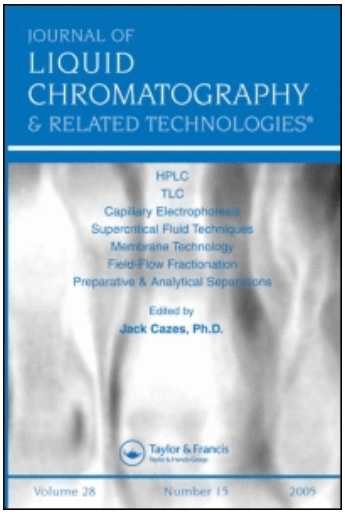

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DETERMINATION OF DEGRADATION PRODUCTS IN NALBUPHINE HYDROCHLORIDE INJECTION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A method for the determination of impurities and degradation products in nalbuphine hydrochloride injection by gradient elution high performance liquid chromatography (HPLC) is reported. Reversed phase gradient elution chromatography was carried out using a mobile phase containing 0.05% trifluoroacetic acid, acetonitrile, and tetrahydrofuran. Validation data for linearity, accuracy, precision, robustness, detection limit, and quantitation limit are presented. The chromatographic system resolves nalbuphine from related substances with corrections made for differences in detector response at the specified wavelength.

INTRODUCTION

Nalbuphine [17-(cyclobutylmethyl)-4,5α-epoxymorphinan-3,6α,14-triol] is a highly efficacious synthetic narcotic agonist-antagonist analgesic of the phenanthrene series. It demonstrates substantially reduced morphine-like side effects at elevated dosage levels and possess a low degree of narcotic abuse potential.

Nalbuphine hydrochloride injection is a parenteral solution consisting of nalbuphine hydrochloride, citric acid, and sodium citrate in sterile water for injection. The preservatives methylparaben and propylparaben are added to inhibit microbial growth in the formulation.

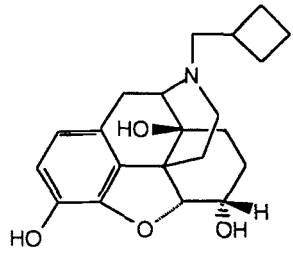
The principal synthetic impurities of nalbuphine are β-nalbuphine and α-nor-14-hydroxydihydromorphine. The primary degradation pathway of 4,5-epoxymorphinans with a hydroxyl group at the 3-position is the oxidation of the phenolic group with subsequent condensation to the dimer.[1, 2] The dimer that forms from nalbuphine is 2,2′-bisnalbuphine. Another degradation product of nalbuphine that has been found in liquid formulations, although at much lower levels, is 10-ketonalbuphine. Structures of nalbuphine and related compounds are shown in Figure 1.

The goal of the present work was to establish a stability-indicating liquid chromatographic method for the determination of the known synthetic impurities and degradation products of nalbuphine in parenteral formulations. Numerous studies concerning the assay determination of nalbuphine in biological fluids have been reported in the literature. Nalbuphine has been determined in urine by gas chromatography[3-5] and in plasma by high performance liquid chromatography using either UV detection[6,7] or electrochemical detection.[8-12] An enzyme immunoassay has been reported for the analysis of nalbuphine in biological fluids and on surfaces.[13] Thin layer chromatography has been used to determine nalbuphine in equine urine.[14] Capillary zone electrophoresis has been used for the determination of nalbuphine in whole blood.[15] A micellar electrokinetic capillary electrophoresis method for the determination of β-nalbuphine and 2,2′-bisnalbuphine in nalbuphine hydrochloride drug substance has been reported.[16] However, no methods were found for the determination of the related substances of nalbuphine using HPLC. The method was developed for the determination of known synthetic impurities and degradation products in parenteral products.

