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Note

High-performance liquid chromatographic analysis of naloxone hydrochloride in injectable solutions

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Naloxone hydrochloride, a potent narcotic antagonist upon parenteral administration, is a synthetic congener of oxymorphone[1].

Previously reported analytical procedures include gas–liquid chromatography (GLC)[2,3], GLC of silyl derivatives[4,5], spectrofluorometry[5,6], UV spectrophotometry[2], and thin-layer chromatography[2,3,6]. None of these methods was adapted for the analysis of naloxone hydrochloride in injectable solutions. A GLC assay method[7] is employed in the analysis of naloxone hydrochloride in injectable solutions by the United States Pharmacopeia. This procedure is time-consuming and requires additional separation and washing steps.

This report discusses a stability-indicating high-performance liquid chromatographic (HPLC) procedure for determination of naloxone hydrochloride in injectable solutions. The method is simple and direct and requires no prior preparation, and at the same time analyzes for methylparaben and propylparaben, preservatives present in the injectable solutions, saving additional labor and time.

EXPERIMENTAL

Reagents

Glass-distilled water and methanol spectroquality from Burdick & Jackson Labs., Muskegon, MI, U.S.A. Ammonium carbonate was analytical grade from Mallinckrodt, St. Louis, MO, U.S.A. Methylparaben, propylparaben and naloxone were USP Reference Standard. Papaverine was from Endo Laboratories, Garden City, NY, U.S.A.

Apparatus

An HPLC system (Model 6000, Micromeretics, Norcross, GA, U.S.A.) equipped with a multiple-wavelength UV detector (Model SF770, Schoeffel, Westwood, NJ, U.S.A.), adjusted at 220 nm and attached to a printer plotter integrator (Model 3385A; Hewlett-Packard, Palo Alto, CA, U.S.A.) was used for the analyses.

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An 0.45-μm filter system from Millipore, Bedford, MA, U.S.A., was used to filter and degas the mobile phase solvents.

Columns

A reversed-phase column, μBondapak®, from Waters Assoc., Milford, MA, U.S.A., 30 × 0.63 cm O.D., with *ca.* 5000 theoretical plates and a 10-μm non-polar packing material consisting of a monomolecular layer of octadecylsilane, was used for the reversed-phase chromatography.

Mobile phase

Methanol containing 0.1% (w/v) aqueous ammonium carbonate (45:55) was used.

Chromatographic conditions

The temperature was ambient. The flow-rate was 3.0 ml/min (inlet pressure *ca.* 3000 p.s.i.g.). The absorbance unit for full scale deflection was 2 × 2, and the chart speed was 0.2 cm/min.

Standard preparation

About 360 mg of methylparaben, 50 mg of propylparaben and 72 mg of naloxone base reference standard (molecular weight ratio of hydrochloride to base, 1:11) were accurately weighed and dissolved in *ca.* 50 ml of methanol in a 200-ml volumetric flask by shaking. Water was added to volume and the solution was mixed well. Then 4.0, 5.0 and 6.0 ml of this standard solution were pipetted into separate stoppered erlenmeyer flasks.

Internal standard preparation

A methanolic solution containing 0.5 mg/ml of papaverine was prepared.

Sample solution

A 5.0-ml volume of the injectable solution was pipetted into a stoppered erlenmeyer flask.

Chromatographic procedure

A 2.0-ml volume of internal standard solution was pipetted into each of the standard sample flasks. A constant-volume loop injected was used to inject 10 μl onto the column. The standard solutions were injected until a precision of $\leqslant 2\%$ was obtained for the peak area ratio of naloxone. To ensure that the same detector response and column conditions were maintained during the analysis time, duplicates of each standard followed by duplicates of the sample were injected, then all three standards were injected at the end of the run.

Naloxone hydrochloride injectable solution containing 0.02 mg/ml of the drug substance was also analyzed via the procedure as described above, with the following modifications.

Standard preparation

About 50 mg of naloxone base were accurately weighed, dissolved and diluted to volume with 0.1 *N* HCl in a 100-ml volumetric flask. Then 4.0 ml of this solution

was pipetted into another 100-ml volumetric flask and diluted to volume with water. Then 8.0, 9.0, and 10.0 ml of this standard solution were pipetted into separate stoppered erlenmeyer flasks.

Internal standard preparation

A methanolic solution containing 0.1 mg/ml of papaverine was prepared.

Chromatographic procedure

A constant-volume loop injection of 100 μl was used. The absorbance unit for full scale deflection was 2.

Calculation

Naloxone hydrochloride, methylparaben and propylparaben were determined either by computer calculation of the least square line or by plotting the peak area ratio of the standards *versus* milligrams per standard. Values of unknown sample concentrations were then determined.

RESULTS AND DISCUSSION

Under the assay conditions described, a linear relationship of ratios of naloxone hydrochloride, methylparaben and propylparaben concentrations *versus* internal standard concentration was obtained over the ranges 1.60–2.40, 7.20–10.8 and 1.00–1.50 for the three compounds, respectively. The correlation coefficient for naloxone was 0.9999. Regression analysis showed that the regression equation was $y = 3.30x - 0.08$, with a standard error of the estimate of y on x of 0.008 and standard errors of the estimate of the intercept and slope of 0.06 and 0.09, respectively. The retention times of naloxone hydrochloride, methylparaben, propylparaben and papaverine were 6.7, 1.8, 4.6 and 9.6 min, respectively (Fig. 1).

