Use of a Nitrogen-Specific Detector for GLC Determination of Plasma Bucainide

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Abstract □ A sensitive and specific GLC determination of plasma bucainide was developed using a nitrogen-specific detector. The method permits the determination of bucainide as low as 5.0 ng/ml of plasma and provides sufficient sensitivity and reproducibility for clinical use.

Keyphrases □ GLC—bucainide, analysis in plasma □ Bucainide—GLC analysis in plasma □ Antiarrhythmic—bucainide, GLC analysis in plasma

Bucainide maleate[1] (I), 1-(*N*-isobutyl)benzimidoyl-4-(*N*-hexyl)piperazine dimaleate, is a novel, potent antiarrhythmic agent. The antiarrhythmic effect was demonstrated in the following animal models: mouse chloroform-induced ventricular arrhythmias, refractory period prolongation in guinea pig isolated atria, aconitine-induced atrial arrhythmias in the dog, ventricular arrhythmias following coronary artery ligation in the dog, and ouabain-induced ventricular tachyarrhythmias in the dog (1). The drug also possesses local anesthetic effects and is a weak central nervous system depressant[2].

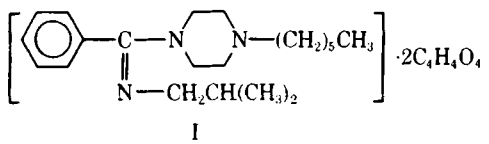
The development of a reliable, specific, and sensitive determination of bucainide in plasma was initiated to support clinical and related studies. Recent advances in GLC detector technology indicated that a nitrogen-specific detector would be feasible. The use of a nitrogen-specific detector for the determination of imipramine and desipramine (2), cocaine (3), and theophylline (4) in biological fluids was reported. Bucainide, due to the three nitrogen atoms on the molecule, appeared to be a promising candidate for this mode of detection.

EXPERIMENTAL

GLC—The gas chromatograph[3] was equipped with a nitrogen-phosphorus detector (NPD). A 1.8-m × 0.2-cm i.d. glass column was packed with 3% phenyl methyl silicone fluid coated on acid-washed, dimethylchlorosilane-treated (80–100-mesh) diatomite support[4]. Retention times and peak areas were measured by a single-channel computing integrator[5].

The gas chromatograph was run in the isothermal mode at a column temperature of 240°. The flow rate of the carrier gas (helium) was 40 ml/min, while the detector gases were set at 100 ml/min for air and 3 ml/min for hydrogen.

The standing current of the detector was calibrated daily by adjustment of the potentiometer so that a 50% full-scale response was obtained on the recorder at an attenuation setting of 64 on the integrator.



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Reagents and Materials—Hexane, acetone, *n*-butyl chloride, and 2-propanol were "distilled-in-glass" grade[6]. The aqueous solution of saturated sodium bicarbonate was filtered[7] before use.

N^1-Hexyl-N^4-benzoylpiperazine maleate[1] (II) was selected as the internal standard. Oxalated dog plasma[8], which was kept frozen in polypropylene containers, was employed for standard curves.

All glassware was kept in chromic acid overnight. After cleaning, the glassware was rinsed with acetone prior to use.

Procedure—Aliquots of 7.5, 15, 30, 60, 90, 120, and 150 μl of bucainide maleate aqueous stock solution (equivalent to 14.8 ng/10 μl as base) were added to 2.0 ml of control dog plasma samples in 15-ml polytef-lined screw-capped tubes. In addition, an aliquot of 50 μl of the internal standard aqueous stock solution (equivalent to 14.1 ng/10 μl as base) was added to each tube. These samples were used to generate a standard curve. An equal aliquot of the internal standard solution was added to all unknown samples.

After an addition of 2.0 ml of saturated sodium bicarbonate solution to each plasma sample, bucainide was extracted with 5.0 ml of *n*-butyl chloride–2-propanol (90:10 v/v) by shaking at 120 oscillations/min for 15 min on a reciprocating shaker[9]. The organic phase was transferred to a second extraction tube containing 5.0 ml of hexane. Bucainide was then extracted with 1 ml of 0.1 *M* H_2SO_4. The addition of hexane to lower the solvent polarity of the organic phase enhanced the extraction of bucainide into acid.

