Determination of stearic acid and 12-hydroxyoctadecanoic acid in PEG-60 hydrogenated castor oil by HPLC-ELSD after alkaline hydrolysis

YU DUAN¹, XIAN-ZHAO LIU², EN-NIAN LI³, ZHONG-JING GUO¹ and XIN-JUN XU¹*

¹ College of Pharmacy, Sun Yat-sen University, Guangzhou 510006, China
² Infinitus (China) Co., Ltd., Jiangmen 529100, China
³ Guangdong Yuansi South Pharmaceutical Biotechnology Co., Ltd., Guangdong 514699, China

ABSTRACT

A rapid and simple method for the determination of stearic acid and 12-hydroxystearic acid in PEG-60 hydrogenated castor oil by high performance liquid chromatography with evaporative light scattering detection was established. The oil sample was first pretreated by alkaline hydrolysis. The analysis was performed on a Zhongpu Develop XD-C18 column (250 mm × 4.6 mm, 5 μm) with gradient elution of methanol and 1% acetic acid aqueous solution at a flow rate of 1.2 mL·min⁻¹ and a column temperature of 40 °C. The drift tube temperature of the evaporative light scattering detection system was set at 40 °C, and the pressure of carrier gas (N₂) was 337 kPa. The regression equation revealed a good linear relation (r = 0.9993–0.9995) during the test ranges (119.1–1190.7 μg·mL⁻¹ for 12-hydroxystearic acid, 10.7–107.4 μg·mL⁻¹ for stearic acid). The detection limits of 12-hydroxystearic acid and stearic acid were 1.1 and 2.5 μg·mL⁻¹, the limits of quantitation were 3.2 and 7.4 μg·mL⁻¹, respectively. And the mean recoveries were 101.5 and 101.0%, the corresponding relative standard deviations (RSDs) were 2.1 and 2.8%, respectively. The RSDs corresponding to repeatability (n = 6) were both less than 2.6% in terms of precision. As to the stability, the test results remained stable after 8 h at room temperature (RSDs were both less than 2.6%). The developed method showed high sensitivity, recovery, repeatability and stability, which indicated that the method could be applied as a quality evaluation method for the determination of stearic acid and 12-hydroxyoctadecanoic acid in PEG-60 hydrogenated castor oil.

KEYWORDS

HPLC-ELSD, hydrogenated castor oil, fatty acids, determination, validation

1. INTRODUCTION

Hydrogenated castor oil, produced by hydrogenation of natural vegetable oil extracted from castor seeds, is a kind of important oil excipient. In the field of medicine, hydrogenated castor oil is often used as an effective surfactant in the preparation of various emulsions to solubilize hydrophobic drugs [1, 2]. In sustained-release formulations, hydrogenated castor oil is often used to prepare sustained-release matrices [3, 4]. It is also used as a thixotropic agent in various ointments and cream preparations to improve the rheological properties of the preparations [5]. In addition, hydrogenated castor oil has also shown excellent performance and great application potential in cosmetics, pesticides, coatings, textiles, printing and dyeing, chemical materials and other industries [6–8].

Hydrogenated castor oil is mainly composed of 12-hydroxystearic acid triglyceride, etc. Fatty acid composition is the main item for quality control of hydrogenated castor oil and
other oil excipients. According to the reported literature, the detection methods of fatty acids include gas chromatography (GC-FID) [9–13], high performance liquid chromatography (HPLC) [14–18], gas chromatography-mass spectrometry (GC-MS) [19–21], liquid chromatography-mass spectrometry (HPLC-MS) [22, 23], near infrared spectroscopy (NIR) [24, 25], nuclear magnetic resonance spectroscopy (NMR) [26] and so on.

Due to the high sensitivity, low detection limit and good repeatability, gas chromatography is the most widely utilized, especially in the current effective quality evaluation standards [27–31]. However, gas chromatography needs to go through the esterification pre-treatment step, which prolongs the analysis time, complicated the analysis method, and may produce the by-products. It is also difficult to ensure the complete esterification.

In addition, although near infrared spectroscopy does not need pre-treatment, the sensitivity of the method is low, the deviation is large, and it is time-consuming and laborious to establish a calibration model. The detectors commonly used in HPLC, such as ultraviolet-visible absorption detector (UV/Vis) and fluorescence detector (FLD), also need to use some new extraction techniques or special pretreatment methods in the determination [14–16]. It is mentioned in a small amount of literature that high-performance liquid chromatography combined with refractive index detector (RID) and electrochemical detector (ECD) can directly detect fatty acids, but gradient elution can not be used, which affects the separation effect and sensitivity of the method [17, 18].

