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Journal of Pharmaceutical and Biomedical Analysis
15 (1996) 221–226

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Stability of revex, nalmefene hydrochloride injection, in injectable solutions

Satya S. Murthy, Harry G. Brittain*

Ohmeda Pharmaceutical Products Division, Inc., 100 Mountain Avenue, Murray Hill, NJ 07974, USA

Received for review 7 December 1995; revised manuscript received 15 April 1996

Abstract

The short-term stability of Revex, nalmefene hydrochloride injection, was determined in a number of diluents commonly employed for intravenous use. An HPLC method was used to follow the potency of the diluted solutions, and was fully validated for its intended concentration range prior to its use. Dilutions of Revex were prepared separately in 0.9% sodium chloride injection, 0.45% sodium chloride injection, 5% dextrose injection, 5% dextrose and 0.45% sodium chloride injection, lactated Ringer's injection, 5% dextrose and lactated Ringer's injection and 5% sodium hydrogencarbonate injection. Each admixture was stored at 4°C, room temperature (21°C) and 40°C, with samples being tested after storage at each temperature for 0, 24, 48 and 72 h. Defining stability as the retention of at least 95% of the initial drug concentration at the end of the storage period, it was concluded that the diluted solutions of Revex were uniformly stable for up to 72 h in all of the injectable solutions maintained at either 4, 21 or 40°C.

Keywords: Revex; Nalmefene hydrochloride; Injection solutions; High-performance liquid chromatography

1. Introduction

Nalmefene hydrochloride (17-(cyclopropylmethyl)-4,5-α-epoxy-6-methylenemorphinan-3,14-diol hydrochloride) (Fig. 1) is a selective narcotic antagonist, and is known commercially as Revex. The pharmacological activity of nalmefene·HCl is mediated via competitive inhibition of opioid receptor sites [1], and is indicated for the complete or partial reversal of opioid depression (including respiratory depression) and for the diagnosis and treatment of opioid overdose. As demonstrated in a variety of preclinical [2,3] and clinical studies [4–6], Revex exhibits a major advantage over naloxone in its longer duration of action.

Revex is currently marketed in ampules at dosage strengths of 1.0 and 0.1 mg ml^{-1}, with the formulation containing 9 mg ml^{-1} NaCl (for isotonicity) and the pH being adjusted to 3.9. In this formulated state, nalmefene·HCl has been found to be fairly stable, and exhibits degradation only at elevated temperatures [7]. The major degradation product has been found to be 2,2′-bisnalmefene (Fig. 1), and this nalmefene dimer is known to be less toxic than nalmefene itself [8].

To increase the database relating to the stability of Revex, a series of studies were conducted to

* Corresponding author.

PII S0731-7085(96)01847-X

evaluate the stability of nalmefene·HCl injection after its dilution with commonly used injectable media. In addition, the analytical method used in the earlier study [7] has been validated over the concentration range existing after dilution in the injectable fluids (i.e. final concentration of 0.01 mg ml^{-1}), and then used to obtain the stability of the drug substance in the various dilution media. Both aspects of this work are described in this paper.

2. Experimental

2.1. Materials

Nalmefene·HCl (lot RL-4913-119D) reference drug substance was obtained from Diosynth (O_{ss}, The Netherlands) and Revex (lot E00H4) from Ohmeda (Murray Hill, NJ). All chemicals required to perform the analytical work were obtained as reagent-grade products from various sources and 0.9% sodium chloride injection, 0.45% sodium chloride injection, 5% dextrose injection, 5% dextrose and 0.45% sodium chloride injection, lactated Ringer's injection, 5% dextrose and lactated Ringer's injection and 5% sodium hydrogencarbonate injection were purchased from Abbott Laboratories (North Chicago, IL).

2.2. Preparation and storage of diluted solutions

Nalmefene·HCl injection samples were pooled from 20 Revex ampules, formulated at a potency of 1 mg ml^{-1}. Aliquots (1 ml) of nalmefene·HCl injection were pipetted from the pooled sample into each of seven 100 ml volumetric flasks, then each flask was diluted to volume with one of the diluent fluids. The final concentration of nalmefene·HCl in each solution was approximately 0.01 mg ml^{-1}, which is the working target concentration of the assay method. Each admixture was prepared in triplicate, so that portions could be stored at three different temperatures. A determination of the nalmefene concentration was made immediately after the preparation of each solution, and subsequent assays were performed after storage for 24, 48 and 72 h at 4°C, room temperature (21°C) and 40°C.

