



**Polymer Laboratories**

# HPLC Analysis of Triterpene Glycosides in Black Cohosh Formulations Using the PLELS 2100 Evaporative Light-Scattering Detector

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Currently, there is a strong initiative to address the need to establish industry-wide standards to help ensure that dietary supplements, such as Black Cohosh, are manufactured consistently as to identity, purity, quality, strength, and composition. As a result, reliable and accurate analytical techniques are required to carry out this initiative.

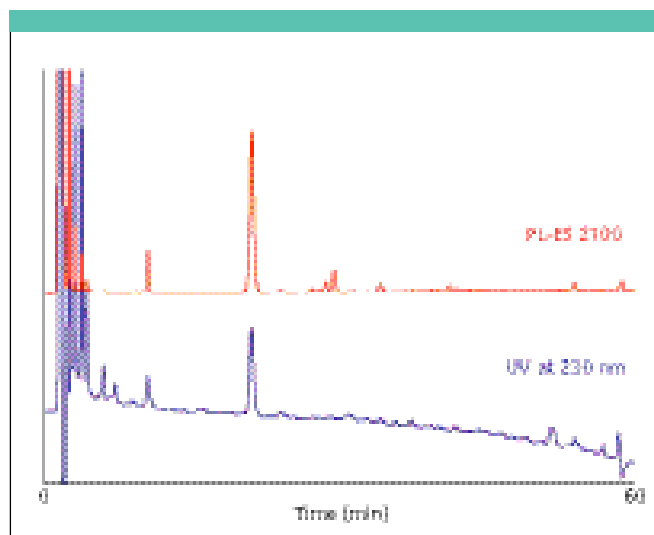
Black Cohosh root can be taken as a dietary supplement to relieve the symptoms of the menopause and hot flashes. Its primary active constituents are the triterpene glycosides, actein, 27-deoxyactein, and cimifugoside, although biologically active substances including alkaloids, flavonoids, and tannins are also thought to contribute to the herb's potency.

Evaporative light-scattering detection is a favourable alternative to UV detection for the analysis of triterpene glycosides, because these compounds possess weak or no UV chromophores, which limits their sensitivity and the ability to run gradient elution on account of the need to analyse at short UV wavelengths (for example, 230 nm). This is highlighted in Figure 1, which shows the analysis of a Black Cohosh sample by UV and ELSD.

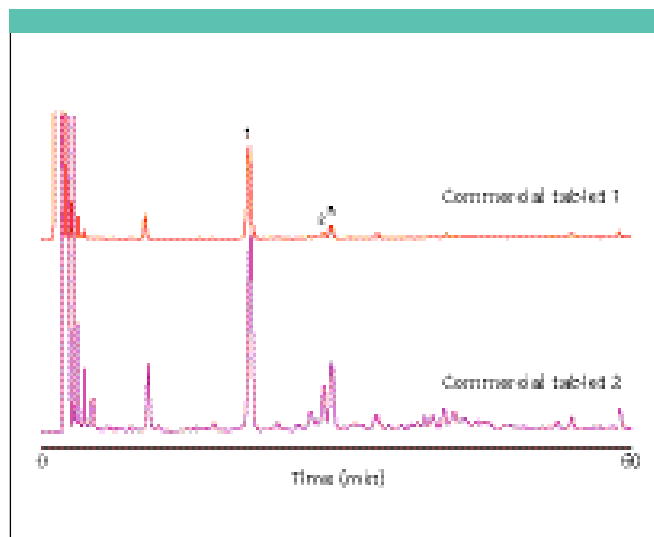
To ensure the potency of Black Cohosh, commercially available extracts from manufacturer to manufacturer are standardized to contain 2.5% triterpene glycosides. However, the analysis of two commercial standardised tablets by ELSD, as shown in figure 2, show differences in their composition and concentration of the active components.

**Table I. Experimental Conditions**

Compounds:	1 Cimicifugoside, 2 Actein, 3 27-deoxyactein
Sample:	Black Cohosh Tablet
Column:	Inertsil C18 5 $\mu$ m 150 $\times$ 4.6 mm
Eluent A:	0.1% Formic Acid in Water
Eluent B:	ACN
Gradient:	30–40% B in 30 min; 40–60% B in 30 min; 60–30% B in 10 min
Flow Rate:	1.0 ml/min
Inj. Volume:	20 $\mu$ l
Detector:	PL-ELS 2100: (neb = 30 $^{\circ}$ C, evap = 50 $^{\circ}$ C, gas = 1.4 SLM) UV, 230 nm



**Figure 1:** Separation of Black Cohosh Tablet using UV detection at 230 nm.



**Figure 2:** Separation of Black Cohosh Tablet using ELS detection.

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