Final Summary Overview, NIJ award 2012-DN-BX-K026

Project Title: "Forensic Chemistry of Substituted 1-Alkyl-3-Acylindoles: Isomeric Synthetic Cannabinoids"

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Purpose of Project:

The goal of this work was to establish an analytical framework for the identification of individual substituted indoles to the exclusion of all other possible isomeric and homologous forms of these compounds. This analytical specificity will be accomplished by 1) the chemical synthesis of complete sets of regioisomeric reference compounds for selected substituted indoles, 2) generation of an analytical profile for each compound, 3) chromatographic studies to separate/resolve all regioisomeric cannabinoids having overlapping analytical profiles, and 4) design and validation of confirmation level analytical methods to identify each compound to the exclusion of other regioisomeric forms.

A variety of 1,3-disubstituted indoles have appeared in clandestine samples in recent years based on designer substituents of the aromatic ring at the 3-position of indole and the nature of the alkyl-group at the indole 1-position. There are numerous compounds from this category already available on the clandestine drug market. These compounds can exist in homologous and isomeric forms often sharing the same mass spectrum, the most common method of confirmation of drug identity in forensic drug analysis.

The project has produced a significant amount of fundamental forensic chemical data resolving numerous issues related to regioisomerism in indole-based synthetic cannabinoids. The results of this project have made available information and data sets to improve the forensic

chemistry knowledge base and produce a scientifically skilled forensic expert available to interact at the interface of the legal system and the science of forensic drug chemistry of synthetic cannabinoids. Twelve peer reviewed scientific publications from this project are in print at this time (see Appendix 1).

Design and Methods:

The 1-alkyl-3-acyl indoles were prepared by established synthetic routes using the general methods reported by Huffman et al. These methods involve either coupling substituted acyl chlorides with 1-alkyl-indoles or by acylation of indole, followed by N-alkylation. The acid chlorides for these syntheses can be obtained by treatment of commercially available substituted carboxylic acids with thionyl chloride or oxaloyl chloride. The required alkyl halides are available directly from commercial sources or can be prepared from the corresponding alcohols by treatment with thionyl chloride or phosphorus tribromide.

The 1-alkyl-2-, 4-, 5-, 6- and 7-acyl indoles were prepared by N-alkylation of the commercially available 2-, 4-, 5-, 6-, and 7-indole aldehydes using n-pentyl bromide under basic conditions. The 1-n-pentyl indole aldehydes were then allowed to react with the appropriate arylmagnesium halide to give the corresponding alcohols, which are subsequently oxidized to the ketones to yield the desired regioisomeric 1-alkyl-2-, 4-, 5-, 6- and 7-acylindoles. The various deuterium-labeled derivatives used in this project were prepared from a variety of deuterated precursor materials commercially available and using the synthetic methods for the unlabeled compounds.

The analytical studies included spectral data on each of the individual compounds, including GC-MS and GC-IR. Additional synthetic studies to label individual portions of the candidate molecules was necessary in order to fully understand and describe the mass spectral fragmentation

chemistry. These labeled compounds (isotopic and homologous labels) were prepared as needed to complete this study. The analytical methods focused on those techniques in routine use in forensic drug chemistry laboratories: GC, GC-MS, IR, GC-IR, and related techniques. Some exact mass GC-TOF-MS studies were used in specific applications for confirmation of the elemental composition of fragment ions. The use of exact mass GC-TOF-MS measurements is significant since the fragmentation process is the same for standard EI-MS and GC-TOF-MS.

Data Analysis:

The analytical data generated in this project is based primarily on gas chromatography with mass spectral and vapor phase infrared detection. The mass spectral fragmentation products generated in electron ionization (EI) experiments were catalogued and characterized using analogue, homolog, and stable isotope labeling techniques. Product ion MS/MS studies provided information on the source and production of a number of fragments while time of flight EI-MS yielded accurate mass and elemental composition of fragment ions.

