APPLICATION NOTE

Liquid Chromatography/Gas Chromatography/Mass Spectrometry

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Analysis of Synthetic Cannabinoids in Seized Drugs by High-Resolution UHPLC/MS and GC/MS

Introduction

The practice of synthesizing novel drugs with slight chemical structure modifications is commonplace for controlled substances. These "designer drugs" are made with the intent of circumventing controlled substance laws, and they present a major challenge to law enforcement laboratories charged with investigating the nature of seized materials. Synthetic cannabinoids represent one of more than twenty classes of designer drugs, under federal control in the United States[1]. Figure 1 shows the chemical structure of Δ9-THC, the principal active component of cannabis, and two representative structures from the group of 23 analyzed in this study.

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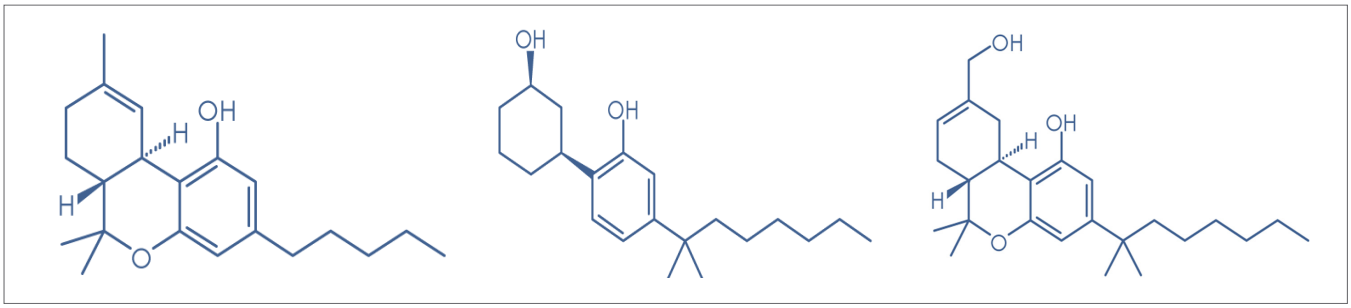

Figure 1. Showing the chemical structures of Δ9-THC, the principal active component of cannabis, and two cannabinoid compounds, designed to mimic the physiological effects of cannabis.

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)[2] sets standards for analysis of seized drugs. Under this guidance, various analytical methodologies are grouped into three categories (designated by SWGDRUG as categories A, B and C) according to technical specificity. Combinations of techniques or hybrids that might be considered include mass spectrometry (MS) (Category A) with a separation technique from category B, such as liquid chromatography (LC) or gas chromatography (GC). However, positive results should be reported only if confirmed by two independent techniques, where hybrid approaches count as a single measurement.[4]

A set of 23 synthetic cannabinoids were analyzed by UHPLC/MS and GC/MS to compare identification abilities. High Resolution (HR) LC/MS offers the advantage of molecular formula determination, while GC/MS provides structurally-significant fragment ions. Chromatographic retention times for the two approaches were compared using TIBCO Spotfire® software[3] to perform principle component analysis (PCA). This analysis demonstrated that retention times were not correlated between the two analytical techniques. This is important when considering regulatory guidance concerning appropriate choices for methodologies applied to the screening and confirmation of seized drug samples.

Methods

Sample Preparation

Synthetic cannabinoid reference standards were purchased from Cayman Chemical (Ann Arbor, MI, USA) and used as received. Lidocaine reference standard was purchased from Sigma-Aldrich® (St. Louis, MO, USA), LC/MS grade water, acetonitrile, methanol and formic acid were purchased from Fisher Scientific™ (Fairlawn, NJ, USA). Samples for GC/MS were prepared from standards or their mixtures at 1 mg/mL or 10 mg/mL in methanol and were diluted with methanol to working concentrations. Samples for UHPLC/MS were prepared in the same fashion, except diluted with injection solvent consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (70:30).

