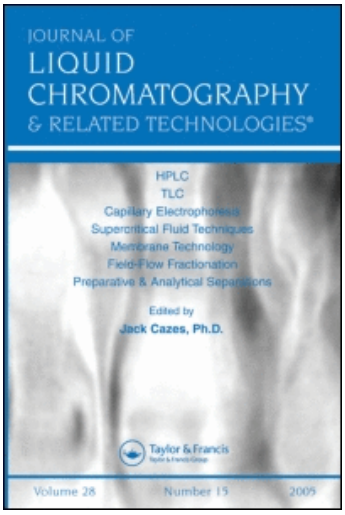

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DETERMINATION OF BRETYLIUM IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, high-pressure liquid chromatographic method for determination of bretylium in pharmaceutical dosage forms is described. The sensitivity of the method is 50 ng with high reproducibility. Exogenous additives from injection, infusion or tablet dosage forms do not interfere with the assay. An increase in the pH of the sample, however, caused a decrease in the peak height of bretylium. Bretylium is a chemically stable compound. Under conditions of either 1.5 N hydrochloric acid, nitric acid, or sodium hydroxide at 25°C and 100°C for a period of up to 48 hours did not influence the outcome of the assay.

INTRODUCTION

Bretylium (o-bromobenzylethyldimethylammonium) is a quaternary ammonium compound which has been shown to possess antiarrhythmic action (1, 2). It was recently released for treatment of ventricular tachycardia or ventricular fibrillation. Bretylium is available in the USA as the tosylate salt for parenteral administration only. Various methods for its determination such as colorimetric (3, 4), paper chromatographic (5), and gas liquid

chromatographic methods (6) have been reported in the literature. These methods are complex, tedious, and require extraction and chemical interaction with other compounds prior to determination. The purpose of this communication is to describe a simple method suitable for rapid determination of bretylium in pharmaceutical dosage forms.

MATERIALS AND METHODS

Reagents:

Acetonitrile was HPLC grade, purchased from Waters Associates (Milford, Mass.). All inorganic chemicals used were AR grade (Mallinckrodt, St. Louis, MO). Bretylium tosylate, 98.7% pure, (Ganes, New York, N.Y.), was used directly without further purification to prepare a stock solution of 1 mg/ml. Standard solutions were made from serial dilutions of the stock solution.

Chromatographic Conditions:

Analyses were carried with a liquid chromatograph consisting of a solvent delivery system (Model M 6000A, Waters Associates, Milford, Mass.), a programmable automatic sampler (WISP Model 710, Waters Associates, Milford, Mass.), a series of two columns packed with microparticulate silica covalently bound with a propylcyano coating (μ-bondapak CN column, Waters Associates, Milford, Mass.), a fixed wavelength ultraviolet detector (Model M-440, Waters Associates, Milford, Mass.), operated at 254 nm and a 10 mv recorder (Model 282, Linear Instruments Co., Irvine, CA). Data acquisition and integration for the determination of peak heights were performed via a laboratory mini-computer (Model 3352D, Hewlett-Packard Instru-

ments, Palo Alto, CA). The mobile phase consisted of acetonitrile: buffered aqueous solution of 0.005 M sodium phosphate monobasic (30/70). The system was operated at ambient temperature and the compounds were eluted with a mobile phase flow rate of 2.0 ml/min.

Assay Procedure:

Four sets of standard solutions of bretylium tosylate in purified water with concentrations ranging from 10 to 400 µg/ml were prepared. One ml of the standard solutions was mixed with 1 ml of the internal standard solution of 2 µg, 2,4-dichloro-bretylium tosylate (a bretylium congener, Arnar-Stone Laboratories, Inc., McGaw Park, IL) in one ml of purified water. The sample was mixed and 20 µl was injected for HPLC analysis.

Effect of pH:

A solution of 1 mg/ml bretylium tosylate in 0.15 M phosphate buffer, pH values of 4, 7, and 8 were prepared. The solutions were assayed as described above.

Analysis of Pharmaceutical Dosage Forms:

Ten ampuls (Bretylol® Injection, Arnar-Stone Laboratories, Inc., McGaw Park, Illinois) each containing 50 mg/ml bretylium tosylate were assayed after dilution with purified water or after preparation of infusion solutions containing NaCl, dextrose, lactose or sucrose. Ten tablets (an experimental dosage form, Arnar-Stone Laboratories, Inc., McGaw Park, IL), each containing 200 mg bretylium tosylate were ground to produce a fine powder. An accurate amount corresponding to one tablet weight was dissolved in 100 ml of purified water. The solution was then centrifuged and the supernatant solution was assayed for bretylium.

