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The Utility of Ultra High Performance Supercritical Fluid Chromatography for the Analysis of Seized Drugs, Application to Synthetic Cannabinoids and Bath Salts

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Final Summary Overview

Final Summary Overview, NIJ award 2014-R2-CX-K009

The Utility of Ultra High Performance Supercritical Fluid Chromatography for the Analysis of Seized Drugs, Application to Synthetic Cannabinoids and Bath Salts

Purpose of Project

The purpose of this project is to investigate the role of ultra high performance supercritical fluid chromatography (UHPSFC) as a separation technique for forensic drug analysis. For this reason the challenging separation of emerging drugs such as synthetic cannabinoids and bath salts will be investigated. Emerging drugs contain similar solutes such as analogues, homologues, positional isomers, and stereoisomers. The latter two classes of compounds can present particularly difficult separation challenges for which UHPSFC appears well suited.

An additional goal of this study is to establish UHPSFC as a viable separation technique (recognized as a Category B test by SWGDRUG) comparable to already established techniques for the separation of emerging drugs. For UHPSFC, validated methods for the determination of synthetic cannabinoids and synthetic cathinones (bath salts) in seized exhibits using UV-PDA detection and single-quad MS detection will be developed.

Project Design

Experiments were designed to answer the question whether UHPSFC is a viable technique for forensic drug analysis, and whether as expected it is particularly well suited, compared to conventional techniques, for the separation of closely related substances (analogues, homologues, positional isomers, and diastereomers), which are present in emerging drug exhibits. The study consisted of three phases. For the first phase, using a UHPSFC instrument equipped with a PDA-UV detector and a single-quad mass

spectrometric detector, optimum chromatographic and detector conditions were established for the separation of selected synthetic cannabinoids and substituted cathinones (Table 1 and Table 2).

Methods

A Waters Acquity UPC^2 system equipped with a PDA-UV detector and a QDA (single quad) MS detector was used for the UHPSFC experiments (Figure 1). The chromatographic conditions, including the effect of co-solvent, additive type, pressure, temperature, and column type (Table 3 and Table 4, Figure 2 and Figure 3) was investigated in order to determine the "optimum" separation conditions (most solutes resolved with resolution $\geq$1) for the UHPSFC separation of synthetic cannabinoids and synthetic cathinones. The experimental steps taken are further summarized as follows:

For synthetic cannabinoids five minute gradient, plus one minute hold separations were carried out, so that the separation used the entire solvent space. UHPSFC separations with a CEL1 column and isopropanol modifier were further fined-tuned by varying gradient steepness (time of gradient), applied backpressure regulator (APBR), and temperature.

For synthetic cathinones five minute gradient, plus one minute hold separations or isocratic separations generally under six minutes were carried out, so that the separation used the entire solvent space. UHPSFC separations with a DIOL column and a methanol modifier were further fined-tuned by varying APBR and temperature.

Peak resolution was determined using standard resolution curves and the ratio of the valley to the height of the peak [1]. Principal component analysis (PCA) was performed on the UHPSFC, GC, and LC retention time data using IBM SPSS Statistics Version 21 (International Business Machines Corporation, Armonk, NY, USA). For PCA analysis the

data were autoscaled. Each variable was adjusted so that it had zero mean and unit standard deviation or variance.

For the synthetic cannabinoids and synthetic cathinones linearity, repeatability, and limits of detection were performed for both UV and MS detection using "optimized" UHPSFC conditions. Twenty simulated synthetic cannabinoid samples were prepared for analysis by adding known amounts of synthetic cannabinoids to ground leaf material (marshmallow, dogrose, beach bean, and honey weed). In addition, twenty simulated synthetic cathinone samples were prepared for analysis by adding known amounts of synthetic cathinones and an adulterant (lidocaine, benzocaine, caffeine, and pancake mix). See appendix S1 and S2 for sample preparation for both types of samples.

The use of two coupled columns in series with different selectivity to enhance the overall resolution of compounds was investigated. A mixture of 15 bath salts, as well as 10 sets of positional isomers of identical mass, were run on six individual columns (<10 minute runs) as well as eight coupled column combinations (Table 5 and Table 6). The Neue selectivity values [2], which determine the orthogonality of the individual columns, are investigated as a means to determine which coupled columns to use. For coupled column separation(s) further optimization was obtained by changing pressure.

Data Analysis

For the separation of synthetic cannabinoids and synthetic cathinones of primary interest was the separation of the positional isomers. These solutes are much more difficult to distinguish via retention time, and MS spectrum. Of secondary interest was the separation of a wide variety of controlled substances, which for the most part have different masses. In

order to best evaluate UHPSFC as a separation technique, it was of interest to compare this chromatographic technique with established techniques such as GC and UHPLC.

For the various UHPSFC systems studied, the CEL1 column with an isopropanol modifier by far gave the best separation of the controlled synthetic cannabinoid JWH-018 and 9 non-controlled positional isomers. The separation was further optimized by varying the gradient steepness parameter (varied time of gradient) and temperature. All ten isomers were resolved (resolution ≥1) at a temperature of 55 degrees (resolution ≥1), in contrast to reversed phase (RP) UHPLC and GC where at best 3 and 4 out of 10 were resolved, respectively (Figure 4 and Figure 5) [3]. Under the same chromatographic conditions, the separation of 11 out of 22 controlled substances, including the separation of the positional isomers JWH-019 and JWH-122, was achieved (resolution ≥1) in under 10 minutes with both UV-PDA and MS QDA detection (Figure 6). For the same set of compounds, GC resolved 18 solutes in a 24-minute temperature-programmed run and UHPLC resolved at best 15 solutes in a 13-minute gradient run, both with a resolution ≥1 (Figure 7) [3]. Not only does UHPSFC exhibit great utility for the separation of positional isomers, it is also excellent for the separation of diastereomers as evidenced by the ability of the above chromatographic system to distinguish CP 47, 497 from its diastereomer Epi CP 47, 497 and the CP 47, 497 C8 homologue from its diastereomer 3-Epi CP 47, 497 C8. Although only useful for possible intelligence value, the AMY1 column with a methanol modifier was able to resolve from each other the individual enantiomers of all of the above solutes (Figure 8). HU-210 is well resolved from its diastereomer using an AMY1 column with an isopropanol modifier (Figure 9), in contrast to GC [4] and HPLC [5] where co-elution occurred.

For synthetic cathinones, the best overall separation of ten sets of positional isomers of identical mass was obtained with a DIOL stationary phase, a methanol modifier, and an

ammonium formate additive under isocratic conditions. Further optimization was accomplished by varying the temperature, whereby 28 out of 34 positional isomers (sum of solutes resolved in each of the ten sets) were separated (resolution ≥1). This was in contrast to GC, RP UHPLC, and hydrophilic interaction UHPLC where at best for these same solutes 22 [6], 27[7], and 7 [7] solutes were separated, respectively [6]. The separation of the $[M-H]^+$ 192 positional isomers by UHPSFC, GC, and UHPLC (RPC and hydrophilic interaction chromatography (HILIC) is shown in Figure 10 and Figure 11, whereby six out of eight, three out of eight, one out of eight, and five out of eight, respectively, are resolved (resolution ≥1). A five-minute isocratic separation of 10 out of 15 (resolution ≥1) controlled synthetic cathinones, by both UV and MS detection employing the "optimized conditions" for the positional isomers is shown in Figure 12. For the same set of compounds, GC, RPC, and HILIC resolved 13 [6] , 12 [7], and 5 [7] solutes, respectively for an, 11-minute temperature-programmed run (Figure 13), an 11-minute gradient run and a 9-minute isocratic run (Figure 14). Although not the focus of this study, chiral separation of the individual "bath salts" using a UHPSFC chiral stationary phase is possible, which could be of intelligence value.

Principal component analysis for the various chromatographic types for the separation of synthetic cannabinoids (Figure 15) and "bath salts" (Figure 16) reveals that UHPSFC systems stand by themselves in a diffuse group, orthogonal to GC and RP UHPLC performed on a C18 column. In addition, for bath salts, UHPSFC is orthogonal to hydrophilic-interaction UHPLC performed on a silica column.

Based on the relatively high resolving power for synthetic cannabinoids and bath salts (particularly for positional and stereoisomers) and orthogonality to GC and UHPLC, UHPSFC belongs in the compendium of methods used for the analysis of seized drugs. The

commonly employed RPC with a C18 column gives poor resolution of positional isomers. In terms of the overall resolution of controlled drugs and the separation of positional isomers, GC in combination with UHPSFC is the method of choice to provide complementary separation methods.

The great utility of employing the complementary detection modes of PDA-UV and electrospray single quad MS for the detection of synthetic cannabinoids and bath salts is shown in Figure 5 and Figure 10, respectively. MS detection offers far superior selectivity over UV detection, especially for non-isobaric compounds. For these solutes, overlapping peaks could be resolved using extracted ion chromatograms from either the $[M+H]^+$ or $[M+H-H_2O]^+$ ions of the analytes of interest. UV and MS data for these solutes, representing synthetic cannabinoids and bath salts, are given in Table 7 and Table 8, respectively. For these analytes, UV and MS libraries were created. UV spectra are particularly useful for determining subclasses of drugs and in distinguishing between positional isomers, particularly when differences occur in benzenoid substitution. JWH-018 has a unique UV spectrum compared to the other positional isomers (Figure 17). For a given mass, most positional isomers of bath salts gave unique UV spectrum (Figure 18-27).

For the synthetic cannabinoids shown in Figure 6, linearity analysis was performed and limits of detection ascertained for both UV and MS detection using the "optimized" UHPSFC conditions previously described (Table 9). For UV detection, excellent linearity was obtained, over two orders of magnitude, with $0.9996 \geq R^2 \geq 1.0000$. For MS detection, less favorable linearity was obtained, well over one order of magnitude for most solutes, with $0.9936 \geq R^2 \geq 0.9998$. For most solutes, MS detection offered lower limits of detection than UV detection, ranging in value from approximately a half to an order of magnitude higher. Run-to-run precision was examined for retention time (UV detection) and peak area (UV and

MS detection) for each of the selected synthetic cannabinoids at three concentrations representing low, moderate, and high linearity concentration ranges (Table 10). Excellent run-to-run precision (0.01≥% RSD≥0.32, 0.18≥% RSD≥2.92, 0.17≥% RSD≥2.97) was obtained for retention time, peak area UV, and peak area MS, respectively. Excellent day-to-day precision (0.12≥% RSD≥0.50) over a one-week period was obtained for retention time (Table 11).

For the bath salts shown in Figure 10, linearity analysis was performed, and limits of detection ascertained for both UV and MS detection using "optimized" UHPSFC conditions previously described (Table 12). For the most part MS detection affords at least a two-order-of- magnitude linearity range. In contrast, UV detection offers at least a one-order-of-magnitude linearity range. MS detection offers lower correlation coefficients than UV detection. For MS detection $0.9900 \geq R^2 \geq 0.9992$, while for UV detection $0.9994 \geq R^2 \geq 1.0000$. In addition, MS detection afforded two to three-orders-of-magnitude lower limits of detection than UV detection. Run-to-run precision was examined for retention time (UV detection) and peak area (UV and MS detection) for each of the selected bath salts, at three concentrations representing low, moderate, and high linearity concentration ranges (Table 13). Excellent run-to-run precision (0.01≥% RSD≥0.55, 0.31≥% RSD≥3.00), (3.55 for 4-MePPP at low linearity concentration), (0.31≥% RSD≥2.86) was obtained for retention time, peak area UV, and peak area MS, respectively. Good day-to-day retention time precision over a one-week period was obtained especially if one takes into account relative retention times ($0.53 \geq$ %RSD ≥ 1.52) (Table 14).

Twenty simulated synthetic cannabinoid samples were prepared for analysis by adding known amounts of synthetic cannabinoids to ground leaf material (marshmallow, dogrose, beach bean, and honey weed). In addition, twenty simulated synthetic cathinone samples

were prepared for analysis by mixing known amounts of synthetic cathinones and an adulterant (lidocaine, benzocaine, caffeine, and pancake mix). Quantitative results for both synthetic cannabinoids and bath salts are shown in Table 15 and Table 16, respectively. For peaks containing single components, quantitation by UV detection resulted in good overall agreement for experimentally determined values in samples versus target values. In general, UV quantitation is more accurate than MS detection. For co-eluting peaks, MS detection provided a good estimate of the quantity of target drug present.

