



ANALYSIS OF ABSINTHE BY SOLID PHASE EXTRACTION AND LC-M/MS: A SIMPLE TEST FOR THUJONE CONCENTRATION

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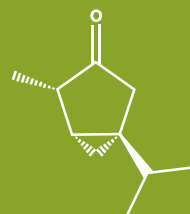
INTRODUCTION:

Absinthe, once a beverage subject to a ban in the United States is now finding a new audience. It is euphemistically called the "Green Fairy" after the color of the liquid. The main constituent of the beverage is Anethole but some samples of the drink may contain α/β Thujone, which is thought to give rise to its hallucinogenic properties. The drink is also known to contain upto 50 % alcohol by volume. The focus of this presentation is to demonstrate a simple and efficient procedure for the analysis of Absinthe which will determine the presence (or absence) of α/β thujone as well as the anethole. This should be of great assistance to those actively testing such drinks and offer an alternative method of analysis.

AIMS:

The focus of this work was to demonstrate the use of both an alternative solid phase procedure, employing a long chain sorbent (C30), and LC-MS/MS in the analysis of an alternative matrix. I.E. Absinthe. This work offers analysts a different route for thujone testing.

α/β Thujone



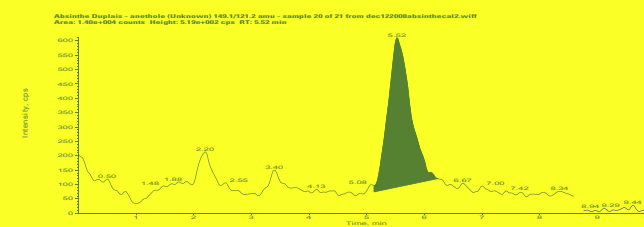
Anethole



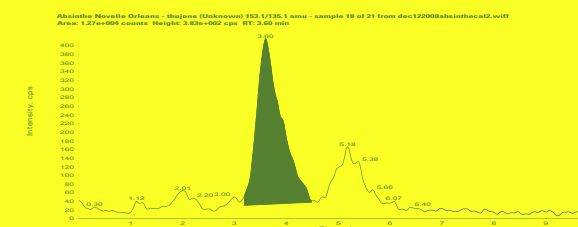
METHODS:

Calibrators and controls were used for extracting anethole and α/β thujone from 50 PC aqueous alcohol samples (0.2 mL). In this method, menthol (IS) was added before dilution with DI water (5 mL) to the calibrators, controls and genuine samples of absinthe(10) and applied to solid phase extraction columns (3 mL containing 25 mg of CEC30 sorbent (UCT, LLC Part #: CEC301(25)3). The columns were conditioned with methanol and DI water (1 mL, respectively). After washing with 0.4 mL of DI water the samples were eluted with 4 x 50 μ L of methanol and the eluates transferred directly to auto sample vials for analysis by LC-MS/MS in positive electrospray (MRM) mode. The quantifying transitions were: Menthol (156.1 \rightarrow 83.1), Thujone (153.1 \rightarrow 135.1), and Anethole (149.1 \rightarrow 121.2), respectively. From the analysis of the calibrators and controls, r² value > 0.995, recoveries > 90% and a limit of detection of 10 μ g/ mL, respectively, were achieved. The method was found to be linear up to 1000 μ g. Tandem mass spectrometry was performed on API 2000 MS/MS, whilst chromatography was carried out with a 50 x 2.1mm (3 μ m) Phenyl column (Selectra[®] UCT, LLC Part #: SLPHY50ID21) for separation of the analytes. A mobile phase consisting of acetonitrile (with 0.1% formic acid) and DI water (with 0.1% formic acid) was used in gradient mode in the analysis.

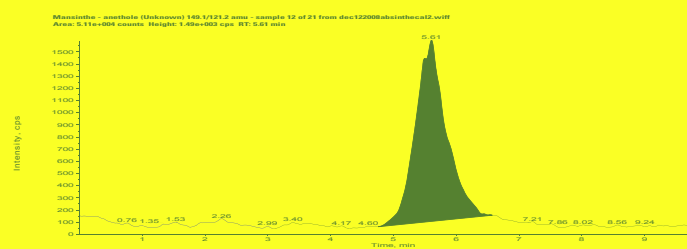
SAMPLE OF AUTHENTIC ABSINTHE NOUVELLE NEW ORLEANS (ANETHOLE)



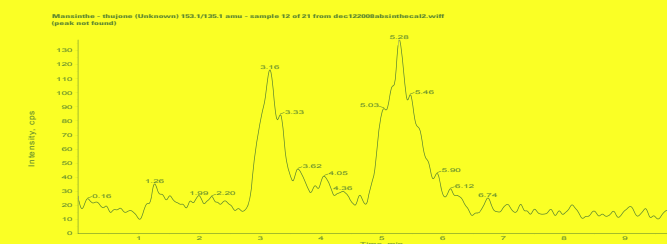
SAMPLE OF AUTHENTIC ABSINTHE NOUVELLE NEW ORLEANS (THUJONE)



SAMPLE OF AUTHENTIC ABSINTHE MANSINTHE (ANETHOLE)



SAMPLE OF AUTHENTIC ABSINTHE MANSINTHE (THUJONE)



RESULTS:

Data is presented in this poster, along with LC-MS/MS chromatograms, showing those samples of genuine absinthe containing thujone and those containing only anethole. The range of α/β thujone concentrations was found to be from 0 to over 750 μ g/ mL (348 to 769 μ g/ mL (n=5)) whilst all of the samples contained anethole at various levels (2480 to 7720 μ g/ mL (n=10)) far exceeding that of the thujone.

Absinthe Type	Alcohol ABV	Anethole/ μ g per mL	Thujone/ μ g per mL
Century	50	1510	769
Absente	55	7220	348
Lucid	62	5705	Not Detected
Grande Absente	65	5590	357
Absinthe MJT	68	7605	Not Detected
Mansinthe	66	7540	Not Detected
Absinthe Hellfrich	68	3645	Not Detected
Absinthe Prototype 37	68	4965	352
Absinthe Nouvelle New Orleans	68	4215	467
Absinthe Duplais	62	2480	Not Detected



CONCLUSIONS:

This simple and efficient procedure for the analysis of Absinthe (especially Thujone) is the first method using both SPE and LC-MS/MS. The use of this procedure should assist those analysts involved in testing Absinthe beverages for the presence of thujone. This method should also help analysts testing pre-ban samples of Absinthe for authenticity, as the presence or absence of the thujone should assist in the establishment of legitimacy of the sample.

