Quality Assessment of Expired Naloxone Products from First-Responders’ Supplies


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Title

Quality Assessment of Expired Naloxone Products from First-Responders’ Supplies

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Abstract

Objective: Naloxone is an opioid receptor antagonist that reverses life-threatening effects of opioid overdose. Since the 1970’s, naloxone products have been developed as injectable solutions, and more recently as nasal sprays. Naloxone products have saved many lives in emergency settings. These products are routinely carried by public safety first-responders including fire fighters (FF), law enforcement officers (LEO) and emergency medical services (EMS). Now, they are also distributed through community access programs to the public. While public safety medications are monitored, those publically distributed are not, so expired products can be possibly found on-hand in an emergency. This study analyzed the quality and stability of expired Naloxone HCl Solutions for Injection, to assess their remaining efficacies and potential risks.

Methods: The samples were collected from EMS or law enforcement training supplies and expired returns, with expiration dates ranging from 1990 to 2018. Using standardized techniques, the remaining naloxone was quantified, and the main degradation products, nornaloxone (also known as noroxymorphone) and other possible species, were monitored and quantified systematically.

Results: Most tested samples were found containing more than 90% of labeled naloxone, including those stored for nearly 30 years. The naloxone degradation was slow, but generally correlated with storage time length. There was no significant amount of degradation products detected across all samples. Nornaloxone was detected from some older samples, but all less than 1%. Therefore, although it is an opioid agonist, the risk caused by nornaloxone should be low.

Conclusion: This quality assessment demonstrates that expired naloxone products may still meet USP standards, even after many years. Further pharmaceutical, clinical and regulatory investigation should be conducted to confirm our findings, especially for new naloxone products with different formulations and routes of administration. Extending the shelf-life of naloxone products may have important financial and public health consequences in addressing future drug shortages and meeting the needs for this critical drug.
Introduction
From 2002 to 2017, opioid overdose deaths have quadrupled in the United States, making opioid abuse a national epidemic\(^1\). Both the use of illegal opioids like heroin and illicitly manufactured fentanyl and overdoses from prescription opioids (such as oxycodone, e.g. OxyContin\(^\text{®}\), and hydrocodone, e.g. Vicodin\(^\text{®}\)) have contributed to the increase in opioid related deaths in the last two decades\(^2,3\). This emergence in the United States has profoundly impacted public health, as well as social and economic welfare.

Naloxone, a potent \(\mu\)-opioid receptor antagonist, is used to reverse the effects of opioid overdose. It crosses the blood-brain barrier (BBB) and antagonizes opioid binding at the receptor level, thus blocks the pharmacological activity of opioids\(^4\). Naloxone reverses the respiratory depression, which is the leading cause of death associated with opioid overdose. Use of naloxone has saved over 26,000 lives since 1996\(^1\).

In April 2018, the Surgeon General released an “Advisory on Naloxone and Opioid Overdose” that emphasized the importance of access to naloxone and suggesting that naloxone products should be carried by members of the public\(^5\). The shelf life of naloxone products are 1-2 years, as promulgated by the manufacturers and approved by the US Food and Drug Administration (FDA)\(^6\). Given this expanded access to the public, it is likely that many doses of naloxone products will expire before being used.

Administration of an expired product that either does not contain sufficient concentration of naloxone or that could contain harmful degradation products would pose risk. Naloxone in public safety supplies is monitored for expiration dates, but once supplies are distributed to the public through community naloxone programs there is limited ongoing awareness of expiration dates and people receiving naloxone through these programs may have expired product on-hand in an emergency. Therefore, it is important to assess the remaining potency and degradation products of expired naloxone to see if it could be used in an emergency. This study analyzed the quality and stability of expired naloxone injectable solutions from expired samples, for benefit-risk assessment looking at the remaining concentration of naloxone and the presence of its known degradadants, including the mu receptor agonist Nornaloxone (noroxymorphone).
Methods

Samples and Sample Preparation

The expired naloxone injection products were collected from training supplies and expired stock from public safety agencies including fire, law enforcement and emergency medical services with expiration dates from 1990 to 2018. Storage conditions for these samples varied and there were no records of the conditions maintained. Samples for testing were selected from common products on the market, with expiration dates spread through the 27 years as evenly as possible. The samples chosen for evaluation were all injectable products, either as prefilled syringes (Mini-I-Jet/Luer-Jet Vial, manufactured by IMS) or ampules of solution (manufactured by Abbot Laboratories). Their manufacture, dosage form, National Drug Code (NDC) and other information are listed in Table 1. New samples were also obtained, which were not expired before testing (March 2018).

