Journal of Chromatography, 257 (1983) 400–404
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,515

Note

Simultaneous determination of hexylcaine hydrochloride and parabens in solution by high-performance liquid chromatography

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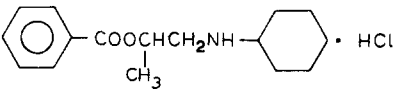
(Received November 10th, 1982)

Hexylcaine hydrochloride [1-(cyclohexylamino)-2-propanol benzoate hydrochloride], supplied as a topical solution, is a pharmaceutically important local anesthetic. Hexylcaine hydrochloride belongs to the benzoic acid ester class of anesthetics such as cocaine, tetracaine and procaine. The analysis of hexylcaine hydrochloride by gas chromatography[1], ion-pairing with methyl-orange[2], and phosphorimetry[3] has been reported in the literature. The United States Pharmacopeial (USP) method[4] of analysis involves column partition chromatography where the absorbance of the final column eluent is measured at 275 nm.

A reversed-phase liquid chromatographic procedure has been developed for the determination of the active ingredient, hexylcaine hydrochloride, and the preservatives, methyl and propylparaben, in a topical solution. This procedure separates hexylcaine hydrochloride from its hydrolysis product benzoic acid, and from methyl and propylparaben and their hydrolysis product *p*-hydroxybenzoic acid.



Hexylcaine hydrochloride

EXPERIMENTAL

Reagents and chemicals

USP reference standard hexylcaine hydrochloride, methylparaben and propylparaben were used in the standard solutions. Benzoic acid (Fisher Scientific, Fairlawn, NJ, U.S.A.) and *p*-hydroxybenzoic acid (Eastman-Kodak, Rochester, NY, U.S.A.) were reagent grade.

High-performance liquid chromatographic (HPLC) grade acetonitrile, *n*-octylamine (Aldrich, Milwaukee, WI, U.S.A.) and reagent-grade phosphoric acid were used in the mobile phase. Denatured ethyl alcohol (95% 3A) was used as the solvent in the parabens standard preparation.

Mobile phase

Octylamine phosphate buffer was prepared by diluting 1.0 ml of *n*-octylamine

0021-9673/83/0000–0000/$03.00

to 1.0 l with deionized distilled water. Phosphoric acid was added with stirring until the pH was equal to 2.9.

The mobile phase was prepared by mixing 200 ml of the octylamine phosphate buffer, 650 ml of deionized distilled water and 150 ml of HPLC-grade acetonitrile. This solution was degassed by vacuum filtration through a 5.0 μm PTFE filter (LSWP 04700, Millipore, Bedford, MA, U.S.A.).

Chromatography

A Varian 5060 high-performance liquid chromatograph, equipped with a Valco loop injector, and an LDC spectromonitor III variable-wavelength detector Model 1204 operated at 254 nm were used. Separation was carried out on a μBondapak CN column (30 × 0.39 cm I.D.) (Waters Assoc., Milford, MA, U.S.A.) at room temperature, with the flow-rate set at 1.4 ml/min.

Standard preparation

A methylparaben stock solution was prepared at a concentration of 1.0 mg/ml by dissolving methylparaben USP reference standard in alcohol.

A propylparaben stock solution was prepared at a concentration of 0.4 mg/ml by dissolving propylparaben USP reference standard in alcohol.

A working standard solution was prepared in deionized distilled water where the final concentration of hexylcaine hydrochloride was 1.0 mg/ml. Aliquots of the methylparaben and propylparaben stock solutions were added to the working standard to yield concentrations of 30 μg/ml of methylparaben and of 4 μg/ml propylparaben.

Sample preparation

The hexylcaine hydrochloride topical solution was diluted to a concentration of 1.0 mg/ml of hexylcaine hydrochloride with deionized distilled water.

RESULTS AND DISCUSSION

With the mobile phase containing acetonitrile, *n*-octylamine and phosphoric acid at a pH of 2.9, the *n*-octylamine acts as a competing base by adsorbing to the unreacted silanol sites[5], and thus greatly improves the peak shape of the hexylcaine hydrochloride. The *n*-octylamine also acts as an ion-pairing reagent with the benzoic acid and the *p*-hydroxybenzoic acid[6]. An increase in the concentration of the *n*-octylamine at a constant pH will increase the retention of the benzoic acid and *p*-hydroxybenzoic acid, while decreasing the retention of hexylcaine hydrochloride, resulting in co-elution of the methylparaben and benzoic acid. The optimal mobile phase was systematically determined by varying its composition until good peak shape was obtained and *p*-hydroxybenzoic acid, benzoic acid and methylparaben were separated. This separation has proven to be difficult using other HPLC systems.