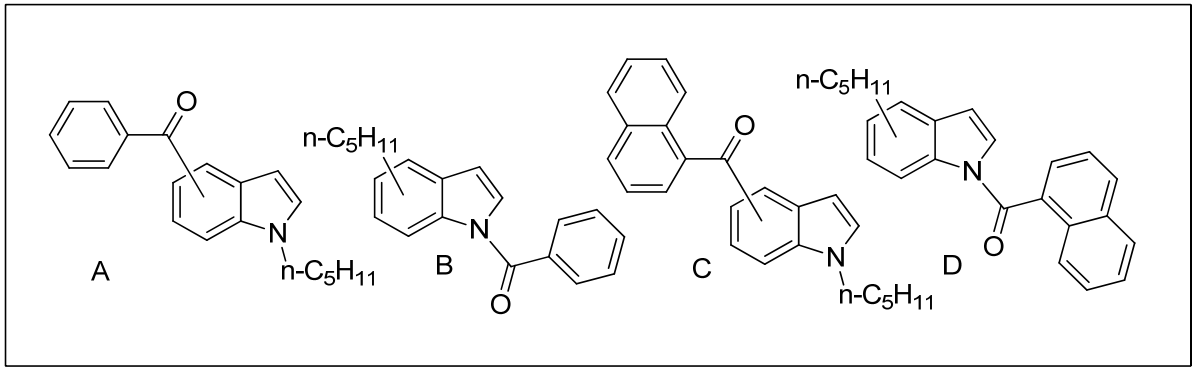
The resolution and separation of the various regioisomeric forms of the compounds in this project were very successful on a number of chromatographic stationary phases. The six regioisomeric substitution patterns of the indole ring (i.e. 1,2; 1,3; 1,4; 1,5; 1,6 and 1,7 disubstituted indoles) as well as the positional isomers of the substituted benzoyl aromatic ring were resolved (both mono- and di-substituted aromatic rings). The inverse isomers (1-acyl-3-alkyl-indoles) were clearly distinguished from the traditional 1-alkyl-3-acylindole synthetic cannabinoids in chromatographic, mass spectral, and infrared experiments.

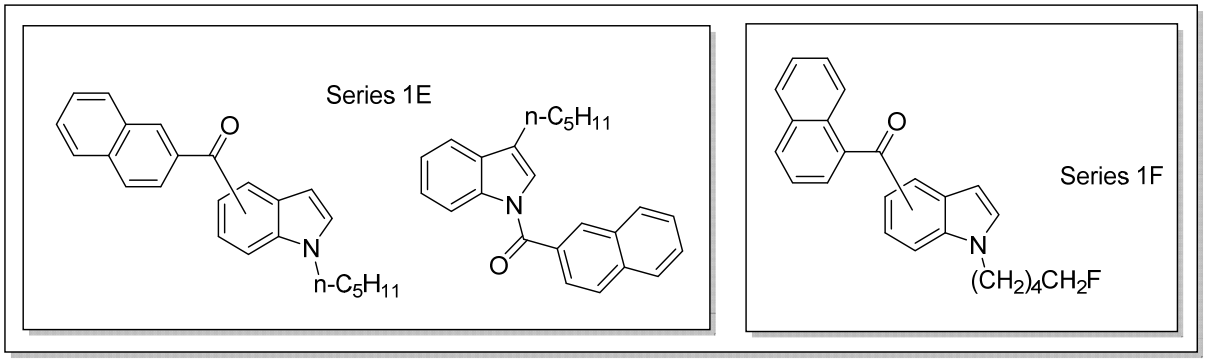
Project Findings:

The structural categories evaluated in this project are indole-based molecules and a general structure for each series (Series 1, 2, and 3) is shown in the following discussion. The targets in Series 1 (a total of six structural categories: 1A, B, C, D, E, and F) are regioisomeric

equivalents of the common 1-alkyl-3-acylindoles and we have completed the synthesis and evaluation of over 28 of the target compounds as well as numerous synthetic intermediates. Furthermore, a number of deuterium-labeled analogues have been evaluated for mass spectral fragmentation confirmation.

Variation in chemical structure in this category includes primarily the position of indole ring substitution of the so-called “head group” (the acyl group) as well as the nature of the acyl group (benzoyl, 1- and 2-naphthoyl) and the nature of the alkyl “tail group” (n-pentyl and 5-fluoro-n-pentyl) while holding the “core” indole ring constant in these compounds. The 3-(1-naphthoyl)-regioisomer in Series 1F is also known as AM-2201.





One of the most obvious results of these structure-analytical property relationship studies were the very different mass spectral properties for the inverse amide compounds (1-acyl-3-alkylindoles) compared to the traditional 1-alkyl-3-acylindoles. However, the elemental composition of all the isomers (inverse and traditional) are identical. While a number of unique

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EI fragment ions occur for the traditional isomers the inverse isomers showed only ions for the naphthyl and naphthoyl ions, m/z 127 and m/z 155 respectively.

