

# TB1071: HPLC Analysis of Triterpene Glycosides in Black Cohosh Formulations using the PL-ELS 2100

## Keywords

HPLC, ELSD, Black Cohosh, Nutraceutical, Acetonitrile, Water

Triterpene glycosides (saponins) are a particular type of molecule 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, which can be taken as a dietary supplement to relieve the symptoms of the menopause and hot flushes. The primary active constituents of the black cohosh root are the triterpene glycosides of actein, 27 deoxyactein and cimifugoside, although biologically active substances including alkaloids, flavonoids and tannins are also thought to contribute to the herb's potency.

Currently, there is a strong initiative to establish industry-wide standards to help ensure that dietary supplements are manufactured consistently with regard to their identity, purity, quality, strength and composition. As a result, reliable and accurate analytical techniques are required to carry out this initiative.

Evaporative light scattering detection (ELSD) is a more favorable 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 230nm). 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 analysis of two commercial, standardized tablets by ELSD, as shown in Figure 2, show differences in their composition and concentration of the active components.

Sample: Black Cohosh Tablet  
Column: Inertsil C18 5µm, 150x4.6mm  
Eluent A: 0.1% Formic Acid in Water  
Eluent B: ACN  
Gradient: 30-40% B in 30 mins, 40-60% B in 30 mins, 60-30% B in 10 mins  
Flow Rate: 1.0ml/min  
Inj Vol: 20µl  
Detectors: PL-ELS 2100 (neb=30°C, evap=50°C, gas=1.4 SLM)  
UV-Vis @ 230nm

Figure 1 - Separation of Black Cohosh Tablet using UV Detection at 230nm

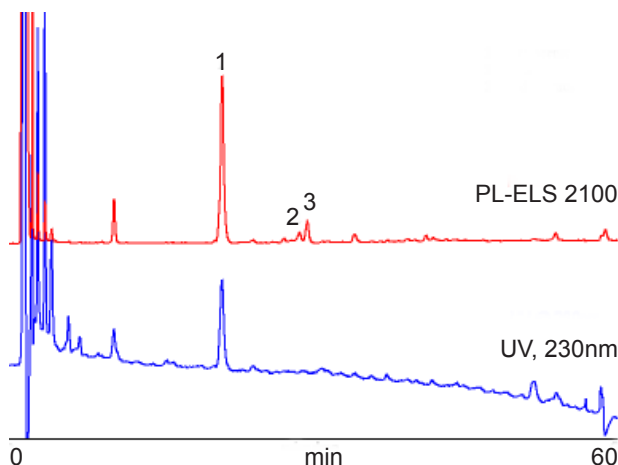
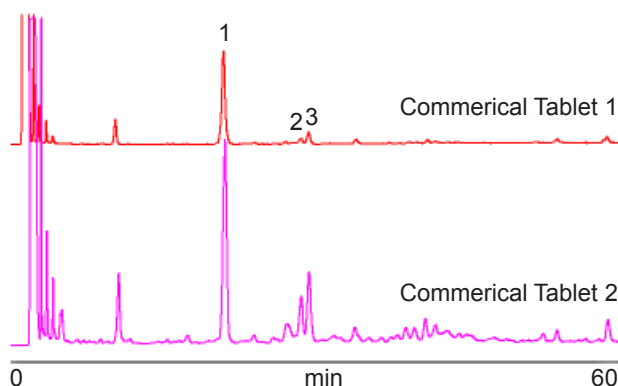


Figure 2 - Separation of Black Cohosh Tablet using ELS Detection



CS946/C/9.06