-2,3-butanediol), and shaking the two-phase mixture for 3 h at 60°C. The methylboronic acid is extracted into the methylene chloride phase and reacted with pinacol to form the cyclic pinacol-boronate ester (Fig. 1). The product formed is hydrolytically and chromatographically stable, and the excess pinacol does not interfere in the chromatography.

## EXPERIMENTAL

### Reagents and chemicals

Methylboronic acid was a characterized product obtained from Bristol-Myers Squibb Pharmaceutical Research Institute (Diagnostics Research and Development Department, Princeton, NJ, USA). Anhydrous pinacol (2,3-dimethyl-2,3-butanediol), 1-butaneboronic acid (gold label) and tetrahydrofuran (anhydrous, gold label) were obtained from Aldrich Chemical. Methylene chloride (HPLC grade) and hydrochloric acid (ACS grade) were obtained from Fisher Scientific. Silyl-8 and Sylon-CT were purchased from Pierce Chemical and Supelco, respectively.

A derivatizing solution was made by dissolving 4.0 g of pinacol in 500 ml of methylene chloride. A methylboronic acid standard stock solution was prepared by dissolving $20 \pm 2$ mg of methylboronic acid in tetrahydrofuran and diluting to 2.0 ml. An internal standard stock solution was prepared by dissolving $20 \pm 2$ mg of 1-butaneboronic acid in tetrahydrofuran and diluting to 2.0 ml.

### Working standard preparation

A 10-ml portion of the derivatizing solution was added to a 20-ml headspace vial (Hewlett-Packard, No. 9301-0716) containing 400 $\mu$l of 1 $M$ hydrochloric acid. Next, 300 $\mu$l of the methylboronic acid stock solution was added, followed by 200 $\mu$l of the internal standard stock solution. The vial was crimp-capped and placed into a heating block set at 60°C. The block was then placed on its side into a mechanical shaker. The shaking and heating was allowed to proceed for three hours. After cooling, a



| Compound | R |
| --- | --- |
| Methylboronic acid *(I)* | CH$_3$ |
| 1-Butaneboronic acid *(II)* | CH$_3$ (CH$_2$)$_3$ |
| Pinacol *(III)* | – |
| Pinacol-boronate Ester *(IV)* | – |

Fig. 1. Reaction of the alkylboronic acids with pinacol.

portion of the lower methylene chloride layer was transferred to fill an autoinjector vial. A 1.0-$\mu$l portion of this solution was then injected into the GC system described below.

### Sample preparation

To the crimp-capped vial containing the lyophilized sample powder, 400 $\mu$l of 1 $M$ hydrochloric acid was added and the sample was dissolved. Next, 200 $\mu$l of the internal standard stock solution and 300 $\mu$l of tetrahydrofuran were added and mixed carefully, allowing the vial to vent by inserting a syringe without plunger through the rubber septum. The rubber septum was removed and the vial contents were then transferred to a 20-ml headspace vial. After adding 10 ml of the derivatizing solution, the vial was crimp-capped and then treated as described under *Working standard preparation*.

### Blank preparation

A 10-ml portion of the derivatizing solution was added to a 20-ml headspace vial containing 400 $\mu$l of 1 $M$ hydrochloric acid and 300 $\mu$l of tetrahydrofuran. The vial was crimp capped and then treated as described under *Working standard preparation*.

### Gas chromatography

A Hewlett-Packard 5890 capillary gas chromatograph, equipped with a split/splitless injection port, a flame ionization detector and 7673A autosampler injector, was used. The fused-silica capillary column used was a HP-5 (5% diphenyl and 95% dimethylpolysiloxane, Hewlett-Packard), 25 m × 0.32 mm I.D. and 1.0 $\mu$m stationary phase film thickness. The oven temperature was maintained at 120°C for 10 min and then ballistically programmed at 70°C/min to 210°C and held there for 4 min. The injector and detector temperatures were maintained at 200°C and 290°C, respectively. Injection was carried out in the split mode, with a split flow of 50 ml/min. The split port liner was a 4 mm I.D. open tube packed with a short glass wool plug (Hewlett-Packard, No. 19251-60540) and Sylon-CT treated after packing. The helium carrier gas head pressure was maintained at 69 kPa (10 p.s.i.g.) and the flowrate of the helium make up gas for the flame ionization detector was 30 ml/min. The GC sensitivity was set at a range of $2^2$ and attenuation of $2^2$.
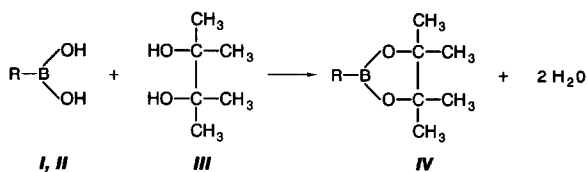
The analysis was carried out by injecting 1.0 μl of the blank, working standard and sample preparations. The preparations are stable at room temperature in capped autoinjector vials for at least 24 h. It is important to condition the column and deactivate the inlet with Silyl-8. This is performed each time the column or the injection liner is changed. The conditioning is performed by setting the oven temperature to 200°C and column head pressure to 103 kPa (15 p.s.i.g) and injecting 5 μl of Silyl-8 with the split off. The signal is allowed to return to its normal level before lowering the oven temperature and head pressure.

