Simultaneous Determination of Chloroprocaine Hydrochloride and Its Degradation Product 4-Amino-2-chlorobenzoic Acid in Bulk Drug and Injection Solutions by High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic method has been developed for the simultaneous determination of chloroprocaine hydrochloride and its hydrolytic degradation product, 4-amino-2-chlorobenzoic acid. Separation is achieved using a μ-Bondapak C_{18} column and the eluant, water–acetonitrile–methanol–glacial acetic acid (74:20:5:1) containing 0.05–0.08% (w/v) sodium 1-heptanesulfonate, at a flow rate of 2 mL/min. Benzoic acid and *p*-nitroacetophenone were used as internal standards. A variable-wavelength UV detector (278 nm) was used for detection of the compounds. The method is simple, accurate, and precise.

Keyphrases □ Chloroprocaine hydrochloride—determination in bulk drug and injection formulations by HPLC separation from synthetic impurities

Chloroprocaine hydrochloride, a local anesthetic of the *para*-aminobenzoic ester class, is formulated as a 2 or 3% aqueous injection solution for caudal and epidural nerve blockage. Like other *para*-aminobenzoic esters, the drug is susceptible to hydrolysis, resulting in the formation of 4-amino-2-chlorobenzoic acid and diethylaminoethanol. The compendial assay (1) for chloroprocaine hydrochloride bulk drug is based on potentiometric titration with perchloric acid, after the addition of mercuric acetate to a glacial acetic acid solution of the drug. The assay is not specific, since any hydrochloride salts of the manufacturing precursors or free bases would also be titrated. The formulation is assayed by UV spectrophotometry following the extraction of the drug as the free base. Although the assay is stability indicating, numerous chloroform extractions of an alkaline sample solution are required. Since the drug is rapidly hydrolyzed in basic solutions, this assay is undesirable. The degradation product, 4-amino-2-chlorobenzoic acid, is determined in a limit test using the Bratton–Marshall reaction (2) of the 4-amino group.

The present study describes a high-performance liquid chromatographic (HPLC) method for the simultaneous determination of chloroprocaine hydrochloride and its potential degradation product, 4-amino-2-chlorobenzoic acid, in bulk drug and injection solutions. It is accurate, precise, and simple, with minimal sample manipulation.

EXPERIMENTAL

The chromatographic system was equipped with a dual piston reciprocating pump[1] at a flow rate of 2 mL/min, a universal injector[2] with 5-μL volume, and a variable-wavelength UV detector at 278 nm[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]. For quantitative analyses, peak area ratios of the sample preparation were compared with those of the standard preparations.

Materials—The eluant was water–acetonitrile[6]–methanol[6]–glacial acetic acid (74:20:5:1) containing 0.05–0.08% w/v sodium 1-heptanesulfonate[7], pH 3.1.

A 1-mg/mL benzoic acid[8] solution in 1:1 methanol–water was used as the internal standard for 4-amino-2-chlorobenzoic acid and a 5-mg/mL solution of *p*-nitroacetophenone[9] in methanol was used as the internal standard for chloroprocaine hydrochloride for the analysis of the bulk drug. The concentration of benzoic acid was increased to 4 mg/mL for the analysis of formulations.

