

# Rapid, high throughput quantitation of thujone in absinthe by UHPLC-MS-MS

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## 1. Introduction

Absinthe is an anise-flavored, distilled alcoholic beverage that was vilified and banned in many countries before the first World War. It was targeted because the beverage was alleged to have hallucinogenic effects; with the compound thujone thought to be the culprit. By the 1990's, the advent of new food safety laws and a renewed interest in absinthe led to a lifting of the ban in many countries, but in

some areas legal limits on thujone levels remain. In order to comply with food and beverage safety laws, a rapid and accurate measurement of thujone in absinthe is required. To accomplish this, a UHPLC-MS-MS method meeting these requirements of selectivity, sensitivity, and speed was developed.

## 2. Materials and Method

A Shimadzu Nexera UHPLC with an LCMS-8040 triple quadrupole mass spectrometer was utilized for this analysis. An authentic standard of  $\alpha,\beta$ -thujone was obtained from PhytoLab (Vestenbergsgreuth, Germany). Fisher LC/MS Grade Water and Methanol were used for

chromatography. Sigma-Aldrich Absolute Ethanol and Fluka Formic Acid were also used. Three brands of absinthe (Grüne Fee, La Sorcière and Grande Absente) were purchased for testing and Smirnoff Triple Distilled Vodka was used as a control.

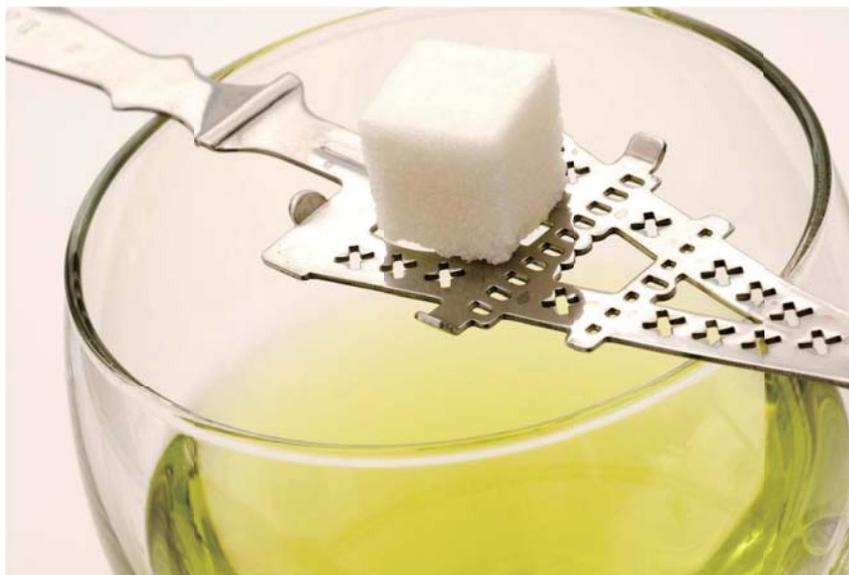
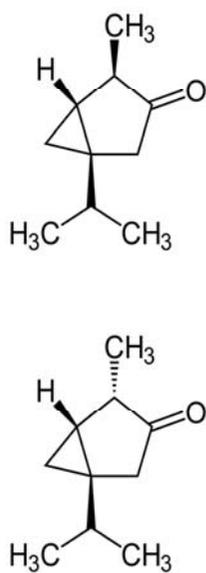


Fig. 1 Chemical Structures of  $\alpha$ -Thujone (Upper Left) and  $\beta$ -Thujone (Lower Left) as well as an example of a typical absinthe cocktail

### Sample Preparation

A standard curve was generated by spiking  $\alpha,\beta$ -thujone into vodka to a known final concentration. Absinthe was diluted 20 times using 40% ethanol in water, or spiked

with 5000 ng/mL  $\alpha,\beta$ -thujone and then diluted 20 times with 40% ethanol in water.