Custom reports were created for both qualitative and quantitative analysis (see appendix S3 and S4 for sample reports). For qualitative analysis, the tentative identification of a synthetic cannabinoid or a bath salt is accomplished by a combination of UV spectra and/or retention time, and MS spectrum base peak. For a given peak the qualitative report includes the UV apex spectrum, peak purity (compares UV spectra across peak), UV library search, MS spectra at peak apex and across the peak, and MS library search.

Protocols were developed for analysts for the analysis of synthetic cannabinoids and synthetic cathinones (appendix S5 and S6). Included are instructions for sample preparation, chromatographic conditions, representative chromatograms, structures, UV data, MS data, linearity data, limits of detection, and Water's instrumental software (Empower 3 files are available from the PI on request).

The use of coupled columns (DIOL + 2-PIC) significantly increased the separation of bath salts, whereby 31out of 34 positional isomers and 15 out of 15 controlled synthetic cathinones were resolved (Figure 12, Figure 28, and Figure 29).

Scholarly Products Produced or in Process

Breitenbach, S.; Rowe, W. F.; McCord, B.; Lurie, I.S*, Assessment of ultra high performance supercritical fluid chromatography as a separation technique for the analysis of seized drugs: Applicability to synthetic cannabinoids, Journal of Chromatography A, 2016, 1440, 201-211.

Rowe, W.F.; Marginean, I.; Carnes (Breitenbach), S.; Lurie, I.S.*, The role of diode array ultraviolet detection for the identification of synthetic cathinones separated by ultra high performance supercritical fluid chromatography. Drug Testing and Analysis, under review.
O'Brien, S.; Carnes, S.; Andrew, E.; Rowe W.F.; McCord, B., Lurie, I.S.* A Comparison of ultra high performance supercritical fluid chromatography, ultra high performance liquid chromatography, and gas chromatography for the separation of synthetic cathinones. manuscript in preparation.

Implications for Criminal Justice Policy and Practice in the United States

The use of UHPSFC would greatly assist the criminal justice system in the adjudication of emerging drug cases. In particular, it would aid in determining which if any controlled substances are present in seized drugs, particularly in the area of emerging drugs.

The use of emerging drugs, which are synthesized to circumvent the controlled substances laws, has greatly increased over the last few years. New structurally-similar compounds, such as synthetic cannabinoids and bath salts, are created by slightly modifying the chemical structure of a controlled substance. For the analysis of these solutes for legal purposes, the desired analytical methodology should have the ability to distinguish between similar solutes (analogues, homologues, positional isomers, and diastereoisomers). In this vein, separation methods such as liquid chromatography (HPLC, UHPLC) and gas

chromatography have played a major role in the analysis of synthetic cannabinoids and bath salts. As evidenced for the analysis of synthetic cannabinoids, this project has demonstrated that UHPSFC has the potential to be superior to UHPLC and/or GC for the analysis of very similar solutes such as positional isomers of synthetic cannabinoids and bath salts and diastereoisomers of synthetic cannabinoids. For the separation of a diverse group for each class of emerging groups, including mainly non- isomeric solutes, UHPSFC has been demonstrated to be orthogonal to GC and UHPLC.

Detection methods also play a major role in the screening and confirmation of these analytes. Single quad MS soft-ionization techniques and UV detection typically used in LC, also provide for UHPSFC molecular mass and complementary structural information. In fact, most positional isomers of bath salts gave unique UV spectrum. UV detection subsequent to UHPSFC separation has proven to be excellent for quantitation of seized drugs. For co-eluting compounds, MS detection can provide a good estimate of the individual solutes. Based on the precision and high resolving-power of UHPSFC for the separation of emerging drugs, such as synthetic cannabinoids and synthetic cathinones, including positional isomers and diastereomers, it is recommended UHPSFC be included in the compendium of techniques for drug analysis, i.e., a SWG Drug category B test.

Appendix

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3. Marginean, I.; Rowe, W.F.; Lurie, I.S., The role of ultra high performance liquid chromatography with time of flight detection for the identification of synthetic cannabinoids in seized drug, Forensic Science Int., 2015, 249, 83-91.

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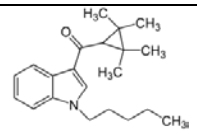
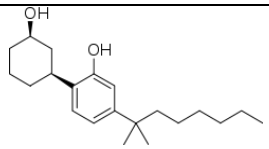
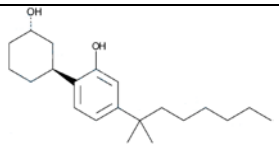
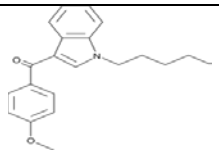
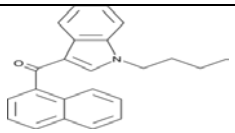
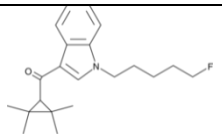
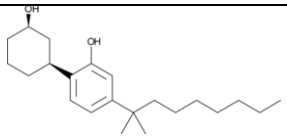
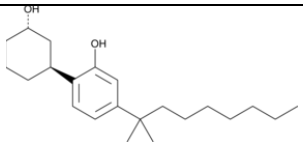
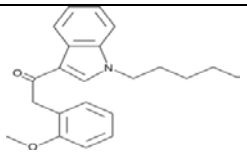
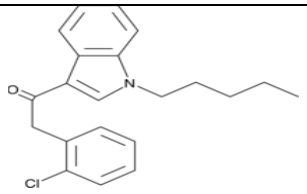
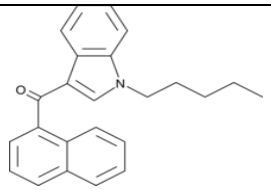
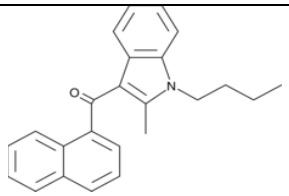
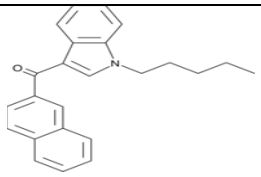
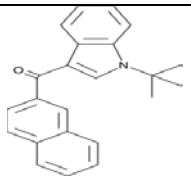
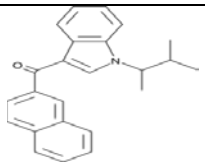
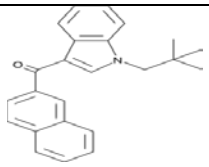
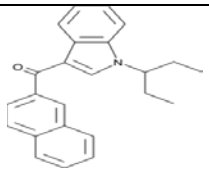
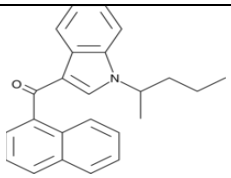
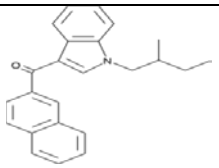
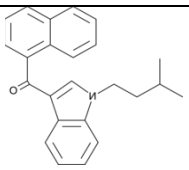
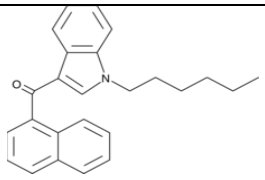
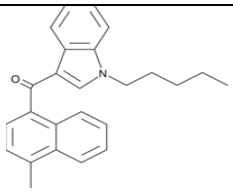
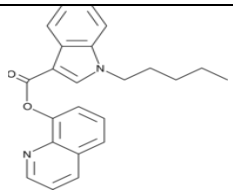
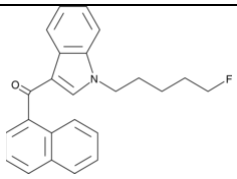
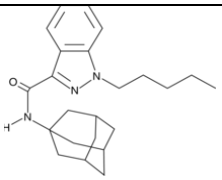
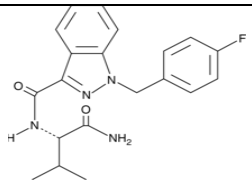
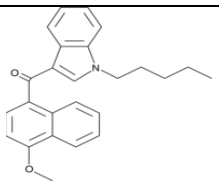
Tables

Table 1- Synthetic cannabinoids

Solute	Structure	Chemical Formula monoisotopic mass
UR-144		$C_{21}H_{29}NO$ 311.2
CP47,497		$C_{21}H_{34}O_2$ 318.3
Epi CP 47, 497		$C_{21}H_{34}O_2$ 318.3
RCS-4		$C_{21}H_{23}NO_2$ 321.2
JWH-073		$C_{23}H_{21}NO$ 327.2
XLR-11		$C_{21}H_{28}FNO$ 329.2
CP47, 497 C8 homologue		$C_{22}H_{36}O_2$ 332.3
3-epi CP47, 497 C8 Homologue		$C_{22}H_{36}O_2$ 332.3
JWH-250		$C_{22}H_{25}NO_2$ 335.2

JWH-203		$C_{21}H_{22}ClNO$ 339.1
JWH-018		$C_{24}H_{23}NO$ 341.2
JWH-016		$C_{24}H_{23}NO$ 341.2
JWH-018 2'-naphthyl isomer		$C_{24}H_{23}NO$ 341.2
JWH-018 2'-naphthyl-N-(1, 1-dimethylpropyl) isomer		$C_{24}H_{23}NO$ 341.2
JWH-018 2'-naphthyl-N-(1, 2-dimethylpropyl) isomer		$C_{24}H_{23}NO$ 341.2
JWH-018 2'-naphthyl-N-(2, 2-dimethylpropyl) isomer		$C_{24}H_{23}NO$ 341.2
JWH-018 2'-naphthyl-N- (1 ethylpropyl) isomer		$C_{24}H_{23}NO$ 341.2
JWH-018 2'-naphthyl-N- (1 methylbutyl) isomer		$C_{24}H_{23}NO$ 341.2

JWH-018 2'-naphthyl-N-(2methylbutyl) isomer		$C_{24}H_{23}NO$ 341.2
JWH-018 2'-naphthyl-N- (3 methylbutyl) isomer		$C_{24}H_{23}NO$ 341.2
JWH-019		$C_{25}H_{25}NO$ 355.2
JWH-122		$C_{25}H_{25}NO$ 355.2
PB-22		$C_{23}H_{22}N_2O_2$ 358.2
AM-2201		$C_{24}H_{22}FNO$ 359.2
AKB-48		$C_{23}H_{31}N_3O$ 365.2
AB-Fubinaca		$C_{20}H_{21}FN_4O_2$ 368.2
JWH-081		$C_{25}H_{25}NO_2$ 371.2

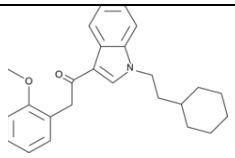
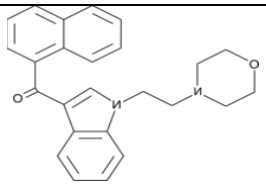
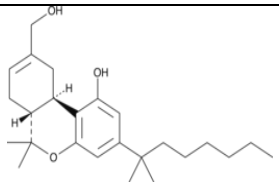
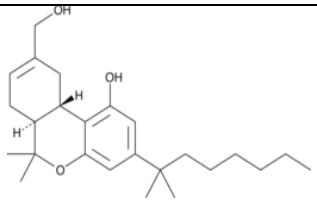
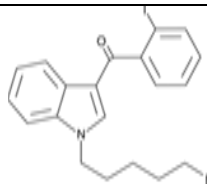
RCS-8		$C_{25}H_{29}NO_2$ 375.2
JWH-200		$C_{25}H_{24}N_2O_2$ 384.2
HU-210		$C_{25}H_{38}O_3$ 386.3
HU-211		$C_{25}H_{38}O_3$ 386.3
AM- 694		$C_{20}H_{19}FINO$ 435.0