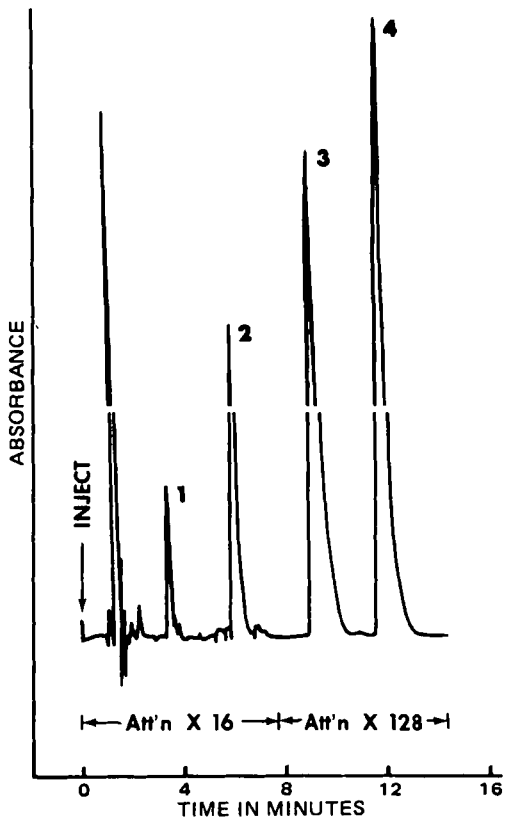

Figure 1—*HPLC analysis of 2% chloroprocaine hydrochloride injection exposed to artificial light for 1 month. Key: (1) 4-amino-2-chlorobenzoic acid; (2) benzoic acid; (3) chloroprocaine hydrochloride; and (4)* p-*nitroacetophenone.*

[1] Model 6000A, Waters Associates, Milford, MA 01757.
[2] Model U6K Universal Injector, Waters Associates, Milford, MA 01757.
[3] Model SF 770 Schoeffel, Westwood, NJ 07675 or Model LC-55, Perkin-Elmer, Norwalk, CT 06856
[4] μ-Bondapak C_{18}, Waters Associates, Milford, MA 01757.
[5] Model 3385A Automation System, Hewlett-Packard, Avondale, PA 19311.
[6] Distilled in Glass, Burdick and Jackson, Muskegon, MI 49442.
[7] Eastman Organic Chemicals, Rochester, NY 14650.
[8] Mallinckrodt, St. Louis, MO 63147.
[9] Aldrich Chemical Co., Milwaukee, WI 53233.

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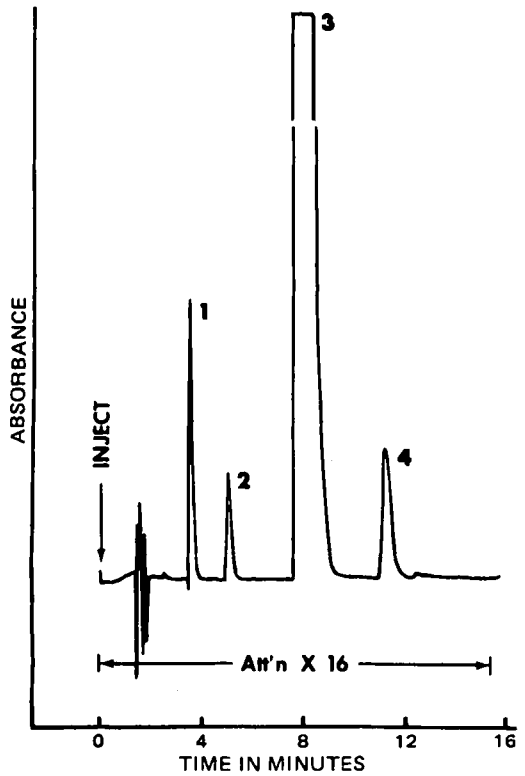

Figure 2—*HPLC analysis of a mixture of chloroprocaine hydrochloride bulk drug and the manufacturing precursors and 4-amino-2-chlorobenzoic acid each spiked at 1% level in the bulk drug. Key: (1) 4-Amino-2-chlorobenzoic acid; (2) 2-chloro-4-nitrobenzoic acid; (3) chloroprocaine hydrochloride; and (4) methyl-2-chloro-4-aminobenzoate.*

Standard Preparation—*4-Amino-2-chlorobenzoic Acid*—Ten milliliters of 0.1 mg/mL 4-amino-2-chlorobenzoic acid reference standard[10] in 1:1 methanol–water and 10.0 mL of 1 mg/mL benzoic acid internal standard solution were pipetted into a 100-mL volumetric flask and diluted to 100 mL with water; this solution was used for the analysis of the bulk drug. For the formulation assay, 20.0 mL of 0.1-mg/mL 4-amino-2-chlorobenzoic acid reference standard solution and 10.0 mL of 4-mg/mL benzoic acid solution were diluted to 100 mL with water.

Chloroprocaine Hydrochloride—One hundred milligrams of chloroprocaine hydrochloride USP reference standard was weighed into a 100-mL volumetric flask; 20.0 mL of *p*-nitroacetophenone internal standard solution was added and diluted to volume with water. This solution was used for the analyses of both bulk drug and injection formulation.