As a universal detector, the evaporative light scattering detector (ELSD) has a unique detection principle. First, the eluent of chromatographic column is atomized to form an aerosol, and then the solvent is evaporated in a heated drift tube, and finally the remaining non-volatile solute particles are detected in a light scattering detection cell. Since the solvent has been completely evaporated before detection, only the analytes with low volatility are detected by scattering the light emitted by the laser light source, the detection response does not depend on the optical characteristics of the sample, but only on the quality of the solute eluting peaks. And the detection is not affected by the solvent, so the high performance liquid chromatography with evaporative light scattering detector (HPLC-ELSD) also has a good resistance to baseline shifting when using the gradient elution method. Due to the above significant advantages, HPLC-ELSD has been widely used in the analysis of saccharide, lipids, amino acids, antibiotics and natural active components [32]. However, it is rarely used in the detection of oil excipients. For the analysis of oil excipients, HPLC-ELSD can direct detect oil components without esterification pretreatment, simple steps and short analysis time. The method has the advantages of simple steps and short analysis time. The sensitivity and selectivity are high and the separation effect is good. Therefore, the HPLC-ELSD also has a good application prospect in the field of oil excipients analysis.

In our study, the content of fatty acids in hydrogenated castor oil after a simple pretreatment of alkaline hydrolysis was determined by HPLC-ELSD for the first time, in order to establish a simple, reliable and applicable method for determination of fatty acids. It also provides a reference for the quality evaluation of other oils.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

12-hydroxystearic acid (purity: 97%) reference substance was purchased from Shanghai Haohong Biopharmaceutical Technology Co., Ltd. (Shanghai, China). Stearic acid (purity: 99%), reference substance and glacial acetic acid (purity: 99.9%) were produced by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). PEG-60 hydrogenated castor oil test sample (batch number: 84050897V0, 12594475L0, abbreviated as 7V0, 5L0) was obtained from SEPPIC (Shanghai) Special Chemicals Co., Ltd. (Shanghai, China). Sodium hydroxide (purity: 96%) was purchased from Tianjin Zhiyuan Chemical Reagent Factory (Tianjin, China). HPLC-grade methanol was produced by Tianjin Kemioiu Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Solution preparation

2.2.1. Preparation of 12-hydroxystearic acid and stearic acid reference solution. 12-hydroxystearic acid and stearic acid were weighed accurately and dissolved in methanol in two different 100 mL volumetric flasks to make a standard solution (1190.7, 107.4 μg·mL⁻¹) which was diluted with methanol to produce a series of concentrations of the standard solution (1190.7, 893.0, 595.3, 297.7, 119.1 μg·mL⁻¹ for 12-hydroxystearic acid and 107.4, 85.9, 53.7, 21.5, 10.7 μg·mL⁻¹ for stearic acid).

2.2.2. Preparation of sample solution and blank solution. The reaction container is the 20 mL headspace vial with lid purchased by CNW Technologies GmbH. 30 mg PEG-60 hydrogenated castor oil sample and 1 mL 1 mol L⁻¹ sodium hydroxide ethanol solution were added to the 20 mL headspace vial, the lid was screwed and sealed. The vial was placed in a water-bath at 90 °C for 30 min with continuous vibration. Then, 1 mL glacial acetic acid and 8 mL methanol are precisely added when the above solution is cooled to room temperature to make the total volume of 10 mL, shake well, and the sample solution is obtained. According to the same operation mentioned above, the blank solution was obtained by using 1 mL methanol instead of the test sample.

2.3. HPLC instrumentation and conditions

The HPLC analysis was carried out on a LC-20A HPLC system (Shimadzu, Japan) equipped with a LT II evaporative light scattering detector (temperature from 25 to 100 °C, gain from 1 to 12, Shimadzu, Japan), an autosampler (Shimadzu, Japan) and a column temperature controller (Shimadzu, Japan). An XD-C18 column (250 mm × 4.6 mm, 5 μm) purchased from Zhongpu Technologies was utilized...
for separation. Methanol and 1% acetic acid aqueous solution were used as the mobile phase under a gradient elution condition at a flow of 1.2 mL min⁻¹ (Table 1). The column temperature was 40 °C. The drift tube temperature of the ELSD system was optimized to 40 °C, the carrier gas was N₂ with a pressure of 337 kPa and the gain of the detector was 4. The injection volume was 20 μL.