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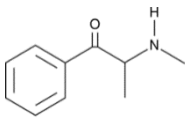
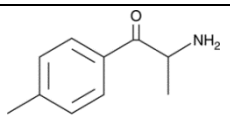
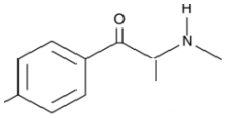
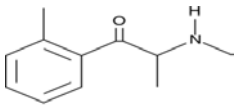
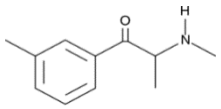
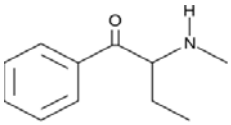
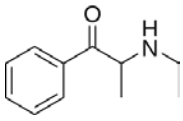
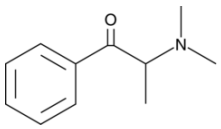
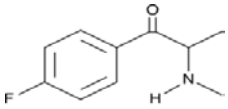
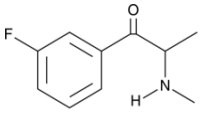
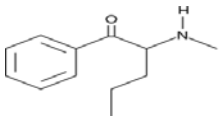
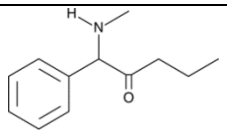
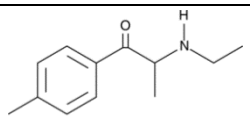
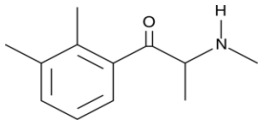
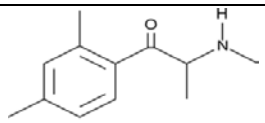
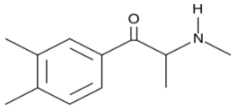
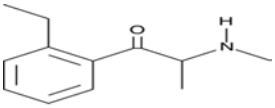
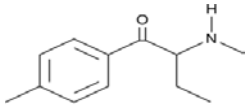
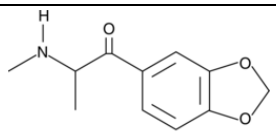
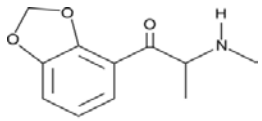
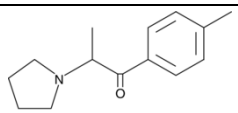
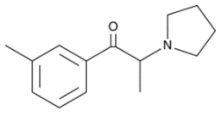
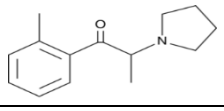
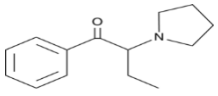
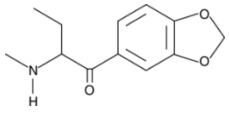
Table 2 - Bath Salts

Solute	Structure	Chemical Formula monoisotopic mass
Methcathinone		$C_{10}H_{13}NO$ 163.1
Nor-mephedrone		$C_{10}H_{13}NO$ 163.1
Mephredrone		$C_{11}H_{15}NO$ 177.1
2-methylmethcathinone		$C_{11}H_{15}NO$ 177.1
3-methylmethcathinone		$C_{11}H_{15}NO$ 177.1
Buphedone		$C_{11}H_{15}NO$ 177.1
Ethcathinone		$C_{11}H_{15}NO$ 177.1
N.N-dimethylcathinone		$C_{11}H_{15}NO$ 177.1
4-fluoromethcathinone		$C_{10}H_{12}FNO$ 181.1
3-fluoromethcathinone		$C_{10}H_{12}FNO$ 181.1
Pentedrone		$C_{12}H_{17}NO$ 191.1

Isopentedrone		$C_{12}H_{17}NO$ 191.1
4-methylethcathinone		$C_{12}H_{17}NO$ 191.1
2,3-dimethylmethcathinone		$C_{12}H_{17}NO$ 191.1
2,4-dimethylmethcathinone		$C_{12}H_{17}NO$ 191.1
3,4-dimethylmethcathinone		$C_{12}H_{17}NO$ 191.1
2-ethylmethcathione		$C_{12}H_{17}NO$ 191.1
4-methylbuphedrone		$C_{12}H_{17}NO$ 191.1
Methylone		$C_{11}H_{13}NO_3$ 207.1
2,3-methylenedioxymethcathinone		$C_{11}H_{13}NO_3$ 207.1
4-MePPP		$C_{14}H_{19}NO$ 217.1
3-MePPP		$C_{14}H_{19}NO$ 217.1
2-MePPP		$C_{14}H_{19}NO$ 217.1
α-PBP		$C_{14}H_{19}NO$ 217.1
Butylone		$C_{12}H_{15}NO_3$ 221.1

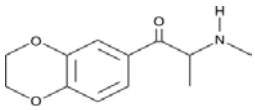
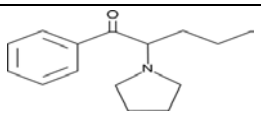
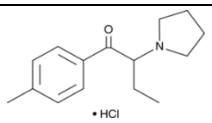
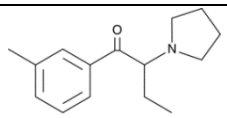
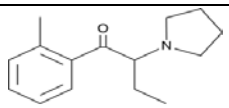
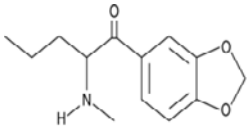
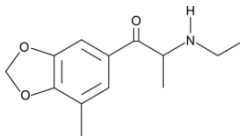
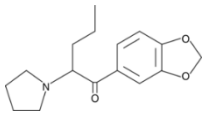
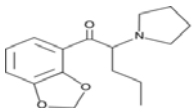
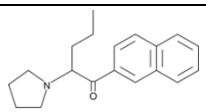
3,4-EDMC		$C_{12}H_{15}NO_3$ 221.1
α-PVP		$C_{15}H_{21}NO$ 231.2
4-MePBP		$C_{15}H_{21}NO$ 231.2
3-MePBP		$C_{15}H_{21}NO$ 231.2
2-MePBP		$C_{15}H_{21}NO$ 231.2
Pentylone		$C_{13}H_{17}NO_3$ 235.1
R-MMC		$C_{13}H_{17}NO_3$ 235.1
MDPV		$C_{16}H_{21}NO_3$ 275.2
2,3-MDPV		$C_{16}H_{21}NO_3$ 275.2
Naphyrone		$C_{19}H_{23}NO$ 281.2

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Table 3- UHPSFC experimental protocol for synthetic cannabinoids. Flow rate 1.25 mL/min, temperature 45°C and APBR 2200 PSI.

Solutes	Columns	Modifiers	Detectors
22 controlled synthetic cannabinoids, JWH-018 and nine positional isomers	2-PIC DIOL DEA 1-AA	methanol	UV (PDA) MS (QDA)
	AMY1 CEL1 CEL2	methanol, acetonitrile, ethanol, isopropanol	

Table 4- UHPSFC experimental protocol for synthetic cathinones. Flow rate 1.25 mL/min, temperature 45°C and APBR 2200 PSI.

Solutes	Columns	Modifiers	Additives	Detectors
15 controlled bath salts and 34 positional isomers of controlled bath salts	2-PIC DIOL DEA	methanol	10 mM ammonium formate, 10 mM ammonium hydroxide	UV (PDA) MS (QDA)
	AMY1 CEL1 CEL2	methanol, acetonitrile, ethanol, isopropanol		

Table 5- Columns (3.0 x 100 mm) used in coupled column experiments

Column	Particle Shape	Particle Size (µm)	Dimensions	Particle Technology
Torus 2-PIC	Spherical	1.7	3.0 x 100mm	BEH[a]
Torus DIOL	Spherical	1.7	3.0 x 100mm	BEH[a]
BEH 2-Ethylpyridine (2-EP)	Spherical	1.7	3.0 x 100mm	BEH[a]
Torus Diethylamine (DEA)	Spherical	1.7	3.0 x 100mm	BEH[a]
CSH Fluoro-Phenyl	Spherical	1.7	3.0 x 100mm	CSH[b]
HSS C_{18} Stable Bond	Spherical	1.8	3.0 x 100mm	HSS[c]

[a]Ethylene bridged hybrid

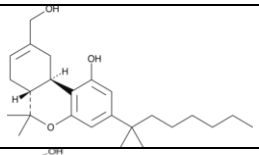
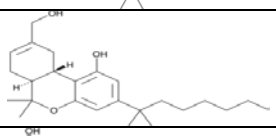
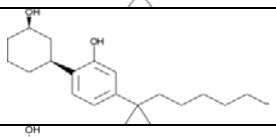
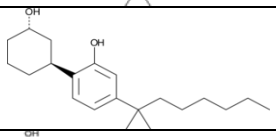
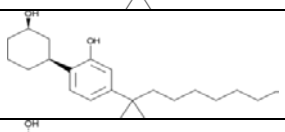
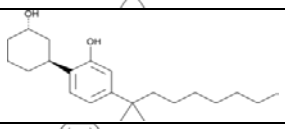
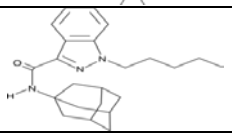
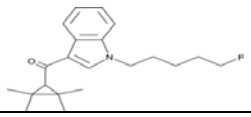
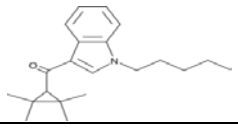
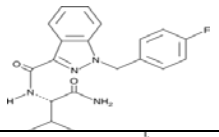
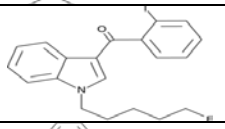
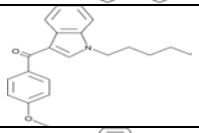
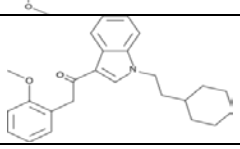
[b]Charged surface hybrid

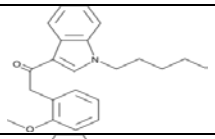
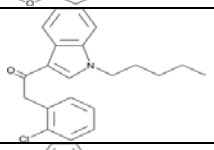
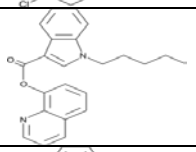
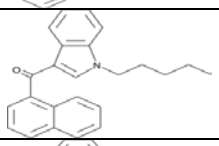
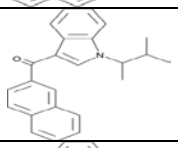
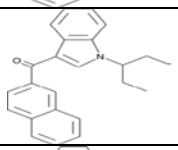
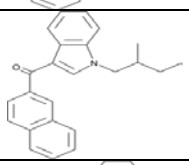
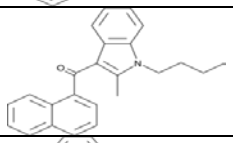
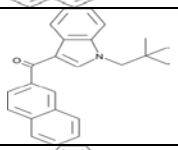
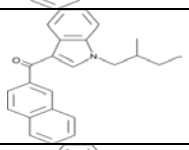
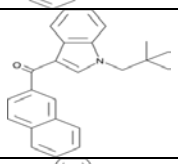
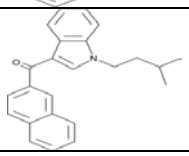
[c]High strength silica

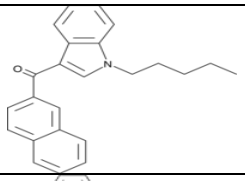
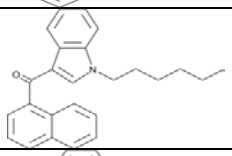
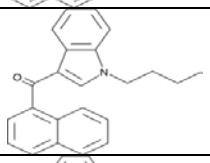
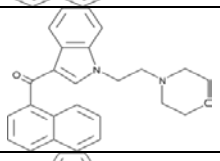
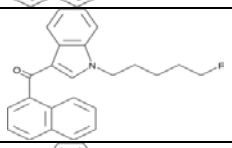
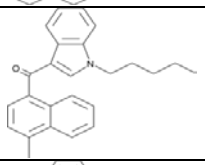
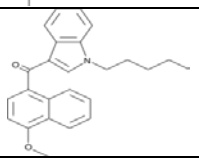
Table 6- UHPSFC experimental protocol for coupled columns. Flow rate 1.25 mL/min, temperature 40°C and APBR 2200 PSI.