The GC-MS properties of the synthetic cannabinoid drug of abuse 1-n-pentyl-3-(1-naphthoyl)-indole (JWH-018) and all 5 of its regioisomeric 1-naphthoyl substituted 1-n-pentylindoles are summarized in this report (The other sets of regioisomeric compounds in Series 1 follow a similar analytical pattern). These compounds have the 1-naphthoyl group attached at each of the possible substituent positions of the indole ring. The six compounds have the same elemental composition $C_{24}H_{23}NO$ and the same substituents attached to the indole ring. The EI-MS show equivalent regioisomeric major fragments resulting from cleavage of the groups attached to the central indole nucleus. The characteristic $(M-17)^+$ fragment ion at m/z 324 resulting from the loss of an OH group is significant in the EI-MS of 1-n-pentyl-3-, 4-, 5- and 6-(1-naphthoyl)-indole regioisomers. Fragment ions occur at m/z 127 and 155 for the naphthyl and naphthoyl cations common to all six regioisomeric substances. Indole-containing fragments yield the cations at m/z 284, 270, 214, and 186 (The structures of these fragment ions are shown in Appendix 2). The unique fragment at m/z 141 observed in the 1,2- and 1,7-isomers results from a rearrangement involving the two indole substituents to yield the $C_{10}H_7CH_2^+$ cation. This unique ion is a combination of the naphthalene ring and a methylene from the pentyl side-chain and represents the base peak for the 1,7-regioisomer. The two major points of EI-MS differentiation of the synthetic cannabinoid JWH-018 from the other five isomers are the high relative abundance of both the m/z 144 ion and the m/z 324 ion in the JWH-018 EI mass spectrum.

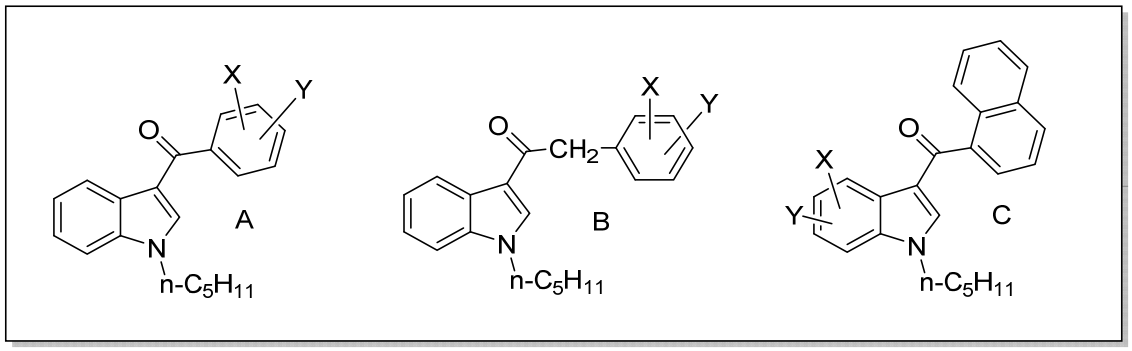
GC separations on a capillary column containing a trifluoropropyl methyl polysiloxane (Rtx-200) stationary phase provided excellent resolution of these six compounds (see Appendix 3). The elution order appears related to the relative distance between the two indole substituents

with the highest retention associated with maximum distance between the groups attached to the indole nucleus in the 3- and 5-substituted indole isomers. Additionally, the 3- and 5-isomers allow for maximum electronic interactions between the indole nitrogen and the carbonyl group in these diarylketones. The 3-position of the indole ring is electron rich due to direct activation (resonance effects) via the indole nitrogen and the 5-position of the indole ring places the carbonyl group in the *para* position relative to the indole nitrogen. The GC separation of the other subsets of regioisomeric equivalents gave the same relative elution order based on the indole ring substitution pattern.

The vapor phase infrared spectra of the twelve 1-*n*-pentyl-2-, 3-, 4-, 5-, 6- and 7-(1- and 2-naphthoyl)-indoles (JWH-018 and eleven regioisomers) were evaluated in GC-IR experiments (see Reference #9 in Appendix 1). These twelve compounds have the same substituents attached to the indole ring, identical elemental composition ($C_{24}H_{23}NO$) yielding identical nominal and accurate masses. These twelve isomers cover all possible positions of carbonyl bridge substitution for both indole (positons 2-7) and naphthalene rings (positions 1 and 2). These spectra show the bridge position on the indole ring is a dominating influence over the carbonyl absorption frequency observed for these compounds. Substitution on the pyrrole moiety of the indole ring yields the lowest carbonyl frequency values for position 2 and 3 giving a narrow range from 1657 to 1654cm^{-1}. Carbonyl absorption frequencies are higher when the naphthoyl group is attached to the benzene portion of the indole ring yielding absorption values from 1674 to 1671 cm^{-1}. The vapor phase infrared spectra for JWH-018 and its inverse isomer are in Appendix 4. The carbonyl absorption frequency is almost 50 cm^{-1} higher for the inverse amide isomer compared to that for the diarylketone JWH-018. This inverse amide regioisomer yields a carbonyl absorption frequency at 1705 cm^{-1}.

