Development and Validation of a Quantitative Method for Identification of Synthetic Cannabinoids

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Abstract

Products such as Spice, K2 Summit, and Serenity Now are labeled as "Not for Human Consumption", but are a part of a growing trend as a way to get around the legality of using marijuana to obtain a high. These products are laced with chemicals known as synthetic cannabinoids, named for their ability to mimic the psychoactive ingredient and therefore effects of marijuana. With the recent scheduling of five of these compounds along with their analogs, there has been a scrambling to find a method that accurately and quickly determines the type and quantity of synthetic cannabinoid in each seized product. This presentation will seek to explain the method used to obtain accurate results using a GC-MS and show how the synthetic cannabinoids in such products can be quantitated using this method.

Introduction

- As early as 2006, these synthetic cannabinoids have been found in herbal incense sold at head shops marketed as a legal substitute for marijuana. [2]
- Bans on the chemicals were placed by the military branches almost immediately, and on March 1, 2011, five of the synthetic cannabinoids were placed on the DEA scheduling as Schedule 1. [3,4]
- Gas chromatography/mass spectrometry (GCMS) has been previously shown to be a good method for the qualitative analysis of the synthetic cannabinoids.
- Quantitative analysis has been limited due to the limited availability of reference standards. [1]

Due to the increasing abuse of these herbal incense products, this presentation will impact the forensic science community by informing attendees about the new developments in the battle against the use and abuse of synthetic cannabinoids as well as a method that has been developed to quantitate the synthetic cannabinoids in each product tested in a quick and accurate way.

Previously, methods have successfully been developed for the qualitative analysis of synthetic cannabinoids, specifically in the products K2 Summit, Atomic Bomb, Space, Spice Gold, and Spice Diamond. The method described in this presentation will facilitate the attendees in quantifying the amount of synthetic cannabinoid(s) in samples of these products, as well as other brands.

Materials & Methods

- **Standards standard curve concentrations with acetonitrile**
  - **+/- CP-47,497 – C8 homolog:** 10 –100 µg/ml.
  - **JWH-018:** 200 - 4000 µg/ml.
  - **JWH-250:** 100 - 750 µg/ml.
  - **AM 2201:** 100 - 1000 µg/ml.
- **Samples**
  - Approximately 10mg or 50 mg, depending on concentration, of previously ground particles of herbal spice product was diluted in 3.0mL of Methanol.
  - Sample extracted for 20 minutes with vortex.
  - Extract filtered through a 0.45 syringe filter.
  - Extract evaporated to dryness.
  - Residue reconstituted with 1mL of acetonitrile.
- **Internal Standard Procaine:** 0.5mg/ml in all samples and standards.

Instrument Settings

- **Agilent 6890 Network Gas Chromatograph - 5973 Network Mass Spectrometer**
  - Zebron ZB-Drug-1 Column 10 m x 0.18 mm x 0.18 µm
  - Split ratio :50:1
  - Injector temperature: 250 °C
  - Oven initial temperature: 100 °C, hold for 0.50 minutes
  - Ramp rate: 40°C /minute
  - Oven final temperature: 200°C, hold for 7.5 minutes
  - Initial pressure: 5.00 psi
  - First ramp rate: 75 psi/min to 15.00 psi, hold for 6.0 minutes
  - Second ramp rate: 150 psi/min
  - Final pressure: 40.00 psi, hold 0.5 minutes
  - Total run time: 12.5 minutes
- **Agilent 6890 Network Gas Chromatograph - Flame Ionization Detector**
  - Zebron ZB-Drug-1column 10 m x 0.18 mm x 0.18 µm
  - Split ratio: 50:1
  - Injector temperature: 250 °C
  - Oven initial temperature: 100 °C for 0.50 minutes
  - Ramp rate: 40 °C/minute
  - Oven final temperature: 280 °C for 7.50 minutes
    - AM 2201 and Cloud 9 - 9.75 minutes
  - Initial pressure: 5.00 psi
  - First ramp rate: 150 psi/min to 15.00 psi, hold for 6.0 minutes
  - Second ramp rate: 150 psi/min
  - Final pressure: 40.00 psi
  - Total run time: 12.5 minutes

Results

Table 1: Retention Times and Concentrations of the Synthetic Cannabinoid found in each sample using GC-FID and GC-MS.

