

Brief Report

A rapid identification of *Radix inulae* and its active component alantolactone in the Tibetan medicine Manuxitang

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Summary

This study sought to establish a more reliable method of identifying the "monarch" or principal drug *Radix inulae* and its active component alantolactone (AL) in the Tibetan medicine Manuxitang. *Radix inulae* and AL in Manuxitang were effectively identified by thin layer chromatography (TLC). AL was quantitatively determined using gas chromatography in the range of 0.1-1.0 µg/mL ($r = 0.9998$). The precision was 1.20% ($n = 6$) with an average RSD of 1.74%. Recovery was in the range of 93.5-98.5% with RSD value of 1.85%. The methods established were simple, accurate, and specific and could be used for quality control of Manuxitang.

Keywords: Manuxitang, *Radix inulae*, Alantolactone, Chromatography, Tibetan medicine

1. Introduction

Tibetan medicine, an important part of traditional Chinese medicine (TCM), is known as a source of sustainable and affordable healing preparations that are effective without lasting negative side effects. More than 3,000 different medicinal materials of traditional Tibetan medicine, including many precious and costly medicinal herbs such as aweto, snow lotus of Tianshan, saffron, and *Solanum muricatum*, grow on the Qinghai-Tibet plateau known as the "Roof of the World".

Traditional medicines are usually prepared by decocting multiple materials, which causes difficulty in identifying their active components and achieving quality control. Qualitative evaluation of a TCM decoction is often challenging since the active compounds in TCM may originally be from single herbs and may also result from the decocting process. There is an urgent need for systematic scientific standards to objectively evaluate their safety and efficacy and to

strictly control their quality based on scientific research and evidence (1,2).

One of the most popular Tibetan medicines is known by the Tibetan name Manuxitang (玛奴西汤) or the Chinese name Siwei Zangmuxiang Tangsan (四味藏木香汤散) and has been widely used in clinical practice for more than 1,000 years. This medicine has proven to have considerable effects on diaphoresis and relief of "exterior syndrome" (signs and symptoms accompanying cold and flu) and has been widely used for the onset of pestis and influenza, chills, headache, joint pain, rheumatoid arthritis, and fever (3). Manuxitang consists of four traditional herbs, namely *Radix inulae* (藏木香; Zangmuxiang), *Tinosporae sinensis* Caulis (宽筋藤; Kuanjinteng), *Rubus niveus* Thunb (悬钩木; Xuangoumu), and *Zingiber officinale* Posc. (干姜; Ganjiang). Manuxitang has already been listed in Volume 1 of the "People's Republic of China medical department drugs standard-Tibetan medicine" in 2005 (4). However, identification and quantitative determination were not included in the standards for Manuxitang.

In a traditional medicine, the principal component is known as the "monarch". *Radix inulae*, the "monarch" of Manuxitang, is the dried roots of *Inula helenium*

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L. or *Inula racemosa* Hook f. (Family Compositae). This medicinal material is widely used in Mongolia, Xinjiang, Tibet, and Qinghai and is frequently used in TCM for abdominal distension and pain, acute enteritis, and bacillary dysentery (5,6). The major active component in *Radix inulae* has been determined to be alantolactone (AL) (7,8). This study attempted to establish a rapid method of identifying the "monarch" or principal drug *Radix inulae* and its active component AL in Manuxitang.

2. Materials and Methods

2.1. Herbs and chemicals

Manuxitang was provided by Qinghai Tibetan Medicine Hospital (batch code: 060101, 060102, 060103). *Radix inulae*, the dried root of *Inula helenium* L. (Composite), was provided by Qianghai Tibetan Medicine Research Institute and identified by the Institute for Drug Control. AL was purchased from China Materia Medica Biological Product Inspection Institute, Beijing, China.

2.2. Thin layer chromatography (TLC)

2.2.1. Sample preparation

Two g of Manuxitang were extracted with 25 mL of trichloromethane by ultrasonication for 30 min. The extract was filtered and then concentrated to 2 mL and the filtrates obtained were used for sample application (9). *Radix inulae*-positive control solutions were prepared from the *Radix inulae* herb using the same extraction methods as for Manuxitang. A *Radix inulae*-negative control was prepared in accordance with the preparation of a Manuxitang formulation without *Radix inulae* following the extraction as described above. AL was dissolved into 2 mL of trichloromethane to prepare an AL control solution.

2.2.2. TLC for *Radix inulae* and AL

About 5 μ L of Manuxitang sample solution, AL control solution, and *Radix inulae*-negative and positive control solutions were spotted on a Silica Gel G plate (Qingdao Haiyang Chemical Plant, Qingdao, China) containing 0.5% sodium carboxymethyl cellulose by means of a semi-automatic sample applicator. After development with a solvent system of petroleum ether (60-90°C)/ethyl acetate (15:3, v/v), the plates were withdrawn from the chambers and dried at room temperature. Color developing was performed by spraying plates with 5% vanillin sulfate solution and then heating them to 105°C until the spots were clear. Thin layer chromatography scanning (TLCS) fingerprint profiles were obtained by both UV absorption at 365 nm and fluorescence detection. Photographic TLC images were also acquired using ReproStar 3.

