Analysis of Stachydrine in *Leonurus japonicus* Using an Agilent ZORBAX RRHD HILIC Plus Column with LC/ELSD and LC/MS/MS

Application Note

Traditional Chinese Medicine

Author

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Abstract

Hydrophilic interaction chromatography (HILIC) was used for the analysis of the polar compound stachydrine from a traditional Chinese medicine. Two isocratic methods were developed using an Agilent ZORBAX Rapid Resolution High Definition (RRHD) HILIC Plus LC column with Agilent evaporative light scattering detection and Agilent 6410 Triple Quadrupole LC/MS. Both methods used acetonitrile/ammonium acetate as the mobile phase, and an appropriate organic composition was optimized individually for the different detectors. Both methods were suitable for the quantitative analysis of stachydrine, but the LC/MS/MS method provided higher sensitivity with low level stachydrine detection in complex samples.

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Introduction

Stachydrine is a major constituent of the traditional Chinese medicine from *Leonurus japonicas* Hoult, which is used to promote blood circulation and dispel blood stasis. The structure of stachydrine is shown in Figure 1. It is a strong polar compound with a quaternary-N, which makes it very difficult to retain on a reversed phase column.

Recently, the HPLC separation technique of hydrophilic interaction chromatography (HILIC) has become popular for polar compound analysis. The retention mechanism of the HILIC mode is unique, because the aqueous phase is used as the strong elution solvent. Due to the weak UV absorption of the compound, both evaporative light scattering and MS detectors were employed.

Experimental

The sample was treated as follows to extract stachydrine for analysis [1]. Weigh 1 g of the dried *Leonurus japonicus* powder, add to 20 mL of 70% ethanol, and extract using sonication for 1 hour. Filter the extract with a 0.45 µm regenerated cellulose membrane filter (p/n 5064-8221) before injection for HPLC. Dilute the sample with acetonitrile to an appropriate concentration before analysis with LC/MS/MS.

The LC/ELSD method was performed with an Agilent 1290 Infinity LC System, including an Agilent 1290 Infinity Binary Pump (G4220A), Agilent 1290 Infinity Autosampler (G4226A), Agilent 1290 Infinity Thermostatted Column Compartment (TCC) (G1316C), and Agilent 1290 Evaporative Light Scattering Detector (ELSD) (G4218A).

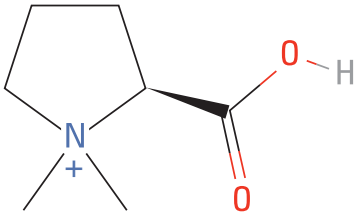

Figure 1. Structure of stachydrine.

LC/ELSD conditions

Column:	Agilent ZORBAX RRHD HILIC Plus, 2.1 × 100 mm, 1.8 µm (p/n: 959758-901)
Mobile phase:	80% Acetonitrile/20% 10 mM ammonium acetate
Flow rate:	0.5 mL/min
Temperature:	30 °C
Sample:	2 µL injection of 0.5 mg/mL standard solution in acetonitrile and prepared extract from *Leonurus japonicus*
ELSD:	gain = 7, filter = 3 s, evaporation temperature = 50 °C

The method for LC/MS/MS used the same Agilent 1290 Infinity LC but coupled to an Agilent 6460 Triple Quadrupole LC/MS.

LC/MS/MS conditions

Column:	Agilent ZORBAX RRHD HILIC Plus, 2.1 × 100 mm, 1.8 µm (p/n: 959758-901)
Mobile phase:	70% Acetonitrile/30% 10 mM ammonium acetate
Flow rate:	0.5 mL/min
Temperature:	30 °C
Sample:	1-µL injection of 10 µg/L standard solution in acetonitrile and prepared extract from Leonurus japonicus diluted with acetonitrile by 1/50,000

MS source parameters

Gas temperatue:	300 °C
Gas flow:	5 L/min
Vaporizer:	350 °C
Nebulizer:	45 psi
Positive capillary:	3,500 V
Sheath gas temperature:	250 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V

ESI acquisition parameters and transitions

Compound	Precursor ion	Product ion	Fragmentor	Collision energy	Polarity
Stachydrine	144	84.1	125	25	Positive
	144	58.1	125	25	Positive

Results and Discussion

The polar compound stachydrine was well retained on the HILIC column with a value of K′ over 5 (Figure 2). Due to the complex components in the sample, the target compounds are usually difficult to separate. However, in this example, an interfering compound was easily coeluted with stachydrine. The mobile phase composition was optimized to achieve the ideal separation (Figure 2). The overlay of standard and sample is shown in Figure 3. The peak of stachydrine in the sample was confirmed using retention time consistency with the standard.

