



B8 The Development of a Polymerase Chain Reaction (PCR) High-Resolution Melt (HRM) Assay for Wormwood (*Artemisia Absinthium*)

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Learning Overview: After attending this presentation, attendees will understand how to develop DNA primers to detect legal high species including artemisia absinthium (wormwood) and the identification of this and other legal high species using a real-time polymerase chain reaction (PCR) high resolution melt (HRM) assay.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by expanding on existing assays that detect legal highs. The focus will be on the development of primers for artemisia absinthium and multiplexing the results with other legal high species to provide a fast and cost-effective way to detect for plant material.

The medicinal properties of wormwood have been celebrated for thousands of years mainly as a way to rid the body of intestinal ringworms but have also been used to treat malaria and repel bugs. During the 18th and 19th Centuries, wormwood was distilled to create the spirit absinthe. Wormwood contains alpha-thujone and beta-thujone, which can cause hallucinations when consumed in concentrated amounts.^{1,2} Although there are herbal medicinal properties of wormwood, doctors and researchers noticed that there was an increase in neurological disorders and epileptic-like episodes that users experienced. Originally believed to stimulate cannabinoid receptors, research has since determined that a high concentration of thujone can block the gamma-aminobutyric acid (GABA) receptors in the brain. Several countries enacted a ban on the sale of absinthe, including the United States in the early 20th Century. In 2007, the United States lifted the ban on absinthe, which has quickly become popular in the bar scene, even prompting a National Absinthe Day. In 2018, *The Baltimore Sun* reported that a local bar has a Happy Hour dedicated to discounted absinthe once a week.³

Even though the sale of absinthe is legal, the Food and Drug Administration monitors the levels of thujone, and limits 10 parts per million of thujone in absinthe. Although thujone is monitored by the Food and Drug Administration, the Drug Enforcement Agency's Controlled Substances Act does not regulate materials that contain the compound. Thujone extracted from wormwood can be ingested in a tea, oil, or smoked as a pellet to experience a "legal high." Obtaining wormwood seeds are legal and can easily be purchased online and in stores throughout the United States.

DNA primers were developed based on the genomic information available, and the assay used real-time polymerase chain reaction high resolution melt (PCR HRM). Radiant Green was used to detect trace biological material from *A. absinthium* and evaluate primer specificity; the melt temperature of the primers was 84.75 ± 0.068 °C. A serial dilution of *A. absinthium* proved that the assay was sensitive to DNA as low as 0.01 ng, and the size of the amplicon was estimated using an agarose gel. The specificity of the assay developed, as well as the results of duplexing this assay against another plant used to obtain a legal high, *Datura stramonium* (jimson weed). In a duplex using LC Green Plus dye, the *A. absinthium* melt temperature is 87.96 °C and the *D. stramonium* melt temperature is 76.20 °C.

Reference(s):

1. Hoeld K. M, Sirisoma N. S, Ikeda T, Narahashi T, Casida J. E. "α- Thujone (the active component of absinthe): γ- Aminobutyric acid type A receptor modulation and metabolic detoxification," *PNAS* 9, no. 8 (April 2000): 3826- 3831, <https://doi.org/10.1073/pnas.070042397>.
2. "Effects of Wormwood And Absinthe: Thujone & Hallucinations." Absinthiana, accessed July 18th, 2018, <https://www.absintheaccessories.org/effects-of-absinthe-thujone-and-wormwood.html>.
3. Wesley Case, "Absinthe is having a moment in Baltimore. Here's what to know about the mythical green spirit," *The Baltimore Sun*, March 5th, 2018, <http://www.baltimoresun.com/entertainment/music/midnight-sun-blog/bs-fe-absinthe-day-rye-20180305-story.html>.

Wormwood, Real-Time PCR, High Resolution Melt