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## Chromatography

Instrument	: Shimadzu Nexera UHPLC system
Column	: Thermo Scientific Hypersil GOLD (2.1 mm ID×100 mm; 1.9 µm)
Mobile Phase A	: LC/MS Grade Water with 0.1% Formic Acid
Mobile Phase B	: LC/MS Grade Methanol (All mobile phases were purchased from Fisher Scientific)
Gradient Program	: 40% B (0-0.5 min) gradient to 95% B (0.5-5.0 min) -95% B (5.0 – 6.0 min) -40% B (6.1-10 min)
Flow Rate	: 0.3 mL/min
Column Temperature	: 40°C
Injection Volume	: 10 µL

## Mass Spectrometry

Instrument	: Shimadzu LCMS-8040 Triple Quadrupole Mass Spectrometer
Ionization	: ESI
Polarity	: Positive
Scan Mode	: MRM
MRM Transitions	: Quantifier (135→107) Reference Ions (135→90.95; 135→76.95)

## 3. Results

Analysis was conducted on three separate days. Commercially available absinthes were diluted 20-fold with 40% ethanol and the concentration of  $\alpha,\beta$ -thujone was calculated from a calibration curve of 5-10,000 ng/mL. The level determined for both unspiked and spiked

absinthe agreed within 10% over the three runs. The calculated concentrations of the spiked absinthe (5 µg/mL spike) also fell within 10% of the expected concentration. The measured carryover from ULOQ to a blank was less than 10% of the LLOQ.

Due to the presence of isobars in the commercially available absinthes, two reference ions were chosen to ensure proper identification in the sample matrix.

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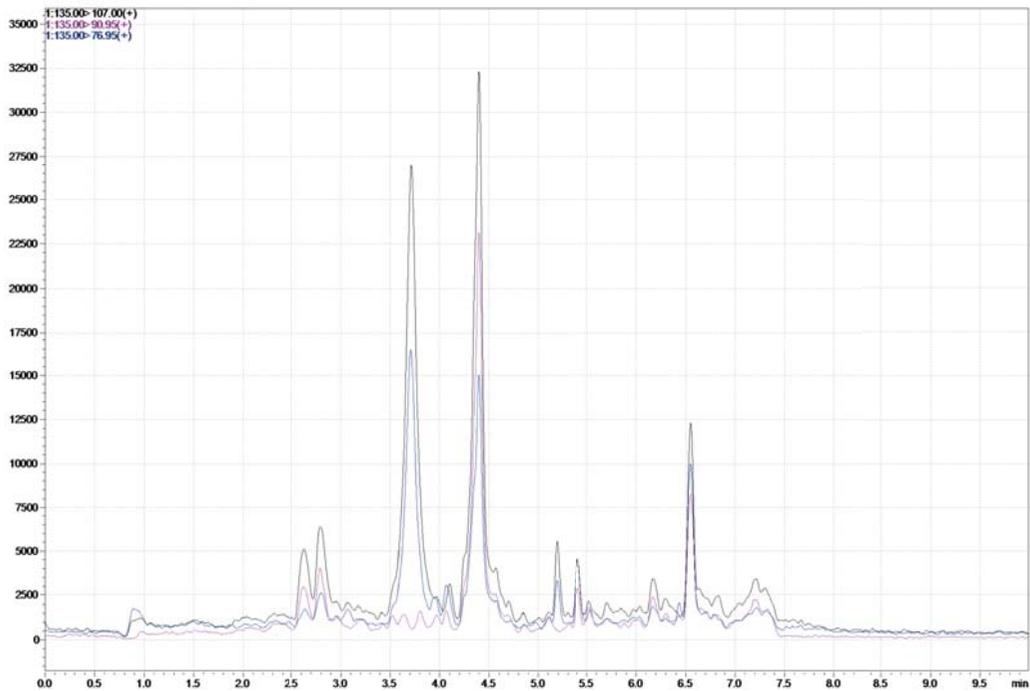
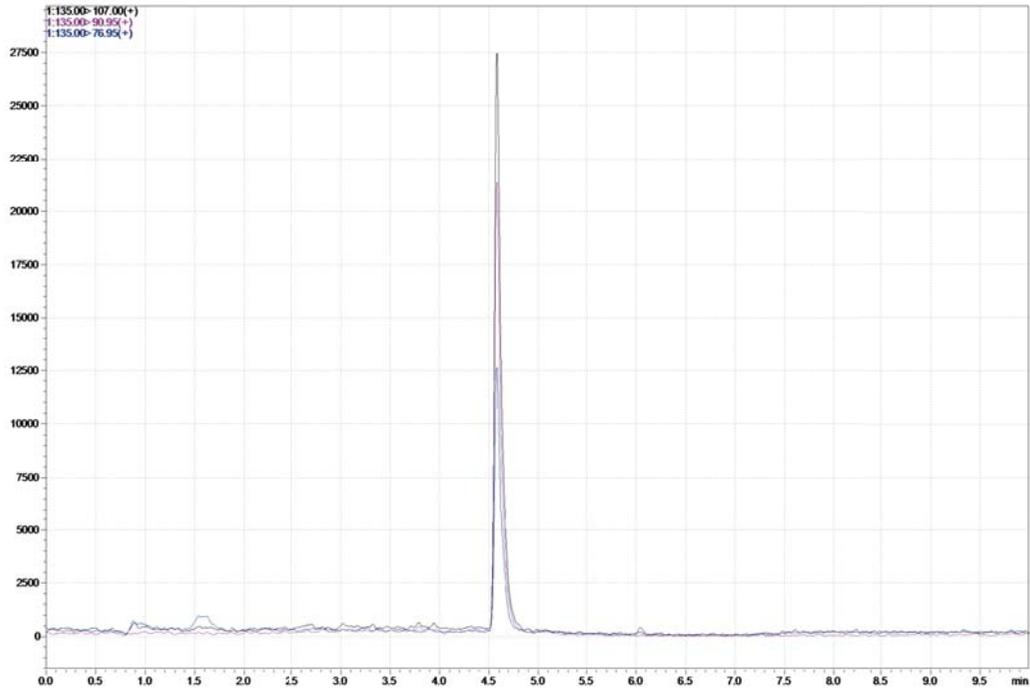