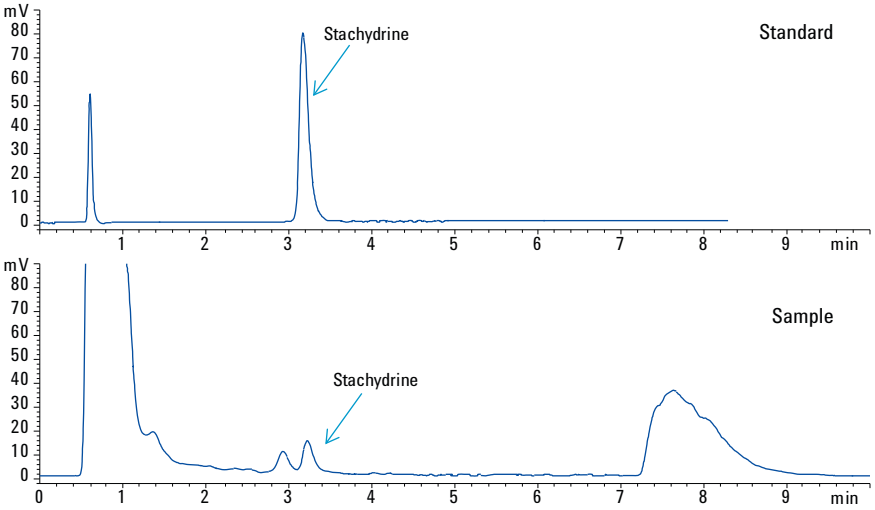

Figure 2. Stachydrine standard and extract from *Leonurus japonicus* analyzed on an Agilent ZORBAX RRHD HILIC Plus, 2.1 × 100 mm, 1.8 µm column with evaporative light scattering detection.

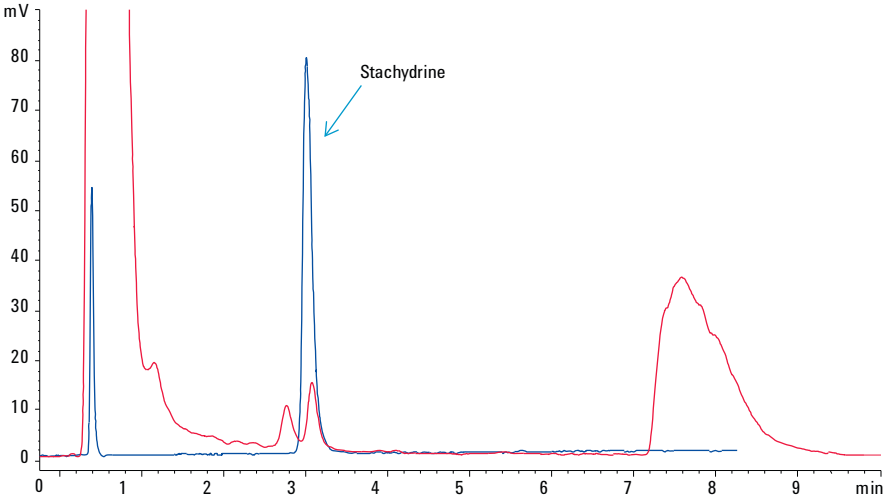

Figure 3. Overlay of stachydrine standard and extract from *Leonurus japonicus*, analyzed on an Agilent ZORBAX RRHD HILIC Plus, 2.1 × 100 mm, 1.8 µm column with evaporative light scattering detection.

