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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF *d*-/*l*-EPINEPHRINE ENANTIOMER RATIO IN LIDOCAINE–EPINEPHRINE LOCAL ANESTHETICS

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SUMMARY

A procedure for the determination of the ratio of *d*- to *l*-epinephrine in lidocaine–epinephrine local anesthetics is described. Epinephrine was isolated via Sep-Pak cartridges and derivatized with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate; the *d*- and *l*-chiral derivatives were separated and measured by high-performance liquid chromatography. About 70 samples of various dosage forms and concentrations from four manufacturers were successfully analyzed by the method.

INTRODUCTION

Epinephrine is a vasoconstrictor that is used to prolong the activity of lidocaine, a local anesthetic, in dental injections. *l*-Epinephrine is several times as biologically active as the racemic mixture[1]. This laboratory received for analysis about 280 samples of local-anesthetic solutions, some of which had been reported to the Food and Drug Administration for apparent therapeutic failure. In addition to analysis for epinephrine, lidocaine, epinephrine sulfonic acid, and decomposition products of epinephrine, a determination of the ratio of *d*- to *l*-epinephrine was requested for 70 of the samples.

The United States Pharmacopeia provides limits for the specific rotation of epinephrine (-50 to $-53.5°$)[2], but this measurement technique is not sensitive enough for analysis of local anesthetics, which usually contain epinephrine at concentrations of 1/100 000 or less. A recently published article[3] described a method for the resolution of derivatized diastereomers of epinephrine in standard solutions by high-performance liquid chromatography (HPLC) that appeared to have the needed sensitivity.

Several modifications were required before the published procedure could be applied to the analysis of commercial dental anesthetic solutions. This paper describes the sample preparation and chromatography that permit resolution of diastereoisomeric derivatives of epinephrine prepared from lidocaine–epinephrine local anesthetics.

EXPERIMENTAL

Reagents

Water was purified with a Milli-Q-Water System (Millipore, Bedford, MA, U.S.A.). Methanol was OmniSolve grade (MCB, EM Science, Gibbstown, NJ, U.S.A.). Dimethylformamide (ChromAR grade) and lead acetate trihydrate, hydrochloric acid, and perchloric acid (all AR grade) were from Mallinckrodt (St. Louis, MO, U.S.A.). Hydrazine hydrate (85% solution) was obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). *l*-Epinephrine bitartrate was from Sigma (St. Louis, MO, U.S.A.), *dl*-epinephrine hydrochloride was from USV Pharmaceuticals (Tuckahoe, NJ, U.S.A.), and lidocaine hydrochloride was from Pfaltz & Bauer (Stamford, CT, U.S.A.). 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) was synthesized[4].

HPLC apparatus and conditions

The HPLC system (Waters Assoc., Milford, MA, U.S.A.) was comprised of a 6000A pump module, a WISP 710B automated injection module, a 440 UV detector (254 nm), and a 730 data module. The reversed-phase column was LiChrosorb RP-18 (10 μm), 250 × 4.0 mm I.D. (Merck, Darmstadt, F.R.G., Cat. No. 50334), with a Waters guard column (Cat. No. 84550) packed with μBondapak C_{18}–Corasil (35–50 μm) (Waters Assoc., Cat. No. 27248).

To prepare the mobile phase, 1.36 g of monobasic potassium phosphate was dissolved in 900 ml of water. The pH was adjusted to 2.92 by dropwise addition of perchloric acid, and the solution was diluted to 1 l. A portion of this aqueous solution (675 ml) was diluted to 1 l with methanol.

The samples were chromatographed with an injection volume of 15 μl, a flow-rate of 2.0 ml/min, and a detector sensitivity of 0.02 a.u.f.s.

Nuclear magnetic resonance (NMR)

A 60-MHz NMR spectrometer (Model T60A, Varian, Palo Alto, CA, U.S.A.) was used. The solvent was deuteromethanol, and tetramethylsilane was used as internal standard.

Thin-layer chromatography (TLC)

TLC sheets (silica gel 60F-254 on aluminium support, Merck, Cat. No. 5534) were developed in methanol–chloroform (1:9) and visualized under shortwave UV light.

Sample preparation

A volume of 10 ml of dental anesthetic solution was transferred to a 15 × 85 mm culture tube, and approximately 40 mg of lead acetate trihydrate was added. The tube was capped, and the contents were mixed on a vortex stirrer and centrifuged at approximately 2000 *g* for 2 min. The supernatant was withdrawn with a 10-ml syringe.

An appropriate number (Table I) of C_{18} Sep-Pak cartridges (Waters Assoc., Cat. No. 51910) were connected in tandem with 7- to 8-mm lengths of 4 mm O.D. glass tubing. The ends of the cartridges were trimmed so that the plastic tubes over-

TABLE I

METHOD PARAMETERS FOR HPLC DETERMINATION OF *d*-/*l*-EPINEPHRINE ENANTIOMER RATIO

Lidocaine (%)	*Number of Sep-Paks*	*Eluate discarded (ml)*	*Eluate collected (ml)*
0.5	2	2	5
1.0	4	4	4
1.5	4	4	4
2.0	4	4	4

lapped the ends of the glass tubes by 3–4 mm. One end of the tandem Sep-Paks was cut short to reduce mixing of eluted fractions.

