Development and Validation of a Method for Detecting Contaminants in Nalmefene Hydrochloride Injections

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Abstract — Nalmefene hydrochloride injection is a first-line drug in emergency management to relieve respiratory depression, but its poor stability limits its clinical application. We aimed to develop and thoroughly validate a detection method for impurities in nalmefene hydrochloride injection and provide a foundation for studies of product stability.

Keywords - validation, detecting contaminants, nalmefene hydrochloride injection

I. INTRODUCTION

Nalmefene hydrochloride[1] is a specific neuroprotective agent and a first-line drug for emergency management to relieve respiratory depression. Due to poor stability in the preparation and storage of this product, the contents of two known impurities (naltrexone hydrochloride and bisnalmefene hydrochloride) regularly exceeded quality standards; for this reason, nalmefene hydrochloride is no longer available in the United States, where it was originally developed. Studies have addressed the clinical application of nalmefene hydrochloride injection, but none have explored ways to improve product stability. The aim of this study was to develop an accurate method for the detection of contaminating substances and to accurately determine the content of two known impurities. This method could be used to guide the preparation and processing of nalmefene hydrochloride and accurately determine the presence of degradation intermediates. The method could also be used to precisely determine product shelf life and optimal storage conditions.

II. EXPERIMENTAL

A. Materials

Nalmefene hydrochloride injection was provided by Changchun Sanshun Pharmaceutical Co. (batch numbers 1203061, 1203071, and 1203081). All sample indexes for these batches were consistent with relevant regulations.

B. Development of a method to detect contaminating substances

The HPLC method employs a Primsphere C18 column (150 × 4.6 mm, 5 μm) and a mobile phase consisting of acetonitrile-phosphate buffer (sodium dihydrogen phosphate 7.8 g, triethylamine 2.0 mL in 1 L of water; pH adjusted to 4.2 ± 0.02 with 85% phosphoric acid, 20:80, v/v).

C. Validation

1) To determine the limit of quantitation[5] (LOQ), the volume of the test solution was accurately measured (0.1 mg/mL) for repeated trials. The LOQs of nalmefene, bisnalmefene, and naltrexone were 30 ng, 30 ng, and 47.5 ng, respectively.

2) To determine the limit of detection (LOD), the volume of the test solution was accurately measured (300 ng/mL)
3) Determination of the correction factors

The standard curve of nalmefene hydrochloride [6,7] was derived by determining the LOQ of nalmefene hydrochloride with five diluted preparations and analysis. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Mass of sample (ng)</th>
<th>1200</th>
<th>600</th>
<th>300</th>
<th>150</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average peak area</td>
<td>521992</td>
<td>262692</td>
<td>136297</td>
<td>66942</td>
<td>33495</td>
</tr>
</tbody>
</table>

The LOQ and LOD of bisnalmefene hydrochloride were used to prepare a standard curve on three high-performance liquid chromatography (HPLC) systems according to the method protocol. The results are shown in Table 2.

4) Specificity

Blanks were generated by testing 100 μl of each of the excipient and mobile phase. Naltrexone hydrochloride and bisnalmefene hydrochloride are two known impurities[8,9] in nalmefene. Aliquots (100 μl) of each of these known impurities were injected into the HPLC system, and chromatograms were recorded. Results are shown in Figure 1, 2,3 and 4.

Table 1: PLOTTING THE STANDARD CURVE TO DETERMINE THE CORRECTION FACTOR OF NALMEFENE HYDROCHLORIDE

Table 2: PLOTTING THE STANDARD CURVE TO DETERMINE THE CORRECTION FACTOR FOR BISNALMEFENE HYDROCHLORIDE

Table 3: PLOTTING THE STANDARD CURVE TO DETERMINE THE CORRECTION FACTOR OF NALTREXONE HYDROCHLORIDE
Fig. 4.: Bisnalmefene hydrochloride

In the absence of impurities, the test sample may be destroyed by bright light, high temperature, humidity, acidic and alkaline hydrolysis, or oxidation. Measurements were performed according to the proposed method, and the numbers and amounts of impurities before and after sample destruction were compared (Table 4).