Solutes	Single Column	Mobile Phase	Coupled Columns	Detectors
15 controlled bath salts and 34 positional isomers of controlled bath salts	2-PIC DIOL DEA 2-EP Fluoro-Phenyl C18	3% 10 mM ammonium formate in methanol, 97% carbon dioxide	DIOL + 2-PIC DIOL + Flouro-Phenyl DIOL + C18 2-PIC + DEA 2-PIC + C18 Flouro-Pheny l+ DEA DEA + C18 C18 + 2 EP	UV (PDA) MS (QDA)

Table 7 - Synthetic cannabinoids UV and MS data

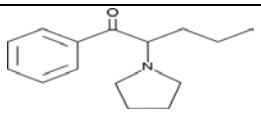
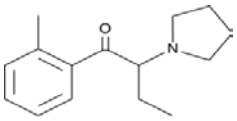
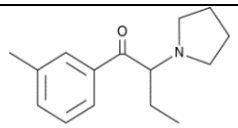
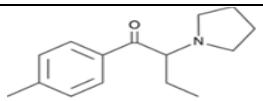
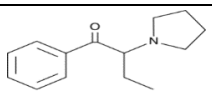
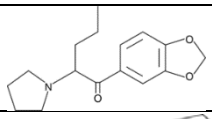
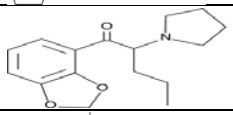
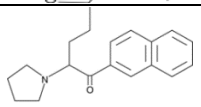
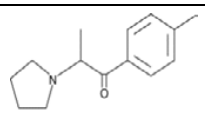
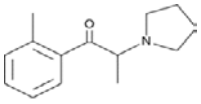
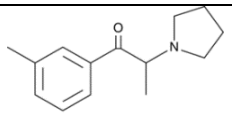
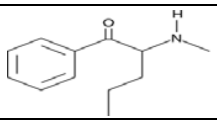
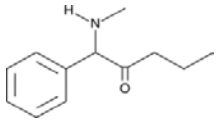
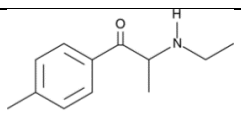
Solute	Structure	UV $_{MAX}$ (nm)	base peak $[M-H]^+$
HU-210		281	387.3
HU-211		281	387.3
CP47, 497		274	301.3[a]
Epi CP47, 497		274	301.3[a]
CP47, 497 C8		274	315.3[a]
3-epi CP47, 497 C8 homologue		274	315.3[a]
AKB-48		266, 276, 300	366.2
XLR-11		215, 242, 296	330.2
UR-144		215, 242, 296	312.2
AB-Fubinaca		264, 270, 277, 300	369.2
AM-694		248, 311	436.1
RCS-4		211, 260, 312	322.2
RCS-8		212, 243, 299	376.2

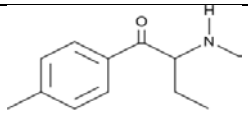
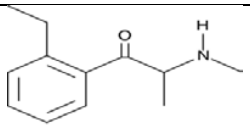
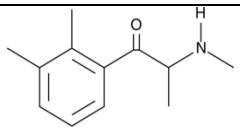
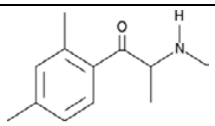
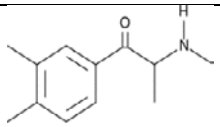
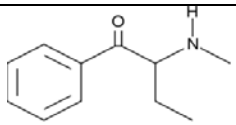
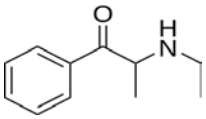
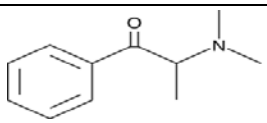
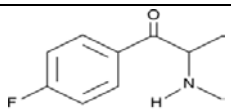
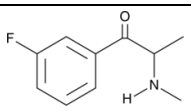
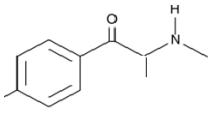
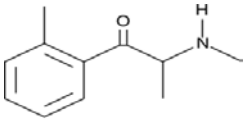
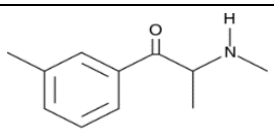
JWH-250		212, 243, 299	336.2
JWH-203		243, 299	340.2
PB-22		214, 292	359.2
JWH-018		215, 244, 308	342.2
JWH-018 2'-naphthyl-N-(1, 2-dimethylpropyl) isomer		213, 244, 316	342.2
JWH-018 2'-naphthyl-N-(1 ethylpropyl) isomer		213, 244, 316	342.2
JWH-018 2'-naphthyl-N-(1 methylbutyl) isomer		213, 244, 316	342.2
JWH-016		215, 244, 314	342.2
JWH-018 2'-naphthyl-N-(1,1-dimethylpropyl) isomer		213, 244, 316	342.2
JWH-018 2'-naphthyl-N-(2 methylbutyl) isomer		213, 244, 316	342.2
JWH-018 2'-naphthyl-N-(2, 2-dimethylpropyl) isomer		213, 244, 316	342.2
JWH-018 2'-naphthyl-N-(3 methylbutyl) isomer		213, 244, 316	342.2

JWH-018 2'-naphthyl isomer		213, 244, 316	342.2
JWH-019		215, 244, 308	356.2
JWH-073		215, 244, 308	328.2
JWH-200		215, 244, 308	385.2
AM-2201		215, 244, 308	360.2
JWH-122		218, 244, 310	356.2
JWH-081		312	372.2

[a][M+H-H2O]$^+$

Table 8 - Synthetic cathinones UV and MS data

Solute	Structure	UV $_{MAX}$ (nm)	base peak $[M-H]^+$
α-PVP		238	232.2
2-MePBP		238, 278	232.2
3-MePBP		243, 284	232.2
4-MePBP		249	232.2
α-PBP		238	218.2
3,4-MDPV		223, 269, 304	276.2
2,3-MDPV		222, 250, 324	276.2
Naphyrone		239, 280	282.2
4-MePPP		249	218.2
2-MePPP		238, 278	218.2
3-MePPP		243, 284	218.2
Pentedrone		239	192.1
Isopentedrone		262	192.1
4-methylethcathinone		250	192.1

4-methylbuphedrone		250	192.1
2-ethylmethcathinone		241, 280	192.1
2,3-dimethylmethcathinone		243, 281	192.1
2,4-dimethylmethcathinone		250	192.1
3,4-dimethylmethcathinone		254	192.1
Buphedone		239	178.1
Ethcathinone		239	178.1
N, N-dimethylmethcathinone		238	178.1
4-fluoromethcathinone		242	182.1
3-fluoromethcathinone		236, 282	182.1
Mephredrone		250	178.1
2-methylmethcathinone		241, 281	178.1
3-methylmethcathinone		243, 286	178.1

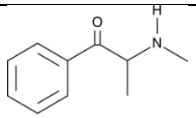
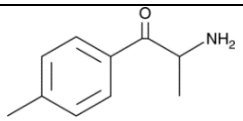
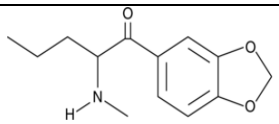
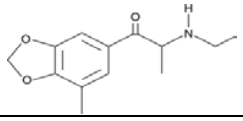
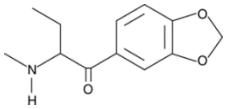
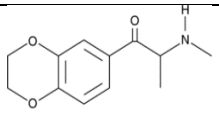
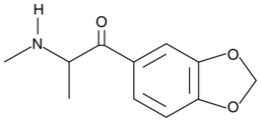
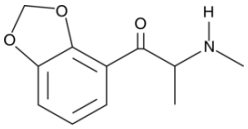
Methcathinone		239	164.1
Nor-mephedrone		249	164.1
Pentylone		224, 270, 304	236.1
R-MMC		228, 277, 304	236.1
Butylone		224, 270, 305	222.1
3,4 EDMC		226,270, 301	222.1
Methylone		224, 271, 304	208.1
2, 3-methylenedioxymethcathinone		222, 249, 326	208.1

Table 9 - Linearity and limits of detection for controlled synthetic cannabinoids

Solute	Detection Mode	Concentration Range µg/mL	R^2	Limit of Detection µg/mL
XLR-11	UV	0.230 – 59	1.0000	0.076
XLR-11	MS	0.230 – 7.4	0.9993	0.002
HU-210	UV	0.460 – 59	0.9999	0.153
HU-210	MS	0.230 – 14.8	0.9976	0.011
JWH-018	UV	0.244 – 62.5	1.0000	0.080
JWH-018	MS	0.244 – 7.8	0.9998	0.004
AKB-48	UV	0.230 – 59	0.9999	0.076
AKB-48	MS	0.057 – 7.4	0.9982	0.033
AM-2201	UV	0.115 – 59	0.9998	0.038
AM-2201	MS	0.115 – 7.4	0.9977	0.005
JWH-200	UV	0.976 – 62.5	0.9996	0.330
JWH-200	MS	0.122 – 7.8	0.9963	0.006
CP 47, 497 C8	UV	0.976 – 250	0.9998	0.330
CP 47, 497 C8	MS	0.488 – 31.2	0.9936	0.024
JWH-203	UV	0.390 – 50	0.9998	0.130
JWH-203	MS	0.097 – 6.25	0.9998	0.012
JWH-081	UV	0.390 – 50	0.9998	0.130
JWH -081	MS	0.097 – 6.2	0.9998	0.007
CP 47, 497	UV	0.976 – 250	0.9999	0.330
CP 47, 497	MS	0.244 – 7.812	0.9986	0.033
RCS-4	UV	0.390 – 50	0.9999	0.130
RCS-4	MS	0.097 – 6.2	0.9960	0.004
RCS-8	UV	0.390 – 50	0.9999	0.130
RCS-8	MS	0.097 – 6.25	0.9992	0.004
UR-144	UV	0.115 – 59	0.9999	0.038
UR-144	MS	0.115 – 14.8	0.9980	0.005
AB-Fubinaca	UV	0.460 – 59	1.0000	0.153
AB-Fubinaca	MS	0.057 – 7.4	0.9974	0.052
JWH-073	UV	0.488 – 62.5	0.9999	0.162
JWH-073	MS	0.122 – 15.6	0.9994	0.007
3-epi CP 47, 497 C8 (1)[a]	UV	1.952 – 250	0.9999	0.650
3-epi CP 47, 497 C8 (1)[a]	MS	0.488 – 31.2	0.9973	0.038
3-epi CP 47, 497 C8 (2)[a]	UV	1.952 – 250	0.9998	0.650
3-epi CP 47, 497 C8 (2)[a]	MS	0.488 – 31.2	0.9975	0.041
PB-22	UV	0.390 – 50	0.9999	0.130
PB-22	MS	0.097 – 6.2	0.9973	0.007
Epi CP 47, 497 (1)[a]	UV	1.952 – 250	0.9999	0.650
Epi CP 47, 497 (1)[a]	MS	0.488 – 31.2	0.9962	0.069
Epi CP 47, 497 (2)[a]	UV	1.952 – 250	0.99991	0.650
Epi CP 47, 497 (2)[a]	MS	0.488 – 31.2	0.9958	0.073
AM-694	UV	0.390 – 50	0.9999	0.130

AM-694	MS	0.097 – 6.2	0.9996	0.007
JWH-122	UV	0.195 – 50	0.9999	0.065
JWH-122	MS	0.097 – 6.2	0.9988	0.003
JWH-019	UV	0.122 – 62.5	0.9997	0.041
JWH-019	MS	0.122 – 7.8	0.9996	0.004
JWH-250	UV	0.244 – 62.5	0.9998	0.081
JWH-250	MS	0.061 – 3.9	0.9974	0.002

[a]individual enantiomer

Table 10 - Run-to-run precision n= 5 for synthetic cannabinoid solutions used for linearity studies.