Stability Testing:

Fifty mg/ml bretylium solutions were prepared in 1.5 N HCl, HNO_3 or NaOH. As control, a similar solution in purified water was used. One series of these solutions was kept at ambient temperature and another series was kept in an oven at 100°C. Samples were taken at 0, 24, and 48 hours. The concentrations of bretylium were determined after the samples were diluted 1:100 with 0.3 M phosphate buffer, pH 6.5.

RESULTS AND DISCUSSION

The tosylate salts of both bretylium and the internal standard undergo hydrolysis prior to elution from the columns. The retention time of toluene sulfonic acid is very short, within 2 minutes and those of bretylium and the internal standard were 10.0 and 12.7 minutes, respectively.

TABLE 1 shows the reproducibility of the HPLC method, utilizing absolute peak height or peak height ratio values. The coefficient of variations of these values averaged 4.08 and 6.46, respectively, with no significant difference between them. There were good linear relationships between either the absolute peak height or the peak height ratio and the concentrations of bretylium in the standard solutions (range 10-400 µg/ml, $r = 0.999$, $n = 40$).

The absolute peak height values of both bretylium and the internal standard decreased in alkaline pH (TABLE 2). At pH 8 this decrease was 34 and 41%, respectively. This decrease may be attributable to a corresponding prolongation of the retention times, 17 and 26%, for bretylium and the internal standard, re-

TABLE 1

Reproducibility of Absolute Peak Height and Peak Height Ratio Values of Bretylium

CONC.	ABSOLUTE PEAK HEIGHT		PEAK HEIGHT RATIO	
µg/ml	Mean [b]	C.V.	Mean [a,b]	C.V.
10	92.0	5.10	0.0432	11.1
25	215.3	3.74	0.102	4.22
50	447.8	9.35	0.195	2.41
100	814.3	4.28	0.376	3.75
200	1596.3	0.822	0.714	4.08
300	2269.0	1.59	1.05	9.59
400	2868.5	3.67	1.42	10.1

[a]Ratio of the bretylium peak to the internal standard peak (2,4 dichlorocongener of bretylium).
[b]Each value represents a mean of 4 determinations.

TABLE 2

Effect of pH on Relative Peak Heights and Retention Times of Bretylium and Internal Standard

	Ratio of Actual Value to Control [a]		
Drug	pH 4	pH 7	pH 8
		Peak Height	
Bretylium (alone)	1.02	0.912	0.699
Bretylium[b]	1.02	0.900	0.671
Internal Standard[b,c]	0.979	0.801	0.587
		Retention Time[d]	
Bretylium (alone)	0.988	1.09	1.23
Bretylium[b]	0.934	1.02	1.17
Internal Standard[b,c]	0.942	1.05	1.26

[a]Control solutions of bretylium tosylate were prepared in purified water.

[b]Bretylium and the Internal Standard were measured simultaneously.

[c]2,4 dichloro congener of bretylium was used as internal standard.

[d]The average retention times of bretylium and internal standard, in the control samples, were 10.0 and 12.7 minutes (n=4), respectively.

spectively. The decrease in the peak height of bretylium and the internal standard at different pH values was not proportional. However, the measurement of absolute peak height appeared to be sufficient.

TABLE 3 summarizes the results of potency of bretylium in ampuls and tablets using standard sample preparation and dilution methods. The potency of the ampuls and tablets was 99.1% and 96.8%, respectively. The coefficients of variation associated with the determination of 10 samples were 3.28 and 4.40%, respectively, for tablets and ampuls, indicating excellent precision of the HPLC method (TABLE 3). TABLE 4 indicates that in the presence of

TABLE 3

Potency Determination of Bretylium Ampuls and Tablets

	Ampuls 250mg	Tablets 200 mg
Mean	99.1 %	96.8 %
Coefficient of Variation	4.40%	3.28 %
n	10	10

TABLE 4

Effect of Acid, Base and Temperature on Bretylium

	Percent of Control[a]				
Sample	0 hr	24 hrs		48 hrs	
		25°C	100°C	25°C	100°C
1.5N HCl	100.0	97.8	100.4	95.3	99.4
1.5N HNO_3	100.0	99.1	100.2	99.1	101.8
1.5N NaOH	100.0	98.7	99.5	95.9	96.7

[a]Every value is an average of two determinations, using the absolute peak height method.

1.5 N HCl, HNO_3 or NaOH at room temperature and at 100°C for a period of up to 48 hours, bretylium was stable with no degradation.

The HPLC procedure described above is reliable for determination of bretylium in pharmaceutical dosage forms. This method applies to simple dissolution and dilution techniques with direct HPLC determination, bypassing reaction with other reagents. Bretylium tosylate is stable under drastic physical conditions.

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