Sample Preparation—For the analysis of the bulk drug, 100 mg of the sample was mixed with 10.0 mL of 1-mg/mL benzoic acid solution and 20.0 mL of *p*-nitroacetophenone internal standard solution and diluted to 100 mL with water. For the formulation, an aliquot of the sample equivalent to 100–120 mg of chloroprocaine hydrochloride was mixed with 10.0 mL of 4-mg/mL benzoic acid internal standard solution and 20.0 mL of *p*-nitroacetophenone internal standard solution and diluted to 100 mL with water.

RESULTS AND DISCUSSION

The retention of chloroprocaine hydrochloride was found to increase with increasing concentration of the ion-pair reagent in the range of 0.02–0.10% in the mobile phase, while a decrease was observed with increase in acidity (1.0% acetic acid *versus* 0.05% sulfuric acid). Neither of the above changes caused any significant change in the retention volumes of 4-amino-2-chlorobenzoic acid and the internal standards.

Figure 1 shows a typical chromatogram of a sample preparation of the formulation exposed to artificial light for 1 month. Diethylaminoethanol is not detectable at the detector wavelength of 278 nm. Peak 1, which is due to 4-amino-2-chlorobenzoic acid, corresponds to ~0.7 mg in 100 mg of the drug (peak 3) in the sample preparation. The recorder has been attenuated after the elution of the benzoic acid peak to scale down the responses of the latter peaks. The concentration of ion-pair reagent in the eluant was 0.08%.

Table I—Statistical Data for the Analysis of Chloroprocaine Hydrochloride Bulk Drug and 2% Injection Solution

	Bulk Drug Purity, % w/w		Formulation, % of Label Claim	
	HPLC	USP XX	HPLC	USP XX
Number of Analyses	9	10	6	6
Chloroprocaine hydrochloride (Mean)	99.3	100.4	100.2	100.7
SD	±0.6%	±0.2%	±1.0%	±2.2%
RSD	±0.7%	±0.2%	±1.0%	±2.2%
4-Amino-2-chlorobenzoic acid	<0.1%	<0.625%[a]	0.67%	<3.0%[a]
SD	—	—	±0.05%	—
RSD	—	—	±7.2%	—

[a] USP XX Limit Test.

The trace component eluting between peaks 1 and 2 was identified as 2-chloro-4-nitrobenzoic acid[11], the starting material for the manufacture of chloroprocaine hydrochloride. Also, the trace component between peaks 3 and 4 was identified as methyl-2-chloro-4-aminobenzoate[11], a manufacturing intermediate. The only other intermediate in the manufacture of the bulk drug, methyl-2-chloro-4-nitrobenzoate[11] does not elute from the column during this chromatography. The levels of the two manufacturing impurities detected in the bulk drug were <0.5%, which was also confirmed by TLC.

Since the HPLC system separates all the manufacturing impurities from chloroprocaine hydrochloride, the procedure was also used for the analysis of the bulk drug. The concentration of the ion-pairing agent was reduced to 0.05% in the mobile phase to enhance the resolution of methyl-2-chloro-4-aminobenzoate from chloroprocaine hydrochloride. Figure 2 shows a chromatogram of the bulk drug spiked with the manufacturing precursors and 4-amino-2-chlorobenzoic acid (degradation product) each at the 1% level in the bulk drug.

The compendial limits for 4-amino-2-chlorobenzoic acid in the bulk drug and formulation are 0.625% and 3%, respectively, as determined by the Bratton–Marshall reaction. As low as 0.1% of the degradation product can be readily quantitated by the HPLC method. The bulk drug used in this study contained <0.1% of the degradation product. Since the drug degrades in solution, separate standard preparations of chloroprocaine hydrochloride and the degradation product are employed for quantitative analyses.

Acidic, basic, and neutral reflux studies of the drug showed 4-amino-2-chlorobenzoic acid as the only UV-active degradation product. The drug was degraded totally in 0.5 M sodium hydroxide, 3% in 0.5 M hydrochloric acid and 12% in water under reflux conditions for 1 h, with excellent material balance between the intact drug and degradation products.