Reagents

Standards were purchased from Sigma-Aldrich® (Cerilliant): Naloxone (N-004 Lot: FN04161601) was 1.0 mg/mL in 1 mL methanol; and Nornaloxone (noroxymorphine, as free base) (N-013 Lot: FE09171501) was 100 𝜇g/mL in 1 mL methanol. Standards and samples were diluted to desired concentrations using a solution of water, acetonitrile, and formic acid (95:5:0.1 by volume).

Liquid Chromatography-Mass Spectrometry (LCMS) Method

Analysis was performed on a Waters Aquity UPLC (Ultra Performance Liquid Chromatograph) system in conjunction with a UV photodiode array detector (PDA) and a tandem quadrupole mass spectrometer detector (TQD). The samples were prepared and maintained in auto sampler at 4°C and were injected at a volume of 10 𝜇L per analysis. Analyte separation was carried out using a UPLC BEH C18 1.7μm, 2.1 x 50 mm column, and mobile phase of Solvent A (water: acetonitrile: formic acid = 95:5:0.1 by volume) and Solvent B (water: acetonitrile: formic acid = 5:95:0.1 by volume). The gradient elution used was as follows: 2% B for 1.0 min; 2-40% B from 1.0 to 4.5 min; 40-95% B from 4.5 to 7.0 min and held at 95% from 7.0 to 8.0 min. From 8.0 to
10.0 min, the column was re-equilibrated back to 2% B. The flow rate was 0.2 mL/min. UV absorbance at 280 nm was used for quantification.

Mass spectrometry scan of MS1 and single ion recording (SIR) was used to monitor naloxone, nornaloxone, and other degradants with the following parameters: capillary voltage at 4.5 kV, cone voltage at 50 kV, extractor voltage at 2 V, RF lens at 0.2 V, source temperature at 92 °C, and desolvation temperature at 350 °C (Figure 3). A shutdown method was developed for overnight runs. Method optimization was determined using standard naloxone and nornaloxone solutions (Figure 4). Naloxone and Nornaloxone had retention times of about 2.9 and 1.2 minutes, respectively. By this developed method, the naloxone in samples was quantified. The main degradation product, nornaloxone, and other possible species were also systematically monitored and quantified.

**Experimental Design**

Calibration curves were prepared using concentrations of 0.1, 0.5, 1, 2, 3, 5, 7, 10, and 20 µg/mL naloxone (Figure 5), to quantify naloxone in the expired samples. All standards, stocks and samples were prepared fresh and stored in -80 °C when necessary. The blank was solvent A, which was injected between samples as needed. The reference standard was 10 µg/mL naloxone, which was prepared fresh daily and injected three times before running samples, for system suitability (precision, resolution, etc.). The expired samples were diluted to 10 µg/mL according to labelled concentration using solvent A. Samples were prepared in triplicate and final concentrations were determined by the area under the curve (AUC) of UV chromatogram at 280 nm. The reference standard and the blank were injected after every six samples for validation.

**Results**

The optimized liquid chromatography method provided good peak resolution, with nornaloxone and naloxone eluting at approximately 1.2 and 2.8 minutes, respectively (Figure 3). The mass spectrometry method produced clear peaks at 328 m/z (m+1) and 310 m/z (loss of “-OH” group) for naloxone and 288 m/z (m+1) and 270 m/z (loss of “-OH” group) for nornaloxone (Figure 2) for analysis and validation. The calibration curve generated for naloxone had an $R^2$ value of 0.99972 (Figure 5) from 0.1 µg/mL to 20 µg/mL.
From most tested samples, more than 90% of naloxone remained as the active pharmaceutical ingredient (API) in the products (Figure 6) which lies above the USP quality standard of 90-110% of labeled amount\(^8\). Their depleted amount approximately correlated with length of storage time (R\(^2\) = 0.5661, Figure 6). While noraloxone was detected from several older samples, its concentration was less than 1% or undetectable in all samples. The limit of detection (LOD) was established at 0.1 µg/mL for both naloxone and noraloxone by UV absorption, and validated by mass spectrometry SIR (Single Ion Recording) of naloxone and noraloxone. No other significant degradation products were detected across all samples by UV and MS scanning between 150 and 750 m/z.

**Discussion**

Our test results implicated that naloxone HCl is highly stable in these injectable products. Environmental and physical factors that impact naloxone stability include pH, light exposure and oxidation, which can be minimized by formulation and packaging\(^9,10\). Of the tested samples, naloxone in ampules were more stable than those packed in prefilled syringes (Figure 6).