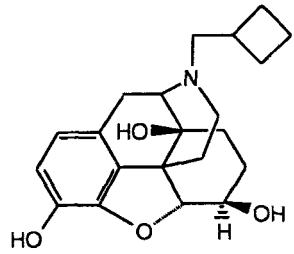
EXPERIMENTAL

Reagents and Materials

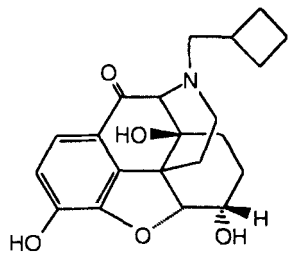
Nalbuphine HCl Injection was formulated at DuPont Pharma (Manati, PR). Nalbuphine reference standard, β-nalbuphine, and α-nor-14-hydroxydihydromorphine were obtained from Mallinckrodt Specialty Chemicals, Inc. (St. Louis, MO, USA). 10-Ketonalbuphine and 2,2′-bisnalbuphine were prepared from nalbuphine.



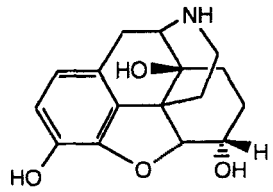
Nalbuphine



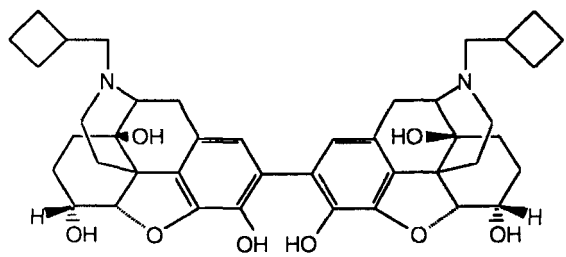
β-Nalbuphine



10-ketonalbuphine



α-nor-14-hydroxydihyromorphine



2,2′-Bisnalbuphine

Figure 1. Structures of nalbuphine and related compounds.

Methanol and tetrahydrofuran (inhibitor-free) were HPLC grade, obtained from EM Science (Gibbstown, NJ). All other reagents were ACS reagent grade or better. All solutions were prepared with HPLC-grade solvents and water from a Milli-Q water system (Millipore, Milford, MA).

Apparatus

Analyses were performed using several chromatographic systems. The first system consisted of a Waters™ Model 600 pump, 600E System Controller, temperature control module, 717plus Autosampler (Milford, MA), and an Applied Biosystem Model 759A Absorbance Detector (Foster City, CA).

The second system consisted of a Waters™ Model 510 pump, 680 System controller, temperature control module, WISP 712 automated sample processor, and a Kratos Spectroflow 757 UV absorbance detector (Ramsey, NJ). Chromatographic data were acquired and processed using Fisons Multichrom™ Data System (ver. 2.01).

Chromatographic Conditions

The method for the parenteral finished product is designed to separate and quantify all degradation products of nalbuphine. The column used was a Zorbax® SB-C8 (5 μm, 150 mm x 4.6 mm) from MAC-MOD Analytical, Inc. (Chadds Ford, PA). Column temperature was maintained at 35°C. The detector wavelength was 280 nm. Mobile phase A was 0.05% trifluoroacetic acid (TFA) in water:acetonitrile (97:3). Mobile phase B was 0.05% TFA in water:tetrahydrofuran:acetonitrile (72:25:3). The mobile phases were filtered, degassed, and pumped at a flow rate of 1.5 mL/min.

The gradient profile was 4% to 28% mobile phase B in 21 minutes, a four-minute hold at 28% B, then four minutes at 100% B before re-equilibrating at initial conditions. The injection volume was 50 μL for all standards and samples. The related substances are measured against nalbuphine base reference standard by comparing peak areas and correcting them with response factors of the known related substances.

Standard and Sample Preparation

Three standard solutions at concentrations of 1.0%, 0.5%, and 0.1% of label strength were prepared from a stock solution of nalbuphine base reference standard in 0.1 N hydrochloric acid (0.09 mg/mL).

Samples were prepared by diluting an aliquot of the finished product to a concentration of 1.0 mg/mL. Mobile phase A was used for the dilution of standards and samples.

