Simultaneous Determination of Tetracaine and Its Degradation Product, *p-n*-Butylaminobenzoic Acid, by High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic method was developed for the simultaneous determination of tetracaine hydrochloride and its hydrolytic degradation product, *p-n*-butylaminobenzoic acid. Separation was achieved using a μBondapak C_{18} column and the eluent, water–acetonitrile–methanol (60:20:20), containing 0.06% (v/v) sulfuric acid, 0.5% (w/v) sodium sulfate, and 0.02% (w/v) sodium heptanesulfonate, at a flow rate of 2 ml/min. Salicylic acid and propiophenone were used as internal standards. The UV detector response at 305 nm was linear for tetracaine hydrochloride in the 0.4–2.0-mg/ml range and for *p-n*-butylaminobenzoic acid in the 0.003–0.02-mg/ml range. The method is simple and precise.

Keyphrases □ Tetracaine—determination by high-performance liquid chromatography □ *p-n*-Butylaminobenzoic acid—determination by high-performance liquid chromatography □ High-performance liquid chromatography—determination of tetracaine, *p-n*-butylaminobenzoic acid

Tetracaine, a widely used local anesthetic, is formulated either as a 1% solution in water or in combination with other *p*-aminobenzoic esters such as procaine and benzocaine. Hydrolytic degradation of tetracaine occurs in solution, resulting in the formation of *p-n*-butylaminobenzoic acid and diethylaminoethanol. *p-n*-Butylaminobenzoic acid is only sparingly soluble in water and potentially can cause crystal deposition in tetracaine hydrochloride injection.

BACKGROUND

To study the ideal pH range for maximum stability of tetracaine hydrochloride in water, it was necessary to determine accurately the intact drug and the extent of *p-n*-butylaminobenzoic acid formation at various pH values. The USP method for the tetracaine hydrochloride assay involves extraction of the drug as its free base and back-extraction into dilute acid followed by spectrophotometry (1). Although theoretically *p-n*-butylaminobenzoic acid can be determined in the aqueous phase after basic extraction of tetracaine, the values are likely to be high due to the greater susceptibility of tetracaine to alkaline hydrolysis during analytical manipulations.

Therefore, it was necessary to develop a simple analytical scheme to determine both the intact drug and its degradation product, preferably by a single approach. The other available methods are measurement of refractive index, GLC, and partition chromatography (2–5), none of which is ideally suited for a simultaneous assay scheme.

This paper describes a high-performance liquid chromatographic (HPLC) method for the determination of both tetracaine and *p-n*-butylaminobenzoic acid in tetracaine hydrochloride injection. The same chromatographic conditions also are applicable to the analyses of several other local anesthetics.

EXPERIMENTAL

The chromatographic system was equipped with a dual-piston reciprocating pump[1], universal injector[2], and a variable-wavelength UV detector[3]. The separation was performed on a 30-cm × 4-mm i.d. column containing microparticulate (10 μm) bonded octadecylsilane material[4]. The chromatographic peaks were electronically integrated and recorded[5].

Eluent—The eluent was water–acetonitrile[6]–methanol[6] (60:20:20) containing 0.06% (v/v) sulfuric acid, 0.5% (w/v) sodium sulfate, and 0.02% (w/v) sodium heptanesulfonate[7] (pH 2.6).

Internal Standard Solutions—A 10-mg/ml salicylic acid[8] solution in methanol–water (1:1) was used as the internal standard for tetracaine, and a 10-mg/ml solution of propiophenone[8] in methanol–water (1:1) was used as the internal standard for *p-n*-butylaminobenzoic acid.

Standard Preparation—The standard solution was prepared by weighing 50 mg of tetracaine hydrochloride USP reference standard into a 50-ml volumetric flask. Five milliliters of a 0.05-mg/ml *p-n*-butylaminobenzoic acid reference standard[9] in methanol–water (1:1), 20.0 ml of

