Evaluation of Ketone-Containing Terpene Compounds using Evaporative Light Scattering Detection

Application Note PHA0313

Keywords

prepELS™II Detector, Evaporative Light Scattering (ELS), High-Performance Liquid Chromatography (HPLC), terpenes, ketones, thujone, camphor, essential oil constituents, volatile compounds

Introduction

Terpenes are naturally occurring compounds whose derivatives are primary constituents of plant essential oils. Many compounds belonging to this large family have notable pharmacological characteristics, ranging from toxic to beneficial.[1] To better understand the pharmacology and toxicology of terpenes, as well as to safely exploit their beneficial properties, a suitable system for isolating and analyzing these compounds is essential. As the structure of terpenes does not include a chromaphore, absorption in the UV spectral range is insufficient, and evaporative light scattering (ELS) detection technology is required.

This application note describes the chromatographic separation and ELS detection of two bicyclic monoterpenoids – camphor and thujone – which contain ketone functional groups. Camphor (Figure 1A) is widely used as a topical analgesic; however, in large doses, this compound also has dangerous neurological effects.[2] Likewise, thujone (Figure 1B), the biologically active ingredient in absinthe, is a well-known neurotoxin that can lead to seizures in a dose-dependent manner, primarily as a result of its antagonistic activity on $GABA_A$ receptors.[3] Use of the Gilson prepELS™II detector following normal phase HPLC yielded adequate signals for both camphor and thujone. The challenges associated with ELS detection of these volatile analytes are discussed.

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Materials & Methods

Materials

- Samples: Camphor – 230 mg (Figure 1A)
  Thujone – 200 mg (Figure 1B)
- Liquid Handler: Gilson GX-271 with Direct Injection Module (1/16")
- Column: Phenomenex – Luna Silica (2), 21.1 mm x 50 mm, 10 µm, 110 Å
- Pump: Gilson 322 Binary with H2 pump heads
- Detector: Gilson prepELS™II

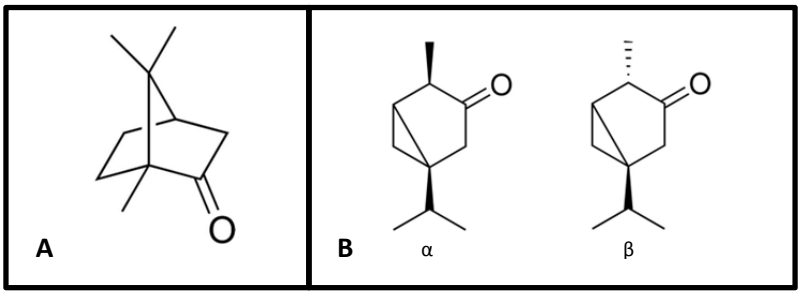

Figure 1. Chemical structures of ketone-containing terpene natural products camphor (A) and α- and β-thujone (B).

Methods

Sample Preparation

Each sample was dissolved in 10 mL of 50:50 Hexane:Isopropyl Alcohol (IPA) to prepare 20-23 mg/mL stock solutions. The stock solution was then further diluted for a working solution of 2 mg/mL.

Normal Phase HPLC Conditions

- Isocratic elution
- Mobile Phase: IPA:Hexane
- Injection Volume: 500 µL (1000 µL partial loop injection)
- Flow rate: 20 mL/min

ELS Detection

- Spray chamber temperature: 0°C
- Drift tube temperature: 22°C
- Split ratio: 20:1
- Gain: Normal
- Filter: 5

Results

Signals were observed for both compounds using the ELS detector, although thujone gave a stronger signal (Figures 2 and 3) than camphor (Figures 4 and 5). In both cases, the signals were markedly above the background signal from the higher IPA concentration (Figures 4 and 5).

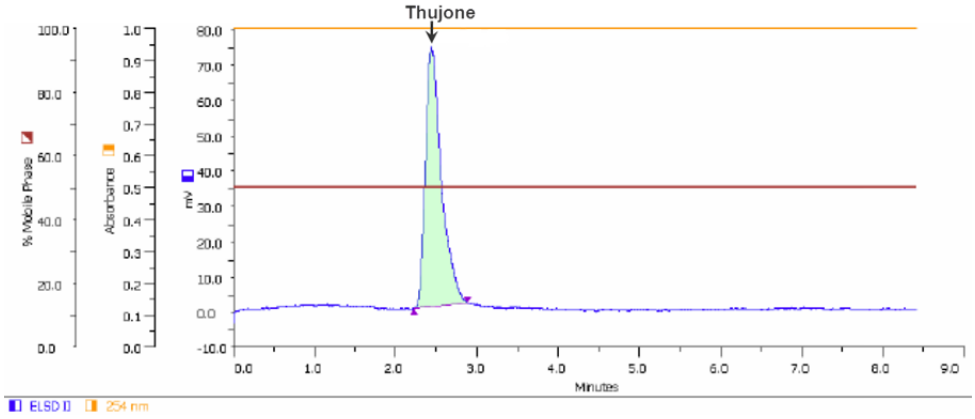

Figure 2. Chromatogram showing the thujone signal when 10 mg was sent to the ELS detector and a 50:50 IPA:Hexane mobile phase was used.

