Alternative to the Thai Herbal Pharmacopoeia Method for Quality Control of Andrographis Capsules

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Jiranuch Jamtaweekul
Prapapun Sukphan

Abstract

Rationale and Objective: Reflux extraction is the standard sample preparation method described in the Thai Herbal Pharmacopoeia (THP) for the assay of andrographolide content in both Andrographis herbal material and capsules, and has been used since 1995. The disadvantages of this method are the consumption of large amounts of volatile and hazardous organic solvents, low extraction efficiency and a time-consuming process. The objectives of this study were to compare the extraction efficiency of ultrasonication and reflux extraction methods and to propose an alternative to the current THP method for quality control of Andrographis capsules.

Methodology: Optimization of extracting solvent and sonication time were investigated. The best condition was further used for comparison of the extraction efficiency with reflux extraction. A total of 30 different lots of Andrographis capsules were tested. The analytical method as described in the THP was revalidated to ensure method performance and data integrity after the sample preparation had been changed.

Results: Ultrasonication with 50% methanol for 15 minutes gave the highest andrographolide content extracted. Changes in sample preparation did not affect the analytical method performance. The contents of andrographolide extracted using ultrasonication were comparable and in most cases they were even higher than those obtained from the reflux method.

Discussion and Conclusion: Ultrasonication is a more efficient, rapid and convenient method and can be used as an alternative sample preparation method to the current monographs in THP for the quality control of both Andrographis herbal material as well as medicinal products.

Keywords: ultrasonication, andrographis capsules, quality
Background and Rationale

Andrographis paniculata (Burmann filius) Nees, also known commonly as “King of Bitters”, is a member of the plant family Acanthaceae, and has been used for centuries as traditional medicine in Asia and Scandinavia for prevention and treatment of fever, dysentery, diarrhea, inflammation, sore throat, and snakebites.\(^1\)\(^\text{-12}\) Furthermore, it is a promising new way for the treatment of several serious diseases, including HIV\(^\text{[13]}\), and numerous symptoms associated with immune disorders.\(^\text{[14-16]}\) The main active constituents of \textit{A. paniculata} (Burmann filius) Nees include flavonoids and diterpenes, especially labdane diterpenes such as andrographolide, dehydroandrographolide and neoandrographolide.\(^\text{[17-20]}\) The local plant name of \textit{Andrographis paniculata} (Burmann filius) Nees in Thailand is “Fa-Tha-Lai” or “Fa-Tha-Lai-Chon”. “Fa-Tha-Lai-Chon” is included in the Thai National List of Essential Medicines and is classified as “Herbal Medicinal Products” for the treatment of diarrhea, sore throat, and common cold.\(^\text{[21]}\) “Fa-Tha-Lai” has been established in the Thai Herbal Pharmacopoeia (THP) since 1995 as herbal material while that of the herbal medicinal product (Fa-Tha-Lai Capsules) has been established since 2004.\(^\text{[22,23]}\) According to the THP monographs “Fa-Tha-Lai” and “Fa-Tha-Lai Capsules”, andrographolide is used as chemical marker for quality control of both herbal material and herbal medicinal product and it is required that the content of andrographolide should be not less than 1.0 %w/w, as determined by HPLC-UV method.\(^\text{[24]}\) However, the standard method described for sample preparation remains unchanged and reflux extraction has been used since 1995 up to now.\(^\text{[22-24]}\)

Reflux extraction is the most widely used conventional technique for the extraction of diterpenoids from \textit{A. paniculata} (Burmann filius) Nees. However, it causes the consumption of large amounts of volatile and hazardous organic solvents, needs long extraction times and consumes more energy. Furthermore, the extraction efficiency of diterpenoids by conventional extraction method is not satisfactory and might not be applicable in routine quality control analysis for most of the Thai traditional medicine manufacturers since they do not equip with laboratory facility to perform reflux extraction. Use of harmful chemicals and large amounts of solvents also cause environmental pollution and health hazards to laboratory personnel. Therefore, it is important to improve conventional extraction technique and establish an efficient, simple and rapid extract method for the assay of andrographolide content in Andrographis herb and herbal medicinal product.

