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Agilent Technologies

# Application Note SI-01239

# High Sensitivity Analysis of Betaine in Chinese Lycium by HPLC with ELSD

## Stephen Bullock

Polymer Laboratories, now a part of Varian, Inc.

#### Introduction

Chinese lycium (wolfberry - *Lycium barbarum* L. and *L. chinense* P. Mill.) is a traditional Chinese medicine that contains a high proportion of polysaccharides, vitamins B and C and 18 amino acids, as well as being a rich source of carotenoids. The fruit has been used for thousands of years and is believed to boost the immune system and enhance vitality, as well as improving eyesight and arthritis. Chinese lycium also contains betaine (trimethylglycine), a methyl donor with similar chemical functions as choline. Betaine's primary benefit is to maintain proper liver function. However, it is also thought to help in reducing blood levels of homocysteine, a product believed to promote atherosclerosis and osteoporosis.

The increasing use of traditional medicines in western society raises the question of their safety, efficacy, mode of action and regulation. For that reason, as with other natural dietary supplements, reliable and accurate analytical techniques are required to ensure traditional medicines are manufactured to the highest purity and quality.

Betaine is a very polar compound that is not retained on traditional C18 columns. In addition, its detection using UV-vis detectors is poor due to its weak UV chromophore, which limits the compound's sensitivity and the ability to run gradient elution on account of the need to analyze at short UV wavelengths. An improvement in sensitivity for betaine is achieved by using the Varian evaporative light scattering detector. In addition to an increase in sensitivity, this detector also gives a more stable, drift-free baseline that improves quantitation precision.

#### Instrumentation

Separation of betaine was achieved with a highly polar zwitterionic HILIC column that functions with a hydrophilic partitioning mechanism. It is a good choice for separating polar and hydrophilic compounds, such as amino acids and carbohydrates that usually have little or no retention in RPLC.

Column: HILIC 5  $\mu$ m, 250 x 4.6 mm Detection: Varian ELSD (neb=40 °C, evap=30 °C, gas=1.6 SLM)

#### Materials and Reagents

Eluent: 25 % 10 mM ammonium acetate, pH 6 : 75 % methanol

# Sample Preparation

Sample: Chinese lycium

#### Conditions

Flow Rate: 1.0 mL/min Injection Volume: 20 µL

#### **Results and Discussion**

The figure shows lycium extracted in water with betaine as a reference.



Figure 1. The presence of betaine revealed by HPLC with Varian evaporative light scattering detection.

## Conclusion

Evaporative light scattering detection is the preferred technique for the analysis of compounds that possess a weak or no UV chromophore, such as betaine. ELSD is capable of detecting any compound that is less volatile than the mobile phase, and is not dependent on the optical properties of the analyte. The combination of HILIC chromatography and the Varian evaporative light scattering detector, operating at low temperature, provides a simple, fast and accurate method for separating and quantifying betaine in complex matrices, such as Chinese herbal medicines.

These data represent typical results. For further information, contact your local Varian Sales Office.

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Application Note SI-01239 / TB1074

Varian, Inc. www.varianinc.com North America: 800.926.3000 – 925.939.2400 Europe: *The Netherlands*: 31.118.67.1000 Asia Pacific: *Australia*: 613.9560.7133 Latin America: *Brazil*: 55.11.3238.0400