After the solution was alkalinized with 3.0 ml of saturated sodium bicarbonate solution, the drug was back-extracted into 5.0 ml of *n*-butyl chloride–2-propanol (90:10 v/v). The organic extract was evaporated[10] to dryness under nitrogen at 30°, and the residue was redissolved with 25 μl of acetone by vigorous vortexing. To avoid evaporation, the sample was kept on ice prior to GLC analysis, which used an aliquot of 5.0 μl.

RESULTS AND DISCUSSION

Solvent Extraction—The extractibility of bucainide and II as a function of pH was studied in separate experiments with *n*-butyl chloride–2-propanol (90:10 v/v) as the solvent. Bucainide, together with *n*-dososane[11] as an internal standard, was spiked into pH 6, 7, 8, 9, 10, 11, and 12 buffers. The organic extracts were evaporated to dryness and redissolved in acetone before detection by flame ionization. The results showed that after an initial increase from pH 6 to 7, no significant increase in bucainide extraction was observed between pH 7 and 12. Similarly, II was found equally extractible over the pH 6–12 range.

The extraction efficiency of bucainide with II as the internal standard was studied by comparing the relative areas of the extracted spiked plasma samples with those of the unextracted reference standards. An average of 98.5% recovery was observed for the range of 7.5–111.8 ng/ml of plasma.

Plasma Standard Curves—Figure 1 is a typical chromatogram of a spiked plasma sample used for the standard curve. The peak at 150 sec represents 66.6 ng/ml of bucainide, whereas the peak at 210 sec represents 35.3 ng/ml of the internal standard.

Four plasma standard curves for the range of 5.6–111.0 ng/ml were analyzed over 3 weeks (Table I). The standard error was 9.1% for 5.6 ng/ml and 1.8% for 111.0 ng/ml. The composite standard curve was determined by regression analysis, the equation of which was: $y = 0.01445x + 0.05788$, where y represents the relative areas of bucainide to II and x is the bucainide concentration in nanograms per milliliter.

[1] Synthesized by the Medicinal Chemistry Department, USV Pharmaceutical Corp., Tuckahoe, NY 10707; purity was certified by the Analytical Research and Development Department.

[2] J. S. Shroff and P. Cervoni, paper presented at the Second Joint Conference of the Chemical Institute of Canada/American Chemical Society, Montreal, Canada, May 1977.

[3] Perkin-Elmer Corp., Norwalk, CT 06582.

[4] OV-17 on Supelcoport, Supelco, Inc., Bellefonte, PA 16823.

[5] Minigrator, Spectra-Physics, Piscataway, NJ 08854.

[6] Burdick & Jackson Laboratories, Muskegon, MI 49442.

[7] Type HA filter of 0.45 μm, Millipore Corp., Bedford, MA 01730.

[8] Pel Freez Biologicals, Rogers, AR 72756.

[9] Arthur H. Thomas Co., Philadelphia, PA 19105.

[10] Organomation Associates, Shrewsbury, MA 01545.

[11] Applied Science Laboratories, State College, PA 16801.

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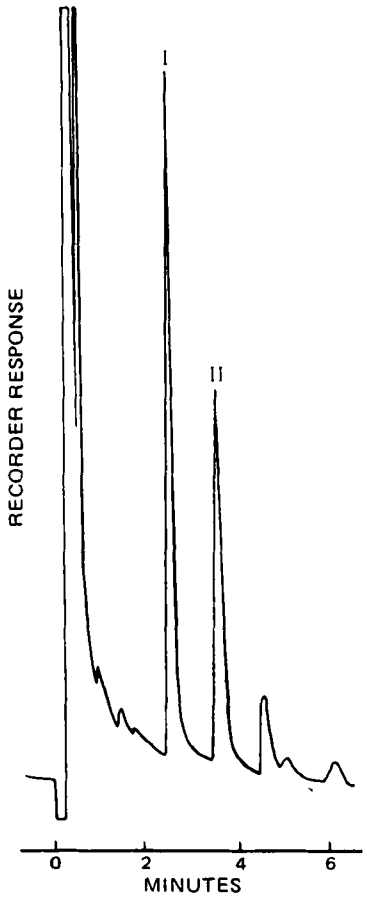