Solute	**Concentration ug/mL**	**% RSD RT**	**% RSD Peak Area UV**	**MS**
XLR-11	59.0	0.07	0.18	1.07
	3.68	0.05	0.79	1.50
	0.460	0.08	1.48	1.08
HU-210	59.0	0.16	0.16	1.48
	3.68	0.13	1.93	0.99
	0.460	0.20	1.39	1.46
JWH-018	62.5	0.04	0.14	0.37
	3.905	0.03	0.81	0.32
	0.448	0.05	1.76	1.17
AKB-48	59.0	0.10	0.61	1.28
	3.68	0.12	0.78	2.97
	0.460	0.06	0.75	2.24
AM-2201	59.0	0.10	0.54	0.56
	3.68	0.06	1.04	1.42
	0.460	0.04	1.63	0.88
JWH-200	62.5	0.07	0.56	0.66
	3.905	0.05	1.52	0.92
	0.448	0.03	2.92	0.85
CP 47, 497 C8	250	0.08	0.39	0.17
	15.62	0.14	0.87	1.93
	1.952	0.16	1.73	1.30
JWH-203	50.0	0.03	0.41	0.79
	3.124	0.03	0.79	0.44
	0.390	0.16	0.65	1.60
JWH-081	50.0	0.03	0.38	0.89
	3.124	0.02	0.42	0.63

	0.390	0.02	0.53	1.05
CP 47, 497	250	0.20	0.38	1.15
	15.62	0.18	1.02	1.87
	1.952	0.23	1.60	1.91
RCS-4	50.0	0.05	0.28	0.76
	3.124	0.02	0.86	0.85
	0.390	0.09	2.32	0.81
RCS-8	50.0	0.02	0.26	0.68
	3.124	0.02	0.87	1.36
	0.390	0.09	2.79	1.73
UR-144	59.0	0.17	0.48	0.94
	3.68	0.08	0.48	2.11
	0.460	0.10	1.83	1.78
AB- Fubinaca	59.0	0.13	0.40	1.69
	3.68	0.06	1.10	2.86
	0.460	0.05	1.26	2.41
JWH-073	62.5	0.09	0.45	1.25
	3.905	0.01	1.16	0.54
	0.448	0.11	0.84	1.56
3-epi CP 47, 497 C8 (1)	250	0.09	0.30	1.36
	15.62	0.21	0.73	1.26
	1.952	0.16	1.78	1.50
3-epi CP 47, 497 C8 (2)	250	0.06	0.59	2.22
	15.62	0.14	1.24	2.08
	1.952	0.32	1.86	1.58
PB-22	50	0.03	0.69	1.30
	3.124	0.03	0.67	0.67
	0.390	0.05	0.54	1.21
Epi CP 47, 497 (1)	250	0.19	0.36	2.77
	15.62	0.21	0.72	2.21
	1.952	0.19	1.54	2.21
Epi CP 47, 497 (2)	250	0.16	0.45	1.89
	15.62	0.17	0.54	1.17
	1.952	0.21	2.08	1.89
AM-694	50	0.05	0.31	0.68
	3.124	0.08	0.51	1.12
	0.390	0.12	0.50	1.31
JWH-122	50	0.03	0.35	0.90
	3.124	0.08	0.52	1.26
	0.390	0.05	0.63	1.25
JWH-019	62.5	0.06	0.49	2.93
	3.905	0.02	0.51	1.09
	0.448	0.04	2.87	0.75
JWH-250	62.5	0.02	0.21	0.31
	3.905	0.03	0.65	0.51
	0.448	0.08	0.25	1.54

Table 11- Day-to-day precision n = 5 for synthetic cannabinoid mix (50 µg/mL each solute)

Solute	% RSD RT
HU-210	0.13
AKB-48	0.31
AM -2201	0.22
JWH-200	0.22
JWH-081	0.29
RCS-8	0.50
AB-Fubinaca	0.27
PB-22	0.26
JWH-122	0.12

Table 12 - Linearity and limits of detection for controlled bath salts

Solute	**Detection Mode**	**Concentration Range µg/mL**	**R^2**	**Limit of Detection µg/mL**
α-PVP	UV	2.5 – 80	0.99980	0.094
α -PVP	MS	0.004 – 0.98	0.9970	0.00039
α -PBP	UV	2.5 – 80	0.99958	0.094
α -PBP	MS	0.004 – 2.96	0.9942	0.0012
MDPV	UV	0.625 – 80	0.99990	0.190
MDPV	MS	0.0013 – 0.98	0.9932	0.00039
Naphyrone	UV	0.625 – 80	0.99980	0.094
Naphyrone	MS	0.0013 – 2.96	0.9950	0.00039
4-MePPP	UV	0.3125 – 80	0.99995	0.094
4-MePPP	MS	0.004 – 2.96	0.9940	0.0012
4-methylethcathinone	UV	0.625 – 80	0.99994	0.094
4-methylethcathinone	MS	0.012 - 80	0.9992	0.0036
Pentedrone	UV	2.5 – 80	0.99940	0.378
Pentedrone	MS	0.036 – 26.6	0.9924	0.011
Buphedrone	UV	0.3125 – 80	0.99993	0.094
Buphedrone	MS	0.012 – 8.8	0.9987	0.0036
4-Fluoromethcathinine	UV	1.25 – 80	0.99980	0.378
4-Fluoromethcathinine	MS	0.004 – 0.98	0.9986	0.0012
3-Fluoromethcathinine	UV	2.5 – 80	0.99957	0.378
3-Fluoromethcathinine	MS	0.004 – 2.96	0.9966	0.0012
Mephedrone	UV	0.625 – 80	0.99992	0.190
Mephedrone	MS	0.012 – 0.98	0.9954	0.0036
Methcathinone	UV	2.5 – 80	0.99948	0.378
Methcathinone	MS	0.012 – 2.96	0.9916	0.0036
Pentylone	UV	1.25 – 80	0.99993	0.190
Pentylone	MS	0.004 – 8.8	0.9961	0.0012
Butylone	UV	0.625 – 80	0.99989	0.190
Butylone	MS	0.0013 – 0.98	0.9911	0.00039
Methylone	UV	1.25 – 80	0.99980	0.378
Methylone	MS	0.004 – 0.98	0.9900	0.0012

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Table 13 - Run-to-run precision n=5 for bath salt solutions used for linearity studies

Solute	Concentration ug/mL		% RSD RT	% RSD Peak Area	
	UV	MS		UV	MS
α-PVP	80	80	0.1	0.35	0.86
	5	0.98	0.05	1.65	2.76
	0.625	0.012	0.07	1.14	2.86
α-PBP	80	80	0.05	0.54	2.24
	5	0.98	0.06	0.55	2.48
	0.625	0.012	0.1	0.81	2.68
MDPV	80	80	0.02	0.31	0.97
	5	0.98	0.07	0.58	1.07
	0.625	0.012	0.09	3	1.1
Naphyrone	80	80	0.09	0.36	0.43
	5	0.98	0.09	0.96	2.73
	0.625	0.012	0.12	1.8	0.79
4-MePPP	80	80	0.09	1.8	0.52
	5	0.98	0.07	0.81	2.32
	0.625	0.012	0.55	3.55	0.95
4-methylethcathinone	80	80	0.01	0.38	0.91
	5	0.98	0.06	1.67	0.9
	0.625	0.012	0.04	1.55	2.11
Pentedrone	80	80	0.09	0.75	1.48
	5	0.98	0.1	2.68	2.41
	0.625	0.012	0.19	----[a]	1.94
Buphedrone	80	80	0.07	0.91	0.73
	5	0.98	0.09	1.52	2.4
	0.625	0.012	0.28	1.13	0.88
4-fluoromethcathinone	80	80	0.07	0.44	0.93
	5	0.98	0.06	2.21	1.78
	0.625	0.012	0.13	2.28	1.53
3-fluoromethcathinone	80	80	0.08	0.47	1.5
	5	0.98	0.09	2.57	1.28
	0.625	0.012	0.14	----[a]	2.03
Mephredrone	80	80	0.02	0.44	0.69
	5	0.98	0.03	0.99	0.31
	0.625	0.012	0.08	0.68	0.9
Methcathinone	80	80	0.06	0.68	1.23
	5	0.98	0.07	0.82	1.07
	0.625	0.012	0.11	----[a]	0.4

Pentylone	80	80	0.1	1.68	0.42
	5	0.98	0.06	1.33	2.08
	0.625	0.012	0.11	2.29	1.54
Butylone	80	80	0.05	2.43	1.06
	5	0.98	0.06	0.56	2.74
	0.625	0.012	0.14	1.81	0.93
Methylone	80	80	0.06	0.52	1.71
	5	0.98	0.03	0.85	1.24
	0.625	0.012	0.16	2.5	1.18

[a]insufficient signal to noise at concentration below linearity range

Table 14 - Day-to-day precision n= 5 for bath salt mix (50 µg/mL each solute)

Solute	% RSD RT (UV detection)	%RSD RRT (Relative to 4-Fluoromethcathinone)
α- PVP	1.74	1.516
MDPV	2.49	1.045
Pentedrone	2.08	0.980
4- Fluoromethcathinone	3.52	0.000
Pentylone	2.27	0.767
Mephedrone	3.86	0.887
Methylone	3.39	0.529

Table 15 - Analysis of simulated synthetic cannabinoid samples

mixture	mix 1			
plant	Marshmallow			
	UV		MS	
compounds	CP 47,497	JWH-018	CP 47,497	JWH-018
Target Concentration (ug/mL)	4	4	4	4
Actual Concentration (ug/mL)				
sample 1	4.74	3.69	4.93	3.72
sample 2	3.95	3.92	4.28	4.02
AVERAGE	**4.34**	**3.81**	**4.60**	**3.87**

mixture	mix 2			
plant	Bay Bean			
	UV		MS	
compounds	JWH-018	JWH-081	JWH-018	JWH-081
Target Concentration (ug/mL)	16	2	16	2
Actual Concentration (ug/mL)				
sample 1	16.44	1.88	14.69	1.90
sample 2	16.54	1.95	14.71	1.93
AVERAGE	**16.49**	**1.91**	**14.70**	**1.91**

mixture	mix 3					
plant	Marshmallow					
	UV			MS		
compounds	RCS-4	JWH-019	JWH-122	RCS-4	JWH-019	JWH-122
Target Concentration (ug/mL)	2	2	2	2	2	2
Actual Concentration (ug/mL)						
sample 1	2.06	2.10	2.06	[a]	[a]	[a]
sample 2	2.07	2.10	1.95	[a]	[a]	[a]
AVERAGE	**2.06**	**2.10**	**2.01**			

mixture	mix 4			
plant	Marshmallow			
	UV		MS	
compounds	JWH-018	AM-2201	JWH-018	AM-2201
Target Concentration (ug/mL)	2	2	2	2
Actual Concentration (ug/mL)				
sample 1	1.91	2.02	[a]	[a]
sample 2	1.87	2.03	[a]	[a]
AVERAGE	**1.89**	**2.02**		

mixture	mix 5			
plant	Marshmallow			
	UV		MS	
compounds	RCS-8	JWH-081	RCS-8	JWH-081
Target Concentration (ug/mL)	4	4	4	4
Actual Concentration (ug/mL)				
sample 1	4.09	3.94	4.09	3.98
sample 2	4.03	3.96	3.99	4.00
AVERAGE	**4.06**	**3.95**	**4.04**	**3.99**

mixture	mix 6			
plant	Bay Bean			
	UV		MS	
compounds	JWH-019	JWH-203	JWH-019	JWH-203
Target Concentration (ug/mL)	4	2	4	2
Actual Concentration (ug/mL)				
sample 1	[b]	[b]	4.11	1.93
sample 2	[b]	[b]	4.02	1.94
AVERAGE			**4.07**	**1.93**

mixture	mix 7			
plant	Damiana			
	UV		MS	
compounds	JWH-250	AKB-48	JWH-250	AKB-48
Target Concentration (ug/mL)	2	2	2	2
Actual Concentration (ug/mL)				
sample 1	1.88	1.94	1.96	2.00
sample 2	1.86	2.06	1.93	2.08
AVERAGE	**1.87**	**2.00**	**1.94**	**2.04**

mixture	mix 8			
plant	Damiana			
	UV		MS	
compounds	HU-210	JWH-250	HU-210	JWH-250
Target Concentration (ug/mL)	2	2	2	2
Actual Concentration (ug/mL)				
sample 1	2.24	1.97	1.85	2.01
sample 2	2.59	2.09	2.17	2.10
AVERAGE	**2.41**	**2.03**	**2.01**	**2.05**

mixture	mix 9			
plant	Damiana			
	UV		MS	
compounds	JWH-250	JWH-203	JWH-250	JWH-203
Target Concentration (ug/mL)	2	8	2	8
Actual Concentration (ug/mL)				
sample 1	[b]	[b]	2.32	8.55
sample 2	[b]	[b]	1.87	7.66
AVERAGE			**2.09**	**8.11**

mixture	mix 10			
plant	Damiana			
	UV		MS	
compounds	JWH-122	RCS-8	JWH-122	RCS-8
Target Concentration (ug/mL)	16	8	16	8
Actual Concentration (ug/mL)				
sample 1	15.40	7.07	13.56	6.75
sample 2	14.75	8.03	13.02	7.53
AVERAGE	**15.08**	**7.55**	**13.29**	**7.14**

mixture	mix 11			
plant	Damiana			
	UV		MS	
compounds	JWH--73	JWH-018	JWH-073	JWH-018
Target Concentration (ug/mL)	4	4	4	4
Actual Concentration (ug/mL)				
sample 1	[b]	[b]	4.13	4.30
sample 2	[b]	[b]	4.21	4.40
AVERAGE			**4.17**	**4.35**

mixture	mix 12			
plant	Bay Bean			
	UV		MS	
compounds	AB-Fubinaca	JWH-122	AB-Fubinaca	JWH-122
Target Concentration (ug/mL)	2	2	2	2
Actual Concentration (ug/mL)				
sample 1	1.98	1.99	2.12	2.02
sample 2	2.08	1.99	2.21	1.98
AVERAGE	**2.03**	**1.99**	**2.17**	**2.00**