Ultrasound-assisted solvent extraction (ultrasonication) is a modified maceration
method where the extraction is facilitated by the use of ultrasound. The plant sample is placed in an ultrasonic bath and ultrasound is used to induce a mechanical stress on the cells through the production of cavitations in the sample. The cellular breakdown increases the solubilization of metabolites in the solvent, improves extraction yields and reduced extraction time. Furthermore, ultrasonic bath is the apparatus available in all quality control testing laboratories with typical laboratory applications of dissolving the standards and degassing of HPLC solvents. There were also some reports on quantitative analysis of andrographolide in Andrographis herb, extracts and dosage forms in which ultrasonication was used frequently. [27-31]

The objectives of this study were to compare the extraction efficiency of ultrasonication and reflux extraction methods and to propose an alternative to the current THP method for quality control of Andrographis capsules.

**Methodology**

**Material**

**Sample**

A total of 30 different lots of Andrographis capsules from 17 manufacturers were used in this study. The strength of Andrographis capsules tested was 250, 350, 400, 450, and 500 mg of powdered form. All samples tested were within their expiration dates at the time of testing.

**Chemicals**

Andrographolide Reference Standard (Lot F01344) was purchased from U.S. Pharmacopoeia, USA. HPLC- and AR-grade of methanol were obtained from Macron Fine Chemicals (USA) while dichloromethane was from Mallinckrodt (USA). Ultrapure water generated by Milli-Q (Millipore Corporation, USA) was used.

**Apparatus**

Reflux extraction apparatus, Rotary evaporator (Eyela, Japan), Water bath (NESLAB, USA), Ultrasonic bath (Elmasonic, Germany), High Performance Liquid Chromatography equipped with UV detector (Waters, USA), Micro balance and analytical balance (Mettler Toledo, Switzerland), Sieve No. 180 (nominal mesh aperture size of 180 mm, Endecotts, England)

**Method**

1. **Sample Preparation**

1.1 **Reflux extraction**

As described in THP[24], remove, as completely as possible, the contents of not less than 20 Fa-Tha-Lai capsules, and grind to No. 180 powder. Transfer about 400 mg, accurately weighed, to a 100 mL round bottom flask. Add 50 mL of a mixture of equal volumes of dichloromethane and methanol,
reflux in a water bath for 30 minutes, and filter. Evaporate the filtrate at 50°C under reduced pressure to dryness and dissolve the residue in sufficient methanol. Transfer quantitatively to a 100 mL volumetric flask, dilute with mobile phase to volume and mix. Filter through a nylon membrane having a 0.45 μm porosity. The filtrates were then analyzed for their andrographolide contents using HPLC.

1.2 Sonication

In order to optimize the ultrasonication extraction conditions, extracting solvent and time were investigated so as to obtain satisfactory extraction efficiency and quantitative results. Different concentrations of solvent (100% and 50% methanol) were investigated using 10 different lots of samples as a preliminary study. Weigh, finely powder and sieve through mesh size No. 180 the contents of not less than 20 capsules. Transfer an accurately weighed quantity of the powder about 400 mg to a 100 mL volumetric flask, add 70 mL of extracting solvent and ultrasonicate for the duration of 5, 10, 15, 20 and 25 minutes at room temperature. Cool to room temperature and dilute with extracting solvent to volume, mix, and filter. The sample solutions were then assayed for their andrographolide content using HPLC.

2. Method validation

The content of andrographolide was determined using HPLC-UV method as described in THP.\textsuperscript{[24]} The separation was performed on a C18 column (4.6 × 150 mm) protected by a C18 guard column (4.0 × 3.0 mm). The mobile phase was a mixture of methanol and water (50:50) at a flow rate of 1 mL/min. Detection was monitored at 224 nm. According to ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology\textsuperscript{[32]}, change in sample preparation is considered as modification of validated method which required partial validation to ensure that the analytical method maintains its characteristics. Therefore, the method was validated for specificity, linearity, accuracy, and precision. Specificity is demonstrated by comparing the representative chromatogram obtained from the sample solution with that of the standard solution. The calibration curves were generated by plotting the peak area against the concentration of andrographolide. Accuracy was evaluated using standard addition method. Three different concentrations (26.2 μg/mL, 44.9 μg/mL and 89.7 μg/mL) of standard were added to a previously analyzed sample solution. Triplicate experiments were performed at each concentration level. The average recoveries were estimated and should be in the range of 97.0 -103.0%. Precision was performed using 6 determinations of the same sample and should be within 2.0 %RSD.