Similar points of isomer differentiation occur in the other series of compounds evaluated in Series 1. Individual peer-reviewed publications are available describing detailed analytical and forensic chemical studies for several of the structural categories in Series 1, see References # 1, 2, 5, 8, 9, and 11 in Appendix 1.

The compounds in Series 2 are traditional 1-alkyl-3-acylindoles with the variation in chemical structure based primarily on the position (and identity) of regioisomeric groups substituted on the acyl head group at the 3-position of the indole ring. Over the course of this project about 50 compounds from this series were synthesized and compared in forensic analytical studies. Series 2A and B represent substituted phenyl groups at the indole 3-position and attached as the benzoyl or phenylacetyl groups. The phenyl-ring substituents evaluated in this study included methyl, methoxy, dimethoxy, and methylenedioxy groups. Additionally, one series (2C) represents JWH-018 having a third substituent on the indole ring (methyl, methoxy, or chloro).



Series 2. Regioisomeric ring substituted equivalents of the 1-alkyl-3-acylindoles.

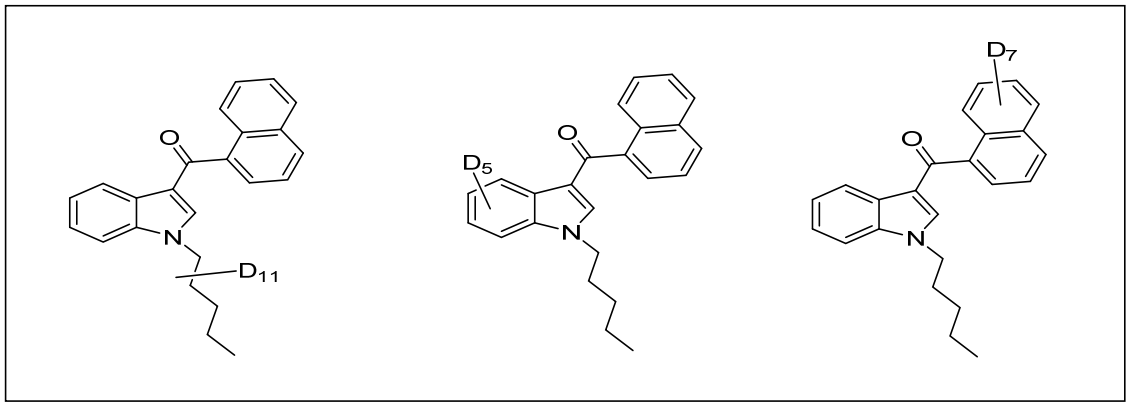
The substituted benzoyl isomers in Series 2A show the characteristic $(M\text{-}17)^+$ fragment ion in all compounds having a methyl or methoxy group substituted *ortho* to the carbonyl group. Thus, this aromatic ring substitution pattern provides a readily available hydrogen radical for migration to the carbonyl oxygen before elimination of the newly formed hydroxyl species. The $(M\text{-}17)^+$ fragment ion is the base peak in the EI-MS for some of the dimethoxybenzoyl isomers.

The EI-MS fragmentation for Series 2B is based primarily on the significant stability of the substituted benzylic cation formed in these phenylacetyl derivatives. In Series 2C the 2-methyl isomer known as JWH-007 is the only isomer not yielding a significant $[M-17]^+$ fragment ion at m/z 338 resulting from the loss of the OH radical. The GC elution order is related to the degree of crowding of the indole ring substituents. The vapor phase infrared spectra in the range of 1700 to 700 cm^{-1} showed unique and characteristic absorption patterns for each of the five regioisomeric compounds, allowing discrimination among these indole ring methylated isomers in this set of cannabinoid compounds.

Individual peer-reviewed publications are available describing detailed analytical and forensic chemical studies for several of the structural categories in Series 2, see References # 3, 6, 7, 10, and 12 in Appendix 1.