mixture	mix 13					
plant	Bay Bean					
	UV			MS		
compounds	RCS-4	JWH-073	JWH-81	RCS-4	JWH-073	JWH-081
Target Concentration (ug/mL)	8	16	4	8	16	4
Actual Concentration (ug/mL)						
sample 1	7.24	15.30	3.81	6.67	14.07	3.80
sample 2	7.55	15.06	3.86	6.71	13.76	3.705
AVERAGE	**7.39**	**15.18**	**3.83**	**6.69**	**13.92**	**3.75**

mixture	mix 14					
plant	Bay Bean					
	UV			MS		
compounds	AB-Fubinaca	RCS-4	JWH-19	AB-Fubinaca	RCS-4	JWH-019
Target Concentration (ug/mL)	2	2	2	2	2	2
Actual Concentration (ug/mL)						
sample 1	1.96	1.89	1.99	2.06	1.96	1.99
sample 2	1.88	2.00	2.07	2.12	2.04	2.09
AVERAGE	**1.92**	**1.95**	**2.03**	**2.09**	**2.00**	**2.04**

mixture	mix 15			
plant	Honey Goat Weed			
	UV		MS	
compounds	CP 47, 497	JWH-018	CP 47, 497	JWH-018
Target Concentration (ug/mL)	2	2	2	2
Actual Concentration (ug/mL)				
sample 1	1.97	2.30	1.99	2.33
sample 2	1.85	2.14	1.97	2.25
AVERAGE	**1.91**	**2.22**	**1.98**	**2.29**

mixture	mix 16			
plant	Honey Goat Weed			
	UV		MS	
compounds	AB-Fubinaca	AM 2201	AB-Fubinaca	AM 2201
Target Concentration (ug/mL)	8	2	8	2
Actual Concentration (ug/mL)				
sample 1	8.10	2.14	7.45	2.13
sample 2	7.18	2.13	6.85	2.10
AVERAGE	**7.64**	**2.13**	**7.15**	**2.12**

mixture	mix 17			
plant	Honey Goat Weed			
	UV		MS	
compounds	JWH-018	JWH-250	JWH-018	JWH-250
Target Concentration (ug/mL)	2	4	2	4
Actual Concentration (ug/mL)				
sample 1	[b]	[b]	2.07	4.05
sample 2	[b]	[b]	2.08	4.58
AVERAGE			**2.07**	**4.31**

mixture	mix 18			
plant	Honey Goat Weed			
	UV		MS	
compounds	JWH-203	JWH-122	JWH-203	JWH-122
Target Concentration (ug/mL)	8	2	8	2
Actual Concentration (ug/mL)				
sample 1	8.90	2.06	8.485	2.04
sample 2	9.18	2.11	8.66	2.00
AVERAGE	**9.04**	**2.08**	**8.57**	**2.02**

mixture	mix 19			
plant	Honey Goat Weed			
	UV		MS	
compounds	JWH-203	RCS-8	JWH-203	RCS-8
Target Concentration (ug/mL)	2	2	2	2
Actual Concentration (ug/mL)				
sample 1	2.03	2.13	2.01	2.10
sample 2	1.96	1.99	1.97	2.00
AVERAGE	**2.00**	**2.06**	**1.99**	**2.05**

mixture	mix 20			
plant	Bay Bean			
	UV		MS	
compounds	AKB-48	JWH-073	AKB-48	JWH-073
Target Concentration (ug/mL)	4	8	4	8
Actual Concentration (ug/mL)				
sample 1	4.30	7.45	4.21	7.29
sample 2	4.31	7.91	4.21	7.65
AVERAGE	**4.30**	**7.68**	**4.21**	**7.47**

[a] no MS data available

[b] no UV data due to coelution

Table 16 - Analysis of simulated synthetic cathinone samples

mixture	mix 1			
matrix	Lidocaine (1 mg/mL)			
	3-fluromethcathinone		Butylone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	75.00	75.00	75.00	75.00
Actual Concentration (ug/mL)				
sample 1	72.08	74.39	72.53	74.28
sample 2	70.93	74.72	71.24	74.48
AVERAGE	**71.50**	**74.56**	**71.89**	**74.38**

mixture	mix 2			
matrix	Pancake Mix (0.5 mg/mL)			
	4-methylethcathinone		α-PBP	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	37.50	37.50	37.50	37.50
Actual Concentration (ug/mL)				
sample 1	37.25	34.67	40.02	36.33
sample 2	37.18	35.66	38.72	36.78
AVERAGE	**37.21**	**35.16**	**39.37**	**36.55**

mixture	mix 3			
matrix	Lidocaine (0.5 mg/mL)			
	Buphedrone		Pentedrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	37.50	37.50	37.50	37.50
Actual Concentration (ug/mL)				
sample 1	37.74	38.87	37.77	40.01
sample 2	38.26	42.30	38.09	43.13
AVERAGE	**38.00**	**40.59**	**37.93**	**41.57**

mixture	mix 4			
matrix	Lidocaine (0.5 mg/mL)			
	Naphyrone		4-methylethcathinone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	37.50	37.50	37.50	37.50
Actual Concentration (ug/mL)				
sample 1	37.08	39.75	36.90	39.74
sample 2	36.74	39.28	36.56	38.93
AVERAGE	**36.91**	**39.51**	**36.73**	**39.33**

mixture	mix 5			
matrix	Benzocaine (1 mg/mL)			
	4-MePPP		Pentedrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	75.00	75.00	75.00	75.00
Actual Concentration (ug/mL)				
sample 1	[b]	75.95	[b]	74.89
sample 2	[b]	74.23	[b]	73.56
AVERAGE		**75.09**		**74.23**

mixture	mix 6			
matrix	Caffeine (1 mg/mL)			
	Butylone		Methedrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	37.50	37.50	37.50	37.50
Actual Concentration (ug/mL)				
sample 1	[b]	34.95	[b]	34.30
sample 2	[b]	35.48	[b]	35.93
AVERAGE		**35.21**		**35.11**

mixture	mix 7							
matrix	Benzocaine (187 ug/mL)							
	Methcathinone		Methylone		Pentylone		α-PVP	
compounds	UV	MS	UV	MS	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	62.50	62.50	62.50	62.50	62.50	62.50
Actual Concentration (ug/mL)								
sample 1	62.75	60.27	64.45	62.10	62.63	61.30	62.31	61.19
sample 2	62.17	59.72	63.30	59.79	61.90	59.58	61.91	59.98
AVERAGE	**62.46**	**59.99**	**63.87**	**60.94**	**62.27**	**60.44**	**62.11**	**60.58**

mixture	mix 8							
matrix	Pancake Mix (187 ug/mL)							
	Methcathinone		Methylone		Pentylone		α-PVP	
compounds	UV	MS	UV	MS	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	62.50	62.50	62.50	62.50	62.50	62.50
Actual Concentration (ug/mL)								
sample 1	65.76	65.98	65.49	65.38	66.45	64.61	65.28	64.39
sample 2	61.01	61.05	59.68	60.00	61.67	59.94	60.59	61.18
AVERAGE	**63.38**	**63.52**	**62.58**	**62.69**	**64.06**	**62.27**	**62.94**	**62.78**

mixture	mix 9							
matrix	Lidocaine (187 ug/mL)							
	Methcathinone		Methylone		Pentylone		α-PVP	
compounds	UV	MS	UV	MS	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	62.50	62.50	62.50	62.50	62.50	62.50
Actual Concentration (ug/mL)								
sample 1	62.57	62.29	60.88	61.34	62.45	60.29	61.54	60.73
sample 2	62.85	61.75	61.97	60.77	62.12	61.44	61.80	60.55
AVERAGE	**62.71**	**62.02**	**61.43**	**61.05**	**62.28**	**60.86**	**61.67**	**60.64**

mixture	mix 10			
matrix	Pancake mix (187 ug/mL)			
	4-fluoromethcathinone		4-MePPP	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	12.50	12.50	62.50	
Actual Concentration (ug/mL)				
sample 1	11.49	[a]	62.61	[a]
sample 2	11.64	[a]	62.70	[a]
AVERAGE	**11.56**		**62.65**	

mixture	mix 11			
matrix	Benzocaine (187 ug/mL)			
	MDPV		Pentylone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	50.00	50.00	25.00	25.00
Actual Concentration (ug/mL)				
sample 1	49.87	52.17	25.42	26.05
sample 2	49.11	50.59	25.16	25.75
AVERAGE	**49.49**	**51.38**	**25.29**	**25.90**

mixture	mix 12							
matrix	Caffeine (187 ug/mL)							
	Methcathinone		Methylone		Pentylone		α-PVP	
compounds	UV	MS	UV	MS	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	62.50	62.50	62.50	62.50	62.50	62.50
Actual Concentration (ug/mL)								
sample 1	63.07	61.36	63.74	61.63	62.70	60.53	64.40	60.72
sample 2	62.63	62.80	63.82	63.21	62.98	63.53	64.87	62.02
AVERAGE	**62.85**	**62.08**	**63.78**	**62.42**	**62.84**	**62.03**	**64.63**	**61.37**

mixture	mix 13			
matrix	Benzocaine (187 ug/mL)			
	α- PVP		Butylone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	25.00	25.00	75.00	75.00
Actual Concentration (ug/mL)				
sample 1	24.89	24.16	74.33	72.42
sample 2	24.10	23.37	72.37	71.25
AVERAGE	**24.50**	**23.76**	**73.35**	**71.83**

mixture	mix 14			
matrix	Pancake Mix (187 ug/mL)			
	Pentedrone		Mephedrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	62.50	62.50
Actual Concentration (ug/mL)				
sample 1	63.71	62.365	63.64	62.05
sample 2	62.46	59.79	62.67	61.13
AVERAGE	**63.08**	**61.08**	**63.15**	**61.59**

mixture	mix 15			
matrix	Caffeine (187 ug/mL)			
	Buphedrone		Pentedrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	25.00	25.00	50.00	50.00
Actual Concentration (ug/mL)				
sample 1	25.90	25.23	52.00	50.56
sample 2	25.04	25.29	50.29	50.44
AVERAGE	**25.47**	**25.26**	**51.14**	**50.50**

mixture	mix 16			
matrix	Pancake Mix (187 ug/mL)			
	MDPV		Butylone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	31.25	31.25
Actual Concentration (ug/mL)				
sample 1	62.98	63.95	31.98	31.97
sample 2	63.64	64.23	32.05	31.16
AVERAGE	**63.31**	**64.09**	**32.01**	**31.56**

mixture	mix 17			
matrix	Lidocaine (75 ug/mL)			
	α- PBP		Buphedrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	25.00	25.00
Actual Concentration (ug/mL)				
sample 1	69.14	64.12	25.78	25.35
sample 2	66.90	62.90	24.91	25.26
AVERAGE	**68.02**	**63.51**	**25.34**	**25.30**

mixture	mix 18			
matrix	Lidocaine (75 ug/mL)			
	4-methylethcathinone		4-MePPP	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	62.50	62.50	12.50	12.50
Actual Concentration (ug/mL)				
sample 1	61.15	59.94	11.78	11.69
sample 2	62.30	59.35	11.70	11.73
AVERAGE	**61.72**	**59.64**	**11.74**	**11.71**

mixture	mix 19			
matrix	Caffeine (75 ug/mL)			
	3-fluoromethcathinone		Naphyrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	12.50	12.50	50.00	50.00
Actual Concentration (ug/mL)				
sample 1	11.53	11.89	49.88	49.07
sample 2	11.61	11.90	49.80	49.26
AVERAGE	**11.57**	**11.89**	**49.84**	**49.17**

mixture	mix 20			
matrix	Caffeine (75 ug/mL)			
	Mephedrone		Pentedrone	
compounds	UV	MS	UV	MS
Target Concentration (ug/mL)	31.25	31.25	25.00	25.00
Actual Concentration (ug/mL)				
sample 1	31.10	29.12	24.92	24.47
sample 2	30.12	30.68	24.00	25.05
AVERAGE	**30.61**	**29.90**	**24.46**	**24.76**

