T2304

NALBUPHINE STABILITY AT 1mg/mL CONCENTRATION

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INTRODUCTION :

Nalbuphine hydrochloride is a semi-synthetic opioïd used as an analgesic.

It has a dual mechanism of action, working as an agonist of κ-receptors and as an antagonist of μ-receptors. At low doses nalbuphine binding to κ-receptors triggers analgesia. At higher dosages, nalbuphine binds to μ-receptors limiting any increase of the analgesic effect or side effects (respiratory depression). These actions are subject to a "ceiling effect" and may not increase proportionately with the dose.

Nalbuphine is of particular interest in pediatrics since it "ceiling effect" may not only limit the therapeutic effect, but also any side effect due to a potential overdose.

To date, no pediatric formulation of nalbuphine is commercially available. We thus intended to prepare a 1mg/mL nalbuphine solution from the commercial vial (10mg/mL) and condition in 2 mL syringes .

OBJECTIVES :

➢To developp and validate an analytical method by HPLC.

➢To carry out the stability study of the diluted conditioned solution.

METHODS :

➢ Forced degradation assay

A standard solution (1mg/mL) of nalbuphine was prepared from the commercial vial (nalbuphine Aguettant® 10 mg/mL, 2 mL) in natrium chloride (0,9%) and subjected to extreme conditions (heat, acid, basic and oxidizing environment). 20 µL of each solution was injected into the HPLC column.

• Heat : The solution was stored twenty hours at 70°C.

• Acid environment : The pH of the standard solution (3,7) was lowered to pH = 1,1, with hydrochloric acid (1N) and then stored twenty hours at room temperature.

• Basic environment : The pH of the standard solution was increased to pH = 11,3 with natrium hydroxide (1N) and than stored twenty hours at room temperature.

• Oxidizing environment : Hydrogen peroxide (3%) was added to the standard solution. The mixture was injected into the LC-UV after 5, 70 and 120 minutes of reaction.

➢ The chromatographic conditions for the analysis were :

Column	C18 Intersil-ODS2 (250mm x 4,6mm, 5µm),
Mobile phase	acetonitrile/purified water (45:55;v/v) and 100 µL triethylamine was added fot 1L of the mixture
Flow rate	1mL/min
Autosampler temperature	4°C
Column oven temperature	25°C
Detection	230 nm
Injection volume	20 µL
Analysis time	10 min

➢ Three diluted solutions were prepared and conditioned in 2 mL syringes (series A, B and C)

➢Half of each serie was stored at –20°C the remaining at +5°C.

➢ Stability was analyzed every seven days for the first month, and every month for eleven months.

➢ A range of calibration was prepared in mobile phase with morphine (0,5 mg in 50 µL) as internal standard (nalbuphine concentration : 0,06 ; 0,08 ; 0,1 ; 0,12 ; 0,14 mg/mL)

➢Six sample solutions were prepared with 1mL of the diluted solution of nalbuphine (1mg/mL), 50 µL of morphine (10 mg/mL) and 8,95 mL of mobile phase.

➢ Each sample was injected three times in the LC-UV.

➢ To evaluate the stability, three parameters were considered : physical appearance of the solution, pH and variation of nalbuphine concentration.

RESULTS :

➢ LC-UV validation

• Specificity :

There was no interference between nalbuphine's signal and the solvents one.

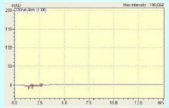


Fig.1 : Solvent's chromatogram

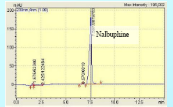
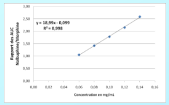


Fig.2 : Nalbuphine's chromatogram

Limit of detection = **0,2 mg/mL**

Limit of quantification = **0,65 mg/mL** (+/- 15 % SD)

• Linearity :



Linearity: +/- 5% SD

•Precision:

The inter-(1) and intra-(2) precisions: +/- 5% SD

Coefficient of variation (1) = **3,62 %**

Coefficient of variation (2) = **1,47 %**

•Accuracy (+/- 5% SD): **0,72 %**

➢ Accelerated degradation conditions :

• degradation with heat, acid environment and basic environment

• nalbuphine is degraded in nalbuphine-N-Oxyde (Tr = 2,5min) with an oxidizing agent

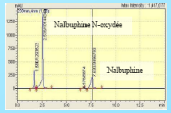
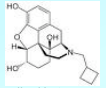
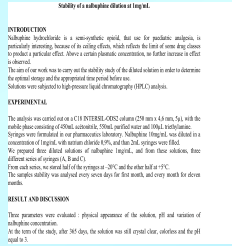


Fig.3 : Nalbuphine after oxidation



Nalbuphine's structure



➢ Physicochemical stability :

• pH : 3,7 for each sample

• Appearance of the solution : all solutions were crystal clear, colorless

• variation of concentration :

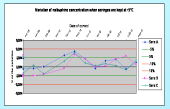


Fig.4 : Concentration measured for the samples kept at +5°C

Fig.5 : Concentration measured for the samples kept at -20°C

DISCUSSION-CONCLUSION :

Nalbuphine was degraded in an oxidizing environment, but the degradation product didn't interfere with the analysis of nalbuphine, because peaks were well separated (nalbuphine retention time : 7,5 min and degradation product retention time : 2,5 min)

At the end of the study (1 year), the solution was still crystal clear, colorless and the pH equal to 3,7. The chromatograms obtained didn't show any degradation product, and the concentrations measured were not significantly different from the nominal values.

This long term stability study did not show any modification of the diluted solution of nalbuphine at 1mg/mL in natrium chloride (0,9%) either at –20°C or at +5°C.

The mass preparation of syringes of diluted nalbuphine is authorized, with a duration of storage of twelve months either at –20°c or at +5°C.

The temperature of preservation was set at +5°C, because we wished to store the syringes under refrigeration in the pediatric wards.