An HPLC method for the determination of thioctic acid in raw material and tablets

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Abstract: Thioctic acid is a vitamin-like antioxidant which is prepared as tablets and injection. The Korean Pharmacopoeia (KP XI) contains monograph for the quality control of raw thioctic acid using ultraviolet-visible spectrophotometry and its formulations using high performance liquid chromatography (HPLC). In British Pharmacopoeia 2013 (BP2013), another HPLC method is used for the assay test of thioctic acid material. For the international harmonization, we present an HPLC method for quantitation of thioctic acid in both raw material and tablets. Method validation was performed to determine linearity, precision, accuracy, system suitability, and robustness. The linearity of calibration curves in the desired concentration range was high ($r^2 = 0.9995$), while the RSDs for intra- and inter-day precision were 0.93 ~ 1.26% and 1.40 ~ 1.76%, respectively. Accuracies ranged from 98.13 ~ 100.00%. Since the system suitability, intermediate-precision and robustness of the assay were satisfactory, this method will be a valuable addition to the Korean Pharmacopoeia (KP XI).

Key words: HPLC, thioctic acid, assay, validation, tablet dosage form

1. Introduction

Thioctic acid (Fig. 1) is chemically described as 5-[(3RS)-1,2-Dithiolan-3-yl]pentanoic acid. Thioctic acid is also known as alpha-lipoic acid. It is a vitamin-like antioxidant that acts as a free-radical scavenger¹ which is prepared as tablets and injection. In the 11th revision of the Korean Pharmacopoeia (KP XI), high performance liquid chromatography (HPLC) is applied for assay test of thioctic acid tablets and injection while assay method of thioctic acid raw material is UV spectrophotometry, which is not as specific as HPLC.² On the other hand, in British Pharmacopoeia 2013 (BP, 2013), an HPLC method is used for the assay test as well as the determination of related compounds but there is no method for the formulations.³ The two HPLC methods in KP XI and BP 2013 are different in

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mobile phase, column and detection, thus, cause difficulties for analyst in routine analysis of thioctic acid in which raw material and preparation should be tested by the same method. The latest version of the United State Pharmacopoeia (USP) and Japanese Pharmacopoeia still have not published monographs for thioctic acid and its preparations.4,5

Since the establishment of the first edition in 1958, the Korean Pharmacopoeia (KP) has been revised 10 times to ensure safety and efficacy of pharmaceutical products through appropriate test methods in accordance with international harmonization. Thus, replacement of non-specific test or conventional methods with updated methods and assurance of laboratory safety and environmental issues are greatly considered in every revision of the KP.

For the above reasons, the objective of the present work is to develop a reliable, simple, affordable HPLC method for quantitation of thioctic acid in raw material and tablets. Validation was conducted following the International Conference on Harmonization (ICH)13 and Korean Food and Drug Administration (KFDA) Validation Protocols.6-8

2. Experimental

2.1. Chemicals and reagents

Thioctic acid material and tablets were supplied by Shinpoong Pharmaceutical (Ansan, Korea). HPLC grade acetonitrile and methanol were obtained from Daejung Chemicals and Metals Co. (Siheung, Korea). Potassium phosphate monobasic and phosphoric acid were purchased from Duksan Pure Chemicals Co. (Ansan, Korea). Purified water was prepared in our laboratory. All other chemicals were of analytical reagent grade.

2.2. Instrumental conditions

Experiments were conducted on Shimadzu HPLC system consisted of following components: DGU – 20A3R Degasser, two LC – 20 AD pumps, SIL – 20A autosampler, SPD-20A UV – Vis Detector, CBM – 20A communication bus module (Shimadzu Corporation, Kyoto, Japan) and CO-965 Column Oven (Jasco Corporation, Tokyo, Japan). In intermediate precision validation, Agilent 1100 HPLC system included G1379A Degasser, G1312 Binary Pump, G1313 Auto-sampler, G1316 Colcom (Column Oven) and G1314AVWD Detector (Agilent Technology, Santa Clara, USA) was used.

For the HPLC condition, a Phenomenex Luna C18 (2) column (250 × 4.6 mm I.D., 5 µm) thermostated at 35 °C was used for the analysis of thioctic acid. Mobile phase included acetonitrile, 0.7 g/L solution of potassium dihydrogen phosphate previously adjusted to pH 3.0 with phosphoric acid and methanol (8:41:51, v/v). Flow rate was 1.2 mL/min. Inject volume was 20 µL. UV detection was at 215 nm.