Table 1

Summary of Linearity Data

Compound	Slope	Correlation Coefficient	Y-Intercept
Nalbuphine	8.52349E+3	0.99990	6.11001E+1
2,2'-Bisnalbuphine	1.03335E+4	0.99992	8.15231E+3

RESULTS AND DISCUSSION

Linearity

Linearity of the standards was demonstrated between 0.5 μg/mL (0.05%) and 20 μg/mL (2.0%) using nalbuphine base reference standard solutions. To demonstrate linearity of 2,2′-bisnalbuphine, solutions were prepared at 0.9, 4.6, and 13.7 μg/mL. Linearity was evaluated by regression analysis of the solution concentrations versus their responses. The linear correlation coefficient for the standards was greater than 0.999 and is acceptable for this method. Correlation coefficients, intercept, and slope values for nalbuphine and 2,2′-bisnalbuphine are found in Table 1. The linear correlation coefficient for 2,2′-bisnalbuphine was greater than 0.999 and demonstrates that peak response is linear in the expected concentration range.

Precision

System precision was evaluated by making replicate injections of the middle standard. Replicate injections (n=5) of standards typically yield relative standard deviations of the peak area of less than or equal to 1.0% (Table 2).

Method precision was evaluated by the analysis of ten replicates of a composite sample. The relative standard deviation was 2.5% (Table 3).

Detection and Quantitation Limits

The detection limit and quantitation limit were determined by injecting solutions prepared from serial dilutions of known concentration until the responses were approximately 3x signal-to-noise ratio and 10x signal-to-noise ratio, respectively.

Table 2

System Precision of Replicate Injections of a Nalbuphine HCl Injection Vial Standard (0.5% Standard)

Injection	Peak Area
1	31192.7
2	30824.6
3	30927.3
4	30723.5
5	30736.4
Mean ± %RSD	30880.9 ± 0.6%

Table 3

Method Precision of Replicate Analysis of Nalbuphine HCl Injection

Replicate	% (w/w) 2,2'-Bisnalbuphine
1	0.36
2	0.34
3	0.34
4	0.34
5	0.36
6	0.34
7	0.34
8	0.34
9	0.34
10	0.35
Mean ± %RSD	0.34 ± 2.5%

The detection limit for nalbuphine and 2,2'-bisnalbuphine was 0.01% and the quantitation limit was 0.05%. While these limits are dependent on the stability and noise of a given detector, routine analysis will permit quantitation of the 0.1% standard when system suitability criteria have been met. The system suitability criteria require that a relative standard deviation for the nalbuphine peak area in six successive standard injections must not exceed 5.0%; the resolution between nalbuphine and β-nalbuphine must be at least 2.0.

Table 4

Percent Recovery of 2,2'-Bisnalbuphine from Fortified Sample

% W/W	Analyst 1 10 mg/mL Formulation	Analyst 2 20 mg/mL Formulation
0.10	105.8	111.7
	103.8	107.4
	106.1	107.6
0.50	107.4	111.5
	105.5	108.7
	106.9	110.3
1.50	108.5	106.3
	107.9	107.0
	108.3	106.5
Mean	106.7	108.2
RSD	1.4%	1.6%

Table 5

Solution Stability of Nalbuphine and 2,2'-Bisnalbuphine in Nalbuphine Hydrochloride Injection

Time (Hours)	Peak Area (mV · sec) Nalbuphine	2,2'-Bisnalbuphine
Initial	30766.5	20372.9
8	31159.4	21186.7
16	32017.2	20829.4
20	31005.4	20924.2
24	30411.1	22184.3
36	31273.1	20612.8
44	30561.9	20798.7
56	30498.1	20618.5
64	30700.1	21131.7
72	31540.9	20671.5