3. Comparison of extraction method

A total of 30 different lots of Andro-
Graphis capsules from 17 manufacturers were assayed for their andrographolide contents using the optimized ultrasonication condition and reflux extraction method. Each sample was extracted in duplicate. The percentage change in the andrographolide content was calculated using the result from reflux extraction method as reference.

Results

1. Optimization of extracting solvent and sonication time

Peak shape problem of andrographolide peak was clearly observed when 100% methanol was used as the solvent of sample solutions. There is no further investigation on the effect of sonication time of this extracting solvent. Optimization of sonication time was then conducted using 50% methanol in order to avoid andrographolide peak shape problem. The effect of sonication time was shown in Table 1. The results showed that the amount of drug extracted increased with the time of sonication, reaching a maximum at 15 minutes for most of the samples tested. The sonication time of 15 minutes was then taken as the best condition for comparing with the reflux method which is a standard preparation method described in the THP.

2. Method validation

Under the described conditions, no peak interferences were found at the retention time of andrographolide in the samples prepared from both reflux extraction and ultrasonication. Chromatograms of sample solutions obtained from the two different extraction techniques

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Content of andrographolide extracted (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sonication time (minutes)</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>1.97</td>
</tr>
<tr>
<td>2</td>
<td>0.85</td>
</tr>
<tr>
<td>3</td>
<td>1.66</td>
</tr>
<tr>
<td>4</td>
<td>2.86</td>
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<tr>
<td>5</td>
<td>1.79</td>
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<td>6</td>
<td>3.91</td>
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<td>7</td>
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<td>8</td>
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<td>9</td>
<td>1.93</td>
</tr>
<tr>
<td>10</td>
<td>0.72</td>
</tr>
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</table>
were comparable, as shown in Figure 1. The method exhibited a good linearity over a concentration range of 20-140 μg/mL with a correlation coefficient (r) of 0.9999, as demonstrated in Figure 2. The recovery and precision results and are summarized in Table 2 and 3, respectively.

3. Comparison of extraction method

Flow chart comparing for sample preparation procedures of reflux and optimized condition of ultrasonication is demonstrated in Figure 3. A total of 30 different lots of Andrographis capsules from 17 manufacturers were assayed for their andrographolide contents using both ultrasonication and reflux.
extraction method. Each sample was extracted in duplicate for each of the extraction method. The contents of andrographolide (%w/w) obtained from the two techniques along with the percentage changes were shown in Table 4.

### Table 2 The recovery results of andrographolide (n=3)

<table>
<thead>
<tr>
<th>Amount of andrographolide added (µg/mL)</th>
<th>Recovery (%)</th>
<th>Average (%)</th>
<th>RSD (%)</th>
<th>Acceptance criteria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>26.2</td>
<td>98.9</td>
<td>99.7</td>
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<td>99.5</td>
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<tr>
<td>44.9</td>
<td>98.0</td>
<td>97.0</td>
<td>97.1</td>
<td>97.3</td>
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<tr>
<td>89.7</td>
<td>97.2</td>
<td>97.0</td>
<td>96.8</td>
<td>97.0</td>
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</table>

### Table 3 Precision results of andrographolide in Andrographis capsules

<table>
<thead>
<tr>
<th>Replicate No.</th>
<th>Andrographolide content (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.786</td>
</tr>
<tr>
<td>2</td>
<td>0.784</td>
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<td>3</td>
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<td>5</td>
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<tr>
<td>Average</td>
<td>0.787</td>
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<tr>
<td>RSD (%)</td>
<td>0.324</td>
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</table>