The main degradation product of naloxone is noraloxone, an opioid agonist, which was detected from some older samples that have been stored over twenty years, but it did not present in a clinically significant amount. Notably, noraloxone is a weaker binder to opioid receptors (Ki= 5.69 nM, 87 nM, 162 nM for µ-, δ-, and κ- opioid receptors respectively)\(^11\) than naloxone (Ki = 0.559 nM, 4.91 nM, 36.5 nM for µ-, δ-, and κ- receptors)\(^12\). Also, noraloxone is reported to have low permeability across the blood brain barrier, with brain-to-plasma partitioning about 1\(^11,13\). Therefore, the noraloxone by-product is of low risk. As no other significant impurities or degradation products were detected by either UV or MS scan, the overall risks from degradation products in naloxone products are low.

Our findings align with several other publications, which reported consistent stability of naloxone\(^9,14,15\). A Shelf Life Extension Program conducted by the US FDA assessed over 100 of
stockpile products for their stability beyond expiration date. Among them, naloxone was considered as one of the “top performers”. Ten lots of Naloxone HCl injection-solution were tested, with an extension time range from 60 to 95 months, at an average of 69 months. Our results confirmed that the formulation within the Naloxone HCl Injection solution is consistently stable for nearly 30 years.

In 2017, the department of health and human services implemented a 5-point strategy to combat the opiate crisis in the United States. Better access to reversal agents is a cornerstone to this 5-point strategy. Meeting this increased demand for naloxone is challenging for many reasons. Maintaining a ready supply is costly for hospitals, public safety, public health and the lay public. The cost of naloxone products has increased significantly from 2014 to 2016, in some cases over 500%, potentially hindering refilling prescriptions due to expiration. This raises public health costs and potentially contributes to the drug shortages.

The implication of effectiveness and safety of a medication beyond its expiration date brings a reasonable solution to potential future drug shortages and costs. Additionally, once we deploy medications including naloxone into an uncontrolled environment, and to people who use drugs, their family and friends, as well as law enforcement, it increases the need for drug stability research under controlled challenging environments, to simulate the real situations in cars, ambulances, garages, or extremes of weather. Also, with new formulations of naloxone becoming available, there must be testing to assure the universality of these findings. Because of the demonstrated apparent stability of naloxone and subsequently its potential ability to maintain a longer shelf life, there is the opportunity to decrease the cost burden to both the public in the distribution of this medication, and increase public access.

Limitations of this study include the variable and unrecorded condition of storage of these old samples. The expired samples were mostly returned from law enforcement, fire department and EMS stock and training supplies, which had been carried by personnel and in police cars, fire trucks and ambulances – in some of these settings without temperature or environmental control. While this likely mimics real-life conditions – people leaving naloxone in their glove
compartments or cars or in medicine cabinets - further investigations, such as accelerated stability tests under simulated extreme conditions and high temperature, should be done to extend shelf-life for various naloxone products in different dosage forms to meet industrial and regulatory requirements. We welcome manufacturers and the FDA to share more data or support more research to confirm our findings.

Conclusion
This quality assessment demonstrates that expired naloxone products may still meet USP standards, even after many years. Further pharmaceutical, clinical and regulatory investigation should be conducted to confirm our findings, especially for new naloxone products with different formulations and routes of administration. Extending the shelf-life of naloxone products may have important financial and public health consequences in addressing future drug shortages and meeting the needs for this critical drug.

The authors report no conflicts of interest.
References


8. USP Monograph, Naloxone HCl Injection Monograph, 2018, USP41-NF36:2856


Table 1. Representative Naloxone Hydrochloride products and their formulations on the US market

<table>
<thead>
<tr>
<th>Dosage Forms</th>
<th>Injectable Solutions</th>
<th>Auto-Injector</th>
<th>Nasal Spray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>Narcan®</td>
<td>Evzio®</td>
<td>Narcan®</td>
</tr>
<tr>
<td>Examples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routes of</td>
<td>Intramuscular</td>
<td>Intramuscular</td>
<td>Nasal Spray,</td>
</tr>
<tr>
<td>Administration</td>
<td>Injection</td>
<td>Injectable;</td>
<td>Metered</td>
</tr>
<tr>
<td>Concentration</td>
<td>2mg/2ml</td>
<td>2mg/0.4ml</td>
<td>4mg/Spray</td>
</tr>
<tr>
<td>And dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>pH 4.0 (by HCL)</td>
<td>NaCl, HCl,</td>
<td>Benzalkonium, NaCl,</td>
</tr>
<tr>
<td>Ingredients</td>
<td>Water</td>
<td>Water</td>
<td>EDTA, -pH 3.5~5.5 (by HCl)</td>
</tr>
<tr>
<td>Approved</td>
<td>24 months</td>
<td>12 months</td>
<td>24 months</td>
</tr>
<tr>
<td>Shelf Life</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>
Table 2. Samples tested in this study for naloxone stability over 27 years