## Quantitation

For quantitation the following equation was used:

$$\text{mg of methylboronic acid per vial} = \frac{C_s R_u}{R_s}$$

Where $C_s$ = mg of methylboronic acid in the working standard preparation vial; $R_u$ = area ratio of methylboronic acid to the internal standard in the sample chromatogram; $R_s$ = area ratio of methylboronic acid to the internal standard in the standard chromatogram.

## RESULTS AND DISCUSSION

Organic boronic acids have been used for the analysis of bifunctional compounds by GC or GC–mass spectrometry (MS) [4–13]. Alkaneboronic acids react with a variety of 1,2- and 1,3-diols, diamines and aminoalcohols to yield five- or six-membered cyclic boronates. On the other hand, characterization of alkaneboronic acids is difficult due to the facile conversion of the acid to the trimeric cyclic anhydride under mild conditions [7,8]. Thus, to determine methylboronic acid in the formulation by GC, a derivatizing reagent was required that would react quantitatively and give a volatile, thermally and hydrolytically stable product with good chromatographic properties. Ethanolamine and diethanolamine derivatives are chromatographically unsuitable while trimethylsilyl esters and alkyl esters are hydrolytically unstable [6]. The method presented in this paper utilizes the reaction (Fig. 1) of methylboronic acid with pinacol, a 1,2-diol, as the basis for the quantification of methylboronic acid in

TABLE I

EFFECT OF TIME ON THE EXTRACTION OF METHYLBORONIC ACID FROM THE FORMULATION AND FORMATION OF THE DERIVATIVE

In this experiment, 1.0 $M$ hydrochloric acid was used instead of 400 μl of 1 $M$ hydrochloric acid used under *Sample preparation*.

| Time (min) | Area ratio[a] | Time (min) | Area ratio[a] |
|---|---|---|---|
| 5 | 0.499 | 90 | 1.311 |
| 15 | 0.902 | 105 | 1.372 |
| 25 | 1.093 | 120 | 1.410 |
| 35 | 1.216 | 150 | 1.450 |
| 45 | 1.325 | 180 | 1.466 |
| 60 | 1.315 | 360 | 1.470 |
| 75 | 1.326 | | |

[a] Area ratio = the area of the methylboronic acid to that of the internal standard.

a formulation that contains several other components.

The addition of hydrochloric acid as described under *Sample preparation* is imperative. Without the acid, (water only), methylboronic acid was only 20% recovered after 3 h at 60°C. The recovery did not improve after allowing the reaction to proceed overnight at room temperature. Table I illustrates the effect of time on the extraction and subsequent derivatization in the presence of 0.1 $M$ hydrochloric acid. The recovery of methylboronic acid was complete after 3 h. At room temperature the reaction went to completion in approximately 20 h. The use of a smaller volume (400 μl) of a more concentrated (1 $M$) hydrochloric acid gave a more reproducible recovery than the 0.1 $M$ hydrochloric acid. An added benefit to the use of the stronger acid was that the extraction mixture looked cleaner and, in fact, after the heating period, the two phases were nearly indiscernible. It is believed that this strongly acidic reaction condition causes the hydrolysis of cyclodextrin to maltosaccharides and glucose, eliminating the possibility of cyclodextrin-methylboronic acid inclusion complex formation [14].

It is important to use fresh tetrahydrofuran of high purity in the preparation of the standard stock solutions. Low-quality tetrahydrofuran can cause the blank to show a large number of peaks, some of which could interfere with the 1-butaneboronic acid derivative. It is advisable that the tetrahydrofuran

be purchased in small bottles sealed with PTFE-lined rubber septa and screw caps. The small volumes reduce the possibility of oxidation of the tetrahydrofuran and make purging of the bottles with nitrogen after use more practical. Protic solvents such as methanol could not be used because they react with boronic acids. Dimethoxypropane, which is commonly used as a water scavenger for moisture sensitive compounds, gives a peak that interferes with the methyl boronic acid derivative. Dimethoxypropane also forms methanol as a result of its reaction with water and the methanol is likely to react with methylboronic acid. Acetone could not be used because of incomplete solubility of 1-butaneboronic acid.