The tandem Sep-Paks were conditioned with methanol and acidified water (pH 4 with hydrochloric acid) as follows. A syringe containing 40 ml of methanol was attached to the long end of the tandem Sep-Paks, and the cartridges and syringe were inverted. Methanol (3–5 ml) was pushed upward through the cartridges to remove trapped air. The cartridges were rotated downward, and the remainder of the methanol was forced through the cartridges while care was taken to ensure that the cartridges were not ejected from the syringe. The cartridges were attached to a syringe containing 25 ml of acidified water. (To prevent introduction of air into the cartridges, the two syringes were laid side by side and the tip of the syringe was filled with liquid.) Acidified water (20 ml) was forced downward through the cartridges.

The cartridges were attached to the 10-ml syringe containing the sample, and similar precautions were taken to prevent introduction of air. The sample was forced downward through the Sep-Paks at the rate of 1 drop/sec. A portion of the eluate (Table I) equal to the void volume was discarded, and an appropriate volume of eluate (Table I) was collected and evaporated to dryness in a 25-ml conical glass vessel on a rotary evaporator equipped with a tap-water aspirator for vacuum and a water bath (50°C).

GITC reagent (2% w/v) in dimethylformamide, 100 μl) was added to the residue in the 25-ml conical flask. A stirring rod and vortex stirrer were used to pulverize the crystals. The stoppered flask was placed in a water bath (50°C). Ten min after the GITC reagent was added, the flask was removed from the water bath and 20 μl of 0.5% (v/v) hydrazine hydrate in dimethylformamide was added. The mixture was transferred to a small, tapered centrifuge tube and centrifuged at 2000 *g* for 2 min. The supernatant was transferred to an HPLC micro injection vial, and 10 min after the addition of hydrazine hydrate solution, a 15-μl sample was injected into the liquid chromatograph.

Calculation

The ratio percentage of *d*-epinephrine in the local anesthetic product can be calculated by

$$d\text{-Epinephrine (\%)} = 100[A_d/(A_d + A_l)]$$

where A_d and A_l are the chromatographic peak areas for the *d*- and *l*-epinephrine derivatives.

RESULTS AND DISCUSSION

Epinephrine reacts with the chiral reagent GITC under mild conditions. The thiourea derivative absorbs light at 254 nm, permitting detection of the diastereomers resolved by HPLC. Hydrazine hydrate is added to consume the excess GITC reagent; the elution time of the GITC-hydrazine hydrate derivative is less than that of the epinephrine-GITC derivative, which shortens the time required per chromatogram[3]. The retention times of the *l*- and *d*-epinephrine derivatives were about 13 and 16 min, respectively (Fig. 1).

Lidocaine hydrochloride–epinephrine injections are aqueous solutions with epinephrine concentrations typically 1/100 000 or less. Concentration of the epinephrine was required to achieve measurable levels. When a concentrated aqueous solution of epinephrine was reacted with GITC, only small amounts of the derivatives were formed. However, when the aqueous standard was evaporated to dryness and the dimethylformamide in the GITC reagent was used as the reaction solvent, the yields increased and the HPLC peaks were greatly enhanced. Björkqvist[5] reported that dimethylformamide seemed to catalyze the reaction and served as a good solvent for the disubstituted urea in a derivatization of amines with phenyl isocyanate.

In typical dental anesthetics lidocaine hydrochloride is present at 1000 times the concentration of epinephrine. At this concentration lidocaine hydrochloride interfered with the formation of the epinephrine-GITC derivative. A chromatographic

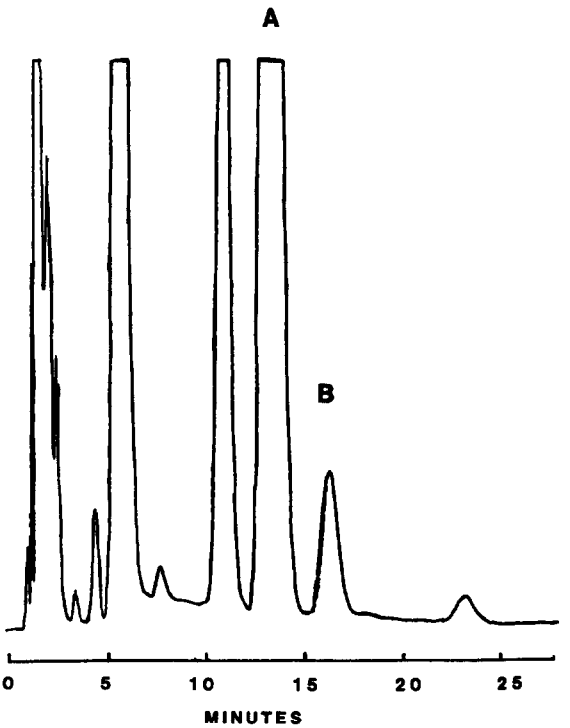

Fig. 1. Chromatogram of GITC derivatives of (A) *l*-epinephrine (95%) and (B) *d*-epinephrine (5%).