### Table 1. Gradient elution condition

<table>
<thead>
<tr>
<th>Time/min</th>
<th>Methanol ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10.0</td>
<td>85%</td>
</tr>
<tr>
<td>10.0–11.0</td>
<td>85→98%</td>
</tr>
<tr>
<td>11.0–25.0</td>
<td>98%</td>
</tr>
</tbody>
</table>

3. RESULTS AND DISCUSSION

3.1. System suitability and analysis

The HPLC chromatograms of blank solution, reference solution and test solution are shown in Fig. 1. The retention...
times of 12-hydroxystearic acid and stearic acid in the standard solution were 8.46 and 19.75 min, respectively and 8.43 and 19.84 min in the sample solution, respectively. The determination of 12-hydroxystearic acid and stearic acid in blank solution and sample solution was not disturbed. Thus, hydrogenated castor oil samples produced 12-hydroxystearic acid and stearic acid by alkaline hydrolysis. In the sample solution, the separation degrees between 12-hydroxystearic acid and its preceding and posterior adjacent peaks were 6.34 and 5.15, respectively, and the separation degrees between stearic acid and its preceding adjacent peaks were 13.13, indicating that the separation effect was good under the conditions. The adaptability of the system was evaluated by five consecutive injections of standard solutions (595.3 μg·mL⁻¹ for 12-hydroxystearic acid and 53.7 μg·mL⁻¹ for stearic acid). The RSD values of the two compounds were less than 2.6%.

### 3.2. Method validation

#### 3.2.1. LOD and LOQ

The reference solution of 12-hydroxy stearic acid and stearic acid was diluted with methanol. The corresponding concentrations of 12-hydroxystearic acid and stearic acid were determined as LOD when the signal-to-noise ratio (S/N) was about 10:1 and LOQ when the S/N was about 3:1. The LOD values of 12-hydroxy stearic acid and stearic acid were 1.1 and 2.5 μg·mL⁻¹, respectively. And the LOQ values of 12-hydroxystearic acid and stearic acid were 3.2 and 7.4 μg·mL⁻¹, respectively (Table 2).

#### 3.2.2. Linearity

Linearity was evaluated by five different concentrations of standard solutions (1190.7, 893.0, 595.3, 297.7, 119.1 μg·mL⁻¹ for 12-hydroxystearic acid and 107.4, 85.9, 53.7, 21.5, 10.7 μg·mL⁻¹ for stearic acid) with each concentration injected in duplicate. The regression equations were calculated in the form of lg Y = a × lg X + b, where Y and X are the peak area and concentration, respectively. The calibration curve obtained is in double log coordinate. The obtained regression equations of 12-hydroxystearic acid and stearic acid were lg Y = 1.3320lg X + 4.7539, lg Y = 1.5290lg X + 4.8329. The two calibration curves showed good linearity and the correlation coefficients were 0.9993 and 0.9995, respectively in the test ranges (Table 2).

#### 3.2.3. Repeatability

Six independent sample solutions prepared from the same batch of PEG-60 hydrogenated castor oil samples (batch No.: 5L0) were injected under the set chromatographic conditions to confirm repeatability, and the results were shown in Table 2. The RSD values of 12-hydroxystearic acid and stearic acid were 1.1 and 1.7%, respectively, indicating that the method has good repeatability.

#### 3.2.4. Recovery

The recovery was determined by standard addition method: low, medium and high levels of standard solutions (80, 100 and 120% of the sample volume, respectively) were added into triplicate sample solutions, and each level was repeated thrice for analysis. The recovery can be calculated as follows: recovery (%) = (C−A)/B × 100%, where A is the amount of original sample, B is the amount of reference standards added, and C is the amount found. The results were shown in Table 2. Recoveries of 12-hydroxystearic acid and stearic acid were in the range of 96.6–105.8% and the RSD values were both less than 2.8%, which indicated that the developed method has high accuracy.

#### 3.2.5. Stability

The stability of the sample was obtained by determining the composition content of the same PEG-60 hydrogenated castor oil sample solution stored at room temperature for 0, 2, 4, 6 and 8 h. The determination results are shown in Table 2. The RSD values of 12-hydroxystearic acid and stearic acid were both less than 2.6%, indicating that the PEG-60 hydrogenated castor oil sample was stable within 8 h after hydrolysis.