The separation of hexylcaine hydrochloride from methyl and propylparaben is shown in Fig. 1. This chromatogram displays the elution order for hexylcaine hydrochloride, parabens and their hydrolysis products.

A linear regression analysis of the data for the five concentration levels of hexylcaine hydrochloride and parabens is shown in Tables I and II. These data show that the method is linear.

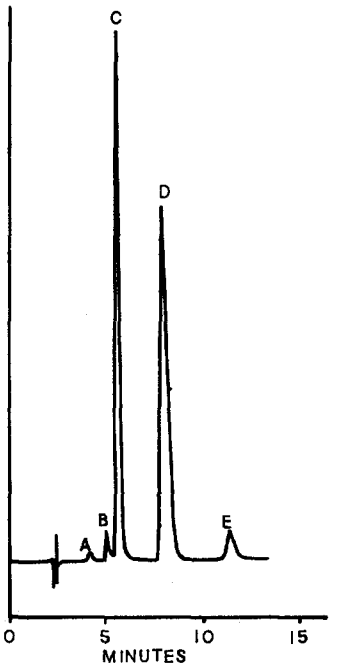


Fig. 1. Separation of hexylcaine hydrochloride from methyl and propylparaben. Peaks: A = *p*-hydroxybenzoic acid; B = benzoic acid; C = methylparaben; D = hexylcaine hydrochloride; E = propylparaben.

TABLE I

LINEARITY AND PRECISION OF THE HPLC METHOD FOR HEXYLCAINE HYDROCHLORIDE

Actual concn. of hexylcaine HCl (mg/ml)	*Observed HPLC results (mg/ml)**	*Recovery (%)*	*Slope*	*Intercept*	*r***
0.50	0.511 ± 0.001	102%	0.977	0.027	0.999
0.80	0.815 ± 0.003	102%			
1.00	1.007 ± 0.002	101%			
1.20	1.206 ± 0.003	101%			
1.50	1.489 ± 0.001	99%			

* Result is based on three replicate injections. The value given is the mean ± S.D.

** Correlation coefficient determined by linear regression analysis.

TABLE II

LINEARITY AND PRECISION OF THE HPLC METHOD FOR PARABENS

Actual concn. of total parabens (mg/ml)	*Observed HPLC results (mg/ml)**	*Recovery (%)*	*Slope*	*Intercept*	*r***
0.0170	0.0167 ± 0.0001	98	1.00	0.00	0.999
0.0272	0.0271 ± 0.0001	100			
0.0340	0.0342 ± 0.0001	101			
0.0408	0.0409 ± 0.0001	100			
0.0501	0.0509 ± 0.0001	100			

* Result is based on three replicate injections. The value given is the mean ± S.D.

** Correlation coefficient determined by linear regression analysis.

TABLE III

HEXYLCAINE HYDROCHLORIDE TOPICAL SOLUTIONS: OBSERVED RESULTS AND LIMITS OF DETECTION

Ingredients	*Limit of detection (20-μl injection)*	*Lot No. 1 age 29 months (mg/ml)*	*Claim (%)*	*Lot No. 2: age 54 months (mg/ml)*	*Claim (%)*	*Lot No. 3: age 64 months (mg/ml)*	*Claim (%)*	*Lot No. 4: age 75 months (mg/ml)*	*Claim (%)*
Hexylcaine hydrochloride	600 ng/ml	49.0	98	46.5	93	45.5	91	45.0	90
Benzoic acid	200 ng/ml	0.67	3.3*	0.85	4.2*	1.16	5.7*	1.30	6.3*
Methylparaben	60 ng/ml	1.38	92	1.35	90	1.33	89	1.33	89
Propylparaben	150 ng/ml	0.14	70	0.14	70	0.14	70	0.16	80
Total parabens	–	1.52	89	1.49	88	1.47	87	1.49	88
p-Hydroxy-benzoic acid	45 ng/ml	0.008	0.5**	0.013	0.9**	0.022	1.5**	0.027	1.8**

* Hexylcaine hydrochloride equivalence (%).

** Total parabens equivalence (%).

The stability-indicating capability of this HPLC procedure is shown in Table III. This table displays a profile of four lots of hexylcaine hydrochloride topical solution from 29 to 75 months in age. As the concentration of hexylcaine hydrochloride decreases with time, a corresponding increase in the concentration of the hydrolysis product benzoic acid is observed. The concentration of the hydrolysis product of the parabens, *p*-hydroxybenzoic acid, also increases with time.

This HPLC procedure is a significant advance over the current methods in the literature. It requires minimal sample preparation, is easier to perform, and requires considerably less time than the pharmacopeial method.

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