Figure 1—*Chromatogram of a control dog plasma sample, which was spiked with the bucainide reference standard and the internal standard. The peaks at 150 and 210 sec represent 66.6 ng of bucainide and 35.3 ng of II/ml of plasma, respectively.*

The standard curve was linear for the range studied (r = 0.986).

Specificity—To study the possible interference due to endogenous materials in the plasma, plasma samples of four different subjects from a clinical study were extracted as already described and analyzed without addition of the internal standard under the established GLC conditions. The samples represent either predose plasma blanks or samples at various time intervals from subjects receiving a placebo. No interference peaks at the retention times of bucainide or II were observed.

Similarly, another set of plasma samples from a multiple-dose clinical study was analyzed by the same procedure without the internal standard. These samples were obtained after the fourth intravenous dose of 50 mg at 10 min and 1, 3, and 6 hr. None of these samples showed any interference peaks at the II retention time.

Subsequently, GLC–mass spectrometry[12] was employed to substantiate further the absence of interference due to either unidentified metabolites or plasma endogenous materials coextracted and cochromatographed with bucainide or II. A set of plasma samples from the same subject at 0 and 15 min and 1, 2, and 3 hr after the fourth intravenous dose was spiked with the internal standard and extracted as described. The pooled extract was analyzed using chemical ionization with isobutane as the reactant gas.

For each fraction, the chemical-ionization mass spectra showed a base peak at m/e 330 and 275, corresponding to the expected protonated molecular ions, MH^+, of bucainide and the internal standard. Minor peaks at m/e 202 for bucainide and m/e 140 for the internal standard also were observed. These minor peaks were the breakdown products of the corresponding MH^+ ions. These assignments were supported by the identical spectra of the respective unextracted reference standards. Based on these studies, the present method was specific for the determination of bucainide in plasma samples from controlled clinical studies when the subjects were free from other medication.

Clinical Samples—A complete profile of the plasma bucainide levels of one subject from a multiple-dose clinical study is shown in Table II. The subject received four intravenous doses of 50 mg at a dose interval of 4 hr. The predose levels of bucainide at Doses 2 and 3 were approximately 5 ng/ml. The levels of the samples taken immediately after Doses 1, 2, 3, and 4 were about 500–1000 ng/ml.

[12] Chemical-ionization mass spectra were obtained using a Varian MAT 112 GLC-mass spectrometer interfaced to a Varian MAT SS-100C data processing system, Varian MAT, Florham Park, NJ 07832.

Table I—Composite Plasma Standard Curve [a]

Plasma Concentration, ng/ml	Mean Relative Area ± *SE*	*SE*, %
5.6	0.11 ± 0.01	9.1
11.1	0.23 ± 0.02	8.7
22.2	0.42 ± 0.03	7.1
44.4	0.65 ± 0.01	1.5
66.6	1.03 ± 0.04	3.9
88.8	1.28 ± 0.07	5.5
111.0	1.70 ± 0.03	1.8

[a] Composite of four standard curves, and each concentration point was determined in duplicate; $y = 0.01445x + 0.05788$ (r = 0.986).

Table II—Plasma Bucainide Levels for One Subject [a]

Sample Time	Dose 1	Dose 2	Dose 3	Dose 4
Predose[b]	0.0	5.2	5.6	—
"Zero"[c]	940.2	497.0	533.9	482.8
10 min	49.5	—	—	52.1
20 min	34.8	—	—	30.7
1 hr	9.3	—	—	27.6
2 hr	10.2	—	—	12.3

[a] Subject received four intravenous doses of 50 mg of bucainide at a dose interval of 4 hr. [b] Predose samples were taken 10 min prior to dosing. [c] Samples were taken immediately after a 2-min intravenous infusion of the drug.

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