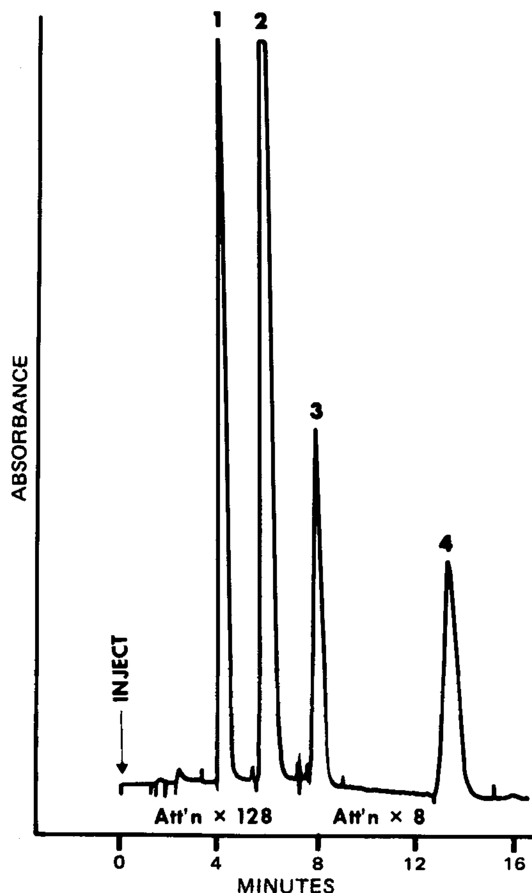

Figure 1—*HPLC analysis of tetracaine hydrochloride injection. Key: 1, salicylic acid; 2, tetracaine; 3, propiophenone; and 4,* p-n-*butylaminobenzoic acid.*

[1] Model 6000A, Waters Associates, Milford, MA 01757.
[2] Model U6K, Waters Associates, Milford, MA 01757.
[3] Model SF 770, Schoeffel, Westwood, NJ 07675, or model LC-55, Perkin-Elmer, Norwalk, CT 06856.
[4] μBondapak C_{18}, Waters Associates, Milford, MA 01757.
[5] Model 3385A automation system, Hewlett-Packard, Avondale, CA 19311.
[6] Distilled in glass, Burdick & Jackson, Muskegon, MI 49442.
[7] Eastman Organic Chemicals, Rochester, NY 14650.
[8] Aldrich Chemical Co., Milwaukee, WI 53233.
[9] Alfred Bader Chemicals, Aldrich Chemical Co., Milwaukee, WI 53233 (characterized and designated Abbott House Reference Standard).

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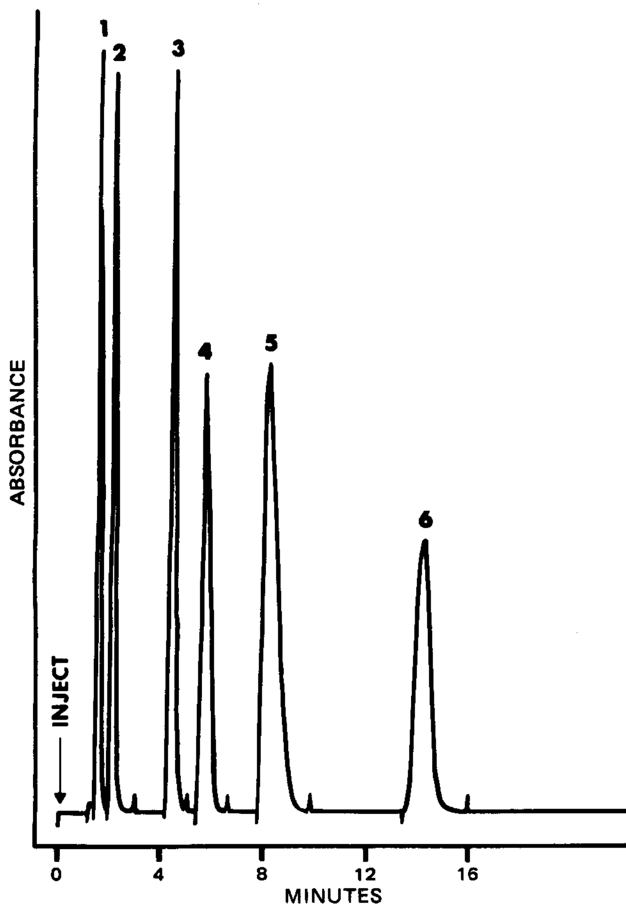

Figure 2—*HPLC analysis of a mixture of local anesthetics. Key: 1, procaine; 2, lidocaine; 3, benzocaine; 4, tetracaine; 5, pramoxine; and 6, butamben.*

the salicylic acid internal standard solution, and 5.0 ml of the propiophenone internal standard solution were pipetted into the flask. The solution was diluted to volume with methanol–water (1:1).

