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Research Article

# LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD TO ASSESS NALOXONE HYDROCHLORIDE PHOTOSTABILITY UNDER ARTIFICIAL LIGHT AND SUNLIGHT EXPOSURE AT ROOM TEMPERATURE

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**ABSTRACT**

**Objectives:** Naloxone is an opioid antagonist indicated for central nervous system and respiratory depression treatment induced by natural or synthetic opioid in adults and neonates whose mothers have received opioids. Although naloxone hydrochloride has been reported to be physically and chemically stable, photostability of naloxone hydrochloride under artificial light, and sunlight has not been reported. Therefore, a method was required for assessment of naloxone hydrochloride photostability.

**Methods:** A high-performance liquid chromatography/mass spectrometry method was established to evaluate the photostability of naloxone hydrochloride. Injections of naloxone hydrochloride in 0.9% sodium chloride were exposed to artificial light and sunlight at room temperature for 192 hrs.

**Results:** Naloxone losses up to 5.26% of its initial concentration when exposed to artificial light at room temperature for 192 hrs, but the degradation increased up to 15.08% under sunlight exposure at room temperature for 192 hrs. The disappearance of naloxone hydrochloride was correlated with the appearance of noroxymorphone degradant.

**Conclusion:** Naloxone hydrochloride is photosensitive and degradation increased as the light intensity increased. Therefore, naloxone intravenous infusion solutions should either be protected from light and/or be frequently replaced when being administered to patients.

**Keywords:** Liquid chromatography/mass spectrometry, Naloxone hydrochloride, Photostability, Noroxymorphone, Intravenous infusion, Electrospray ionization.

## INTRODUCTION

Different classes of drugs have been developed to control different types of pain, nevertheless none of these classes has replaced or compete with opioid analgesics to manage moderate to severe pain [1-4]. Therefore, opioids are the first line treatment for cancer and postoperative pain. Unfortunately, opioid analgesics have serious possible side effects such as constipation, nausea, vomiting, drowsiness, and respiratory depression [5-12]. Thus, an opioid antagonist know as naloxone hydrochloride (N-allyl-noroxymorphone) is indicated for complete or partial reversal of central nervous system and especially respiratory depression induced by over-dosage or intoxication of natural or synthetic opioids. Moreover, it can reverse the dysphoric, delusional, and hallucinatory properties of synthetic opioid [13,14]. In addition, naloxone hydrochloride has been successfully administered by different routes. However, due to rapid onset and dose titration, the intravenous (i.v.) route seems to be the ideal route for naloxone hydrochloride administration [15,16]. Therefore, continuous infusion of naloxone hydrochloride is recommended for neonate, whose mothers have received opioids, to treat respiratory depression and the infusion can be continued for 2-5 days if the respiratory depression recurred [17,18]. However, five impurities have been indicated for naloxone hydrochloride by British Pharmacopeia including, noroxymorphone, which is the starting material for the synthesis of naloxone hydrochloride [19]. Noroxymorphone may cause nausea, vomiting, abdominal pain, and constipation [20]. Therefore, naloxone hydrochloride photostability should be indicated and the levels of noroxymorphone present should be controlled. Although several methods are reported in the literature for the determination of naloxone hydrochloride concentration in blood, urine and solution samples, few studies have been established to evaluate naloxone hydrochloride stability beyond 24 hrs in case of continuous occurrence of opioid symptoms [20,21]. Moreover, most of the designed studies have used complicated and sensitive detectors which are not regularly used in analytical laboratories [22,23]. For example, one of the reported methods involves the use of electrochemical detectors, which is highly sensitive to the quality of water used in the mobile phase [24]. Although naloxone hydrochloride is known to be a photosensitive compound, the previously reported literatures investigated only the effects of heat and humidity as the main factors affecting naloxone hydrochloride infusion stability [16,20,21,25,26]. Thus, a simple stability indicating method was designed to investigate the effect of artificial light and sunlight exposure on naloxone hydrochloride in 0.9% sodium chloride at room temperature for 192 hrs.

## METHODS

### Chemical and materials

Naloxone hydrochloride (0.4 mg/ml) ampoules were a purchased from HIKMA Pharmaceutical (Amman). Isotonic sodium chloride solution for injection was supplied by Kuwait Saudi Pharmaceutical Industry Company (Kuwait). Naloxone hydrochloride dehydrate and high-performance liquid chromatography (HPLC) grade water were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile HPLC grade was supplied by LiChroslov (Darmstadt, Germany). Formic acid (99-100%) purchased from by Surechem Products Ltd. (16 Maitland Road, Ipswich, England). Plastic syringes were purchased from Sensecure (Loughborough, England).