One major goal of the project for the Series 3 compounds was to evaluate the unique fragment ion at m/z 324 in the EI-MS for JWH-018. The issue concerns the source and mechanism for this loss of 17Da from the molecular ion in this compound. These labeled compounds needed for this study are shown below and these compounds represent the deuterium labeling of each region of the JWH-018 molecule. We prepared the deuterated analogues for the 3 regions of the JWH-018 molecule and evaluated the mass spectral properties of these derivatives. Appendix 5 details the observed masses associated with the major fragment ions for the regionally-deuterated analogues. The goal of this work was to observe the resulting mass shifts to determine the source of the hydrogen eliminated along with the oxygen in the fragmentation process. A loss of $(M-18)^+$ from the appropriately labeled analogue would identify the molecular source of the hydrogen. High resolution mass spectrometry shows the $(M-17)^+$ ion in JWH-018 to be the loss of an OH group and mass spectral studies on the 3 regionally-

deuterated derivatives show the hydrogen lost in this fragmentation to come from the 8-position of the naphthalene ring. The data presented in Appendix 5 concerning the mass shifts associated with the regionally-deuterated analogues also confirms the proposed fragment ion structures described for JWH-018 and related compounds outlined in Appendix 2.



Series 3. Structures for the deuterium-labeled regions of JWH-018.

Structural analogues such as the benzoyl-indoles and the 2-naphthoyl isomers show essentially no $(M-17)^+$ fragment ions and confirm that the hydrogen lost in this fragmentation comes from the 8-position of the naphthalene ring. This $(M-17)^+$ fragment is unique to the 1-naphthoyl group of compounds and is most abundant when the 1-naphthoyl group is substituted at the 3-position of indole. This is the subject of the manuscript published in "Rapid Communications in Mass Spectrometry," publication #4. Other crowded ring compounds from Series 2A also undergo an analogous fragmentation process to yield ions at $(M-17)^+$. Further confirmation for the effects of an appropriately positioned hydrogen for intramolecular migration comes from the mass spectral studies on the compounds in Series 2A. The substituted benzoyl isomers in Series 2A show the characteristic $(M-17)^+$ fragment ion in all compounds having a methyl or methoxy group substituted *ortho* to the carbonyl group.

The project has produced a significant amount of fundamental forensic chemical data for indole-based synthetic cannabinoids. The specific details of numerous individual project goals are described in the publications listed in Appendix 1.

Implications for criminal justice policy and practice:

This project has added a significant amount of fundamental analytical scientific information to the forensic chemistry knowledge base. The information generated in this project primarily used common analytical tools available to the practicing forensic scientist. A proactive investigation of the forensic analytical chemistry of these potential designer substances was the objective of this project. The resulting analytical data and methods represent important advancements in forensic drug chemistry and provide the forensic chemistry community with significant fundamental chemical information for the substituted indole class of compounds. This fundamental information includes gas chromatographic separations and elution order, mass spectra with confirmation of fragmentation mechanisms/structure, and infrared spectra for mass equivalent regioisomers. The relentless development of new designer substances of synthetic origin creates challenges in forensic drug identification due to the commercial availability of a variety of mass equivalent precursor substances. Regioisomeric forms of synthetic substances of equivalent elemental composition and yielding regioisomeric fragment ions of equal elemental composition present unique challenges in forensic drug identification using mass-based analytical methods (mass spectrometry). The results of this project have made available information and data sets to improve the forensic chemistry knowledge base and produce a scientifically skilled forensic expert available to interact at the interface of the legal system and the science of forensic drug chemistry of synthetic cannabinoids.

Appendix 1.

Bibliography of Project Publications:

1-Jack DeRuiter, Karim M. Abdel-Hay, Forrest Smith and C. Randall Clark "Analytical Differentiation of 1-Alkyl-3-acylindoles and 1-Acyl-3-alkylindoles: Isomeric Synthetic Cannabinoids," Analytical Chemistry, 86, 3801-3808 (2014).

2-Forrest T. Smith, Jack DeRuiter, Karim M. Abdel-Hay and C. Randall Clark "GC-MS Evaluation of the Six Benzoyl-Substituted-1-pentylindoles: Isomeric Synthetic Cannabinoids," Talanta, The International Journal of Pure and Applied Analytical Chemistry, 129, 171-182 (2014).

3-Karim M. Abdel-Hay, Jack DeRuiter, Forrest Smith, Tarek S. Belal and C. Randall Clark, "GC-MS Analysis of the Regioisomeric Methoxy- and Methyl-benzoyl-1-Pentylindoles: Isomeric Synthetic Cannabinoids," Science and Justice, 55, 291-298 (2015).

4-Amber Thaxton, Tarek S. Belal, Forrest Smith, Jack DeRuiter, Karim M. Abdel-Hay and C. Randall Clark, "Mass Spectral Studies on 1-n-pentyl-3-(1-naphthoyl)-indole (JWH-018), three Deuterium Labeled Analogues and the Inverse Isomer 1-naphthoyl-3-n-pentylindole," Rapid Communications in Mass Spectrometry, 29, 871–877 (2015).

