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THE DETERMINATION OF BIFUNCTIONAL COMPOUNDS

IX*. A SELECTIVE REACTION FOR THE DETERMINATION OF GUAIFENESIN IN PLASMA BY GAS CHROMATOGRAPHY

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SUMMARY

Guaifenesin after extraction from plasma with an organic solvent can be selectively derivatized with 2,4-dichlorobenzeneboronic acid and determined by gas chromatography with electron-capture detection. The detection limit for guaifenesin was 15 ng ml^{-1} for a 2.0-ml plasma sample. The mass spectra of the boronate derivatives of guaifenesin and mephenesin, used as internal standard, show good molecular ions with characteristic modes of fragmentation useful for their identification.

INTRODUCTION

Guaifenesin [guaiacol glyceryl ether, 3-(*o*-methoxyphenoxy)-1,2-propanediol] is a widely used expectorant and demulcent in many asthma- and cough-preventive medicines. Other uses are as a muscle relaxant [1], as a hypocholesteremic reagent [2,3], as an agent for reducing platelet adhesiveness [4] and as a general equine anesthetic [5—7].

Guaifenesin has been determined in pharmaceutical formulations by direct current polarography after nitration [8], by a colorimetric reaction with formaldehyde in sulfuric acid—methanol [9], by gas chromatography (GC) without derivatization [10] and as its acetate [11] and trimethylsilyl ether [12] derivatives and by high-performance liquid chromatography [13,14]. In cough syrups these methods provide adequate detection limits for the relatively high concentrations (e.g. 20 mg ml^{-1}) of drug in the formulations but lack the necessary sensitivity required to determine trace levels in plasma. The

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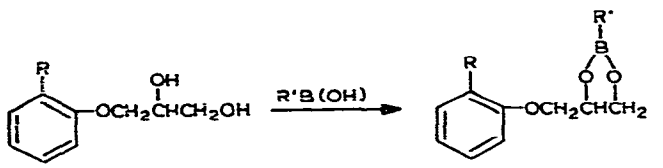
blood levels of guaifenesin in dogs [15] and rabbits [16] has been studied at rather high dose levels using relatively non-selective spectroscopic methods for the analysis. Maynard and Bruce [17] have described a sensitive method for the determination of guaifenesin in plasma by solvent extraction and derivatization of one of the hydroxyl groups of the propanediol side chain with heptafluorobutyric anhydride for analysis by GC with electron-capture detection. This method provided adequate sensitivity for monitoring the fate of the drug (plasma half-life 1.00 h) in volunteers given oral doses of 600 mg. However, the non-specific nature of the derivatization reaction produces many detected peaks in the plasma chromatogram often making quantitation difficult. Also, the anhydride must be freshly purified to avoid extraneous peaks, all traces of moisture must be absent during the derivatization reaction and all traces of the reagent must be removed prior to injection. The presence of excess reagent in the solution injected into the gas chromatograph causes a large disruption of the detector baseline in the region of the chromatogram where guaifenesin elutes.

One approach to simplifying the analysis of guaifenesin in biological fluids would be to take advantage of the bifunctional nature of the molecule and employ a chemically specific reagent to carry out the derivatization reaction. The boronic acids are suitable for this purpose as they form thermally stable derivatives with diols but do not produce derivatives stable to GC with monofunctional compounds [18]. Also if boronic acids containing electron-capturing groups are employed then advantage can be taken of the high sensitivity and selectivity of the electron-capture detector [19—24]. Thus by a combination of reagent specificity, chromatographic resolving power and detector selectivity it should prove possible to determine guaifenesin at therapeutic levels in a complex biological fluid like plasma, employing the minimum number of sample isolation and purification steps.

The method for the analysis of guaifenesin described in this paper uses mephenesin as internal standard and a simple extraction from plasma into an organic solvent with selective derivatization of the drug in the extract prior to gas chromatography with electron-capture detection.



R = OCH_3, guaifenesin
R = CH_3, mephenesin

R' = 2,4- dichlorobenzene
R' = 3,5-bis(trifluoromethyl) benzene
R' = 4-bromobenzene
R' = 4-iodobutane

EXPERIMENTAL

Reagents

Guaifenesin and mephenesin were obtained from Ciba-Geigy (Basle, Switzerland). 2,4-Dichlorobenzeneboronic acid, 3,5-bis(trifluoromethyl)benzenobo-

ronic acid and 4-iodobutaneboronic acid were obtained from Lancaster Synthesis (St. Leonard Gate, Lancaster, Great Britain) or from the Alfa Products Division, Ventron Corp. (Danvers, MA, U.S.A.). 4-Bromobenzeneboronic acid was obtained from Aldrich (Milwaukee, WI, U.S.A.). Ethyl acetate and hexane were of nanograde quality and dichloromethane was certified ACS grade.