2.3. Nalmefene assay method

The nalmefene content in each solution was determined using an isocratic HPLC method, based on separation on a Primsphere C_{18} column. This assay has been shown to be stability-indicating [7], and uses a mobile phase consisting of acetonitrile–0.05 M phosphate buffer (20:80, v/v). The buffer contained 0.2% triethylamine, and the pH was adjusted to 4.2 with 85% phosphoric acid. The mobile phase flow rate was 1.0 ml min^{-1}, and analyte detection was effected by measuring the UV absorbance at 210 nm.

In a typical analysis run, the sample sequence consisted of duplicate injections of the working standard, followed by duplicate injections of each sample preparation. Each chromatogram was run for at least 17 min prior to the next injection, and the peak areas associated with each duplicate injection were averaged to obtain a response for that sample. After 10 sample injections had been made, the standard was injected in duplicate. Finally, a duplicate standard injection was made at the conclusion of the sample sequence. The criteria for sample acceptance was that the mean areas of the bracketing standards had to agree to within 2%.

The concentration nalmefene (expressed as the free base in units of mg ml^{-1}) in each sample was calculated by using the equation

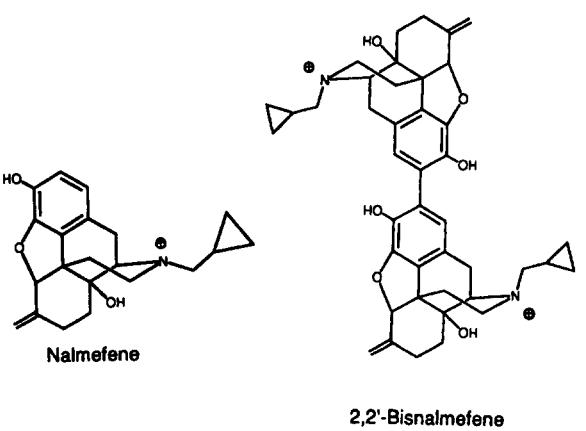

Fig. 1. Structures of nalmefene hydrochloride and its degradation product 2,2'-bisnalmefene.

Table 1
Summary of accuracy data

Target concentration (%)	Concentration taken (mg ml^{-1})	Concentration found (mg ml^{-1})	Recovery (%)
50	0.005	0.00492	98.40
75	0.0075	0.00739	98.53
100	0.010	0.00986	98.60
125	0.0125	0.0123	98.40
150	0.015	0.0148	98.67
Average			98.5
SD			0.12
RSD			0.12

Table 2
Summary of precision data

No.	Concentration (mg ml^{-1})				
	0.005 mg ml^{-1} Spiking level	0.0075 mg ml^{-1} Spiking level	0.010 mg ml^{-1} Spiking level	0.0125 mg ml^{-1} Spiking level	0.015 mg ml^{-1} Spiking level
1	0.00493	0.00740	0.00986	0.01231	0.01481
2	0.00492	0.00740	0.00986	0.01231	0.01480
3	0.00491	0.00740	0.00987	0.01233	0.01483
4	0.00492	0.00738	0.00985	0.01232	0.01482
5	0.00492	0.00739	0.00985	0.01233	0.01484
Mean	0.00492	0.00739	0.00986	0.01232	0.01482
SD	0.0000071	0.0000089	0.0000084	0.000010	0.000016
RSD (%)	0.14	0.12	0.085	0.081	0.11

[a] Average RSD = 0.11%.

$$\text{nalmefene (mg ml}^{-1}) = (A_{Sam}/A_{Std}) \times [W(1.0 - \%H_2O)\,P/100] \times (339.4/375.9)$$

where A_{Sam} = average peak area of nalmefene·HCl in the sample solution, A_{Std} = average peak area of the nalmefene·HCl standard solution, W = mass of standard taken (mg), $\%H_2O$ = water content of the standard, expressed as a fraction, 339.4/379.5 = conversion factor from nalmefene hydrochloride salt to the free base and P = purity factor of the nalmefene·HCl reference standard, expressed as a fraction.