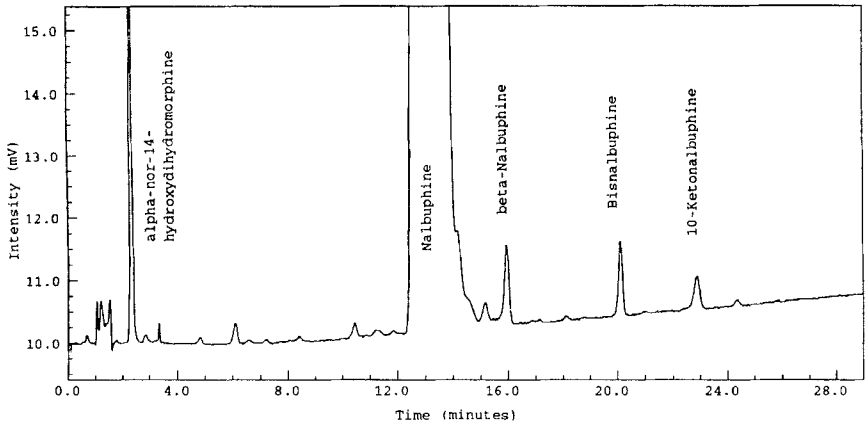


Figure 2. Representative chromatogram illustrating the separation of nalbuphine and related substances.

Determination of Response Factors

The response factor is defined here as the reciprocal of the peak area response of the related substance peak relative to that of the parent compound. Response factors at 280 nm were determined by making injections of known concentrations of the degradation products of nalbuphine hydrochloride and calculating the slope of the regression line. The ratio of the slope for nalbuphine to the slope for each degradation product is the response factor for the degradation product. These response factors were then used to compensate for the differences in UV absorptivity between the standard and the degradation products.

The calculated response factors are 0.86 for 2,2′-bisnalbuphine and 0.51 for 10-ketonalbuphine.

Accuracy

Recovery of 2,2′-bisnalbuphine was conducted using nalbuphine hydrochloride injection 10 mg/mL and 20 mg/mL formulations. Samples were fortified with 2,2′-bisnalbuphine at the 0.10%, 0.50%, and 1.50% levels. Quantitation was accomplished versus nalbuphine standards using the proper response factor. Testing was performed in duplicate by different analysts on different equipment and on different days. Accuracy was represented by the recoveries after correction for 2,2′-bisnalbuphine in the unfortified samples.

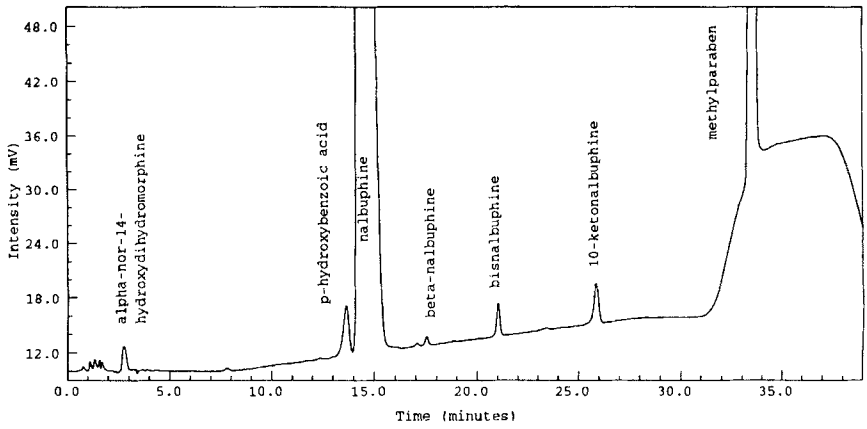


Figure 3. Chromatogram of fortified nalbuphine hydrochloride formulation with 2% increase in tetrahydrofuran concentration in mobile phase.

