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Investigation of 4,5-epoxymorphinan degradation during analysis by HPLC

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Abstract

Compounds of the 4,5-epoxymorphinan series have been shown to degrade in solution to the corresponding 2,2′-dimers when stored in amber glass HPLC vials. A colorant in the glass has been shown to catalyze the degradation. Although amber glass is routinely used to protect solutions from light degradation, it should not be used without evaluating its effect on sample stability. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: 4,5-Epoxymorphinan; Naloxone; Nalbuphine; Oxymorphone; Degradation; Reversed-phase HPLC

1. Introduction

Naloxone, nalbuphine, and oxymorphone (Fig. 1) are compounds in the 4,5-epoxymorphinan series, and have narcotic agonist or antagonist properties. Naloxone is a narcotic antagonist that prevents or reverses the effects of opioids including respiratory depression, sedation, and hypotension. Nalbuphine and oxymorphone are potent narcotic analgesics used for the relief of moderate to severe pain.

The primary degradation product of 4,5-epoxymorphinans having a hydroxyl group at the 3-position is the 2,2′-dimer. This forms from the oxidation of the phenolic group with subsequent condensation to the dimer [1]. It has been reported that pH and oxygen contribute to the degradation of this class of compounds [2,3]. In alkaline or neutral solution, degradation occurs rapidly at room temperature, while acidic solutions are relatively stable. Elevated temperature, autoclaving in particular, causes the highest level of degradation [4]. Noroxymorphone is another degradation product that is formed in solution. Structures of these compounds are shown in Fig. 2.

An investigation into the degradation of these compounds began when inconsistencies were found between degradation product results obtained from different laboratories for the same drug product lots. Inconsistent results were obtained for the degradation products noroxymor-

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phone and 2,2′-bisnaloxone in a neonatal formulation of naloxone hydrochloride injection (0.02 mg/ml). Initially, the analytical method was suspect, but the analytical parameters were evaluated and determined to be acceptable. Compounds of the 4,5-epoxymorphinan series are known to be light sensitive, and precautions were taken to minimize light exposure of the samples during testing. In one laboratory, colorless vials were covered with foil to protect samples from ambient light; amber glass HPLC sample vials were used in the other laboratory. A constituent of the amber glass was found to have caused degradation of these compounds to the 2,2′-dimers. Degradation did not occur in foil-covered colorless glass vials. Similar degradation products were found for other compounds of like chemical structure.

2. Experimental

2.1. Reagents and standards

Naloxone, oxymorphone, and nalbuphine standards were obtained from Mallinckrodt Specialty Chemicals, Inc. (St. Louis, MO). The dimers of

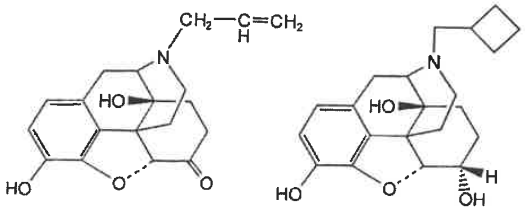
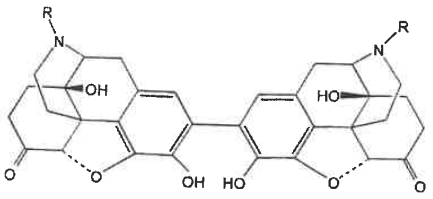
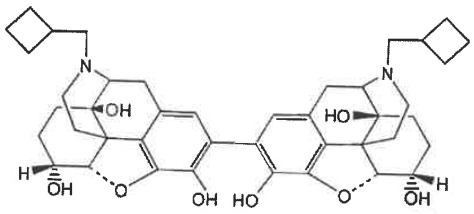


Fig. 1. Chemical structures of naloxone, nalbuphine, and oxymorphone.

