Application Note

Traditional Medicines, Dietary Supplements, Botanicals

Analysis of Polar Compounds in Plant Material

Quantitation of Stachydrine in Chinese Motherwort (*Leonurus japonicus*) by HILIC

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Abstract

The alkaloid stachydrine is the primary active ingredient in Chinese Motherwort (*Leonurus japonicus*), one of the 50 fundamental herbs used in Traditional Chinese Medicine (TCM). The highly polar nature of stachydrine means it is largely unretained by reversed-phase chromatography, and the coelution of numerous other polar compounds in the plant matrix makes the analysis of stachydrine very challenging.

Hydrophilic Interaction Chromatography (HILIC) is a simple, powerful solution for the analysis of polar compounds. The Agilent InfinityLab Poroshell 120 HILIC-Z column can separate and quantitate stachydrine directly from plant extract. The column combines an advanced zwitterionic phase chemistry, and superficially porous particles provide excellent peak shape and resolution while operating at low backpressures.

Introduction

Separation of stachydrine from *Leonurus japonicus* was achieved using the Agilent InfinityLab Poroshell 120, 2.7 µm HILIC-Z. An evaporative light scattering detector (ELSD) was used for detection because of the weak UV signal of stachydrine. Both isocratic and gradient methods were developed.

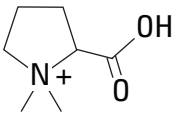

Figure 1. Stachydrine structure.

Experimental

Reagents and Chemicals

All reagents were HPLC grade or higher. HPLC grade acetonitrile was bought from J. T. Baker (Center Valley, PA, U.S.A.). Water was purified using an EMD Millipore Milli-Q Integral System (Darmstadt, Germany.) Ammonium acetate was purchased from were from Sigma-Aldrich (St. Louis, MO, USA). Stachydrine hydrochloride and *L. japonicus* powder was provided by Shanghai Nature Standard (Shanghai, China).

Equipment and Materials

- Agilent InfinityLab fittings
  - **Column front:** Quick Connect fitting (p/n 5067-5965)
  - **Column back:** Quick Turn fitting (p/n 5067-5966)
- Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 µm pore size (p/n 5190-5265)
- Agilent Vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716)
- Agilent bonded screw cap, PTFE/red silicone septa (p/n 5190–7024)
- Agilent vial insert, 250 µL, deactivated glass with polymer feet (p/n 5181–8872)
- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, 3 ports, 1 vent valve (p/n 5043-1219)
- Eppendorf pipettes and repeater
- Sonicator (VWR, Radnor, PA, USA)
- Vortexer and multitube vortexers (VWR, Radnor, PA, USA)

Instrumentation

- Agilent 1260 Infinity II binary pump (G7112B)
- Agilent 1260 Infinity II vialsampler (G7129C)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A)
- Agilent 1290 Infinity II ELSD (G7102A)
- Ultralow dispersion kit (5067-5189)
- Agilent OpenLAB software

Sample Preparation

The stachydrine hydrochloride standards were dissolved in 70 % ethanol to a concentration of 0.5 mg/mL, and injected with no further sample preparation.

Plant extract was prepared as follows:

1. Weigh 1 g of dried *L. japonicus* powder.
2. Add 25 mL of 70 % ethanol in a conical flask, and weigh the total vessel.
3. Reflux the solution for 2 hours, and weigh again after cooling down.
4. Add 70 % ethanol to replace lost weight.
5. Shake well, and filter the extract through a 0.2 µm PTFE membrane syringe filter (p/n 5190-5265).
6. If necessary, dilute to the desired concentration with acetonitrile.

Mobile Phase Preparation

Ammonium formate was weighed and diluted to 10 mM concentration in water. To prevent degradation and microbial growth, buffers were prepared 1 L at a time, and replaced regularly.

Instrument Conditions

Parameter	Value
HPLC	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm (p/n 685775-924)
Mobile phase A	10 mM ammonium acetate in water
Mobile phase B	Acetonitrile
Flow rate	0.30 mL/min
Column temperature	30 °C
Injection volume	2 µL
ELSD	
Nebulizer temperature	40 °C
Evaporator temperature	40 °C
Gas flow rate	1.6 SLM
Data rate	40 Hz