5-Amber Thaxton, Tarek S. Belal, Forrest Smith, Jack DeRuiter, Karim M.Abdel-Hay and C. Randall Clark, "GC-MS Studies on the Six Naphthoyl-Substituted 1-Pentyl-Indoles: JWH-018 and Five Regioisomeric Equivalents," Forensic Science International, 252, 107–113 (2015).

6-Karim M. Abdel-Hay, Jack DeRuiter, Forrest Smith, Amsha S. Alsegiani, Amber Thaxton-Weissenfluh and C. Randall Clark, "GC-MS Differentiation of the Six Regioisomeric Dimethoxybenzoyl-1-pentylindoles: Isomeric Cannabinoid Substances," Journal of Pharmaceutical and Biomedical Analysis, 125, 360-368 (2016).

7-Tarek S. Belal, Amber Thaxton-Weissenfluh, Jack DeRuiter, Forrest Smith, Younis Abiedalla, Karim M. Abdel-Hay and C. Randall Clark, "Differentiation of Methylated Indole Ring Regio-isomers of JWH-007: GC-MS and GC-IR Studies," Journal of Forensic Chemistry, 7, 1-9 (2018).

8-Amber Thaxton-Weissenfluh, Amsha S. Alsegiani, Younis Abiedalla, Jack DeRuiter, Forrest Smith and C. Randall Clark, "Analytical Studies on the 2-Naphthoyl Substituted-1-n-Pentylindoles: Regioisomeric Synthetic Cannabinoids," Journal of Chromatography B, 1077-1078, 77-84 (2018).

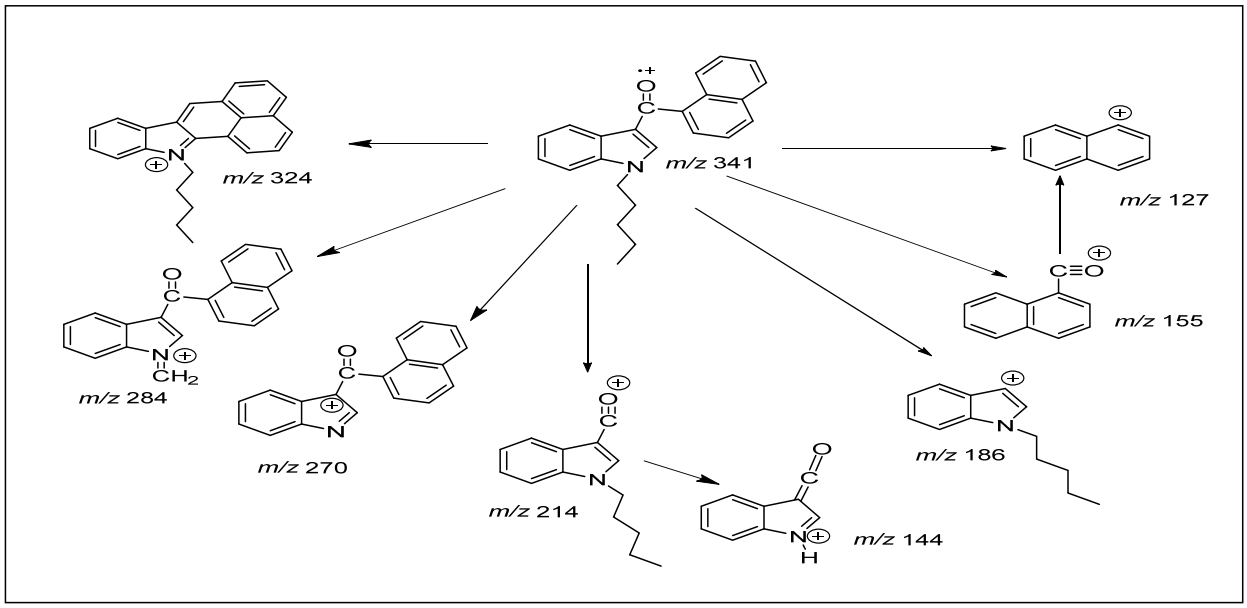
9-Lewis W. Smith, Amber Thaxton-Weissenfluh, Younis Abiedalla, Jack DeRuiter, Forrest Smith and C. Randall Clark, "Correlation of Vapor Phase Infrared Spectra and Regioisomeric Structure in Synthetic Cannabinoids," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 196, 375-384 (2018).