Standard solutions

Guaifenesin. Guaifenesin (25.00 mg) was dissolved in 50.00 ml of distilled water; 100 μl of this solution was diluted to 50.00 ml with water to give a final concentration of 5 μmole l^{-1}.

Mephenesin. Mephenesin (25.00 mg) was dissolved in 50.00 ml of distilled water; 185 μl of this solution was diluted to 10.00 ml with water to give a final concentration of 50 μmol l^{-1}.

2,4-Dichlorobenzeneboronic acid. 2,4-Dichlorobenzeneboronic acid (25.00 mg) was dissolved in 50.00 ml of ethyl acetate; 1.00 ml of this solution was diluted to 25.00 ml with ethyl acetate to give a final concentration of 105 μmole l^{-1}.

Buffer solution pH 6.5. The buffer solution was prepared by mixing 100.00 ml of 0.3 *M* Na_2HPO_4 with 29.00 ml of 1.0 *M* NaH_2PO_4.

Plasma samples. Plasma samples were obtained from the Texas Medical Center Blood Bank and fortified by making standard additions of the dilute aqueous guaifenesin standard. The plasma-containing drug samples were then divided into 2.00-ml aliquots and frozen at —20°C until used.

Extraction and derivatization

To a thawed 2.00-ml plasma sample in a 13.0 × 1.5 cm I.D. Pyrex PTFE-lined screw-capped culture tube was added 50 μl of internal standard (mephenesin, 50 μmole l^{-1}), 0.20 ml of phosphate buffer (pH 6.5) and 10.00 ml of hexane—dichloromethane (4:1). The tube was shaken mechanically for 10 min and centrifuged. A 5.00-ml aliquot of the organic phase was withdrawn by pipette and evaporated to a residue with a stream of nitrogen at approximately 50°C in a conical tipped culture tube. To the residue was added 1.00 ml of the 2,4-dichlorobenzeneboronic acid solution, the solution mixed thoroughly and the capped tube heated for approximately 5.0 min at 50°C. The solution was evaporated to dryness with nitrogen, 200 μl of hexane added and after mixing 1.0—2.0 μl was injected into the gas chromatograph.

Gas chromatography

For GC a Varian 3700 gas chromatograph equipped with a flame ionization and a pulse-modulated constant-current ^{63}Ni electron-capture detector was used. For analysis a 1.5 m × 0.2 cm I.D. glass column packed with 3% OV-17 on Gas-Chrom Q (100—120 mesh) and a nitrogen flow-rate of 30 ml min^{-1} was used. The oven temperature was 240°C isothermal, the injection temperature 270°C and the electron-capture detector temperature 350°C. For the calculation of Kovats indices a 100 cm × 0.2 cm I.D. nickel column packed with 3% SE-30 on Gas-Chrom Q (100—120 mesh) at 140°C and a nitrogen flow-rate of 60 ml min^{-1} was used.

For gas chromatography—mass spectrometry (GC—MS), a Hewlett-Packard

5992A mass spectrometer equipped with a single-stage glass-jet separator and a 6 ft. × 0.4 cm I.D. glass column packed with 3% OV-1 on Gas-Chrom Q (100—120 mesh) and operated with a helium flow-rate of 30 ml min^{-1} was used. Electron-impact mass spectra were recorded at an ionization potential of 70 eV.

RESULTS AND DISCUSSION

The solvent system hexane—dichloromethane (4:1) was selected for the extraction of guaifenesin and mephenesin as it is efficient while at the same time minimizing the amount of co-extractants and water which interfere in the chromatographic step. Guaifenesin and mephenesin are quantitatively derivatized by reaction with a slight excess of the boronic acid in ethyl acetate for either 15 min at room temperature or 5 min at 50°C. The Kovats indices for the boronate derivatives are given in Table I. All derivatives have good peak shape on GC and are stable in solution for at least two days at room temperature. The identity of the derivatives was confirmed by GC—MS. The electron-impact mass spectra of the 2,4-dichlorobenzeneboronate of guaifenesin (Fig. 1) and mephenesin (Fig. 2) are typical of all the derivatives studied. Characteristic modes of fragmentation are observed in all mass spectra with a prominent molecular ion and a base peak at *m/e* 108 for mephenesin and *m/e* 124 for guaifenesin (Fig. 3).