Results

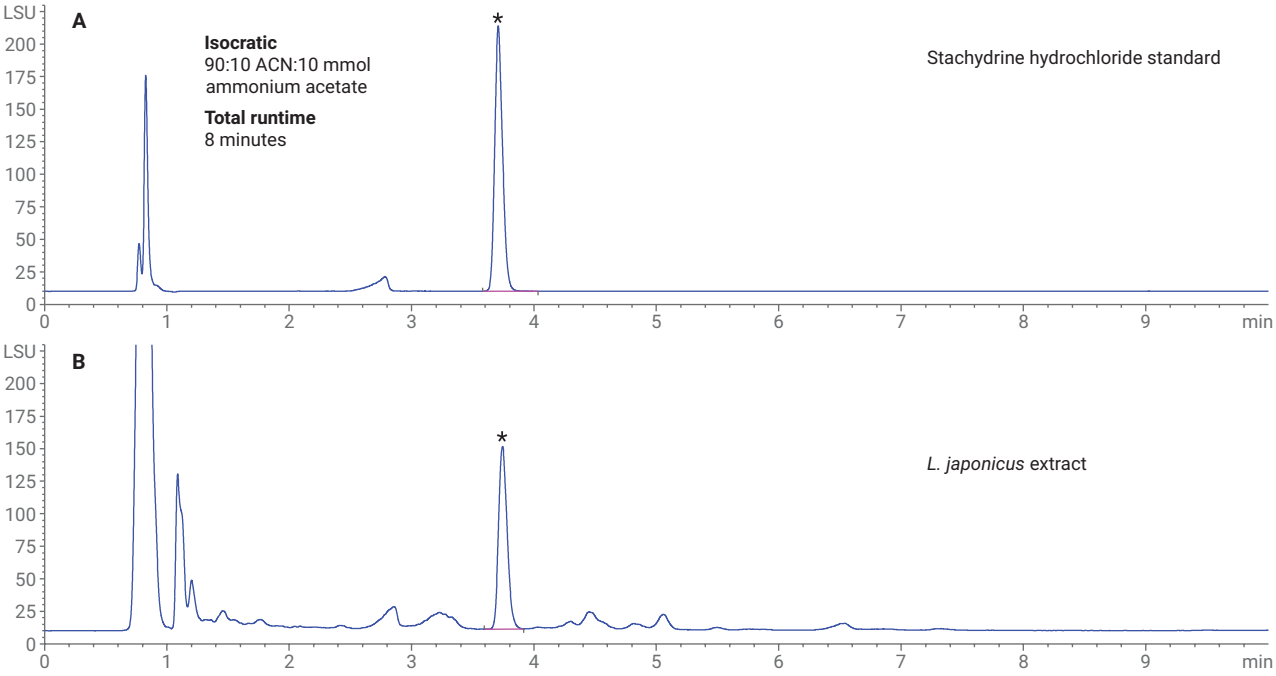

Figure 2. Comparison between plant sample (B) and standard (A) under optimized isocratic conditions.

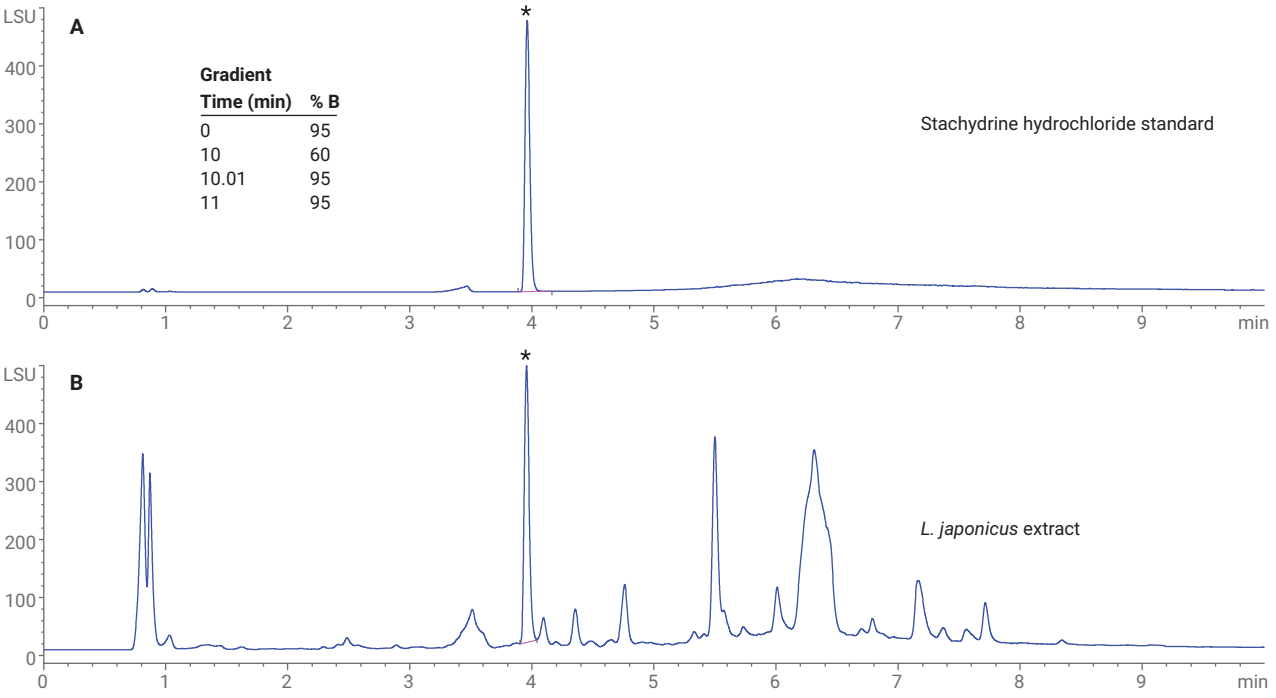

Figure 3. Comparison between plant sample (B) and standard (A) under optimized gradient conditions.

Conclusions

Stachydrine was successfully separated from an extract of *L. japonicus*. Both isocratic and gradient methods were developed, which fully resolved stachydrine from the polar and nonpolar compounds that were also present in the extract.

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Printed in the USA, April 10, 2018
5991-8617EN

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