[a] no MS data available
[b] no UV data due to coelution

Figures

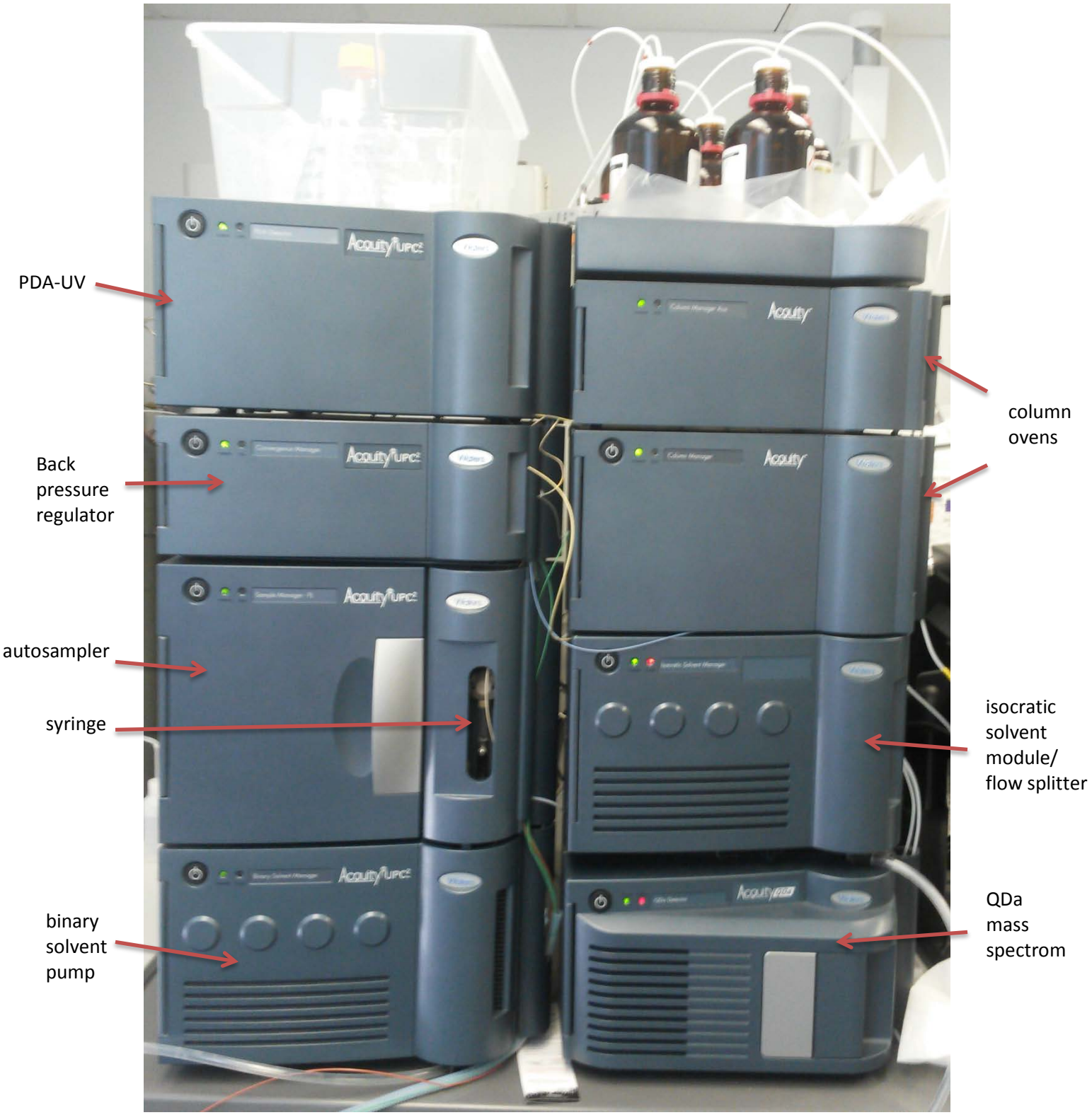
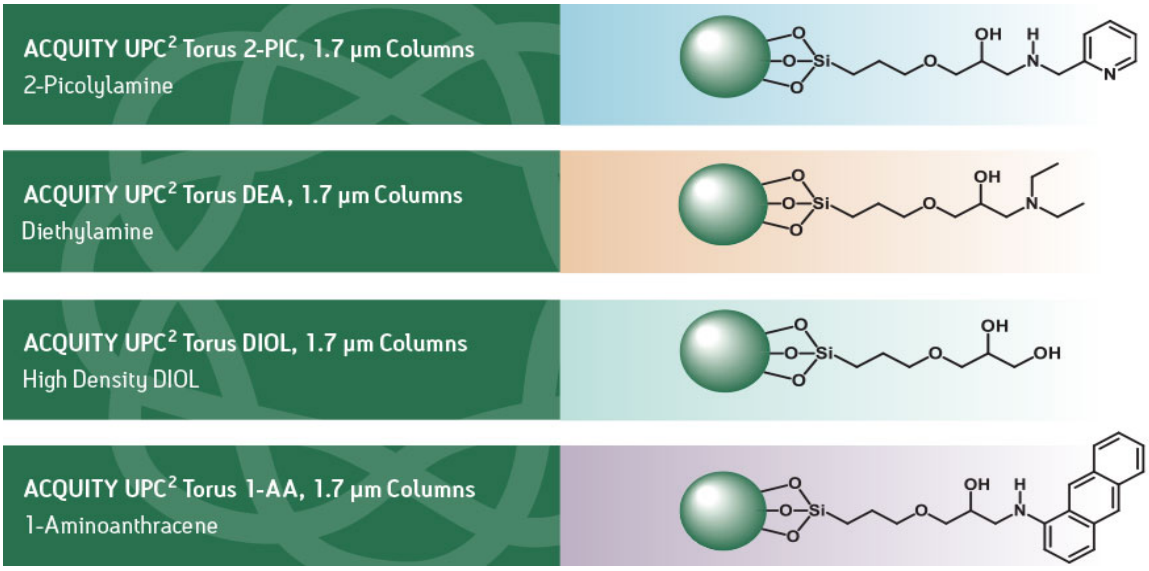


Figure 1

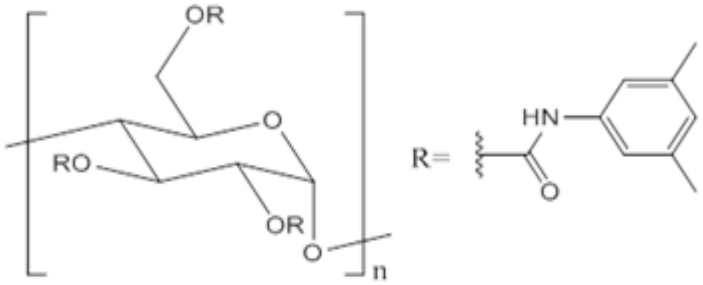
UHPSFC Waters Achiral Columns
3.0 mm x 100 mm



http://www.waters.com/waters/en_US/ACQUITY-UPC2-Trefoil-and-Torus-Columns/nav.htm?cid=134696052&locale=en_US

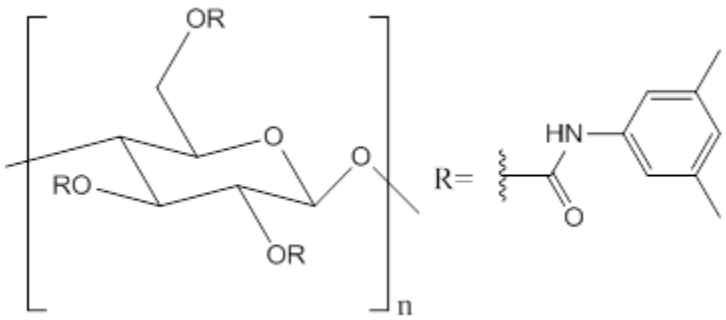
Figure 2

UHPSFC Waters Chiral Columns
Acquity UPC2 Trefoil
3.0 mm x 150 mm



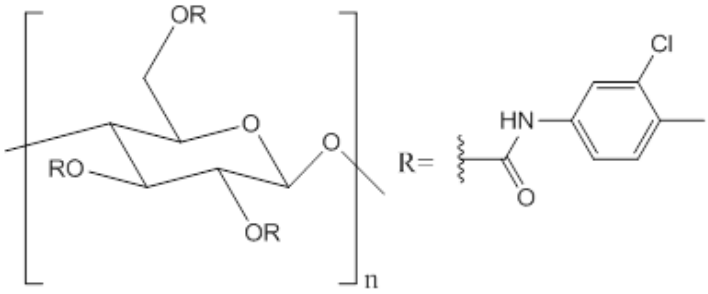
AMY1: Amylose tris-(3,5-dimethylphenylcarbamate)

Chiral polysaccharide coating on silica particle



CEL1: Cellulose tris-(3,5-dimethylphenylcarbamate)

Chiral Selectors

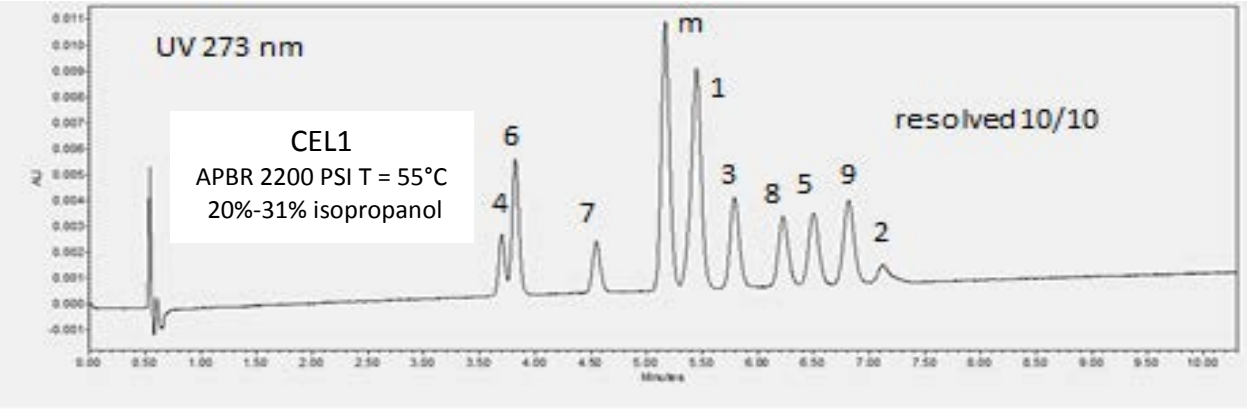


CEL2: Cellulose tris-(3-chloro-4-methylphenylcarbamate)

Courtesy Waters

Figure 3

UHPSFC JWH-018
Positional Isomers



1) JWH-016 m) JWH 018 2) 2'-napthyl isomer 3) 2'-napthyl-N-(1,1-dimethylpropyl) isomer
4) 2'-napthyl-N-(1,2-dimethylpropyl) isomer 5) 2'-napthyl-N-(2,2-dimethylpropyl) isomer
6) 2'-napthyl-N-(1 ethylpropyl) isomer 7) 2'-napthyl-N-(1 methylbutyl) isomer
8) 2'-napthyl-N-(2 methylbutyl) isomer 9) 2'-napthyl-N-(3 methylbutyl) isomer