The accuracy and precision of the HPLC assay are demonstrated in Table I. The standard deviation based on ten assays for naloxone hydrochloride, 0.4 or 0.02 mg/ml, methylparaben and propylparaben were ± 0.4 or ± 0.9, ± 0.7 and $\pm 1.8\%$, respectively. Under the conditions of assay, as little as 0.16 mg/ml, 0.72 mg/ml and 0.08 mg/ml of naloxone hydrochloride, methylparaben and propylparaben, respectively, can be quantitatively determined. This was demonstrated with standards with concentration ratios in the ranges 0.8–3.2, 3.6–14.4 and 0.5–2.0 for naloxone hydrochloride, methylparaben and propylparaben, respectively. The results (Table I and Fig. 1) indicate that using a single injection, it is possible to separate active ingredients and the two parabens present in commercial injectable solutions. While naloxone hydrochloride is assayed, methylparaben and propylparaben, present as preservatives in the injection, can be assayed simultaneously.

As shown in Table II, results obtained with the HPLC procedure compared favorably with those of the USP XX method in the analysis of commercial naloxone hydrochloride injectable solutions. (Narcan® and Narcan®–Neonatal Injections, Endo Labs.)

To prove the stability-indicating character of the HPLC procedure for naloxone hydrochloride, degraded samples were prepared and analyzed. A stock solution of naloxone hydrochloride in alkaline medium was prepared and degraded by the action

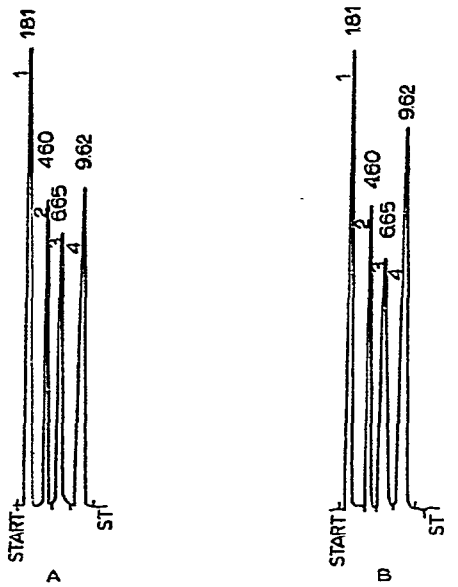

Fig. 1. HPLC scans of naloxone hydrochloride. (A) From commercial injectable solution. (B) From standard solution. Peaks: 1 = methylparaben; 2 = propylparaben; 3 = naloxone hydrochloride; 4 = papaverine.

TABLE I

RECOVERY RESULTS OF NALOXONE HYDROCHLORIDE, METHYLPARABEN AND PROPYLPARABEN

	*Assay results**	
	% of added	*Standard deviation (%)*
Naloxone hydrochloride (0.4 mg/ml)	100.3	±0.4
Naloxone hydrochloride (0.02 mg/ml)	100.8	±0.9
Methylparaben	99.9	±0.7
Propylparaben	99.8	±1.8

* Mean of ten assays.

TABLE II

RESULTS OF ANALYSIS OF NALOXONE HYDROCHLORIDE INJECTION

	Amount claimed (mg/ml)	*% of claim* found*		*Standard deviation (%)*	
		HPLC	*USP*	*HPLC*	*USP*
Naloxone hydrochloride	0.4	95.5	94.3	±0.004	±0.004
Naloxone hydrochloride	0.02	100.8	101.2	±0.009	±0.015
Total parabens	2.0	99.5**	98.0***	±0.009	±0.005

* Mean of ten assays.

** Parabens reported as total of methylparaben and propylparaben.

*** Parabens analyzed as total of methylparaben and propylparaben by UV procedure.

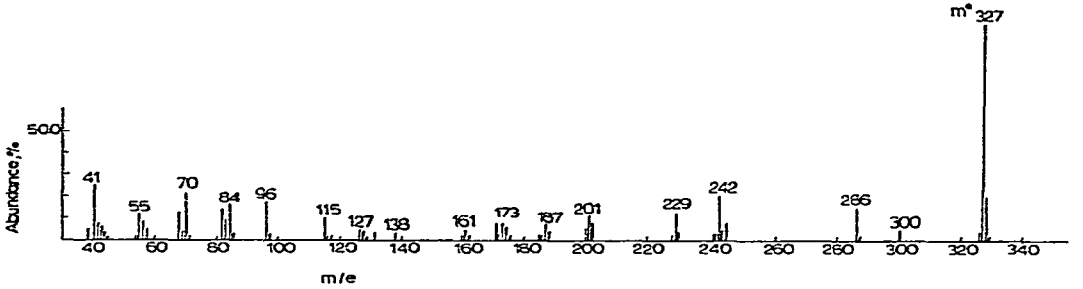

Fig. 2. Mass spectrum of naloxone (normalized).

of heat and oxygen to 50% of its original value as ascertained by the USP GLC procedure[7]. This solution was injected on to the liquid chromatograph using the described method. The peak eluting at the same retention time (6.7 min) of naloxone USP reference standard was collected. No other peaks were detectable. The collected sample was evaporated to dryness under vacuum on a water bath at 40°C and the residue was analyzed by mass spectrometry. (Model 21-492; DuPont, Wilmington, DE, U.S.A.). The mass spectrum showed a molecular ion at *m/e* 327, and a fragmentation pattern identical with that of the naloxone USP reference standard (Fig. 2).

If degradation of the parabens were to occur, the degradation product (*p*-hydroxybenzoic acid) elutes with the solvent and thus is separated from the components analyzed.

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