#### 3.2.6. Determination of 12-hydroxystearic acid and stearic acid in samples

The established HPLC-ELSD method was applied to the determination of 12-hydroxystearic acid and stearic acid in two batches of hydrogenated castor oil. As shown in Table 3, the RSD values were all less than 1.7%. The results show that the established method is simple, effective and accurate.

<table>
<thead>
<tr>
<th>Items</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12-hydroxystearic acid</td>
</tr>
<tr>
<td>LOD (μg·mL⁻¹)</td>
<td>1.1</td>
</tr>
<tr>
<td>LOQ (μg·mL⁻¹)</td>
<td>3.2</td>
</tr>
<tr>
<td>Calibration curves</td>
<td>lg Y = 1.3320lg X + 4.7539</td>
</tr>
<tr>
<td>Linear range (μg·mL⁻¹)</td>
<td>119.1–1190.7</td>
</tr>
<tr>
<td>Correlation coefficient, r</td>
<td>0.9995</td>
</tr>
<tr>
<td>Repeatability (n = 6)</td>
<td>1.1</td>
</tr>
<tr>
<td>Recovery (n = 9) (%)</td>
<td>101.5 (RSD% = 2.1)</td>
</tr>
<tr>
<td>Stability (RSD%)</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Percentage content (%)</th>
<th>RSD (%)</th>
<th>Percentage content (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7V0</td>
<td>20.3</td>
<td>0.6</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td>5L0</td>
<td>20.2</td>
<td>1.3</td>
<td>2.3</td>
<td>1.7</td>
</tr>
</tbody>
</table>
3.3. Discussion

3.3.1. Selection of mobile phase ratio. It is found that increasing the proportion of methanol in the methanol-1% acetic acid aqueous solution mobile phase can accelerate the separation process, but reduce the degree of separation and increasing the proportion of aqueous solution in the mobile phase will make the separation better, but the retention time will be prolonged and the peak shape will become worse. Considering the above factors, setting the elution gradient as shown in Table 1 and the flow rate of 1.2 mL min$^{-1}$ can achieve complete separation of compounds and save time, which is satisfactory. Besides, adding 1% acetic acid to the water can improve the peak shape and protect the chromatographic column.

3.3.2. Optimization of ELSD parameters. We adopted a single factor optimization method. The carrier gas pressure was fixed at 337 kPa, and the injection of the reference solution was used to investigate the effect of drift tube temperature on the response signal at 35, 40, 45 and 50 °C. The results showed that with the increase of drift tube temperature, the number of theoretical plates increased, the column efficiency became higher and the baseline became flat. But excessively high drift tube temperature setting can increase background noise and cause the loss of decomposition or volatilization of the sample, which is not conducive to the detection of samples. The optimal temperature is the lowest temperature that produces acceptable noise on the basis of the basic evaporation of the mobile phase. Considering comprehensively, the drift tube temperature is selected as 40 °C.

3.3.3. Optimization of alkaline hydrolysis conditions. Considering the influence of hydrolysis time and temperature on the hydrolysis process, the alkaline hydrolysis was carried out under different temperature conditions of 65, 75, 85, 90 and 95 °C (hydrolysis time was 30 min) and different time conditions of 10, 20, 30, 40, 50 min (hydrolysis temperature was 90 °C). The results of the single factor optimization experiment showed that the content of 12-hydroxystearic acid and stearic acid increased with the increase of hydrolysis temperature, and stopped growing when the temperature rose to 85 °C or above, as shown in Fig. 2. In the actual determination, in order to ensure the complete hydrolysis reaction, the hydrolysis temperature was set to 90 °C. The results of hydrolysis time were shown in Fig. 3. The content of the two compounds increased with the extension of hydrolysis time, but stopped when the hydrolysis time reached 30 min or above. Therefore, the optimum hydrolysis conditions were determined to be 1 mol L$^{-1}$ NaOH anhydrous ethanol solution, temperature of 90 °C and reaction time of 30 min.

4. CONCLUSIONS

The HPLC-ELSD method was successfully used to determine the content of 12-hydroxystearic acid and stearic acid in hydrogenated castor oil by gradient elution for the first time. The method has been verified to have high selectivity and good repeatability, recovery and stability. Compared with the GC commonly used in the current quality standards of oil excipients, it has the advantages of no esterification pretreatment, simple steps and short analysis time. This method opens up a new idea for the quality control of oil excipients and the quantitative analysis of fatty acids.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. Zhu Longping from Sun Yat-sen University for his help in the instrument.

REFERENCES