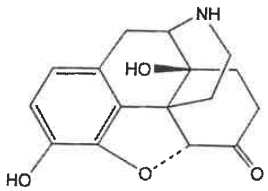


2,2′-Bisnaloxone: R = $CH_2CH{=}CH_2$

2,2′-Bisoxymorphone: R = CH_3



2,2′-Bisnalbuphine



Noroxymorphone

Fig. 2. Chemical structures of degradation products.

these compounds (i.e., 2,2′-bisnaloxone, 2,2′-bisnalbuphine, and 2,2′-bisoxymorphone) were prepared by the oxidation of the parent compounds and were isolated as the free bases. The acetonitrile and methanol were HPLC grade obtained from EM Science (Gibbstown, NJ). All solutions were prepared using water from a Milli-Q® water system (Millipore, Milford, MA). All other reagents were ACS reagent grade.

The formulations contain the active drug substance, hydrochloric acid for pH adjustment, sodium chloride for isotonicity, and water for injection, USP. The nalbuphine formulation also includes sodium citrate and citric acid. All formulations are packaged in 1-ml ampuls.

Naloxone, oxymorphone, and nalbuphine standards were prepared by weighing the pure bases into volumetric flasks and diluting to volume with 0.1 N hydrochloric acid. The concentration of naloxone was 0.02 mg/ml, which is the concentration of active in the formulation in which the degradation was first observed. Nalbuphine and oxymorphone solutions were prepared at 1 mg/ml.

2.2. Equipment

The chromatographic system used at Site 1 was a Hewlett-Packard model 1050 solvent delivery system, a variable-wavelength UV–visible detector, a variable-volume injector, and a column oven (Palo Alto, CA). The system used at Site 2 consisted of a Waters® (Milford, MA) Model 600 pump, a Waters® 600E Multisolvent Delivery System, a Waters™ 717plus Autosampler, a Waters® column oven, and an ABI Model 759A Absorbance Detector (Foster City, CA). All chromatographic data were collected with a Multichrom™ Data System from Fisons.

Atomic emission using ICP was performed with the Plasma Spec ICP 2.5 (Leeman Labs, Inc., Lowell, MA).

2.3. Chromatographic conditions

For the analysis of naloxone hydrochloride, an isocratic mobile phase consisting of 0.1 M phosphate buffer (pH 3.5) and acetonitrile (90:10, v/v) containing 0.1% triethylamine was used. Samples were analyzed on a Waters™ 30-cm × 7.8-mm i.d. μBondapak® Phenyl column. The column was equilibrated at 40 °C. The flow rate was 2.0 ml/min and column effluent was monitored at 220 nm. The samples were transferred directly from the dosage container to the HPLC vial with no further dilution. A 1-ml injection volume was used.

The HPLC method used in these studies for naloxone hydrochloride had been validated. The linear correlation coefficient for the standard curve was 0.9996. The RSD of the mean of nine injections was 3.2%. Recovery studies were performed using placebos fortified with authentic samples of noroxymorphone and 2,2′-bisnaloxone. Triplicate samples at concentrations of 0.5, 2.0, and 4.0% of label strength were prepared by a single analyst on three separate days. A fourth set of samples were prepared by a second analyst and analyzed on a different HPLC system using a different column. The mean recovery of the method was $100.9 \pm 7.4\%$ for noroxymorphone and $100.2 \pm 4.8\%$ for 2,2′-bisnaloxone. Noroxymorphone and 2,2′-bisnaloxone response factors were determined and used to calculate levels in the formulations. The quantitation limit is 0.055 μg/ml (0.28% of label strength). The detection limit is 0.024 μg/ml (0.12% of label strength).

The method for nalbuphine hydrochloride used a 30-min linear gradient composed of 0.05% trifluoroacetic acid (TFA), acetonitrile, and tetrahydrofuran. Samples were analyzed on a 15-cm × 4.6-mm i.d. Zorbax® SB-C8 column. The column was equilibrated at 35 °C. The flow rate was 1.5 ml/min and column effluent was monitored at 280 nm. A 50-μl injection was used.

For the analysis of oxymorphone hydrochloride, the method uses a gradient mobile phase consisting of 0.01 M sodium heptane sulfonate and methanol containing 0.1% triethylamine. Samples were analyzed on a 25-cm × 4.6-mm i.d. Zorbax® Phenyl column. The column was equilibrated at 45 °C. The flow rate was 1.6 ml/min and the detector wavelength was 280 nm. The injection volume was 150-μl.