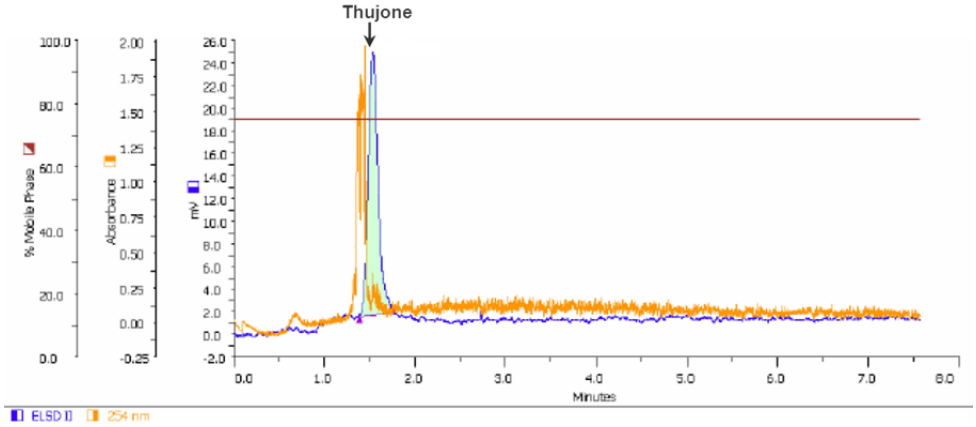

Figure 3. Chromatogram showing the thujone signal when 1 mg was sent to the ELS detector and a 75:25 IPA:Hexane mobile phase was used, indicating the optimal conditions for the sample working solution (2 mg/mL).

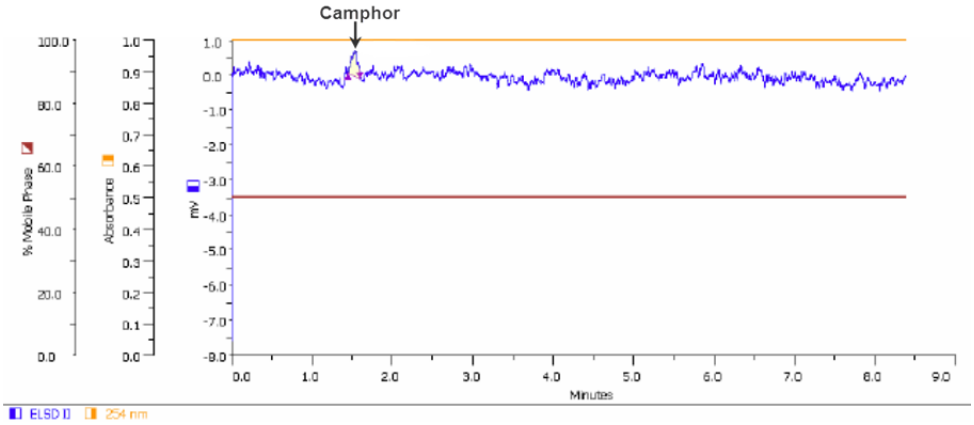

Figure 4. Chromatogram showing the camphor signal when 10 mg was sent to the detector and a 50:50 IPA:Hexane mobile phase was used.

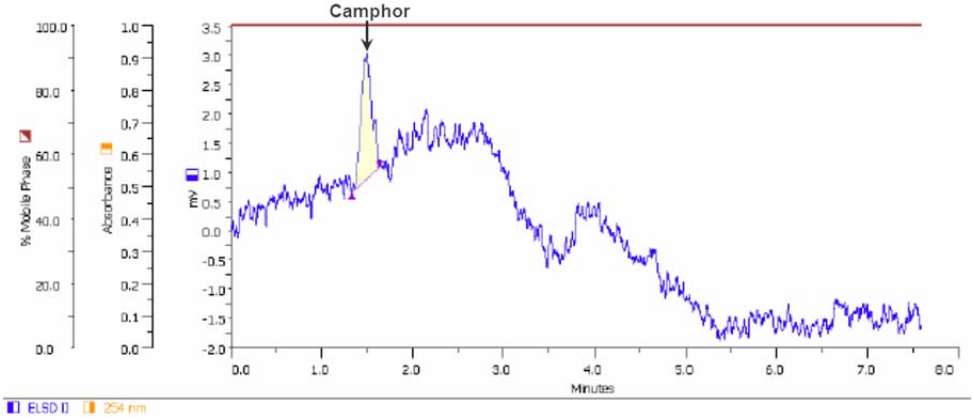

Figure 5. Chromatogram showing the camphor signal when 10 mg was sent to the detector and a 75:25 IPA:Hexane mobile phase was used, indicating the optimal conditions for the sample stock solution (20 mg/mL).

Summary

Using evaporative light scattering detection, both thujone and camphor gave acceptable responses, which improved as the percentage of IPA in the mobile phase increased. The resulting signals were relatively low for the large sample load; however, ELS detectors are not ideally suited for highly volatile compounds. Given the volatility of terpenes, it is likely that these compounds had begun to evaporate within the prepELS™II detector drift tube and before reaching the detector's laser. Increasing the IPA content in the mobile phase did provide an optimal set of conditions for ELS detection confirmation of both thujone and camphor. Thus, ELS detection constitutes a good method for analyzing the presence of sample components with limited UV absorbance. In the future, signals could be further improved by replacing the IPA component of the mobile phase with a more volatile substance, such as diethyl ether.

References

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