<table>
<thead>
<tr>
<th>Expiration Date</th>
<th>Years after Expiration</th>
<th>Package</th>
<th>NDC</th>
<th>Labeled Concentration (mg/ML)</th>
<th>Concentration from tests (mg/ML)</th>
<th>Difference from the labeled amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-1990</td>
<td>27.5</td>
<td>Mini-I-Jet</td>
<td>0548-1469-00</td>
<td>1</td>
<td>0.9352</td>
<td>-6.48%</td>
</tr>
<tr>
<td>May-1992</td>
<td>25.5</td>
<td>Mini-I-Jet</td>
<td>0548-1469-00</td>
<td>1</td>
<td>0.8930</td>
<td>-10.70%</td>
</tr>
<tr>
<td>Oct-1997</td>
<td>20.08</td>
<td>Mini-I-Jet</td>
<td>0548-1469-00</td>
<td>1</td>
<td>0.9465</td>
<td>-5.35%</td>
</tr>
<tr>
<td>Jul-1999</td>
<td>18.34</td>
<td>Mini-I-Jet</td>
<td>0548-1469-00</td>
<td>1</td>
<td>0.9906</td>
<td>-0.94%</td>
</tr>
<tr>
<td>Sep-2003</td>
<td>14.17</td>
<td>Ampule</td>
<td>0074-1212-01</td>
<td>0.4</td>
<td>0.4092</td>
<td>2.29%</td>
</tr>
<tr>
<td>May-2004</td>
<td>13.5</td>
<td>Ampule</td>
<td>0074-1212-01</td>
<td>0.4</td>
<td>0.4057</td>
<td>1.43%</td>
</tr>
<tr>
<td>Jun-2011</td>
<td>6.42</td>
<td>Luer-Jet Vial</td>
<td>76329-3369-1</td>
<td>1</td>
<td>1.0082</td>
<td>0.82%</td>
</tr>
<tr>
<td>Dec-2011</td>
<td>5.83</td>
<td>Luer-Jet Vial</td>
<td>76329-3369-1</td>
<td>1</td>
<td>0.9952</td>
<td>0.48%</td>
</tr>
<tr>
<td>May-2014</td>
<td>3.5</td>
<td>Luer-Jet Vial</td>
<td>76329-3369-1</td>
<td>1</td>
<td>1.0100</td>
<td>1.00%</td>
</tr>
<tr>
<td>Apr-2015</td>
<td>2.58</td>
<td>Luer-Jet Vial</td>
<td>76329-3369-1</td>
<td>1</td>
<td>0.9884</td>
<td>-1.16%</td>
</tr>
<tr>
<td>Nov-2015</td>
<td>2</td>
<td>Luer-Jet Vial</td>
<td>76329-3369-1</td>
<td>1</td>
<td>1.0196</td>
<td>1.96%</td>
</tr>
<tr>
<td>Mar-2018</td>
<td>0</td>
<td>Luer-Jet Vial</td>
<td>76329-3369-1</td>
<td>1</td>
<td>1.0034</td>
<td>0.34%</td>
</tr>
</tbody>
</table>
Figure 1. Naloxone degrades to Noraloxone (Noroxymophone)
Figure 2. Mass spectrum of Normaloxone (top) and Naloxone (bottom) ions

1.267 Extracted – TQ 1: MS1 Scan 1: 150.00-700.00 ES+, Centroid, CV=Tune (Uncalibrated - 5000.0 is outside the calibration range of 200.00-2000.00 Da/sec)

2.874 Extracted – TQ 1: MS1 Scan 1: 150.00-700.00 ES+, Centroid, CV=Tune (Uncalibrated - 5000.0 is outside the calibration range of 200.00-2000.00 Da/sec)
Figure 3. Method Development: UPLC-UV Chromatograms of Naloxone samples: USP Standards of 10 μg/mL naloxone (Green), USP standard: 10+10 μg/mL naloxone & Nornaloxone (Blue), standard: 1+1 μg/mL naloxone & Nornaloxone (Red), and an from expired naloxone product (diluted to 10 μg/mL by labeled value, Black).
Figure 4. A Naloxone sample (Red, Expiration in May 1990, prepared as 10ug/ml Naloxone according to the label), comparing with 1 μg/mL Normaloxone Standards (Green, Normaloxone peak at 1.25 min of retention time), and blank of sample preparation solvent (Blue)
Figure 5. Calibration Curve for Naloxone Quantification by external standards.

\[ y = 8574.9x - 1045.1 \]
\[ R^2 = 0.99972 \]
Figure 6. Naloxone Stability Over 27 Years. Tested samples are products packed in prefilled syringes of 1.0 mg/mL solution (by IMS, blue circles), or ampules of 0.4 mg/mL solution (by Abbott) (red triangles)