2.3. Sample preparation

Standard solution: 100 mg of thioctic acid was dissolved in 20 mL solvent mixture which contained equal volumes of acetonitrile and a 0.7 g/L solution of potassium dihydrogen phosphate previously adjusted to pH 3.6 with phosphoric acid to obtain a 5.0 mg/mL standard solution. This solution was diluted to make 1.0 mg/mL standard solution.

Sample solution: 20 tablets were weighed and powdered. A quantity of the powder containing the equivalent of 250 mg of thioctic acid was transferred to a 50 mL volumetric flask. Solvent mixture was added to about 50 % of the capacity of the flask. For completely dissolution of thioctic acid, the content of the flask was sonicated for 5 min, cooled to room temperature and diluted with solvent mixture to volume. Resultant solution was quantitatively diluted so that a concentration of 1.0 mg/mL thioctic acid was obtained. A portion of this solution was passed through a 0.45 µm membrane filter as the sample solution.
2.4. Validation studies
Method was validated accordingly to ICH Q2 (R1) guideline with regard to linearity, precision, accuracy and robustness.

Calibration curves were prepared by taking appropriate volume of thioctic acid stock solution and diluting with solvent mixture to obtain final concentrations of 0.2; 0.5; 1.0; 1.5; 2.0; 2.5 mg/mL and used for evaluation of the linearity, accuracy, precision. Linearity was estimated by coefficient of determination ($r^2$) of the regression lines from 6 repeated analyses of the desired concentration range. Precision (relative standard deviation, RSD %) of the method were assessed by six analyses in a day (Intra – day) and in three different days (Inter – day) of standard solutions at concentrations corresponding to 80, 100, 120 % of analysis concentration (0.8; 1.0 and 1.2 mg/mL). Accuracy was expressed as recovery rates evaluated by standard addition method: three concentrations (0.8; 1.0 and 1.2 mg/mL) were spiked into 1.0 mg/mL sample solution. The experiments were performed in triplicate.

2.5. Application of the method
This analytical method was applied to quantitate the content of thioctic acid in tablets. The study was conducted on 6 samples prepared from tablets as mentioned above. The amount of thioctic acid in sample was calculated by following expression:

$$\text{Thioctic acid (C}_8\text{H}_{14}\text{O}_2\text{S}_2\text{) (mg)} = m \times \frac{A_T}{A_S} \times 1000$$

Where $m$ (g) is the amount of thioctic acid weighed, $A_S$ (µAU*s) is area of standard, $A_T$ (µAU*s) is area of sample.

3. Results and Discussion
3.1. Chromatography
A Phenomenex Luna C18 (2) column (250 × 4.6 mm I.D., 5 µm) thermostated at 35 ºC was used for the

Fig. 2. Typical chromatograms of (a) blank solvent mixture, (b) 1.0 mg/mL thioctic acid standard solution, (c) sample solution prepared from tablets. Condition: Luna C18 (2) column (250 × 4.6 mm I.D., 5 µm), mobile phase: acetonitrile : 0.7 g/L KH$_2$PO$_4$ pH 3.0 : methanol (8:41:51), flow rate: 1.2 mL/min, inject volume: 20 µL, detection at 215 nm.
analysis of thioctic acid. Mobile phase included acetonitrile, 0.7 g/L solution of potassium dihydrogen phosphate previously adjusted to pH 3.0 with phosphoric acid and methanol (8:41:51, v/v). Flow rate was 1.2 mL/min. Inject volume was 20 µL. UV detection was at 215 nm. Typical chromatogram was shown in Fig. 2(b).

3.2. Linearity
Calibration curves showed good linearity in the concentration range 0.2 ~ 2.5 mg/mL (Table 1). The equation of the calibration line obtained is: $y = 7933.2x + 134690$. The coefficient of determination was 0.9995.

3.3. Precision
The precision of the method was assessed by determining the intra-day assay relative standard deviation (RSD %) of the analysis (n = 6) of standard solutions at three concentrations (0.8; 1.0 and 1.2 mg/mL). Three replicates of each concentration were analyzed on each of three consecutive days. Results obtained are shown in Table 2. The intra-day precision for each concentration was 0.92 ~ 1.26 % and the inter-day precision was 1.41 ~ 1.76 %.