The method may also be used to assay the content of either the 1.0 or 0.1 mg ml^{-1} presentations of Revex, as long as aliquots of the neat formulation are diluted to 0.01 mg ml^{-1} prior to analysis.

2.4. Absorbance and pH measurements

The pH of the solution was determined using a glass combination electrode, which was calibrated using two standards. Absorbances at 400 and 600 nm of the diluted nalmefene solutions were determined using a single-beam spectrophotometer. The absorbance and pH were measured immediately after preparation of the solutions and after storage for 72 h.

3. Results and discussion

3.1. Validation of the assay method

To establish the validity of the analytical methodology for the anticipated nalmefene concentration range, the method was validated ac-

Table 3
Summary of linearity data

No.	Nalmefene concentration taken (mg ml^{-1})	Nalmefene concentration found (mg ml^{-1})
1	0.005	0.00492
2	0.0075	0.00739
3	0.010	0.00986
4	0.0125	0.01232
5	0.015	0.01482
Slope		0.9892
Intercept		−0.000030
Correlation coefficient		0.9999964

cording to its performance characteristics of accuracy, precision and linearity. The method was not evaluated with regard to its limits of detection and quantitation since these criteria are irrelevant for the determination of a major constituent in a formulation.

3.1.1. Accuracy of the method

Placebo solutions of the Revex formulation were spiked with known concentrations of nalmefene at target concentrations of 50, 75, 100, 125 and 150% of the intended working concentration of the method (0.01 mg ml^{-1}). The concentration of each solution was determined according to the method procedure, and the accuracy was evaluated as the percentage recovery from these solutions. These data are reported in Table 1. The average recovery over the range studied was 98.5%.

3.1.2. Precision

The precision of the analytical procedure was evaluated through multiple analyses of the solutions prepared for the accuracy work. Five samples at each concentration level were independently prepared and analyzed according to the analytical procedure, and the results of these analyses are given in Table 2. The precision of the assay values (as measured by the relative

Table 4
Stability of Revex in various injectable solutions

Solution	Storage temperature (°C)	Initial nalmefene concentration (mg ml^{-1})	Percentage of initial concentration remaining		
			24 h	48 h	72 h
0.9% Sodium chloride	4	0.01020	98.5	97.6	97.6
	21	0.01020	99.0	97.6	97.6
	40	0.01015	99.5	98.5	98.0
0.45% Sodium chloride	4	0.01020	98.5	97.1	97.6
	21	0.00995	100.5	99.5	99.0
	40	0.01005	100.0	99.0	97.5
5% Dextrose	4	0.00995	99.0	99.5	98.9
	21	0.00990	99.5	100.0	98.5
	40	0.01005	97.5	96.5	97.0
5% Dextrose and 0.45% sodium chloride	4	0.01015	99.5	98.5	98.5
	21	0.01005	100.5	100.0	99.5
	40	0.01000	100.5	100.0	100.0
Lactated Ringer's	4	0.00990	101.0	101.5	100.5
	21	0.01000	100.5	100.0	99.5
	40	0.00990	102.5	102.5	101.0
5% Dextrose and Lactated Ringer's	4	0.01010	101.0	99.5	99.0
	21	0.01020	99.0	98.0	98.5
	40	0.01010	100.5	99.5	100.0
5% Sodium hydrogencarbonate	4	0.00980	99.5	99.5	101.0
	21	0.00975	100	99.5	100.00
	40	0.00970	100.5	99.5	99.0