### Instrumentation

The HPLC system (Waters 2690 Separation Module) used in this analytical method consisted of a Waters 600E multisolvent delivery

system pump, a Waters Ultra WISP 715 auto-injector, and a Waters 996 diode-array detection system. Chromatographic separation was performed using Waters Symmetry, C-18 (5 µm, 150 mm × 4.6 mm i.d) column.

**Liquid chromatography-mass spectrometry (LC-MS)**

Tandem MS was performed by a Waters Alliance 2695 Separations Module HPLC, equipped with a quaternary pump and an automatic interfaced to a Micromass Quattro micro API (triple quadrupole) MS equipped with a Z-spray electrospray (ESI) ionization source was used. Nitrogen as drying, as well as nebulizing gas, was generated from pressurized air in a NG-7 nitrogen generator. The nebulizing gas flow was set to 50 L/h and the gas flow desolvation to 550 L/h. The optimized values were: Capillary voltages, 4.5 kV; extractor voltage, 2 V; source temperature, 100°C; desolvation temperature, 400°C; and multiplier, 650 V.

**pH measurement**

The pH of the injection was measured prior to sample analysis with a pH meter that was calibrated with buffers at pH 4 and 7.

**Chromatographic conditions**

Mobile phase comprised filtered and degassed 0.04% v/v formic acid in water and acetonitrile in proportion of 65:35 v/v and pumped at a flow rate of 1 ml/minutes. Samples were analyzed at a wavelength of 260 nm and were injected at 10 µm injection volume. In LC-MS, the same conditions as were used in HPLC but at a flow rate of 2 ml/minutes. LC was directly attached to the triple quadrupole MS via ESI.

**Sample preparation**

A stock solution containing 1 mg/ml of naloxone hydrochloride dehydrate dissolved in 0.9% sodium chloride solution was prepared and the linearity of response around the nominal content in the injection was achieved with six concentrations ranging from 25% to 200% diluted with 0.0.4% formic acid in water:acetonitrile (65:35 v/v). Duplicate injections composed 0.2 mg/ml naloxone hydrochloride in 0.9% sodium chloride solution were prepared and stored at 22°C under artificial light and sunlight exposure. Samples were analyzed at 0 minutes, 24, 48, 96, and 192 hrs.

## RESULTS

Figs. 1 and 2 shows the chromatograms obtained for naloxone hydrochloride after sunlight exposure for 24 hrs and 4 days, respectively. After 192 hrs under room temperature, injection solutions exposed to artificial light were prone to degradation up to 5.26%, while under sunlight exposure naloxone hydrochloride was degraded up to 15.08%. Tables 1 and 2 shows the set of data obtained for naloxone hydrochloride exposed to artificial light and sunlight, respectively. There was a loss of naloxone hydrochloride in the sample with a corresponding increase in the degradant peaks. Thus, MS was used to identify the degradant peak. Fig. 3 shows a full scan ESI MS of the degradant peak obtained in Fig. 2.

## DISCUSSION

The calibration curve for naloxone hydrochloride was linear over the range between 25% and 150% of the stated content for naloxone hydrochloride in 0.9% sodium chloride infusion with $R^2$ of 1.00. Method precision was tested by preparing 10 samples containing 0.2 mg/ml of naloxone hydrochloride and analyzed using previously mentioned chromatographic conditions. The relative standard deviation of peak area obtained for naloxone hydrochloride was ±0.9%. Degradation of naloxone hydrochloride was noticeable under artificial light and sunlight exposure at room temperature. Under the examined conditions, one degradant peak is clearly eluted before naloxone hydrochloride peak. Noroxymorphone peak is formed and it becomes more prominent after 48 hrs at room temperature under artificial light and sunlight exposure. As shown in Fig. 3, identification of degradant to be nornaloxone (noroxymorphone) [19,20] was performed using tandem MS. Visual inspection of naloxone hydrochloride injection solution showed that the appearance remain unchanged for 192 hrs under artificial light exposure at room temperature (22°C), while a noticeable color change from colorless solution once prepared to a yellow-brownish solution

**Table 1: Stability data obtained for naloxone hydrochloride (0.2 mg/ml) in in 0.9% sodium chloride under artificial light exposure at room temperature for 192 hrs**

| Time (hrs) | Naloxone hydrochloride | | RSD% |
|---|---|---|---|
| | % Remaining syringe 1 | % Remaining syringe 2 | |
| 0 | 100 | 100 | 0 |
| 4 | 98.59 | 98.54 | ±0.03 |
| 24 | 97.81 | 96.88 | ±0.7 |
| 48 | 97.75 | 96.58 | ±0.9 |
| 96 | 96.70 | 96.34 | ±0.3 |
| 192 | 95.83 | 94.74 | ±0.8 |