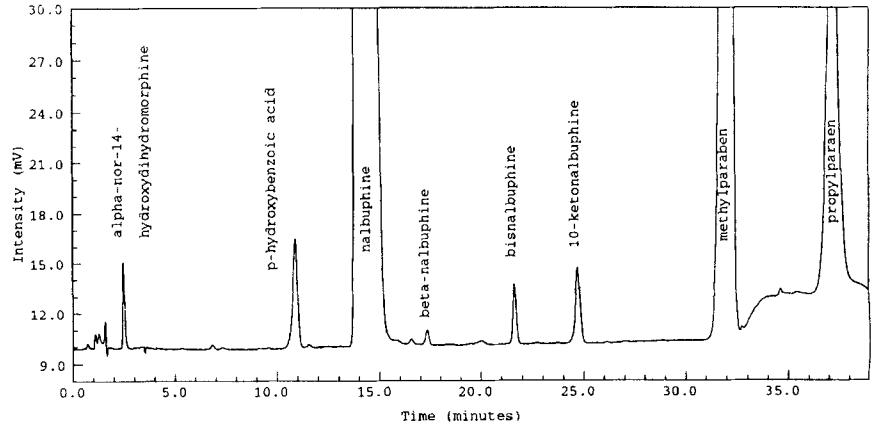


Figure 4. Chromatogram of fortified nalbuphine hydrochloride formulation with 2% increase in acetonitrile concentration in mobile phase.

Recovery data based on peak area calculations for 2,2′-bisnalbuphine are presented in Table 4. The average recovery for 2,2′-bisnalbuphine from a fortified sample was 107.6±1.9%, which is acceptable accuracy for this method.

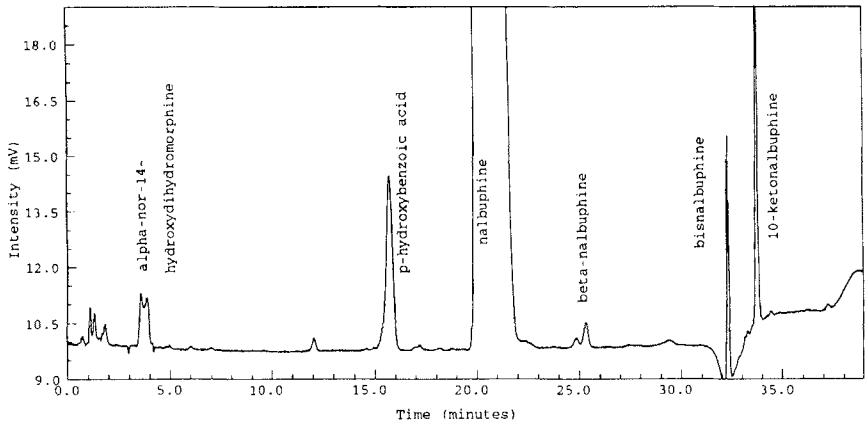


Figure 5. Chromatogram of fortified nalbuphine hydrochloride formulation with 1% decrease in acetonitrile concentration in mobile phase.

Solution Stability

Solution stability was evaluated by measuring the peak responses and chromatographic profiles of a standard and a sample preparation over 72 hours. Peak areas of nalbuphine and 2,2′-bisnalbuphine did not change significantly over the 72 hours of the study. Areas were between 98.8% and 104.1% of initial for the nalbuphine standard and between 100.0% and 108.9% of initial for 2,2′-bisnalbuphine (Table 5). There was no observable degradation in either the standard or sample chromatographic profiles over the course of the study.

Specificity

The chromatographic separation of nalbuphine, 2,2′-bisnalbuphine, α-nor-14-hydroxydihydromorphine, β-nalbuphine, and 10-ketonalbuphine under test method conditions is shown in Figure 2. All related substances are well resolved from each other and from nalbuphine. There was no interference from formulation component peaks.

Robustness

The robustness of the method was evaluated by varying the organic composition of the mobile phases. Changes in the retention of nalbuphine and related substances resulting from changes in the mobile phase composition are

shown in representative chromatograms in Figures 3 through 5. Modifications to the mobile phases demonstrated that if the solvent strength increases by a small amount, the retention of the peaks decrease, but adequate resolution is maintained. However, if the solvent strength is decreased, the last eluting degradation product, 10-ketonalbuphine, may be eluted during the change in gradient, which makes quantitation difficult.

CONCLUSION

The described gradient elution HPLC method has been shown to be linear, precise, accurate, and specific. The standard and sample preparations were found to be stable for 72 hours at ambient temperature. All known degradation products are well resolved from each other and from nalbuphine. Thus, the method is accurate and reliable for the determination of nalbuphine degradation products in nalbuphine hydrochloride injection.

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