Chloroprocaine hydrochloride formulations are discolored on prolonged storage under natural light. To study the nature of the photodegradation product, a formulation contained in a sealed vial was exposed to UV radiation for 45 min. A large peak was seen eluting at the same retention volume as the trace component seen between peaks 2 and 3 in Fig. 1. Preparative chromatography followed by mass spectrometry suggested the new component to be due to 2-hydroxyprocaine. This was confirmed by further hydrolyzing the UV degradation product in dilute carbonate solution to produce 4-aminosalicylic acid, the retention volume of which was in good agreement with that of an authentic material. The amount of the photodegradation product produced was found to be <0.1% after 1 month exposure to artificial light, as is apparent in Fig. 1, although the formulation was slightly discolored.

Linear detector responses were obtained when 50–200 mg of chloroprocaine hydrochloride and 0.25–5 mg of 4-amino-2-chlorobenzoic acid were analyzed. The correlation coefficients were 0.9999 in both cases, and the response *versus* concentration plots essentially passed through the origin.

A placebo formulation containing 0.4% sodium chloride and 0.2% sodium bisulfite in water for injection was spiked simultaneously with chloroprocaine hydrochloride at 12–28 mg/mL and 4-amino-2-chlorobenzoic acid at 0.2–1 mg/mL; 5 mL of each of the spiked solutions were taken through the procedure. The average recovery of chloroprocaine

[10] Pfaltz and Bauer, Inc., Stamford, CT 06902 (Characterized and designated Abbott House Reference Standard).

[11] Orgamol, S.A. Evionnaz (Valais) Suisse.

hydrochloride was found to be 99.8% ($n = 5$, $CV = \pm 0.7\%$) and that of 4-amino-2-chlorobenzoic acid, 101.0% ($n = 5$, $CV = \pm 0.7\%$). The reproducibility of the method was determined using a typical lot of bulk drug and a 2% formulation by three analysts over a 6-d period. The results and the statistical data are presented in Table I along with the results obtained by the corresponding assays prescribed by USP.

In conclusion, ion-pair reverse-phase chromatography permits the simultaneous analysis of chloroprocaine hydrochloride and its degradation product, 4-amino-2-chlorobenzoic acid, in bulk drug and injection formulations. The method is specific for the analysis of the bulk drug and stability indicating for the drug in injection solutions.

REFERENCES

(1) "U.S. Pharmacopeia XX; National Formulary XV," U.S. Pharmacopeial Convention, Rockville, Md., 1979, p. 135–136.

(2) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

Adsorption of Sulfonylureas onto Activated Charcoal *In Vitro*

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Received September 3, 1982, from the *Department of Clinical Pharmacology, University of Helsinki, Paasikivenkatu 4, SF-00250 Helsinki 25, Finland.* Accepted for publication December 23, 1982.

Abstract □ Adsorption of carbutamide, chlorpropamide, tolazamide, tolbutamide, glibenclamide (glyburide), and glipizide onto activated charcoal was compared *in vitro* using different charcoal-to-drug ratios. Maximal binding capacities of different sulfonylureas were 0.45–0.52 g/g of charcoal at pH 7.5. The affinity of the second generation derivatives, glibenclamide and glipizide, was considerably higher than that of the first generation derivatives. The affinity of sulfonylureas to charcoal was higher at pH 4.9 than at pH 7.5. Poor water solubility of sulfonylureas at pH 1 prevents the adequate testing in these conditions. Contrary to what has appeared previously, activated charcoal effectively adsorbs different sulfonylureas and can be used to possibly prevent their gastrointestinal absorption.