separation on tandem Sep-Paks was developed to remove the lidocaine hydrochloride from the injection solution. As the local anesthetic solution passed through the Sep-Paks, epinephrine eluted continuously while lidocaine was retained up to its "break through" point, as determined by HPLC examination of sequential fractions. Sample collection was stopped before lidocaine began to elute. An initial fraction equal to the void volume was discarded to minimize the amount of liquid to be evaporated. Care was taken not to overload the Sep-Paks with lidocaine; to avoid channeling, the Sep-Paks should not be squeezed. The Sep-Paks were prepared for reuse by a two-step procedure: First, they were washed with acidified water, and then with methanol, to remove the lidocaine; second, they were reconditioned by a wash with acidified water.

To verify the chromatographic pattern, the compounds retained on the cartridges were eluted with methanol and examined by TLC and NMR, which showed that lidocaine [R_F = 0.56, δ:7.08 (3H, s), 2.19 (6H, s)] and methylparaben [R_F = 0.78, δ:7.85 (2H, d, J = 4.5 Hz), 6.79 (2H, d, J = 4.5 Hz), 3.80 (3H, s)] were the sole organic materials retained on the cartridges.

Sodium bisulfite, an antioxidant added to the dental injections, may react with epinephrine[6] to yield 1-(3,4-dihydroxyphenyl)-2-methylaminoethane sulfonic acid (epinephrine sulfonic acid). Solutions of epinephrine formulated with sodium metabisulfite in ampoules and in the absence of oxygen have been reported to be stable for 7 years at 15°C without significant formation of epinephrine sulfonic acid[7–10]. When 5 ml of a local-anesthetic sample from which the lidocaine had been removed was concentrated to 0.5 ml under nitrogen in a room-temperature water bath, the epinephrine was converted to its sulfonic acid derivative, as shown by HPLC. In further tests, a standard solution of epinephrine was combined with a sodium bisulfite solution and evaporated to dryness; when the resulting compound was treated with GITC, the chromatogram of the reaction mixture showed that no GITC derivatives of *d*- or *l*-epinephrine were formed. This problem was solved by precipitation of metabisulfite from the local-anesthetic solution with an excess of lead acetate as the initial step in the analysis.

Sodium chloride or a small amount of residual water had no effect on the GITC reaction. Heating the reaction mixture actually increased the yield of the GITC derivatives. In the procedure presented here, lidocaine, methylparaben, and metabisulfite salts were removed, and the derivatization of the remaining residue was conducted at about 50°C.

Two formulations (about 20 samples) contained an unknown interfering compound that eluted at the same retention time as the GITC derivative of *d*-epinephrine. A slight modification of the mobile phase [aq. buffer–methanol (70:30)] allowed resolution of the interfering material and the GITC derivatives of *d*- and *l*-epinephrine.

Control solutions containing known added amounts of lidocaine hydrochloride, *dl*-epinephrine hydrochloride, and *l*-epinephrine were prepared with ratios of *d*- to *l*-epinephrine from 0 to 0.4 (0–40% *d*-isomer). Least-squares linear-regression analysis of the chromatogram peak areas *vs.* percent *d*-epinephrine gave a correlation coefficient of 0.99995, an intercept of 1.25% *d*-epinephrine, and a slope of 0.973. Three replicate assays of *dl*-epinephrine hydrochloride standard yielded 50.44, 50.35, and 51.42% *d*-epinephrine.

This method was used with success to assay approximately 70 samples (four

manufacturers) of local-anesthetic solutions containing lidocaine hydrochloride and epinephrine hydrochloride. The survey results, to be published elsewhere, showed that even after the expiration date had passed, most samples contained 5% or less of the *d*-epinephrine isomer.

REFERENCES

1 R. F. Doerge, in C. Wilson, O. Gisvold and R. Doerge (Editors), *Textbook of Organic Medicinal and Pharmaceutical Chemistry*, Lippincott, Philadelphia, PA, 7th ed., 1977, p. 436.
2 *United States Pharmacopeia*, 20th rev., United States Pharmacopeial Convention, Inc., Rockville, MD, 1980, p. 278.
3 N. Nimura, Y. Kasahara and T. Kinoshita, *J. Chromatogr.*, 213 (1981) 327.
4 N. Nimura, H. Ogura and T. Kinoshita, *J. Chromatogr.*, 202 (1980) 375.
5 B. Björkqvist, *J. Chromatogr.*, 204 (1981) 109.
6 T. Higuchi and L. C. Schroeter, *J. Amer. Chem. Soc.*, 82 (1960) 1904.
7 D. Szulczewski and W. Hong, in K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. 7, Academic Press, New York, 1978, pp. 193–229.
8 K. Backe-Hansen and G. Holst, *Acta Pharm. Suecica*, 3 (1966) 269.
9 P. Lundgren and S. Strom, *Acta Pharm. Suecica*, 3 (1966) 273.
10 K. Backe-Hansen, A. Drottning, A. M. Vennerod and K. Briseid-Jensen, *J. Pharm. Pharmacol.*, 15 (1963) 804.