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Studies on the dynamic accumulations of *Sophora alopecuroides* L. Alkaloids in different harvest times and the appropriate harvest time

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ABSTRACT

A sensitive and accurate method for the simultaneous determination of five alkaloids, namely 9α-hydroxymatrine (M1), matrine (M2), sophoridine (M3), oxymatrine (M4), alopecurin A (M5) in different parts (seed, legume, stem, and root) and different harvest times of *Sophora alopecuroides* L. was developed by high performance liquid chromatography (HPLC) with photodiode array detector (PDA) for the first time. The separation by gradient elution was achieved on Scienhome Kromasil C_{18} (4.6 × 250 mm, 5 μm) column at 30 °C with acetonitrile (A)/0.1% phosphatic acid + 0.1% triethylamine (B) as the mobile phase. The detection wavelength was 205 nm. The optimized method provided a good linear relation ($r \geq 0.9993$ for all the target compounds), satisfactory precision (RSD values less than 2.3%) and good recovery (96.4–103.6%). The limits of detection ranged between 0.11×10^{-3} and 4.70×10^{-3} μg for the different analytes. The method was successfully applied to analysis and quality control of alkaloid extracts from the traditional Chinese herbal drugs of *S. alopecuroides* L.

1. Introduction

Sophora alopecuroides L. (Leguminosae, *Sophora* Linn.), a traditional Chinese herbal medicine, is widely distributed in the northwest of china, especially in Xinjiang. Phytochemical investigations show that there exist more than twenty chemical compounds, belonging to alkaloids, flavonoids, volatile oil, organic acid, amino acid, protein, saccharide, *etc*. Among these chemical constituents, the principal bioactive constituents of *S. alopecuroides* L. are quinolizidine alkaloids, which have been shown to exhibit sedative, depressant, analgesic, hypothermic, antipyretic, and cardiotonic activities [1], especially anti-tumor [2] and improve the immunity [3]. Two types of quinolizidine alkaloids, matrine-type and pyridone quinolizidine bases, are found in *Sophora* species and can be used for identification of *Sophora* species in commercial preparations [4]. Hence, sensitive, rapid and specific methods for analyzing quinolizidine alkaloids are of great interest.

Several analytical methods such as high performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and capillary electrophoresis (CE) have been used to analyze this crude drug for the presence of quinolizidine alkaloids [5–10]. However, TLC lacks quantitative precision, presented CE method is applied in non-aqueous phase, which limited their application. HPLC analyses of these alkaloids have been reported elsewhere: under uncontrolled conditions it has not been possible to obtain satisfactory selectivity or reproducibility and also takes longer analyze time. Therefore, it is necessary to establish a rapid and effective method for the quantitative analysis of these alkaloids.

This paper reports an approach that gives an acceptable chromatogram for separation of the major quinolizidine alkaloids. The RP-LC method is suitable for quantitative analysis and can be used as an effective tool to evaluate herbal medicines. In this study, an RP-LC method was developed for simultaneous detection and quantification of the five bioactive compounds, namely 9α-hydroxymatrine (M1), matrine (M2), sophoridine (M3), oxymatrine (M4), alopecurin A (M5) (Fig. 1) in *S. alopecuroides* L., while the samples of its different growing periods were analyzed for identifying the best collecting time. Five alkaloids were separated and quantitatively determined. Up to now, there is no published quality standards for *S. alopecuroides* L., although laboratories in various universities are engaged in the analysis of *S. alopecuroides* constituents. It is necessary to establish standards for the quality control of *S. alopecuroides* L. phytomedicines.

2. Experimental

2.1. Plant material

The samples of *S. alopecuroides* L. in different harvest times were collected from Shihezi (Xinjiang, China), and identified by Profes-