Keyphrases □ Activated charcoal—adsorption, sulfonylureas, carbutamide, chlorpropamide, tolazamide, tolbutamide, glibenclamide, glipizide □ Sulfonylureas—adsorption to activated charcoal □ Adsorption—sulfonylureas, activated charcoal

The capacity of activated charcoal to adsorb chemicals has been recognized for centuries, and in many countries charcoal is generally used as an antidote for intoxication. Maximal amounts of drugs adsorbed by charcoal of good quality are on the order of 100–1000 mg/g of charcoal (1–4). Decker *et al.* (4) have concluded from their *in vitro* studies that compounds insoluble in aqueous acidic solution, such as tolbutamide, are not adsorbed to any measurable extent onto activated charcoal. Others have cited this and extended the conclusions to include other sulfonylureas as well. Recently, claims have been made that activated charcoal is ineffective (5, 6) or even contraindicated (7) as an antidote in poisonings caused by tolbutamide or sulfonylureas in general. This paper reports the adsorption of six commonly used sulfonylureas onto activated charcoal *in vitro*.

EXPERIMENTAL

Material—Activated charcoal[1], carbutamide[2], chlorpropamide[3], tolazamide[4], tolbutamide[5], glibenclamide[2] (glyburide), and glipizide[4] were used as received. Dichlormethane, acetonitrile, isopropyl alcohol, and methanol were HPLC grade. All other reagents were analytical grade quality and were used as received.

Preparation of Drug Solutions—Solutions containing 700 mg/L of various sulfonylureas (or glipizide 360 mg/L, glibenclamide 100 mg/L) were prepared in 50 mM phosphate buffer as follows. The solutes were first dissolved in a small amount of 0.1 M NaOH, then phosphate buffer (pH 6.5) was added and the pH was adjusted to 7.5 with 1 M NaOH. Gilbenclamide, which is very sparingly soluble, was first dissolved in an ethanol–sodium hydroxide solution.

In addition, carbutamide and chlorpropamide were dissolved in two phosphate–acetate buffers, pH 7.5 and 4.9, containing 50 mM phosphate and 40 mM acetate. The sulfonylureas were maintained in the aforementioned solutions for several days at room temperature and at 4°C.

Adsorption Studies—The adsorption studies were carried out at room temperature (20–24°C) either at pH 7.5 or 4.9 (phosphate or phosphate–acetate buffers; see Preparation of Drug Solutions). A solution of 20 mL of sulfonylureas in different concentrations and 20 mg of charcoal were mixed in 30 mL stoppered glass tubes. When the effect of pH was studied, 16 mg of charcoal was added to 25 mL of the drug solution to achieve lower charcoal-to-drug ratios. The charcoal-to-drug ratio varied from 0.91:1 to 27:1. The tubes were shaken for 10 min, after which time the solutions were allowed to stand for an additional 10 min and then centrifuged (1800×*g* for 10 min). The drug concentration in the supernatant was determined.

Determination of Sulfonylurea Concentrations—The UV absorption of sulfonylureas could not be used for direct determination of these drugs because of the unpredictable amount of impurities absorbing at the UV range eluted from charcoal. The drug concentrations were therefore determined by HPLC[6] with some modification of the original procedure (8). The impurities from the charcoal did not interfere with the HPLC assay. Aliquots of the supernatant were diluted to constant volume with buffer and acidified with hydrochloric acid. The drugs were extracted with dichlormethane-containing internal standard (carbutamide or tolbutamide). The organic phase was separated and evaporated in a nitrogen stream. The residue was dissolved in methanol, and samples of this were injected into the chromatograph. The separation was performed with a C-18[7] column which was eluted with 50 mM phosphate buffer–acetonitrile, 55:45, pH 3.9 at a flow rate of 2.5 mL/min. For analysis of glibenclamide 10% isopropyl alcohol was added to the mobile phase to shorten the retention time. The sulfonylureas were monitored with a variable-wavelength UV detector[8] at 230–240 nm, depending on the optimal ratio of absorption of the derivatives and the internal standard.

The coefficient of variation between runs for the whole process comprising incubations with activated charcoal and drug determination was

[1] Carbomix (Norit A), Medica Ltd, Helsinki.
[2] Orion Ltd, Helsinki.
[3] Farmos Group Ltd, Turku.
[4] Medica Ltd, Helsinki.
[5] Hoechst AG, Frankfurt.

[6] HPLC: M6000A Chromatography pump and 6UK injector; Waters Associates, Milford, Mass.
[7] μBondapak C_{18}; Waters Associates, Milford, Mass.
[8] SF 770 Spectroflow; Schoeffel, Westwood, N.J.

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