All mobile phases were filtered through a 0.45-μm filter and degassed with helium.

2.4. Glass vials

Clear glass Type I, Class A and Type I, Class B HPLC vials were obtained from Kimble Glass, Inc. (Vineland, NJ), Waters® (Milford, MA), and Wheaton Glass (Millville, NJ). Polypropylene vials were obtained from Sunbrokers (Wilmington, NC). Amber glass Type I vials were obtained from Hewlett-Packard.

Table 1
Interlaboratory results for 2,2′-bisnaloxone in 0.02 mg/ml naloxone hydrochloride ampuls

Lot	% 2,2′-Bisnaloxone (w/w)	
	Site 1	Site 2
A	1.15	0.69
B	1.72	0.66

3. Results and discussion

3.1. Investigation of glass type

Stability samples of the naloxone hydrochloride neonatal formulation (0.02 mg/ml) were tested by laboratories at two sites. The levels of 2,2′-bisnaloxone levels were consistently higher at one site, while the results from both sites for noroxymorphone, the other known degradation product of naloxone, were statistically equivalent (Table 1). A typical chromatogram is shown in Fig. 3.

The method parameters were reviewed for discrepancies between the two sites. Sample preparation was not an issue as the formulation is transferred from the original container to the HPLC vial without dilution. The injection volume was 1 ml, but was not suspect due to the consistency of the noroxymorphone results between sites. Since the method was isocratic, inconsistencies that may occur between different types of HPLC systems were ruled out. The only method difference between the two sites was the type of HPLC vial used. Site 1 used vials made of amber glass while Site 2 used colorless glass vials of type N-51A. Experiments were undertaken to investigate the effect of the different types of glass on the sample solutions.

A solution of naloxone base was prepared at 0.02 mg/ml in 0.1 N HCl. Aliquots of the solution were placed in both amber and colorless glass HPLC vials. At designated timepoints, the solutions were injected onto the HPLC under normal method conditions. The results for 2,2′-bisnaloxone are shown in Table 2. The concentration of 2,2′-bisnaloxone remained constant in the solution stored in the volumetric flask and in the colorless glass HPLC vials. There was a definite increase with time in the amount of 2,2′-bisnaloxone stored in the solution in amber glass vials. After the 4-h sample in amber glass was injected, the solution was then transferred to a colorless glass vial and injected again after standing in colorless glass for an additional 19 h. The level of 2,2′-bis-

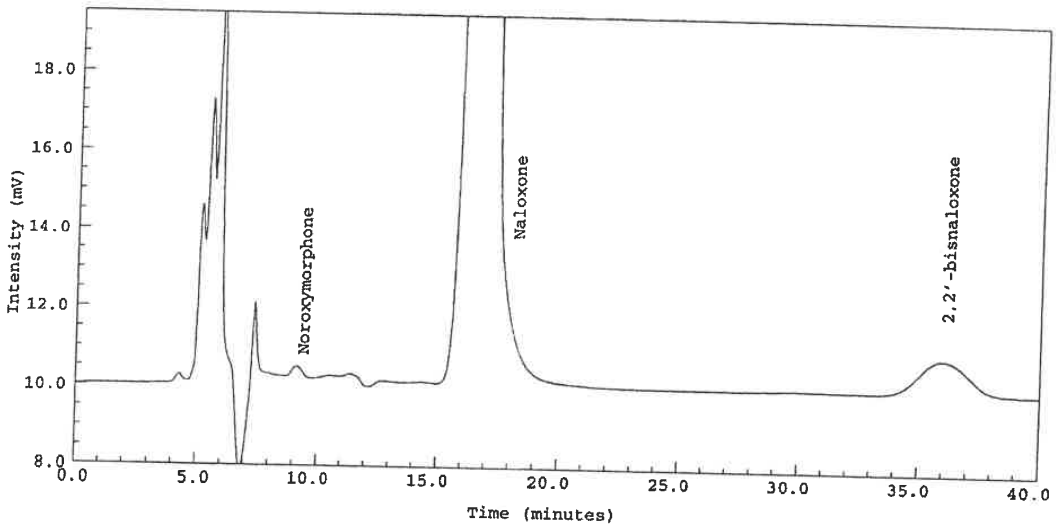


Fig. 3. Chromatogram of naloxone hydrochloride 0.02 mg/ml with typical degradation.