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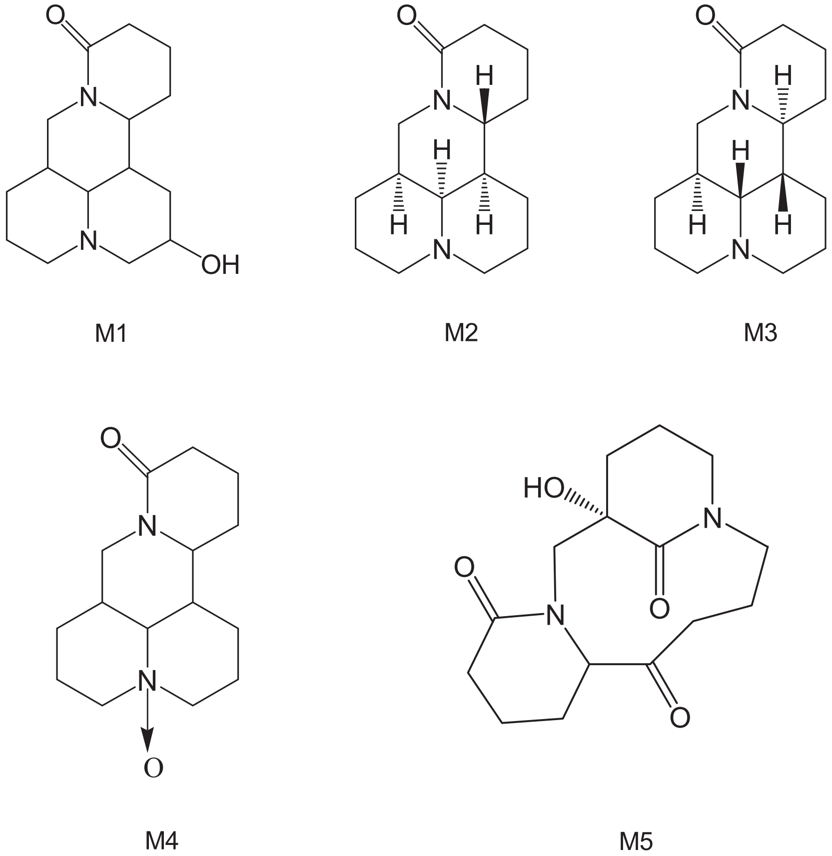

Fig. 1. Structures of compounds M1–M5.

sor Yong Tan (Shihezi University, Xinjiang, China). The voucher specimen (no. 20070816006) is deposited in School of Pharmacy faculty, Shihezi University. For the analysis, samples were dried and pulverized into powder.

2.2. Reference standards and solvents

The reference standard of M1–M5 quinolizidine alkaloids was isolated previously from the total alkaloids of *S. alopecuroides* L. by author, structures of which were elucidated by comparison of spectral data (UV, IR, MS, ^{1}H NMR and ^{13}C NMR) with the literature data [11–13]. The purity of each reference standard was determined to be above 98% by LC analysis based on a peak area normalisation method, detected by HPLC-PDA and confirmed by LC–ESI-TOF-MS and NMR spectroscopy.

Acetonitrile (HPLC-grade) and methanol (HPLC-grade) were purchased from Fisher Scientific Co. (Franklin, USA). LC grade water was prepared using a redistilled water equipment. Phosphoric acid (analytical grade) was purchased from Tianjin Guangfu Chemical Reagent Co. Ltd. (Tianjin, China). Deionised water for LC analysis was purified using a Milli-Q system (Milford, MA, USA). Other solvents from Tianjin Guangfu Chemical Reagent Co. Ltd. (Tianjin, China) were all of analytical grade.

2.3. Instrumentation and chromatographic conditions

A Waters 2695 Alliance HPLC system with Waters 2998 PDA detector was used. The analysis was performed on Scienhome Kromasil C_{18} column (4.6 mm × 250 mm, 5 μm) at a column temperature of 30 °C, using (A) acetonitrile and (B) 0.1% phosphatic acid + 0.1% triethylamine as mobile phase with the gradient elution procedure show in Table 1. The flow rate was 1.0 ml/min and the detection wavelength was 205 nm. The injection volume was 10 μl and the alkaloids derivatives were well separated in chromatographic conditions above.

2.4. Preparation of reference standard

The stock reference standard solution was prepared by dissolving an appropriate amount of each reference compound in acetonitrile. Concentrations of stock reference standard solutions for five compounds were (M1) 9α-hydroxymatrine 1, 038 $\mu g\,ml^{-1}$, (M2) matrine 994 $\mu g\,ml^{-1}$, (M3) sophoridine 760 $\mu g\,ml^{-1}$, (M4) oxymatrine 2, 677 $\mu g\,ml^{-1}$ and (M5) alopecurin A 1, 088 $\mu g\,ml^{-1}$. These solutions were respectively stored in a refrigerator at 4 °C. Then before LC injection, accurately take 6 μl 9α-hydroxymatrine, 10 μl matrine, 675 μl sophoriding, 351 μl oxymatrine and 2.6 μl alopecurin A were placed in a 2 ml flask with stopper, diluted with acetonitrile to make sure the volume reached 2 ml as the mixed reference standard solution.

Table 1
Time program of the gradient elution.

Time (min)	Flow (ml/min)	Acetonitrile (%)	0.1% phosphatic acid + 0.1% triethylamine (%)
0	1	1	99
10	1	1	99
25	1	8	92
40	1	20	80
50	1	22	78
53	1	95	5
60	1	95	5