2.3. Gas chromatography

2.3.1. Preparation of sample solutions

Two g of Manuxitang were crushed to obtain a fine powder and then mixed well. After screening, the powder was accurately weighed, dissolved with 30 mL ethyl acetate, and then ultrasonicated for 30 min. The mixture was filtered through a 0.45 μ m membrane filter and the filtrate obtained was used as the sample solution. The negative control solution (without *Radix inulae*) was prepared using the same method.

2.3.2. Gas chromatography conditions

A Finnigan Trace GC Ultra gas chromatographic system (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with an SUPELCO WAXTM10 column (30 m \times 0.25 mm \times 0.25 μ m), a flame ionization detector, and a GCD-500G high-purity hydrogen generator were used for gas chromatography. The oven temperatures were controlled as follows: the temperature was initially 50°C and then raised to 250°C at a rate of 20°C/min, kept for 6 min, and then raised again to 260°C at a rate of 5°C/min and kept for 5 min. The temperature of the injector block was set at 260°C. Samples (1.0 μ L) were injected in split injection mode (10:1). Helium was used as the carrier gas at a flow rate of 1.0 mL/min.

2.3.3. Method validation

2.3.3.1. *Calibration curves.* A stock standard solution of AL (1 mg/mL) was prepared with ethyl acetate. Working standard solutions ranging from 0.1-1.0 mg/mL of AL were prepared from various aliquots of stock standard solution and then diluted to 1 mL with ethyl acetate to give a final appropriate analyte concentration. Six-point calibration curves were acquired by plotting the peak area with respect to the concentration of the calibration standards.

2.3.3.2. *Precision.* Same-day precision was estimated by six replicate injections of standard solutions at the concentrations of 0.1, 0.50, and 0.9 mg/mL on the same day. A relative standard deviation (RSD) within 5% was the criterion for acceptability of data.

2.3.3.3. *Recovery.* Recovery studies were conducted by spiking one batch of Manuxitang (batch code: 060101) with 1.00 mg/mL of recovery standard solution. Two mL of standard solution were added to separate aliquots of the Manuxitang powder. The spiked samples were then extracted, processed, and quantified in accordance with the established method ($n = 6$).

3. Results and Discussion

On TLC analyses, *Radix inulae* and AL were both successfully separated by development on the plate (Figure 1). The Manuxitang sample solution (lane 2) produced a purple spot, which developed at the same position as that produced by control AL (lane 4), and

some spots with colors differing from those produced by the *Radix inulae*-positive control solution (lane 3). R_f values of *Radix inulae* and AL were 0.18 and 0.54, respectively. These two spots were not observed with the *Radix inulae*-negative control solution (lane 1).

When Manuxitang was analyzed by gas chromatography under the optimal conditions as

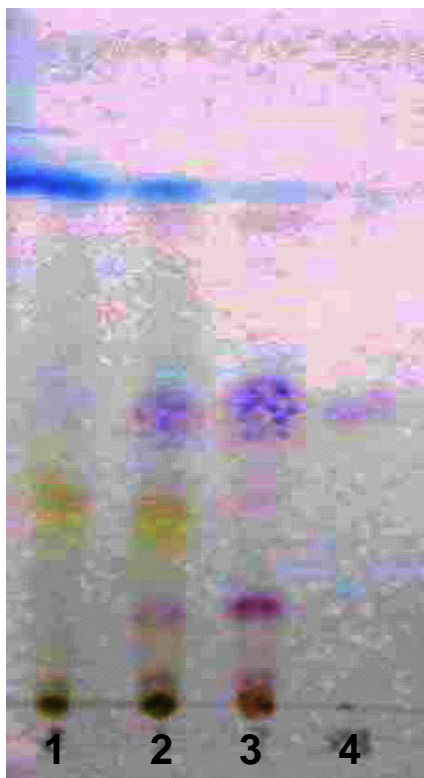


Figure 1. A typical TLC profile for *Radix inulae* and AL. Mobile phase: petroleum ether (60-90°C)/ethyl acetate (15:3, v/v). Lane 1, negative control; lane 2, Manuxitang sample; lane 3, *Radix inulae*-positive control; lane 4, AL-positive control.

described in the Materials and Methods, AL was successfully separated and detected at a retention time of 16.8 min (Figure 2). The peaks in Manuxitang were identified by retention times and the contents were calculated by the areas of the peaks. The AL content in three batches of Manuxitang was 4.33, 4.21, and 4.30 mg/mL, respectively.