The mobile phase for HILIC separations usually involves high organic and volatile additives, such as ammonium acetate and formic acid. High organic phases have higher volatility than traditional reversed phase liquid chromatography (RPLC) mobile phases, making HILIC well suited for applications with mass spectrometers [2]. Previous work demonstrated that the HILIC mode allowed more sensitive MS detection, due to more efficient spraying and desolvation in the ESI-MS source, as a result of the volatile mobile phase used.

Using LC/MS/MS, a product ion from stachydrine was monitored during the separation. The product ion selected is unique to stachydrine, which makes it easy to be chromatographed. Figure 4 shows multiple reaction monitor (MRM) chromatograms of standard and sample; only one peak was found. LC/MS/MS is, therefore, suitable for quantitative analysis of stachydrine in complex samples. The sub-2 µm Agilent ZORBAX RRHD column used with LC/MS/MS provided a narrow peak, which helped to enhance sensitivity.

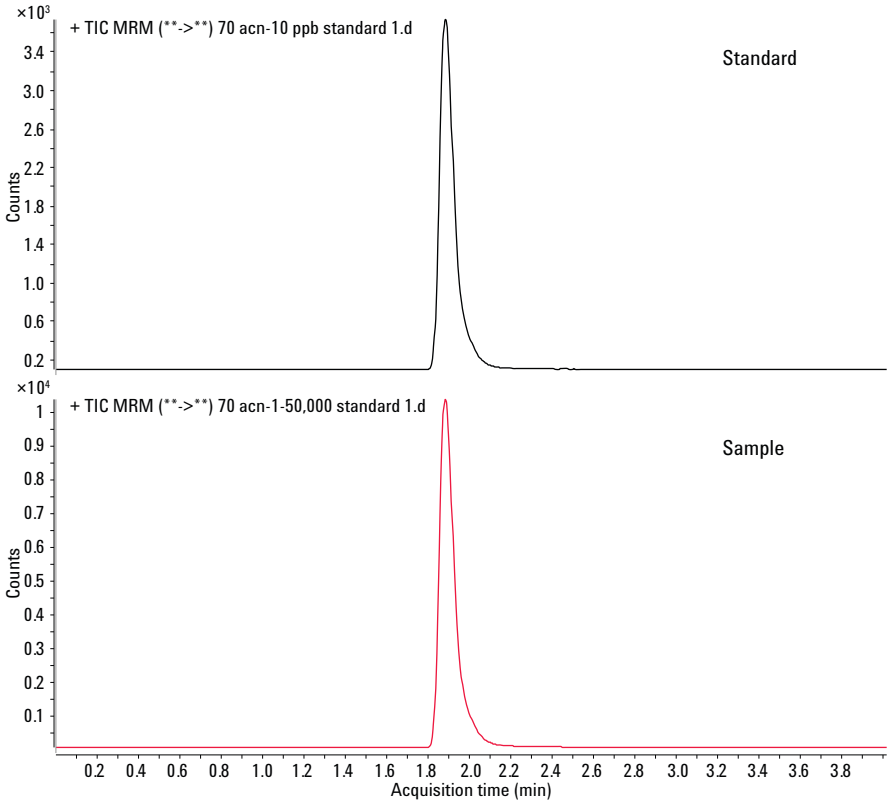

Figure 4. MRM chromatograms of stachydrine standard and extract from *Leonurus japonicus* analyzed on an Agilent ZORBAX RRHD HILIC Plus, 2.1 × 100 mm, 1.8 µm column with LC/MS/MS.

Conclusion

The polar compound stachydrine was well retained on the Agilent ZORBAX RRHD HILIC Plus column and was easily separated in the sample for quantitative analysis. An LC/ELSD method was suitable for the routine analysis of stachydrine, but an LC/MS/MS method provided higher sensitivity and permitted the detection of low levels of stachydrine in complex samples.

References

1. Leonuri Herba. *China Pharmacopoeia*, Part 1, 272 (2010).
2. Anne E. Mack. "Comparing HILIC and RPLC of Morphine Using Agilent ZORBAX RRHD columns with UHPLC/MS", Application Note, Agilent Technologies, Inc., Publication No. 5991-0245EN (2012).

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