RSD: Relative standard deviation

**Table 2: Stability data obtained for naloxone hydrochloride (0.2 mg/ml) in in 0.9% sodium chloride under sunlight exposure at room temperature for 192 hrs**

| Time (hrs) | Naloxone hydrochloride | | RSD% |
|---|---|---|---|
| | % Remaining syringe 1 | % Remaining syringe 2 | |
| 0 | 100 | 100 | ±0 |
| 4 | 98.16 | 94.43 | ±2.7 |
| 24 | 88.04 | 88.73 | ±0.6 |
| 48 | 87.01 | 86.89 | ±0.1 |
| 96 | 86.01 | 85.71 | ±0.3 |
| 192 | 85.68 | 84.91 | ±0.6 |

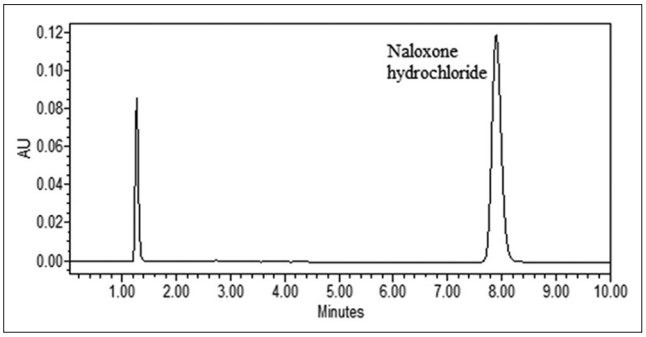


**Fig. 1: High-performance liquid chromatography analysis of 0.2 mg/ml naloxone hydrochloride in 0.9% sodium chloride after 24 hrs of sunlight exposure at room temperature**

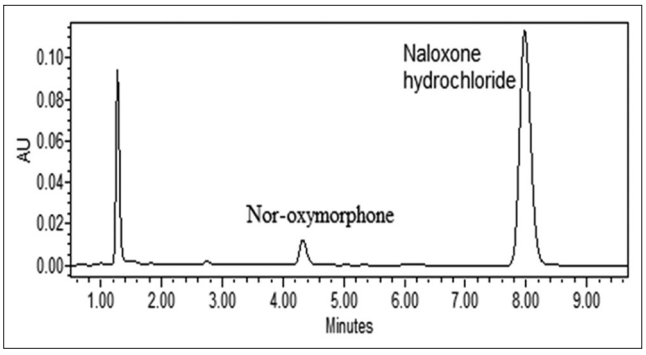


**Fig. 2: High-performance liquid chromatography analysis of 0.2 mg/ml naloxone hydrochloride in 0.9% sodium chloride after 4 days of sunlight exposure at room temperature**

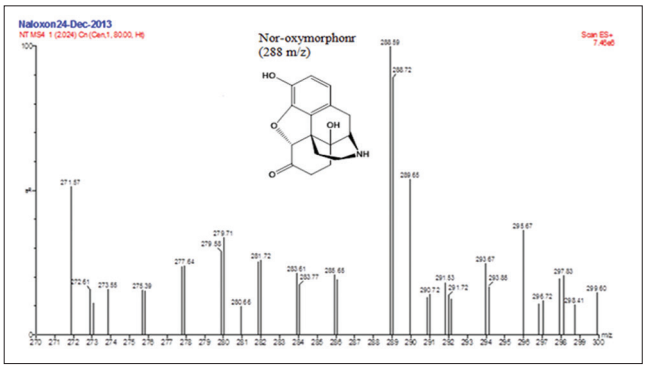


**Fig. 3: Full scan electrospray ionization mass spectrum of noroxymorphone**

was performed due to extensive degradation after 7 days sunlight exposure at room temperature. Apart from the color change, the pH of naloxone hydrochloride injection solution remains stable over 192 hrs, and there was no evidence of particulate formation.

## CONCLUSION

The naloxone hydrochloride shown in the current study is considerably stable under artificial light exposure at room temperature (22°C) for 192 hrs, while it shows more degradation under sunlight light exposure at room temperature. Moreover, the study showed that naloxone hydrochloride degradation is directly proportional to the amount of light exposed to it. Thus, naloxone hydrochloride i.v. infusion should be administered in a controlled light radiation and the infusion preferably changed if the infusion continued for more than 24 hrs.

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