For evaluation of the quantitative applicability of the method, the following parameters were analyzed under optimum conditions. (i) The linear relationship between the concentration of the active compounds (X) and the corresponding peak areas (Y) was investigated. The regression equation for AL was: $Y = 9.0 \times 10^7 X + 2.0 \times 10^6$ in the range of 0.1-1.0 $\mu\text{g/mL}$ ($r = 0.9998$). (ii) The limit of detection (LOD), a concentration that generates a signal-to-noise ratio of 3, was determined to be 10 $\mu\text{g/mL}$ for AL. (iii) Precision was measured by performing same-day experiments of six replicate injections of standard solutions at three different concentrations. The precision (RSD) for AL was 1.20% with an average RSD of 1.74%, indicating good precision at low, medium, and high concentrations of the compound. (iv) Recovery was in the range 93.5-98.5% for AL, with RSD values of 1.85%, which met the criterion for acceptability of accuracy (95-105%) at the analyte concentration.

The last few years have seen a tremendous increase in regulatory activities regarding the herbal industry, and demand for analytical methods that can help to ensure safety and quality has been growing at an accelerated pace (10-12). Due to the complexity of components in Chinese herbs and especially in their preparation, however, these methods suffer from limitations such as being material-intensive and time-consuming. The present study established a relatively simple,

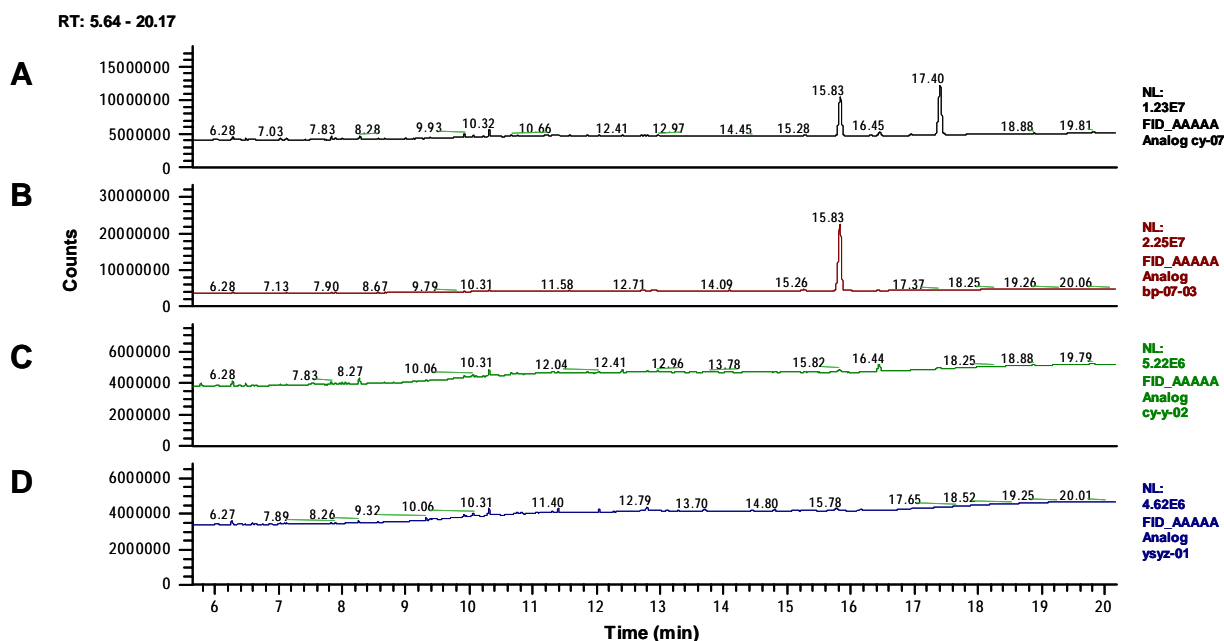


Figure 2. Gas chromatograph analyses of AL in Manuxitang. A, Manuxitang sample; B, AL control; C, negative control; D, solvent only.

convenient, and effective method of determining AL, a major active component in *Radix inulae* (7,8), and used it for the quality control of a Manuxitang decoction.

TLC is often the method of choice for routine compositional analysis and quality screening of medicinal plants when many samples have to be compared, when flexibility is important, and when rapid qualitative and semi-quantitative data are needed with a low cost per sample. The present data showed that the identification of *Radix inulae* and its active component AL in a Manuxitang decoction could be achieved by TLC, although TCL analysis was semi-quantitative. In contrast, gas chromatography analysis is a simple, sensitive, and reproducible method of determining AL in *Radix inulae* and Manuxitang. Additionally, the proposed method may also be suitable for the quality control of TCM containing *Radix inulae*.

Acknowledgements

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