Single Run Analysis of St John's Wort Tablets by HPLC with ELSD

Stephen Bullock
Polymer Laboratories, now a part of Varian, Inc.

Introduction

St John's Wort *(Hypericum perforatum L.)* has been used extensively in Europe for the relief of mild neuralgia and to help reduce the effects of depression, mild anxiety and nervous tension. Although St John's Wort has been the focus of a number of studies, doubt remains as to its mode of action. The herb contains a complex mixture of chemicals, including hypericin and hyperforin, which are believed to be the two main active ingredients in St John's Wort. Hyperforin, in particular, has been noted as a potent uptake inhibitor of the neurotransmitters serotonin (5-HT), norepinephrine, dopamine and GABA, which are believed to be responsible for feelings of anxiety, depression and related conditions.

The US FDA classifies St John's Wort as a dietary supplement and, as a result, the herbal product can be sold without requiring studies on dosage, safety or effectiveness. However, work has shown that St John's Wort can interact with certain drugs, and these interactions can be dangerous depending on the dose of the herb. Therefore, the need to monitor and characterize herbal formulations is paramount, because the strength and quality of St John's Wort products are often unpredictable. Products can differ in content not only from brand to brand, but also from batch to batch.

While HPLC has proved an ideal method of separating the complex components within St John's Wort, detection methods such as fluorescence[1] are too specific to reveal all of the components within the formulation. UV[2] detection is less specific but the optical differences between compounds within St John's Wort require multiple wavelength analysis, which requires multiple chromatographic runs.

A better alternative for the detection of St John's Wort by HPLC is to use the Varian evaporative light scattering (ELS) detector, which is universal and not dependent on the optical properties of the compound. Consequently, the instrument detects all of the components within St John's Wort in a single chromatographic run, if the separation has been optimized to separate the individual compounds within the formulation.

The advantanges of the Varian ELS detector are highlighted in the isolation and quantification of hypercin and hyperforin in different herbal formulations.

Instrumentation

Column: C18 5 µm, 150 x 4.6 mm
Detection: Varian ELSD (neb=30 °C, evap=50 °C, gas=1.6 SLM)

Materials and Reagents

Eluent A: 0.1 % Ammonium formate, adjusted to pH 2.5
Eluent B: Acetonitrile

Sample Preparation

Sample: St John's Wort tablets

Conditions

Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Gradient: 50-95 % B in 10 min, hold 5 min

Results and Discussion

The chromatographic profile varies at different evaporator temperatures due to the loss of semi-volatile components within the St John's Wort formulation. For the analysis of the active ingredients, hypericin and hyperforin, 50 °C is the optimum temperature. However, some of the minor peaks show a reduction in response (Figure 1).

The Varian ELS detector can be used to compare the composition of St John's Wort tablets from different manufacturers, as shown in Figure 2.

These chromatograms show the differences in composition between standardized St John's Wort tablets from different manufacturers. The ratio of hypericin to hyperforin varies between the tablets, as well as the number and concentration of minor peaks. This shows how the Varian ELS detector can provide a fast, cost effective method for characterizing St John's Wort extract.

Peak Identification
1. Hypericin
2. Hyperforin
3. Adhyperforin

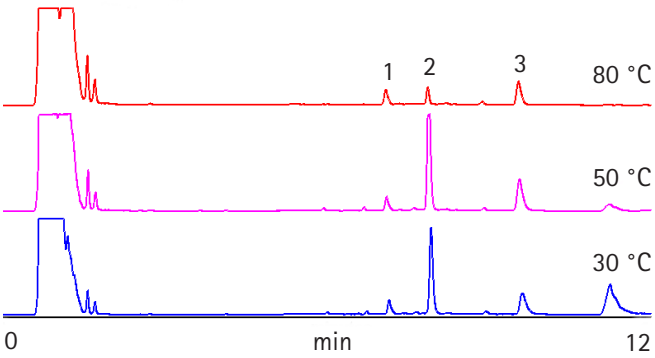

Figure 1. Chromatographic profile varies at different evaporator temperatures and some of the minor peaks show a reduction in response.

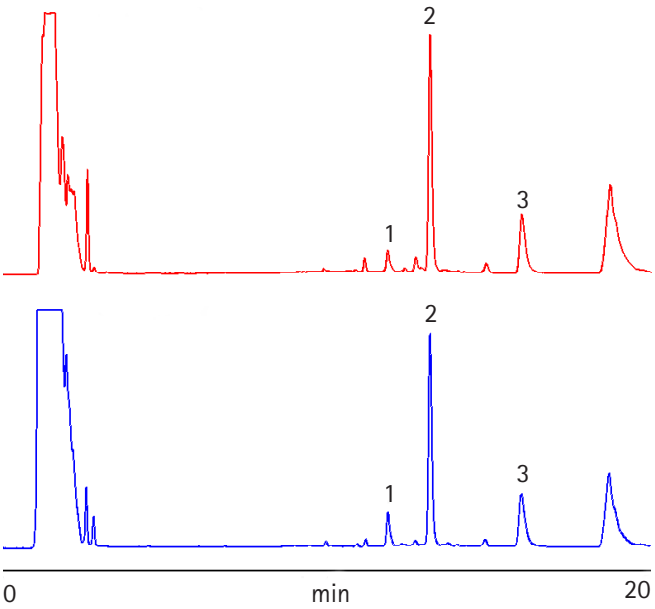

Figure 2. Differences in composition of St John's Wort tablets from two different manufacturers revealed by Varian ELSD.

Conclusion

ELSD is capable of detecting any compound that is less volatile than the mobile phase. The Varian ELS detector can evaporate highly aqueous eluents at 25 °C, facilitating the detection of semi-volatile compounds. For complex mixtures, such as St John's Wort, which contain compounds of differing volatilities, operating the Varian ELS detector at low temperature offers several advantages over standard detction methods.

References

[1]Draves, A. and Walker, S. 2000. Determination of hypericin and pseudohypericin in pharmaceutical preparations by HPLC with fluorescence detection. J. Chromatogr. B, 749: 57-66

[2]Ang, C.Y. et al. 2002. Determination of St John's Wort components in dietary supplements and functional foods by liquid chromatography. J. AOAC Int., 85: 1360-1369

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Application Note SI-01228 / TB1063