### Discussion

The choice of a particular method for extraction of drug from herbal formulations is most commonly determined by the solubility characteristics of the drug as well as the type of formulation. Andrographolide is easily dissolved in methanol, ethanol, acetic acid, and acetone but has limited solubility in ether and water, thus polar solvent can be used to extract the drug. Extraction solvent and time are the two crucial factors that can affect the efficiency of ultrasonic extraction. Therefore, these two factors were investigated in our experiment. When 100% methanol is used as extracting solvent and the filtrate was then analyzed for their andrographolide contents using HPLC, peak shape problem of andrographolide was observed. This is probably due to mismatch of sample solvent and mobile phase. In order to eliminate solvent effect, mobile phase itself (50% methanol) is then used as the solvent of sample solutions. Optimization of sonication time was investigated using 50% methanol and the duration of 15 minutes gave the highest amount of andrographolide extracted. There is no noteworthy difference in the amount of drug extracted after 20 minutes. The sonication time of 15 minutes was then taken as the best condition.
Figure 3  Flow chart for sample preparation procedures of ultrasonication and reflux extraction

Table 4  The content of andrographolide (%w/w) obtained from sonication and reflux methods

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Andrographolide content (%w/w)</th>
<th>Percentage change</th>
<th>Sample No.</th>
<th>Andrographolide content (%w/w)</th>
<th>Percentage change</th>
</tr>
</thead>
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<td>Sonication</td>
<td></td>
<td>Reflux</td>
<td>Sonication</td>
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<td>1</td>
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<td>5.79</td>
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<td>4.47</td>
<td>18</td>
<td>1.95</td>
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<tr>
<td>4</td>
<td>1.80</td>
<td>1.77</td>
<td>-1.48</td>
<td>19</td>
<td>1.78</td>
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<td>5</td>
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<td>1.69</td>
<td>0.61</td>
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<td>6</td>
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<td>1.17</td>
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<td>7</td>
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<td>11</td>
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<td>2.89</td>
<td>2.97</td>
<td>26</td>
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</tr>
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<td>19.67</td>
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<td>2.23</td>
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<tr>
<td>13</td>
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<td>9.28</td>
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<td>1.86</td>
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<td>28.71</td>
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<tr>
<td>15</td>
<td>0.87</td>
<td>0.95</td>
<td>9.16</td>
<td>30</td>
<td>1.44</td>
</tr>
</tbody>
</table>
to prepare the sample solutions for comparing the extraction efficiency with that of reflux extraction method which is a standard preparation method described in the THP.

According to ICH Q2(R1) Validation of Analytical Procedures, change in sample preparation requires partial validation to ensure that the analytical method maintains its characteristics. Change in sample preparation did not have an effect on the analytical method performance and data integrity as confirmed by validation results on specificity, linearity, accuracy and precision. All validated parameters were within the acceptance criteria.

The contents of andrographolide extracted using ultrasonication were comparable and in most cases they are even higher than those obtained from reflux method. These results indicated that the extraction efficiency of reflux method using a mixture of methanol and dichloromethane is not as efficient as that obtained from ultrasonication with 50% methanol. However, there were two samples that reflux method gave higher content of extracted andrographolide. These were further investigated and it was found that the particle sizes of sample powder were not uniform even they were finely ground and sieved through mesh size No.180, small stem parts were also observed. Although reflux extraction is the standard sample preparation method described in THP, it has various drawbacks, such as time-consuming, labor-intensive and multi-step process that requires concentration by solvent evaporation before chromatographic analysis. Long sample preparation times limit the number of samples, and multi-step procedures are prone to introduce errors and loss of analytes. The significant advantages of ultrasonication over reflux method are reduction of organic solvent consumption, elimination of additional concentration step and improvement in extraction efficiency. Therefore, ultrasound-assisted extraction is an attractive alternative to conventional extraction techniques because it is easy, inexpensive, fast and efficient. The proposed sample preparation technique was also applied to determine the andrographolide content in Andrographis herbal materials as well as Andrographis extract capsules. It is found that raw materials have satisfied quality in terms of andrographolide content (1.64-2.14 %w/w) and the extract capsule contains andrographolide as high as 7.38 %w/w.

