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Application Note SI-01236

Accurate Analysis of Triterpene Glycosides in Black Cohosh by HPLC with ELSD

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Introduction

The Varian evaporative light scattering detector is universal and its response is not dependent on the optical properties of the compound. Consequently, its good discriminating power and sensitivity is well suited to compounds such as triterpene glycosides that possess weak or no UV chromophores.

Triterpene glycosides (saponins) are molecules that have a four- or five-ring planar-base containing 30 carbon atoms (aglycone) with various attachments of sugar molecules such as glucose, galactose, glucuronic acid or xylose. The great complexity of triterpene glycoside structures arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these sugar moieties. The isolation, analysis and structural determination of triterpene glycosides demands accurate and sophisticated techniques due to their unique chemical nature. The task of isolating these compounds from plant material is further complicated by the presence of many closely related substances in the plant tissue and by the fact that most of the saponins lack a chromophore.

HPLC is typically used as part of the purification process to isolate and identify triterpene glycosides in plant material, in order to achieve high purity extracts. Once the saponins have been purified, analytical techniques such as MS, NMR and infrared spectroscopy are used to elucidate their structure.

Triterpene glycosides occur in a number of plant species, such as black cohosh root (*Cimicifuga racemosa* (L.) Nutt.), which is taken as a dietary supplement in the belief that it relieves symptoms of the menopause and hot flushes. The primary active constituents of black cohosh root are the triterpene glycosides actein, 27 deoxyactein and cimifugoside, although biologically active substances including alkaloids, flavonoids and tannins are also thought to contribute to the herb's potency.

Instrumentation

Column: C18 5 μ m, 150 x 4.6 mm

Detection: Varian ELSD (neb=30 °C, evap=50 °C, gas=1.4 SLM);

UV-vis, 230 nm

Materials and Reagents

Eluent A: 0.1 % Formic acid in water

Eluent B: ACN

Sample Preparation

Sample: Black cohosh tablet

Conditions

Flow Rate: 1.0 mL/min

Injection Volume: 20 μ L

Gradient: 30-40 % B in 30 min, 40-60 % B in 30 min, 60-30 % B in 10 min

Results and Discussion

Evaporative light scattering detection is a better alternative than UV detection for the analysis of triterpene glycosides because these compounds possess weak or no UV chromophores, thus limiting their sensitivity and the ability to run gradient elution on account of the need to analyze at short UV wavelengths (eg 230 nm).

This is highlighted in Figure 1, which shows the analysis of a black cohosh sample by UV and ELSD. To ensure the potency of black cohosh, commercially available extracts from manufacturer to manufacturer are standardized to contain 2.5 % triterpene glycosides.

However, the determination of two commercial, standardized tablets by ELSD, as shown in Figure 2, reveals differences in the composition and concentration of the active components.

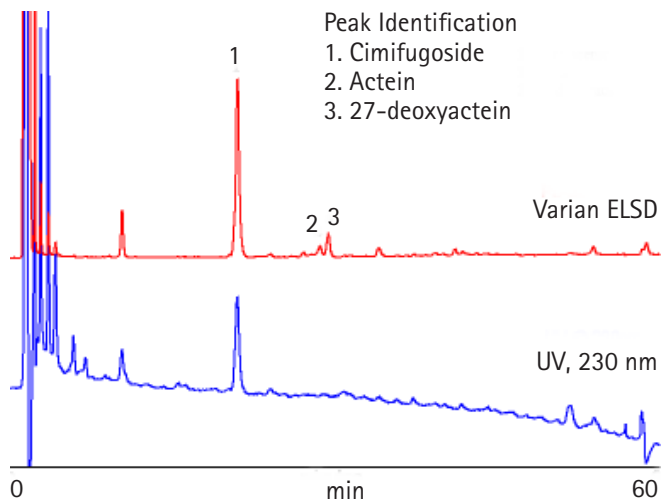


Figure 1. Separation of black cohosh tablets showing the superiority of the Varian evaporative light scattering detector over UV detection.

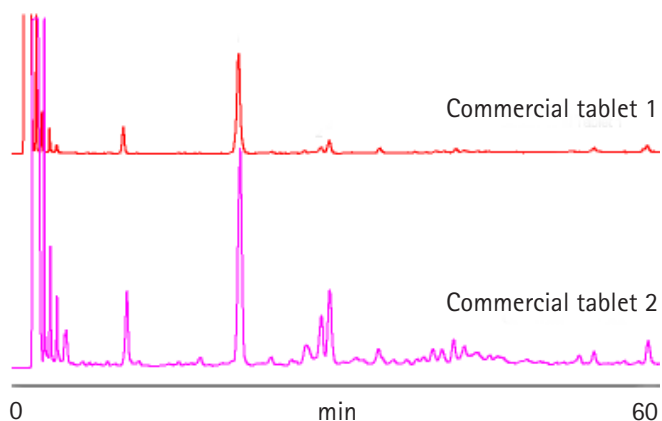


Figure 2. Highlighting the differences in active components in black cohosh tablets from different manufacturers using the Varian evaporative light scattering detector.

Conclusion

There is pressure to establish industry-wide standards to help ensure that dietary supplements are manufactured consistently with regard to their identity, purity, quality, strength and composition. Reliable and accurate analytical techniques are required to meet these objectives. HPLC with Varian evaporative light scattering detection are ideal for the elucidation of active ingredients in dietary supplements, providing important quality control data for industry, regulators and consumers.

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

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