Sample	Retention time (minutes)		Synthetic Cannabinoid	Concentration (µg/mL)	
	FID	MS		FID	MS
Spice Gold	6.553	5.832	+/- CP 47,497 (C8) homolog	198	157
Spice Diamond	6.552	5.830	+/- CP 47,497 (C8) homolog	181	140
K2 Summit	9.604	8.470	JWH-073	-	-
	10.429	9.062	JWH-018	566	638
Atomic Bomb	10.462	9.091	JWH-018	1165	1158
Serenity Now Red	10.520	9.127	JWH-018	3905	2768
Serenity Now Blue	10.417	9.045	JWH-018	773	665
Space	10.453	9.112	JWH-018	991	749
Flawless	10.414	9.067	JWH-018	378	436
Baked	7.966	7.315	JWH-250	414	421
Cloud 9	12.649	10.559	AM2201	336	306

Table 2: Curve Validation for GC-FID and GC-MS

Standard Concentration	Concentration from Curve		Percent Error	
	FID	MS	FID	MS
JWH-018 600 µg/mL	692 µg/mL	531 µg/mL	15.4%	11.5%
JWH-250 400 µg/mL	341 µg/mL	245 µg/mL	14.8%	38.8%
+/- CP-47,497– C8 homolog 325 µg/mL	321 µg/mL	268 µg/mL	1.4%	17.5%
AM-2201 900 µg/mL	856 µg/mL	810 µg/mL	4.9%	10.0%

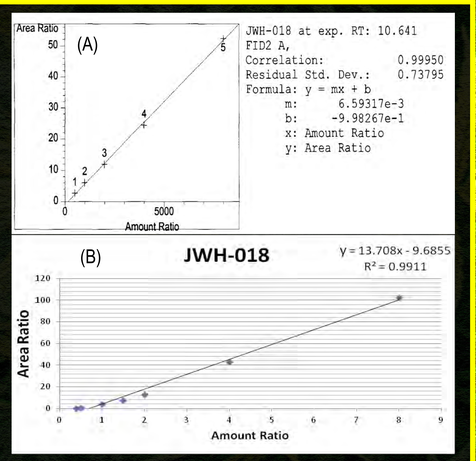

Figure 1: Typical Calibration Curves for (A) GC-FID and (B) GC-MS

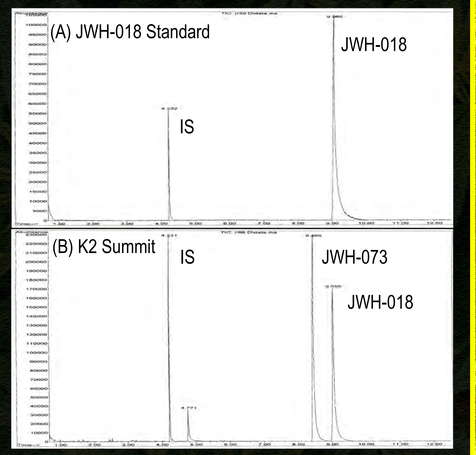

Figure 2: Typical Chromatogram of a (A) standard synthetic cannabinoid and a (B) herbal spice product

Discussion

- Methanol extraction was found to be the most favorable extraction method due to its relative availability, speed, and ability to extract more completely.
- Tetracosane was originally intended to be the internal standard, but the study showed Procaine to give more consistent and reliable results.
- Standards should be made on the same day as the run and should be kept in a freezer at -20°C until use and after use if kept for future analysis. The differences seen in the accuracy of the calibration curves and in the variation of the concentrations of the synthetic cannabinoids in the samples between the GC-FID and the GC-MS measurements are proof of the degradation of the synthetic cannabinoids due to time and heat.

Conclusions

- The findings of this study show that quantification of synthetic cannabinoids in the herbal spice products is able to be done through use of the GC-FID or GC-MS and the described methods.

References

1. Drug profile: Synthetic cannabinoids and 'Spice'. EMCDDA. www.emcdda.europa.eu/publications/drug-profiles/synthetic-cannabinoids (accessed May 3, 2011)
2. Understanding the 'Spice' phenomenon. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). 2009
3. Spice Product. www.erowid.org/chemicals/spice_product/spice_product.shtml (accessed May 3, 2011)
4. U.S Drug Enforcement Administration. (2011, March 1). Chemicals Used in "Spice" and "K2" Type Products Now Under Federal Control and Regulation. Retrieved May 3, 2011, from www.justice.gov/dea/pubs/pressrel/pr030111.html
5. Uchiyama, N., Kikura-Hanajiri, R., Ogata, J., Goda, Y. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. Forensic Science International - 20 May 2010 (Vol. 198, Issue 1, Pages 31-38, DOI: 10.1016/j.forsciint.2010.01.004)

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