10-Amber Thaxton-Weissenfluh, Tarek S. Belal, Jack DeRuiter, Forrest Smith, Younis Abiedalla, Logan Neel, Karim M. Abdel-Hay and C. Randall Clark, "GC-MS and GC-IR Analysis of the Methoxy-1-n-pentyl-3-(1-naphthoyl)-indoles: Regioisomeric Designer Cannabinoids," Journal of Chromatographic Sciences, https://doi.org/10.1093/chromsci/bmy059, in press.

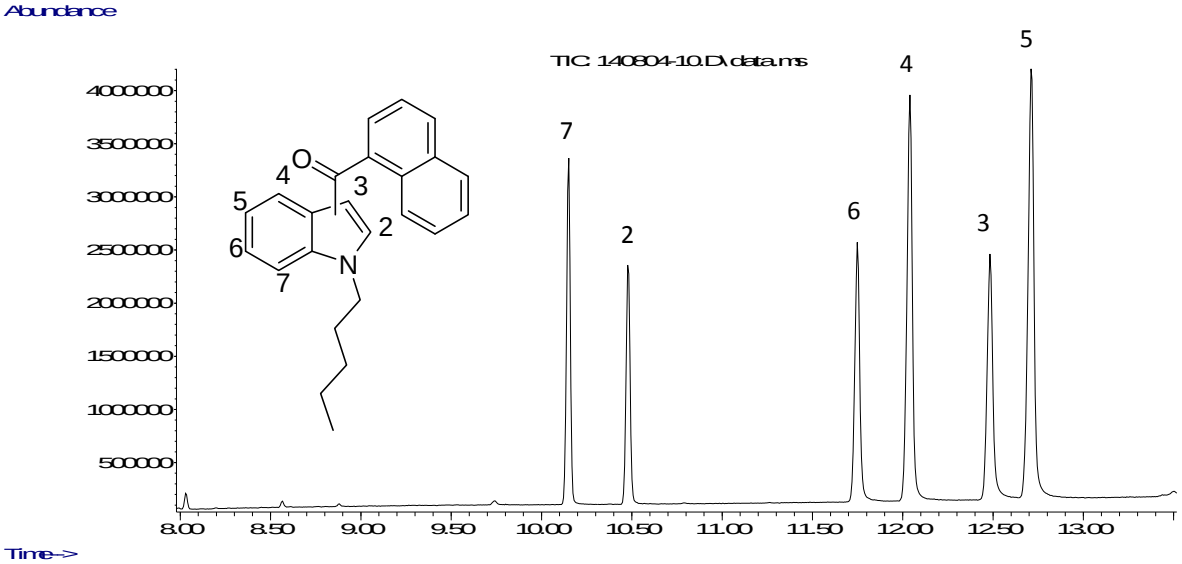
11-Jack DeRuiter, Forrest Smith, Younis Abiedalla, Logan Neel and C. Randall Clark, "GC-MS and GC-IR Analysis of Regioisomeric Cannabinoids Related to 1-(5-fluoropentyl)-3-(1-naphthoyl)-indole," Journal of Forensic Chemistry, 10, 48-57 (2018).

12- Karim M. Abdel-Hay, Tarek S. Belal, Amber Thaxton-Weissenfluh, Jack DeRuiter, Forrest Smith, Younis Abiedalla and C. Randall Clark, "GC-MS and GC-IR Analysis of the Chloro-1-n-pentyl-3-(1-naphthoyl)-indoles: Regioisomeric Designer Cannabinoids" Applied Spectroscopy, accepted for publication (2018).

Appendix 2.
Structures of the major mass spectral fragment ions for JWH-018 and related isomers.



Appendix 3.
Capillary GC separation of JWH-018 and five regioisomeric equivalents.