The plasma extracts show several peaks on GC in the area of the chromatogram where guaifenesin and mephenesin boronate derivatives elute. The exception is the 2,4-dichlorobenzeneboronate derivatives which elute in a relatively empty region of the chromatogram and are not interfered with. Fig. 4 shows a chromatogram for a plasma blank and a plasma sample containing approxi-

TABLE I

KOVATS INDICES ON SE-30 FOR THE BORONATE DERIVATIVES OF GUAIFENESIN AND MEPHENESIN

Boronate derivative	Kovats index	
	Guaifenesin	Mephenesin
2,4-Dichlorobenzeneboronate	2489	2420
3,5-Bis(trifluoromethyl)benzeneboronate	2081	1972
4-Bromobenzeneboronate	2455	2357
4-Iodobutaneboronate	2319	2210

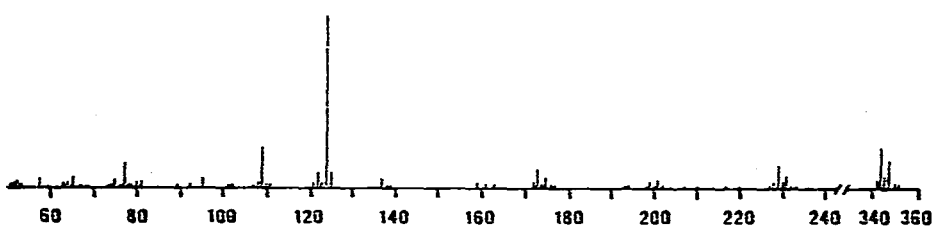


Fig. 1. Electron-impact mass spectrum of the 2,4-dichlorobenzeneboronate of guaifenesin at 70 eV.

Fig. 2. Electron-impact mass spectrum of the 2,4-dichlorobenzeneboronate derivative of mephenesin at 70 eV.

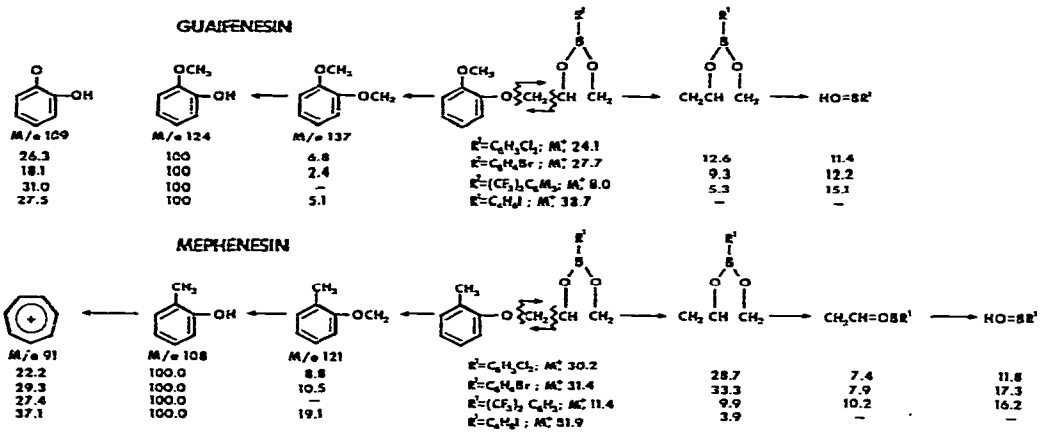
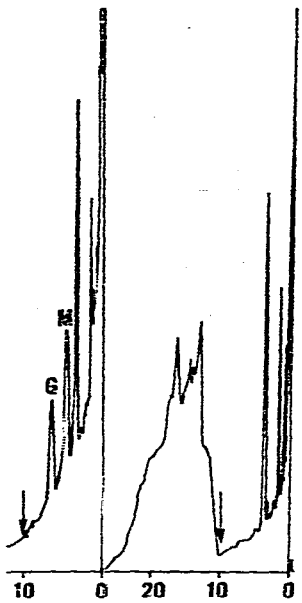


Fig. 3. Characteristic modes of fragmentation under electron-impact of the 2,4-dichlorobenzeneboronate derivatives of guaifenesin and mephenesin.

mately 1.00 ng each of guaifenesin (G) and mephenesin (M) derivatives. The arrow marks the point where the column temperature was increased from 240°C to 290°C ballistically and maintained at the higher temperature for 10 min. This treatment was required to rapidly elute less volatile plasma constituents and excess reagent from the column before the next injection could be made. This treatment does not damage the column or adversely affect the long-term stability of the detector.