3.4. Accuracy (Recoveries)
Results of recovery studies by standard addition method were ranged from 98.34 % to 98.49 % for material. For tablets, recoveries were from from 98.13 % to 100.00 % (Table 3). This also suggested that there was no interference from excipients in determining content of thioctic acid in tablets.

3.5. System suitability, robustness and intermediate precision
Relative standard deviations of retention time, peak areas and number of theoretical plates, symmetric factor were measured after 6 repeats of 1.0 mg/mL solution analyses to evaluate system suitability of method (Table 4). RSD % of retention time and peak areas were 0.11 % and 0.06 %, respectively. The number of theoretical plates was 5894 and tailing factor was 1.21.

Robustness of the method was checked by making small deliberate changes in the ratio of phosphate buffer in mobile phase (41 ± 1 %) and flow rate (1.2 ± 0.1 mL/min). In both case, except changes in retention time, the results of method were not affected: RSD % of peak area (n = 6) was not more

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fusidate sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>$y = 7933.2x + 134690$</td>
</tr>
<tr>
<td>Range (mg/mL)</td>
<td>0.2 ~ 2.5</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9995</td>
</tr>
<tr>
<td>Number of data points</td>
<td>6</td>
</tr>
<tr>
<td>Slope ± SD</td>
<td>7933.2 ± 33.27</td>
</tr>
<tr>
<td>Intercept ± SD</td>
<td>134690 ± 42801.84</td>
</tr>
</tbody>
</table>

SD: Standard deviation

Table 2. Results of precision (intra/inter-day) validations of the proposed method

<table>
<thead>
<tr>
<th>Conc. (mg/mL)</th>
<th>Intra-day (n=6)</th>
<th>Inter-day (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSD (%)</td>
<td>Accuracy (%)</td>
</tr>
<tr>
<td>0.8</td>
<td>1.26</td>
<td>98.99</td>
</tr>
<tr>
<td>1.0</td>
<td>0.92</td>
<td>100.04</td>
</tr>
<tr>
<td>1.2</td>
<td>1.18</td>
<td>100.46</td>
</tr>
</tbody>
</table>

Table 3. Recovery tests for thioctic acid tablets (n = 3)

<table>
<thead>
<tr>
<th>Added conc. (%)</th>
<th>Recovery Mean (%)</th>
<th>RSD (%)</th>
</tr>
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<tbody>
<tr>
<td>80</td>
<td>99.94</td>
<td>0.43</td>
</tr>
<tr>
<td>100</td>
<td>100.00</td>
<td>1.07</td>
</tr>
<tr>
<td>120</td>
<td>98.13</td>
<td>0.03</td>
</tr>
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</table>

Table 4. System suitability data (n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Retention time (RSD %)</td>
<td>0.11</td>
</tr>
<tr>
<td>Peak area (RSD %)</td>
<td>0.06</td>
</tr>
<tr>
<td>Plate number</td>
<td>5894</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Table 5. Contents of thioctic acid tablets (n = 6)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Claimed value</th>
<th>Assay Content (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet A</td>
<td>600 mg</td>
<td>98.73</td>
<td>0.80</td>
</tr>
</tbody>
</table>

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than 0.3 %, number of theoretical plates were more than 4000 and symmetric factor was not less than 1.18 and not more than 1.78.

Intermediate precision was studied by using Agilent HPLC system. Results showed that there was a decrease in retention time - about 0.3 minutes earlier compared to Shimadzu system. Asymmetric factor was 0.6 and the number of theoretical plates was about 4642. RSD % of peak area was 0.60 %.

3.6. Application

This analytical method was applied to quantify the content of thioctic acid in tablets. The results of assay test in 6 samples of commercial tablets were recorded in Table 5. The average content of thioctic acid in the formulation was 98.73 %, RSD % of samples was 0.80 %. A typical chromatogram of sample is shown in Fig. 2(c).

4. Conclusions

The above proposed study describes a simple HPLC method for the determination of thioctic acid in raw material and tablets. The method was validated and found to be sensitive, accurate and precise.

Acknowledgements

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References