Table 1. Instrumental methodologies

Flexar System: FX-15 UHPLC Pump, Autosampler and Column Oven	
Mobile phase A	Water containing 0.1% formic acid
Mobile phase B	Acetonitrile with 0.1% formic acid
Sample injection	5-µL per injection
Flow rate	300 µL/min.
Column temperature	35 °C
Columns:	
Brownlee SPP C18	2.1 x 150 mm x 2.7 µM, p/n N9308405
Brownlee SPP phenyl-hexyl	2.1 x 150 mm x 2.7 µM, p/n N9308486
Brownlee SPP pentafluorophenyl (PFP)	2.1 x 150 mm x 2.7 µM, p/n N9308470
PerkinElmer Clarus® 680 Gas Chromatograph and Injector	
Carrier gas	Helium
Column	PerkinElmer Elite 5-MS (30 m x 250 µm ID x 0.25 µM film thickness), p/n N9316282
Injection	1-µL splitless
Injector temperature	250 °C
Gradient	80 °C for 2.5 min., 15 °C/min. ramp, hold 320 °C for 6.5 min.
Mass Spectrometry Instrument and Software Parameters	
LC/MS	PerkinElmer AxION® 2 TOF mass spectrometer
	Chromera® v. 4.4.1 software for data processing
	TOF driver 6.1 for instrument control
	Ultraspray™ 2 Dual Probe electrospray source
	Lock mass solution with added Lidocaine
GC/MS	PerkinElmer Clarus SQ8C single quadrupole
	Turbomass V 6.1 software
	Electron impact ionization
PCA Data Analysis	
PerkinElmer TIBCO Spotfire®	

Results

Figure 2 shows the responses from the mixture of 23 synthetic cannabinoids analyzed using LC/MS with three different UHPLC columns and by GC/MS.