The response of the electron-capture detector is markedly temperature dependent, a phenomenon arising from the mechanism of the electron-capture process itself [25]. The 2,4-dichlorobenzeneboronate derivative of guaifenesin captures electrons by a dissociative mechanism giving its maximum response to the detector at high detector temperatures (Fig. 5, B). This should be compared to the 3,5-bis(trifluoromethyl)benzeneboronate derivative of guaifenesin (Fig. 5, C) which captures electrons by a non-dissociative mechanism with its maximum detector response being obtained with low detector temperatures. With a detector temperature of 375°C, the minimum detectable quantity of guaifenesin as its 2,4-dichlorobenzeneboronate derivative was $25 \cdot 10^{-12}$ g.

A linear calibration curve was obtained for guaifenesin added to plasma using mephenesin as internal standard over the investigated range 0.1—2.0 ng (Fig. 6). Each point is the average of five independent determinations and the bars on the graph represent one standard deviation. At the 0.1-ng level the relative standard deviation was 12% and at the 1.0-ng level it was 7%.



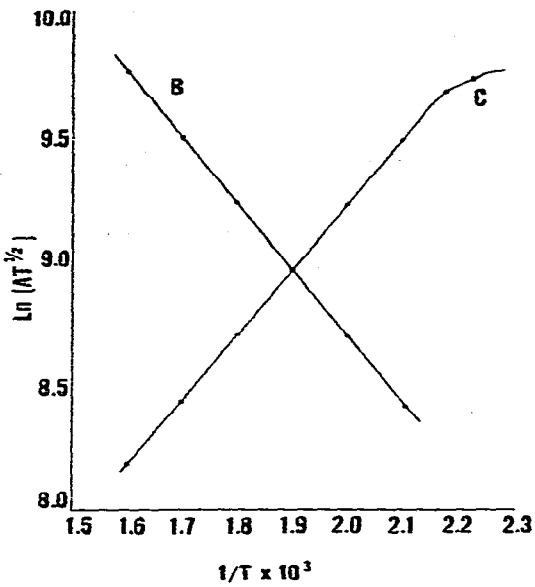


Fig. 4. Gas chromatogram with electron capture detection of a plasma blank and a plasma sample containing approximately 1.0 ng each of guaifenesin (G) and mephenesin (M) as their 2,4-dichlorobenzeneboronates.

Fig. 5. Temperature dependence of the electron-capture detector response for the 2,4-dichlorobenzeneboronate of guaifenesin (B) and the 3,5-bis(trifluoromethyl)benzeneboronate of guaifenesin (C). A = peak area for a fixed mass of derivative and T = detector temperature in °K.

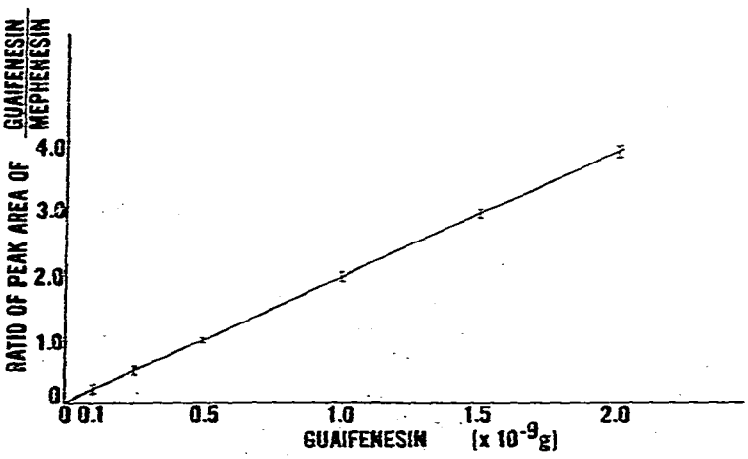


Fig. 6. Calibration curve for guaifenesin in plasma.

The detection limit corresponds to a concentration of approximately 15 ng ml^{-1} of guaifenesin in plasma assuming a 2.0-ml sample. This detection limit is adequate for pharmacological studies of this drug. However, lower detection limits could be obtained if desired by making appropriate adjustments to the experimental procedure described here.

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