Table 2
Results for 2,2′-bisnaloxone in 0.02 mg/ml naloxone hydrochloride solution in clear and amber glass

Sample	Time	Condition	% 2,2′-Bisnaloxone (w/w)
I	Time zero	Clear glass volumetric	0.13
I	24 h	Clear glass volumetric	0.13
II	Time zero	Type I class A colorless vial	0.11
II	23 h	Type I class A colorless vial	0.15
III	4 h	Amber glass vial	0.28
III	19 h	Amber glass vial	0.77
III	24 h	Amber glass vial	0.85
IV	23 h	4 h in amber vial, then 19 h in colorless glass vial	0.54

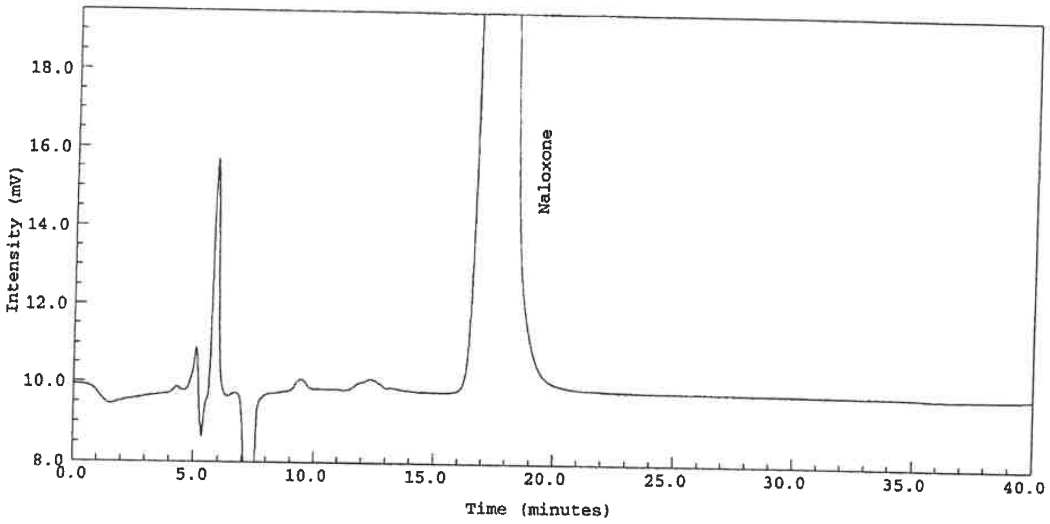


Fig. 4. Chromatogram of naloxone solution stored in amber glass after addition of EDTA solution.

naloxone increased, indicating that something had been leached out of the glass and was causing further degradation in solution.

3.2. EDTA experiment

Since iron oxide is used as a colorant in amber glass, an experiment was performed using EDTA as a complexing agent to determine if Fe^{+3} in the glass was leached into solution at low pH, catalyzing the oxidation. A solution of EDTA disodium salt was prepared at 0.2 mM in colorless glass. A solution of naloxone base was prepared at 0.02 mg/ml in colorless glass. Aliquots (0.25 ml) of the EDTA solution were added to several amber HPLC vials. The naloxone solution was then added to each vial. The vials were periodically shaken over several hours, then analyzed for 2,2′-bisnaloxone. The 2,2′-bisnaloxone peak was not detected (Fig. 4).

3.3. Determination of iron in solution

The iron content of the solution of naloxone hydrochloride that was stored in amber glass for 4 h was determined by atomic emission using inductively coupled plasma spectroscopy. The level was 0.3 ppm. The level of iron in the solution stored in

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