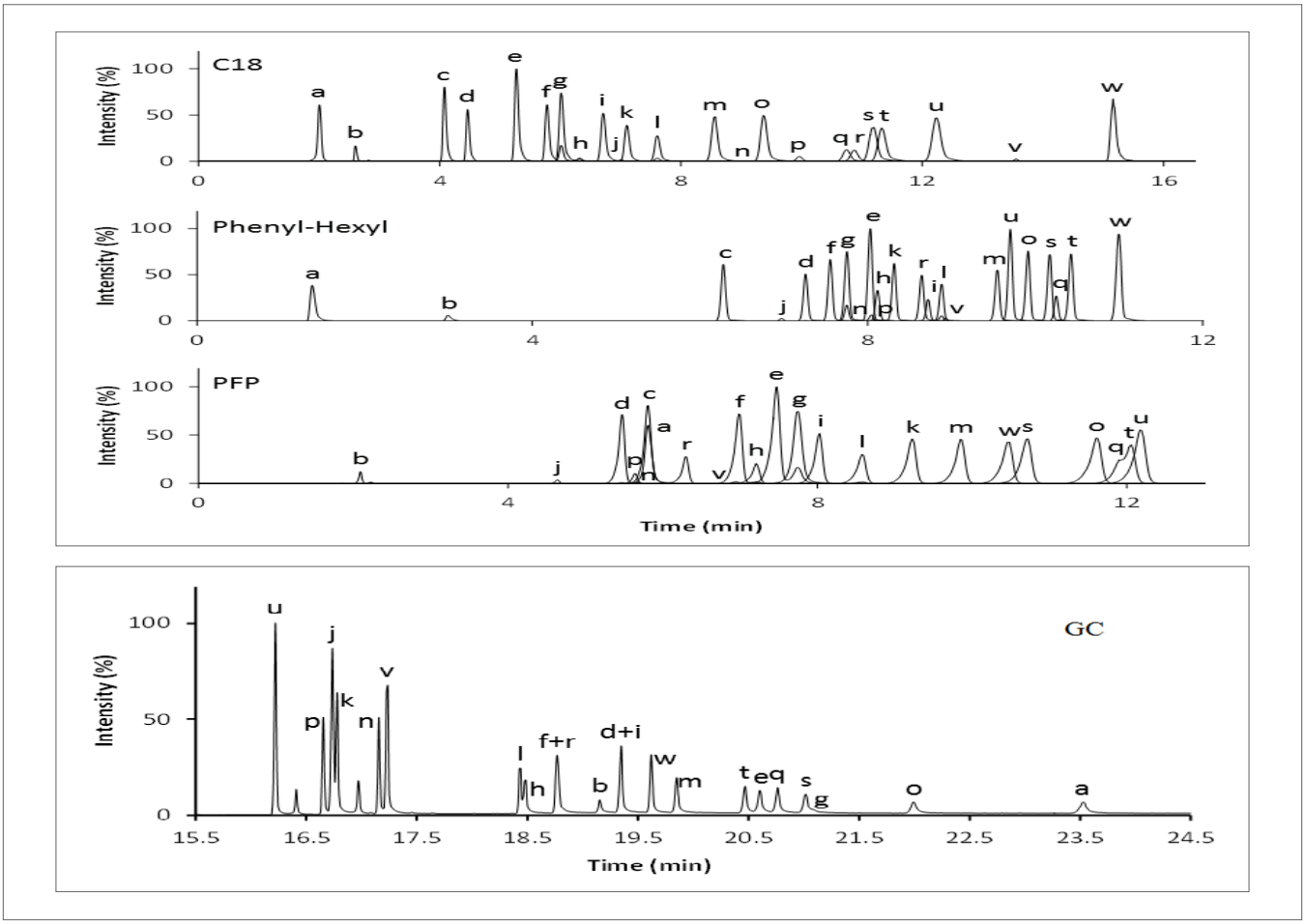

Figure 2. Showing UHPLC/MS (upper three traces) and GC/MS (lowest trace) responses for 23 synthetic controlled cannabinoids.

The LC/MS data shows excellent peak separation on each of the three stationary phases and each is adequate for screening purposes. The use of photodiode array (PDA) detectors has been shown to be applicable to these compounds [4,5]; however, these compounds are rarely found in pure form and consequently the potential of overlapping unexpected signals could lead to ambiguous results. The selectivity of mass spectrometry offers added confidence in the ability to distinguish among structurally similar compounds. In addition, the power of accurate mass measurement using time-of-flight (TOF) mass spectrometry adds another dimension in this regard, coupled with the ability to re-interrogate TOF data sets for information on unexpected and unknown observed species.

The ability of GC and LC separation methodologies to comply with SWGDRUG guidelines, and good laboratory practice, requires a demonstration that the results from the two techniques are non-correlated. One method of making such an assessment is the use of principle component analysis (PCA), which distinguishes differences in data sets by the means of score plots.

Figure 3 shows a score plot comparing the retention times observed with the three SPP UHPLC columns with the GC retention times.

This analysis indicates that the three UHPLC column data sets are significantly correlated, which is expected given their similar separation mechanisms. However, the GC data are highly non-correlated with the UHPLC data, both for the individual columns and as a group of data.

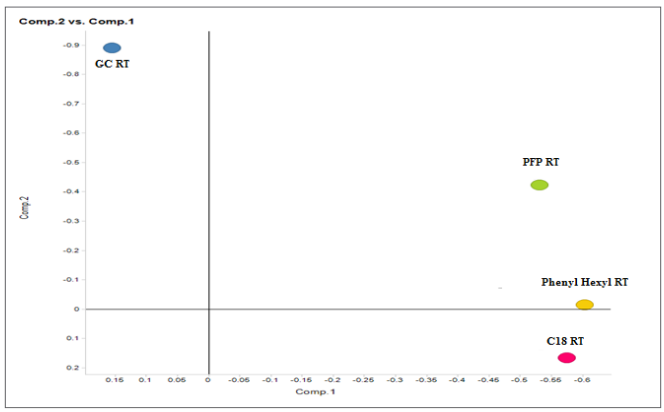

Figure 3. PCA analysis: Score plot comparing retention times from three different UHPLC/MS analyses with GC/MS analysis.

Conclusion

This data indicates that UHPLC/TOF/MS and GC/MS may be used in the analysis of synthetic cannabinoids. PCA analysis indicated a lack of correlation between the retention times, which conforms to SWGDRUG requirements and therefore would allow one technique to be used for preliminary screening, and the other to be used for secondary analysis to confirm any positive results that arise. The LC analysis was faster, while GC offered higher chromatographic resolution, suggesting the use of LC/MS as the preliminary screening technique and the workflow of choice.

Footnote

Drs. Ira Lurie, Ioan Marginean and Walter Rowe, Dept. Forensic Sciences, The George Washington University, Washington DC, originally presented this data at the meeting of the American Association of Forensic Sciences, Orlando, FL, February 2015.

Additional information and analysis can be found in reference 6, which was completed prior to the publication of this note.

References

1. Title 21 United States Code Controlled Substances Act, Part B, Section 812.
2. http://swgdrug.org.
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4. B. K. Logan, L.E. Reinhold, A. Xu, F.X. Diamond Forensic Sci., 57 (2012) 1168-1180.
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6. I. Margininean, W.F. Rowe, I.S. Lurie Forensic Sci. Int. 249 (2015) 83-91

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