Sample Preparation—The sample formulation contained 1% tetracaine in a solution of sodium chloride and acetone sodium bisulfite in water. Five milliliters of the formulation, 20 ml of the salicylic acid internal standard solution, and 5 ml of the propiophenone internal standard solution were pipetted into a 50-ml volumetric flask and diluted to volume with methanol–water (1:1). For analysis of formulations with suspected crystal deposition, it is necessary to homogenize the sample prior to analysis.

Analysis—The following chromatographic conditions were used for the analysis: flow rate, 2 ml/min; detector wavelength, 305 nm; and injection volumes for tetracaine hydrochloride and *p-n*-butylaminobenzoic acid, 5 and 50 μl, respectively. Quantitative analysis was accomplished by comparing the peak area ratios of the standard preparations to those of the sample preparations.

RESULTS AND DISCUSSION

At the pH of the chromatographic eluent, both tetracaine and *p-n*-butylaminobenzoic acid were ionized and formed ion-pairs with heptanesulfonate. Figure 1 shows a typical chromatogram resulting from a 50-μl injection of the sample preparation. The linearity of the detector response was established for 5-μl injections of tetracaine hydrochloride in the 0.4–2.0-mg/ml range (*y*-intercept, 0.017; correlation coefficient, 0.9997) and for 50-μl injections of *p-n*-butylaminobenzoic acid in the 0.003–0.02-mg/ml range (*y*-intercept, 0.01; correlation coefficient, 0.9999). Since the peak area ratio for tetracaine analysis obtained from a 50-μl injection fell beyond the linear working range, the assay was performed using a 5-μl injection.

The accuracy and reproducibility of the method were demonstrated by replicate analyses of a simulated formulation that contained 10.0 mg of tetracaine hydrochloride/ml and was spiked with 0.050 mg of *p-n*-butylaminobenzoic acid/ml. The data generated from these analyses show that the method is accurate and precise.

For eight replicate analyses of a 10-mg/ml tetracaine hydrochloride injection spiked with 0.050 mg of *p-n*-butylaminobenzoic acid/ml, the results were: for tetracaine hydrochloride, 10.0 ± 0.12 mg/ml (*SD*), *RSD* = ±1.2%; and for *p-n*-butylaminobenzoic acid, 0.050 ± 0.0005 mg/ml (*SD*), *RSD* = ±1.0%.

Standard addition experiments also were performed to demonstrate method accuracy. Four formulations were prepared, containing tetracaine hydrochloride at levels ranging from 4 to 20 mg/ml and *p-n*-butylaminobenzoic acid at levels ranging from 0.03 to 0.2 mg/ml. These four formulations were diluted and analyzed according to the experimental conditions; recoveries of tetracaine hydrochloride varied from 98.3 to 101.4% with an average recovery of 99.5 ± 1.4%. Recoveries of *p-n*-butylaminobenzoic acid varied from 100.6 to 104.0% with an average recovery of 102.2 ± 1.7% for the four formulations; the tetracaine hydrochloride bulk drug used to prepare the formulations for the standard addition experiments contained <0.1% of *p-n*-butylaminobenzoic acid, which resulted in slightly higher recoveries of the added degradation product.

p-n-Butylaminobenzoic acid has low solubility in aqueous solution, which may result in crystal deposition of the degradation product in tetracaine hydrochloride injection subjected to long-term storage. No crystallization has been observed in actual formulations. However, when simulated formulations were subjected to hydrolytic degradation at high temperature (85°), crystal deposition occurred at concentrations of >0.6 mg of *p-n*-butylaminobenzoic acid/ml in the pH 3.15–5.15 range.

Figure 2 shows a chromatogram obtained from a mixture of six common local anesthetics analyzed with the same HPLC conditions. In the absence of either sulfuric acid or sodium sulfate in the eluent, butamben and pramoxine eluted together and the peaks had certain asymmetry. Slight increases in the capacity factors for the different solutes could be obtained by decreasing the salt concentration; this finding was in agreement with published data on salt effects (6).

Ion-pair reversed-phase chromatography permits simultaneous analysis of tetracaine hydrochloride and its hydrolytic degradation product. The described system has utility in analyzing several other local anesthetics with which it may be coformulated.

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