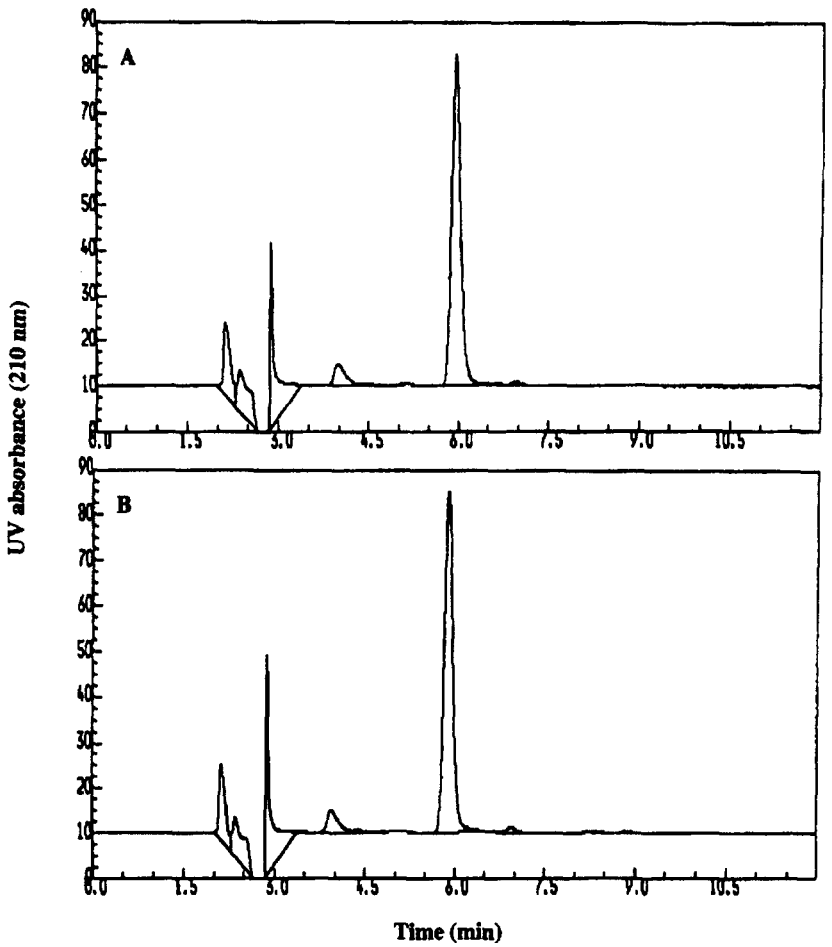

Fig. 2. HPLC traces of Revex after dilution with 0.9% sodium chloride injection, (A) immediately after preparation and (B) after storage for 72 h at room temperature. The slight degradation of the drug substance is indicated by the small 2,2′-bisnalmefene peak located at a retention time of ca. 7 min.

standard deviation of the various independent preparations) was 0.096%.

3.1.3. Linearity

To evaluate the linearity of the method, linear regression analysis was used to determine the relationship between the analytical results and known concentrations of nalmefene in test solutions. The results are summarized in Table 3. The correlation coefficient of 0.999977 obtained during this portion of the validation work indicates that the assay methodology is characterized by acceptable linearity.

3.2. Absorbance and pH measurements

The absorbance of each sample was measured at 400 nm to detect any yellowing of the solution and at 600 nm to detect any turbidity. It was found in each instance that all absorbance readings were less than 0.03, indicating that none of the diluted preparations discolored or formed insoluble substances during the storage period. The pH of each solution remained invariant to within 0.3 units of the initial value in all solutions, except for 5% sodium hydrogencarbonate, where the pH was found to have in-

creased by 0.8 units at the end of the 72 h storage time.

3.3. Results of the stability studies

As shown in Table 4, the concentration of nalmefene in all injectable media never dropped below 97% by the end of the 72 h storage periods. Where the nalmefene content was found to decrease slightly from the initial value, the missing mass was estimated to be accounted for as the principal degradant, 2,2′-bisnalmefene. Owing to the very low concentration of drug substance in the diluent solutions (0.01 mg ml^{-1}), all detectable amounts of 2,2′bisnalmefene formed were judged to be less than the limit of quantitation and therefore unreportable.

In Fig. 2, HPLC traces are shown which correspond to assays conducted immediately after dilution with 0.9% sodium chloride injection and after storage for 72 h at room temperature. This particular slight degradation represented the worst instance observed, and would not be considered as an impediment to using the solution. The 2,2′-bisnalmefene degradant is known to be less toxic than the drug substance itself [8].

4. Conclusion

Defining stability as the retention of at least 95% of the initial drug concentration at the end of the storage period, it is concluded that the diluted solutions of Revex were uniformly stable for up to 72 h in all of the injectable solutions maintained at either 4, 21 or 40°C

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