Fig. 2 Typical chromatograms for a standard (top) and an absinthe sample (bottom), illustrating why 2 reference ions are monitored.

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Table 1 Average calculated concentration of  $\alpha,\beta$ -thujone in three commercially available absinthe brands analyzed on three separate days

Absinthe Brand	Average Calculated Concentration $\alpha,\beta$ -thujone ( $\mu\text{g/mL}$ )	% CV
Grüne Fee-uns spiked	2.38	2.4%
Grüne Fee-5 $\mu\text{g/mL}$ spike	6.96	5.1%
La Socière-uns spiked	3.99	2.6%
La Socière-5 $\mu\text{g/mL}$ spike	8.82	3.6%
Grande Absente-uns spiked	0.558	8.5%
Grande Absente-5 $\mu\text{g/mL}$ spike	5.20	6.3%

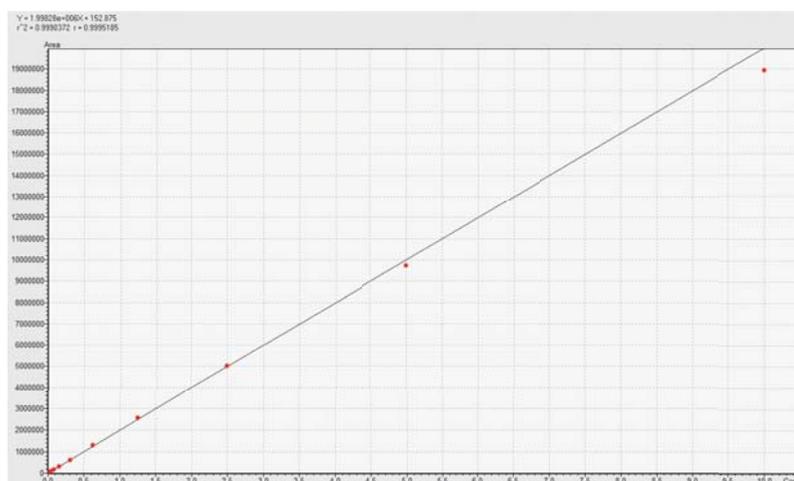


Fig. 3 Calibration Curve for  $\alpha,\beta$ -thujone spiked into vodka at concentrations ranging from 5-10,000 ng/mL

## 4. Conclusions

A rapid dilute-and-shoot method enabling the analysis of  $\alpha,$   $\beta$ -thujone in commercially available absinthes has been developed.

This method could be utilized to ensure that absinthes sold in the US conform with the FDA guideline of containing less than 10 ppm thujone.

## 5. References

Abass K. Et al. Xenobiotica (2011) 41(2):101-111