The accuracy of the method was established by analyzing portions of a placebo formulation (*i.e.* the formulation without methylboronic acid) spiked with varying amounts of methylboronic acid. As shown in Table II, added methylboronic acid was quantitatively recovered. The table also shows that the precision of replicate preparations is excellent. As shown in Table III for two levels of spiking, excellent precision was obtained for replicate injections of the same sample preparation.

Figs. 2–4 show typical chromatograms for a blank preparation (without the internal standard), working standard preparation and a typical sample preparation respectively. The retention times for methylboronic acid derivative, pinacol and 1-butaneboronic acid derivative are 3.3, 4.1 and 10.5 min, respectively.

TABLE II

RECOVERY OF METHYLBORONIC ACID FROM THE FORMULATION

The two values shown for each level of spiking represent replicate preparations, as each level was spiked in duplicate.

| Added (mg/vial) | Found (mg/vial) | Recovered (%) |
|---|---|---|
| 1.00, 1.00 | 1.06, 1.06 | 106.0, 106.0 |
| 2.00, 2.00 | 2.03, 1.99 | 101.5, 99.5 |
| 3.00, 3.00 | 3.05, 2.97 | 101.7, 99.0 |
| 4.00, 4.00 | 4.06, 4.08 | 101.5, 102.0 |
| 5.00, 5.00 | 5.00, 5.01 | 100.0, 100.2 |

TABLE III

REPRODUCIBILITY OF REPLICATE INJECTIONS AT TWO LEVELS OF SPIKING

| Replicate No. | mg/vial found for level 1 | mg/vial found for level 2 |
|---|---|---|
| 1 | 2.00 | 4.03 |
| 2 | 2.01 | 3.97 |
| 3 | 2.01 | 3.93 |
| 4 | 2.01 | 3.93 |
| 5 | 2.01 | 3.91 |
| Mean | 2.01 | 3.95 |
| R.S.D.[a] | 0.2% | 1.2% |

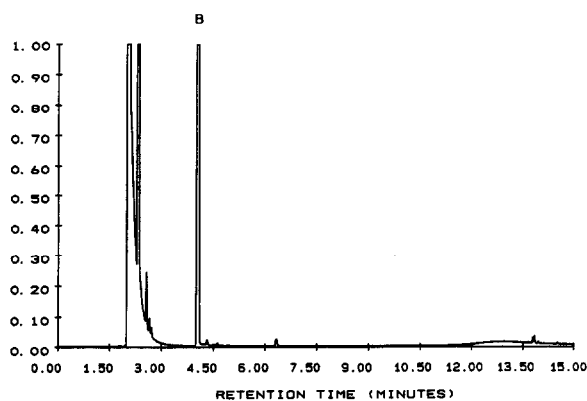[a] R.S.D. = relative standard deviation.



Fig. 2. A chromatogram of a blank preparation without the internal standard. Peak B is due to pinacol.
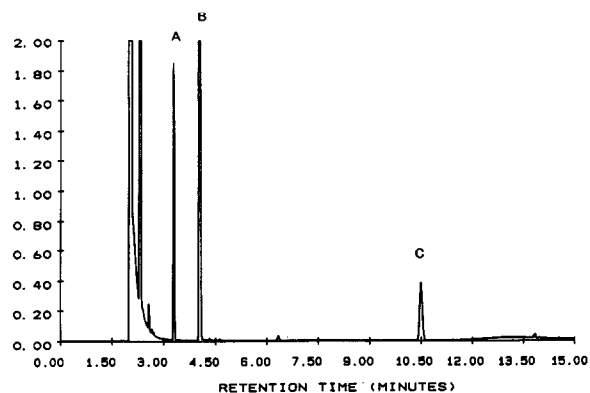


Fig. 3. A chromatogram of a working standard preparation. Peaks A, B and C are due to the methylboronic acid derivative, pinacol and 1-butaneboronic acid derivative, respectively.
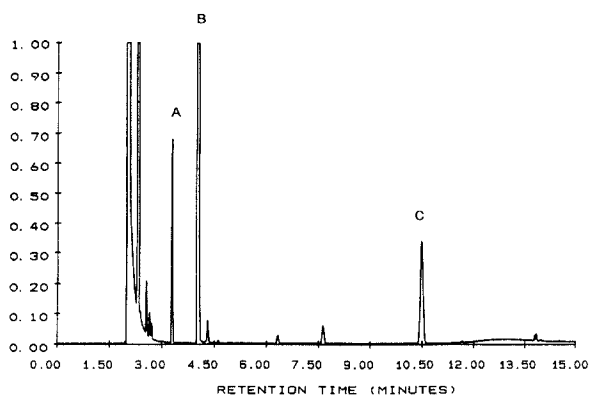
Fig. 4. A chromatogram of a typical sample preparation. Peaks A, B and C are due to the methylboronic acid derivative, pinacol and 1-butaneboronic acid derivative, respectively.

## ACKNOWLEDGEMENT

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