**Conclusion**

Two sample preparation techniques were compared in this study. The results clearly indicated that ultrasonication was better than reflux extraction method because it provides higher extraction efficiency, was more economical, more convenient, easily
operated and rapid. The study showed that ultrasonication can be a viable alternative to the current THP method for quality control of both Andrographis herbal material as well as medicinal product.

Acknowledgement

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References


บทคัดย้อย

วิธีทางเลือกที่ดีควบคุมคุณภาพแก่ผลิตภัณฑ์ทางกายภาพตามคำมั่นความสุขในไทย

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ผลการและวัสดุประสงค์: รัฐไทยเป็นการเตรียมตัวอย่างตามวิธีคุมความสุขในคำมั่นความสุข

กรมไทยสำหรับการศึกษา, วิจัยและพัฒนา/tree, หรือยุทธศาสตร์การพัฒนา ที่ปรึกษาบัณฑิต ป.ศ. 2538 วิธีคุมคุณภาพผลิตภัณฑ์ที่เหมาะสมและอันดับความในปริมาณสุข

ประสิทธิภาพในการสั่งค่า และใช้ความมั่นคง มั่นคงของวัสดุประสงค์เพื่อแก้ไขปัญหาระบบการพัฒนาของ

วิธีคัดเลือกตัวบ่งชี้ มั่นคงการใช้ผลิตภัณฑ์ความสุข และสะดวกเป็นวิธีทางเลือกในการเตรียมตัวอย่างเพื่อ

ควบคุมคุณภาพแก่ผลิตภัณฑ์ทางกายภาพตามคำมั่นความสุขไทยยุคปัจจุบัน

ระเบียบวิธีโน: หาผลการวิเคราะห์และระบุคุณภาพที่เหมาะสมที่สุดในการเตรียมตัวอย่างโดยใช้

กลิ่นเสียงความสุข แล้วนำมาปรับคุณภัณฑ์ของผลิตภัณฑ์ไทยหลักโดยตลอดกลับงาชุด

ที่ปรึกษาบัณฑิต อันวุฒิ 30 ตัวอย่าง วิธีการคัดเลือกผลิตภัณฑ์ที่เหมาะสมในการเตรียมความสุข

ในไทยแล้วเกินกว่าการตรวจสอบความสุขโดยข้อศึกษาเพื่อให้เกิดวิธีดังกล่าวข้อด้วยมีประสิทธิภาพ

และมีความสุขต่อเนื่องซึ่งยังคงมีการเปลี่ยนแปลงวิธีการเตรียมตัวอย่าง

ผลการคัดเลือก: การเตรียมคู่อย่างโดยใช้ผลิตภัณฑ์ความสุข 15 มาตร์ และมีผลทั่วไป 50%

เป็นตัวอย่างที่ปรับผลิตภัณฑ์ที่เหมาะสมที่สุดให้สุข ผลการเปลี่ยนแปลงวิธีเตรียมตัวอย่างไม่มีผล

การทดสอบประสิทธิภาพของวิธีการดังกล่าว ปรับผลิตภัณฑ์ที่เหมาะสมให้การใช้ผลิตภัณฑ์ความสุข

ที่มีให้ผลคือและเหมาะสมกว่าการเตรียมด้วยวิธีผลิตภัณฑ์

ผลการและรายละเอียด: การเตรียมตัวอย่างโดยใช้ผลิตภัณฑ์ความสุขเป็นวิธีที่มีประสิทธิภาพ รวดเร็ว

และระยะเวลาการใช้วิธีผลิตภัณฑ์ สามารถนำมาใช้เป็นวิธีทางเลือกในการเตรียมตัวอย่างเพื่อควบคุมคุณภาพยา

แก่ผลิตภัณฑ์ทางกายการตามคำมั่นความสุขไทยยุคปัจจุบันได้

คำสำคัญ:  กลิ่นเสียงความสุข, ยา, ผลิตภัณฑ์, คุณภาพ