### TABLE 4 DESTRUCTIVE TESTING AND CONTAMINANTS

<table>
<thead>
<tr>
<th>Condition</th>
<th>Nalmefene hydrochloride (%)</th>
<th>Bisnalmefene hydrochloride (%)</th>
<th>Number of theoretical plates for main peak</th>
<th>Separation between main peak and front/tail peaks</th>
<th>Total peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undestroyed product</td>
<td>0.12</td>
<td>0</td>
<td>4361.439</td>
<td>11.654</td>
<td>43702581</td>
</tr>
<tr>
<td>Product destroyed by light</td>
<td>0.14</td>
<td>0</td>
<td>3069.461</td>
<td>9.177</td>
<td>44531695</td>
</tr>
<tr>
<td>Product destroyed by alkali</td>
<td>0.138</td>
<td>0.016</td>
<td>3301.844</td>
<td>1.22</td>
<td>44442844</td>
</tr>
<tr>
<td>Product destroyed by high</td>
<td>0.56</td>
<td>0.614</td>
<td>4573.976</td>
<td>2.819</td>
<td>42140879</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product destroyed by acid</td>
<td>0.018</td>
<td>0.025</td>
<td>3361.309</td>
<td>1.264</td>
<td>43262668</td>
</tr>
<tr>
<td>Product destroyed by oxidation</td>
<td>0.897</td>
<td>1.22</td>
<td>3666.219</td>
<td>1.971</td>
<td>42953890</td>
</tr>
</tbody>
</table>

III. INVESTIGATION AND RESULTS

A. Development of a method to detect contaminating substances

The chromatography column was C18 (150 × 4.6 mm, 5 μm). The mobile phase was acetonitrile-phosphate buffer (20:80). The mobile phase was prepared by adding 2 mL trimethylamine and 7.8 g sodium dihydrogen phosphate in a final volume of 1 L in water, and then adjusted to pH 4.2 ± 0.02 with 85% phosphoric acid. HPLC was performed with a detection wavelength of 210 nm, flow rate of 1.0 mL/min, and a sample volume of 100 μl.

B. Validation

1). To determine the limit of quantitation (LOQ)

The RSDs of the test results for each contaminating substance were consistent with expectations, indicating that the LOQs for nalmefene, bisnalmefene hydrochloride, and naltrexone hydrochloride were accurate and reliable.

2). To determine the limit of detection (LOD)

The results showed that the RSDs for each substance were consistent with requirements, indicating that the LODs for nalmefene, bisnalmefene hydrochloride, and naltrexone hydrochloride were accurate and reliable.

3). Determination of the correction factors

The slopes of the standard curves for nalmefene hydrochloride, bisnalmefene, and naltrexone were 4.3308, 1.963, and 3.880, respectively. The calculated correction factors for bisnalmefene hydrochloride and naltrexone hydrochloride were 2.206 and 1.12, respectively.

4). Specificity

The test results showed that degradation and impurities could appear under acidic, basic, oxidizing, high-temperature, and light conditions. Oxidation generated the maximum degree of degradation. The peaks of each...
degradation product were well separated from the main peak. The proposed chromatographic conditions provided strong specificity and could be used to detect contaminating substances.

IV. DISCUSSION

When the correction factors of bisnalmefene hydrochloride and naltrexone hydrochloride—two known impurities—were calculated, the results showed the sample concentration would be close to the LOQ, requiring the preparation to be accurate and reproducible. At least three complete measurements are required and the calculation should be based on the average of three measurements in order to ensure the accuracy of the correction factor for the impurity, thereby ensuring the accuracy of the determination.

V. CONCLUSIONS

Five methods were used for nalmefene hydrochloride sample destruction, which led to the appearance of additional impurity peaks; this is important for understanding the degradation or aggregation pathways occurring in the sample. The peak area for the impurity should account for at least 20% of the main peak area; otherwise, the destructive strength should be increased.

REFERENCES

[2] Nalmefene Hydrochloride injection YBH14222008 SFDA Quality standard
[4] Nalmefene Hydrochloride injection YBH11242008 SFD Quality standard