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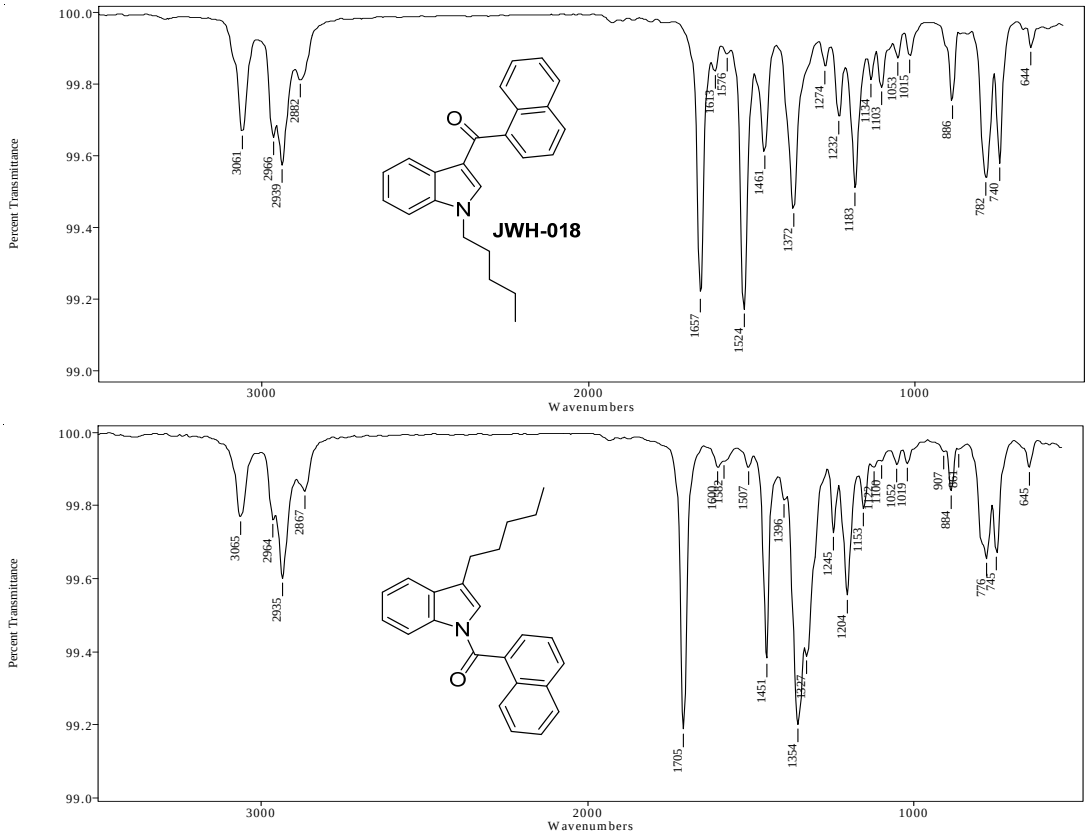
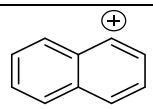
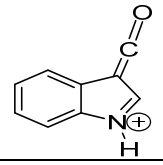
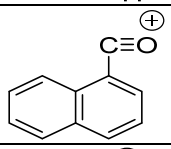
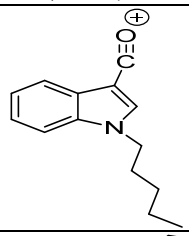
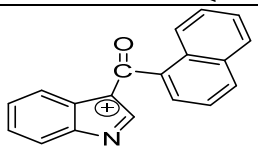
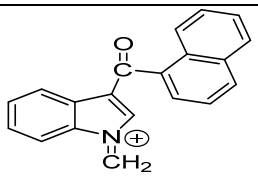
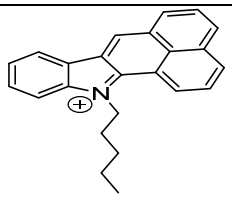
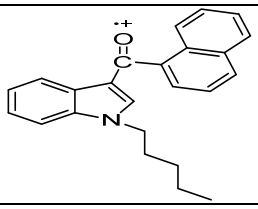
Appendix 4.

Vapor phase infrared spectra for JWH-018 and the inverse amide isomer.



Appendix 5: Summary of the mass to charge ratios of the fragment ions characteristic of the EI-MS of 1-n-pentyl-3-(1-naphthoyl)-indole and the three deuterium-labeled forms.

Fragment ion	**Unlabeled**	**D_{11} n-pentyl**	**D_5 indole**	**D_7 naphthyl**
	*m/z* 127	*m/z* 127	*m/z* 127	*m/z* 134
	*m/z* 144	*m/z* 145	*m/z* 149	*m/z* 144
	*m/z* 155	*m/z* 155	*m/z* 155	*m/z* 162
	*m/z* 214	*m/z* 225	*m/z* 219	*m/z* 214
	*m/z* 270	*m/z* 270	*m/z* 275	*m/z* 277
	*m/z* 284	*m/z* 286	*m/z* 289	*m/z* 291
	*m/z* 324 $[M-17]^+$	*m/z* 335 $[M-17]^+$	*m/z* 329 $[M-17]^+$	*m/z* 330 $[M-18]^+$
	*m/z* 341 $[M]^{+\cdot}$	*m/z* 352 $[M]^{+\cdot}$	*m/z* 346 $[M]^{+\cdot}$	*m/z* 348 $[M]^{+\cdot}$