Figure 4

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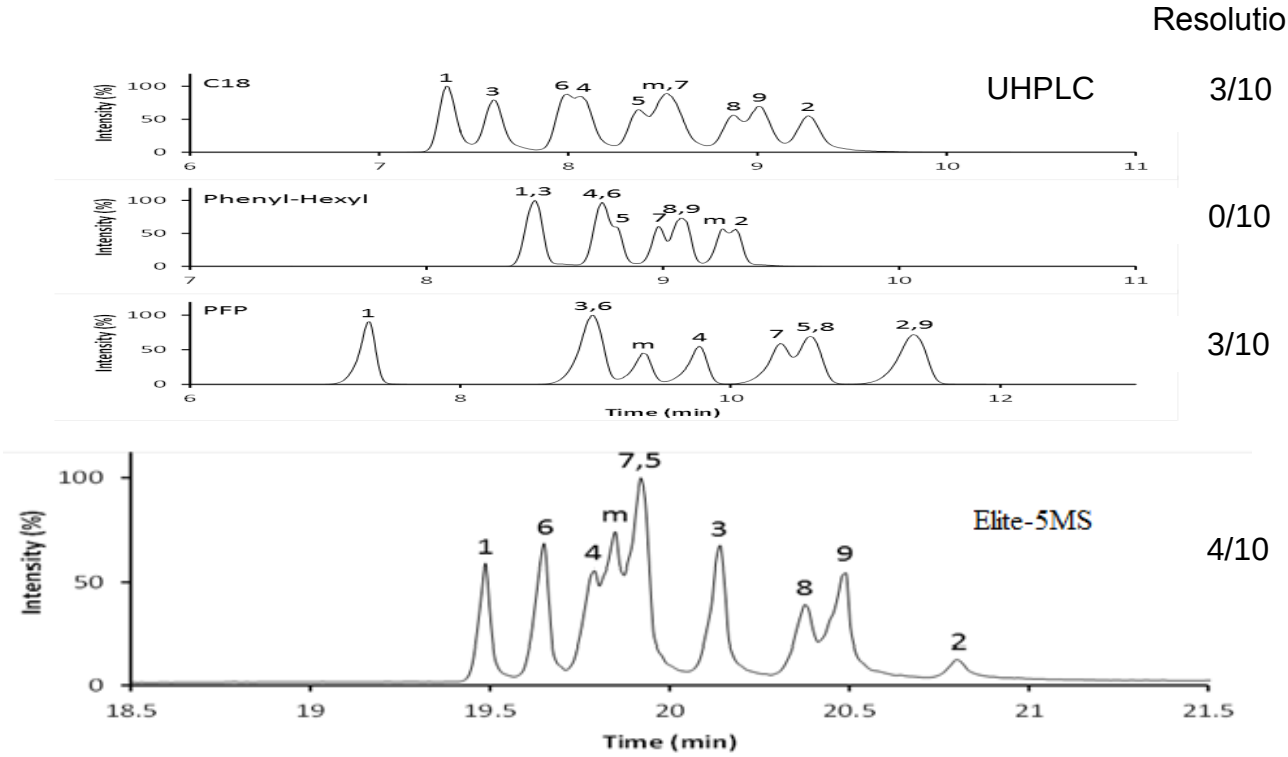
UHPLC VS GC
JWH-018 Positional Isomers



1) JWH-016 m) JWH-018 2) 2'-napthyl isomer 3) 2'-napthyl-N-(1,1-dimethylpropyl) isomer
4) 2'-napthyl-N-(1,2-dimethylpropyl) isomer 5) 2'-napthyl-N-(2,2-dimethylpropyl) isomer
6) 2'-napthyl-N-(1 ethylpropyl) isomer 7) 2'-napthyl-N-(1 methylbutyl) isomer
8) 2'-napthyl-N-(2 methylbutyl) isomer 9) 2'-napthyl-N-(3 methylbutyl) isomer)

*See reference #3 for chromatographic conditions

Figure 5

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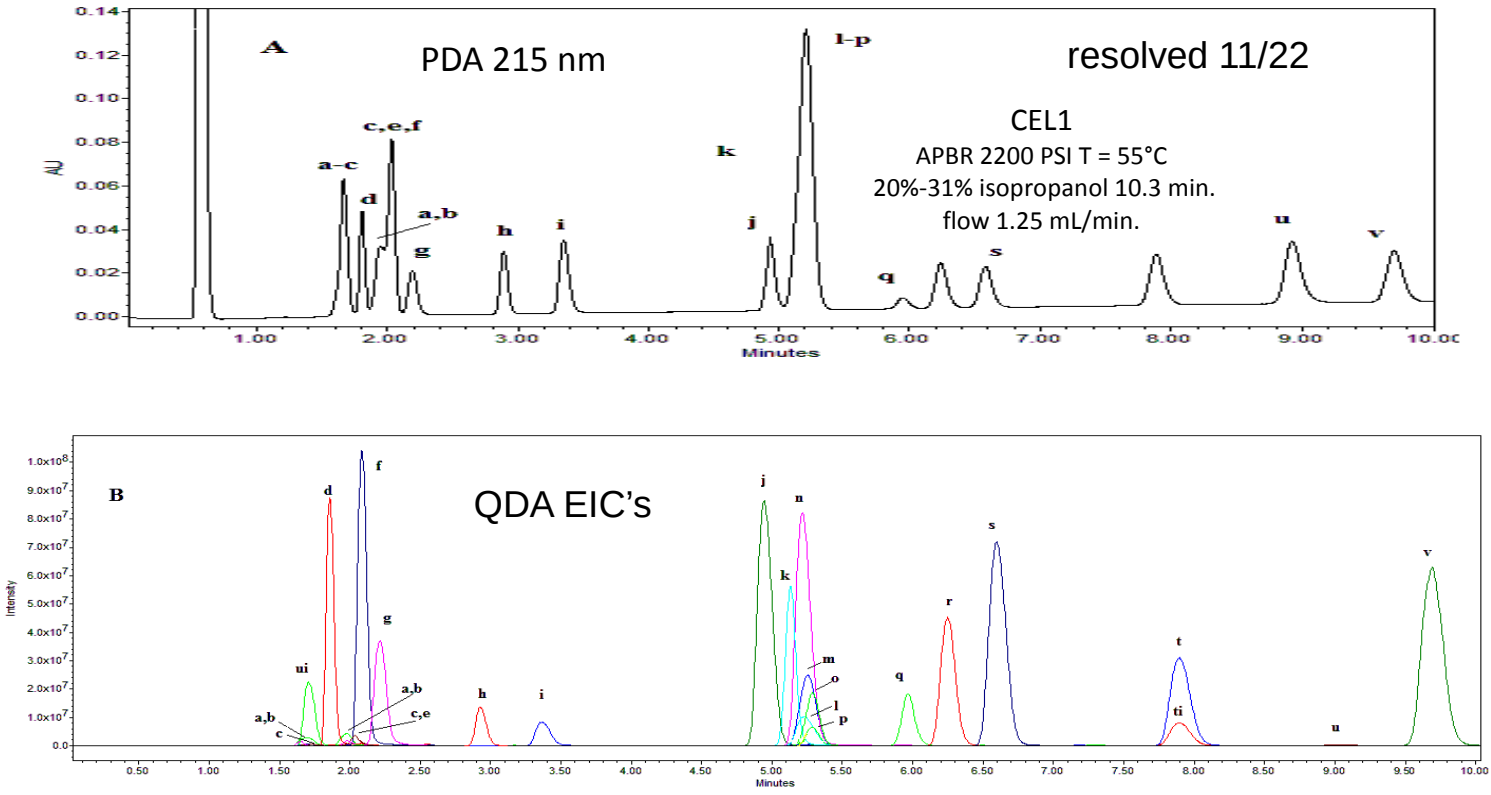
UHPSFC Controlled Synthetic Cannabinoids



a) CP47, 497 b) Epi-CP47, 497 c) 3-epi CP47, 497 C8 d) UR-144 e) CP47, 497 C8
f) XLR-11 g) HU-210 h) AKB-48 i) AB-Fubinaca j) RCS-4
k) AM-694 l) JWH-203 m) JWH-018 n) JWH-250 o) JWH-073
p) JWH-019 q) JWH-122 r) AM-2201 s) RCS-8 t) PB-22
ti) PB-22 u) JWH-200 v) JWH-081 ui) JWH-081 possible breakdown product

Figure 6

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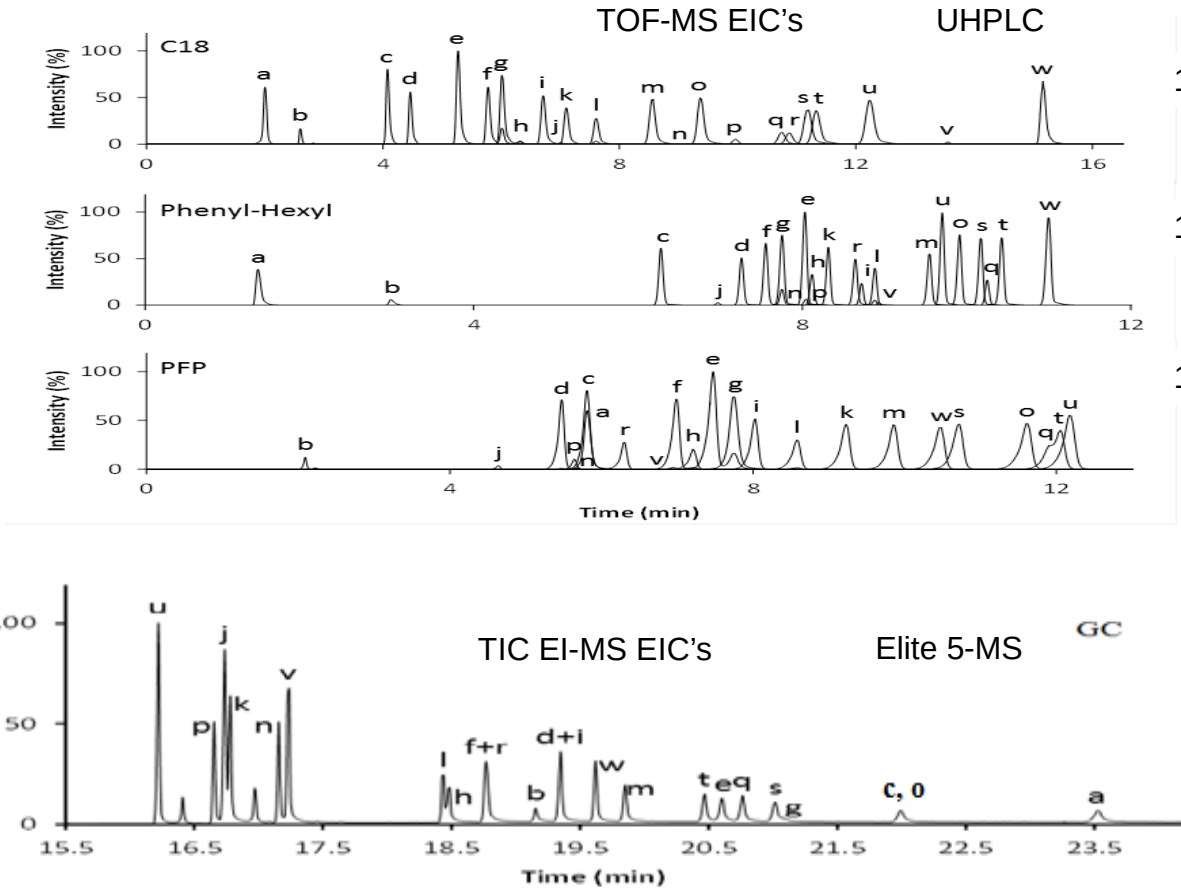
UHPLC VS GC
Synthetic Cannabinoids

Resolution ≥1



a) JWH-200 b) AB-Fubinaca c) 5-Fluoro PB-22 d) AM-694 e) AM-2201 f) RXS-4
g) PB-22 h) JWH-250 i) JWH-073 j) CP-47 497 k) XLR-11 l) JWH-203
m) JWH-018 n) CP47, 497 homologue o) JWH-081 p) Epi- CP47, 497
q) JWH-122 r) HU-210 s) RCS-8 t) JWH-019 u) UR-144
v) 3-epi-CP47, 497 C8 w) AKB-48

*See reference #3 for chromatographic conditions.

Figure 7

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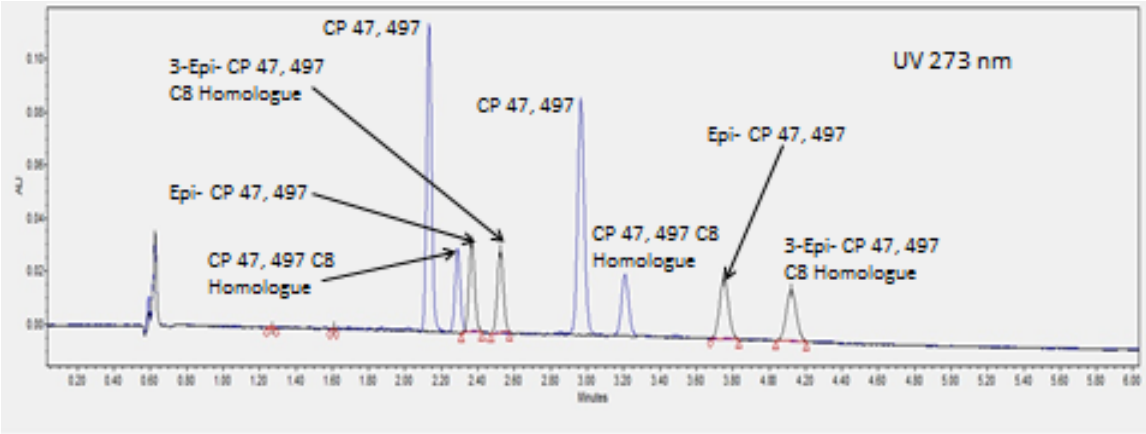
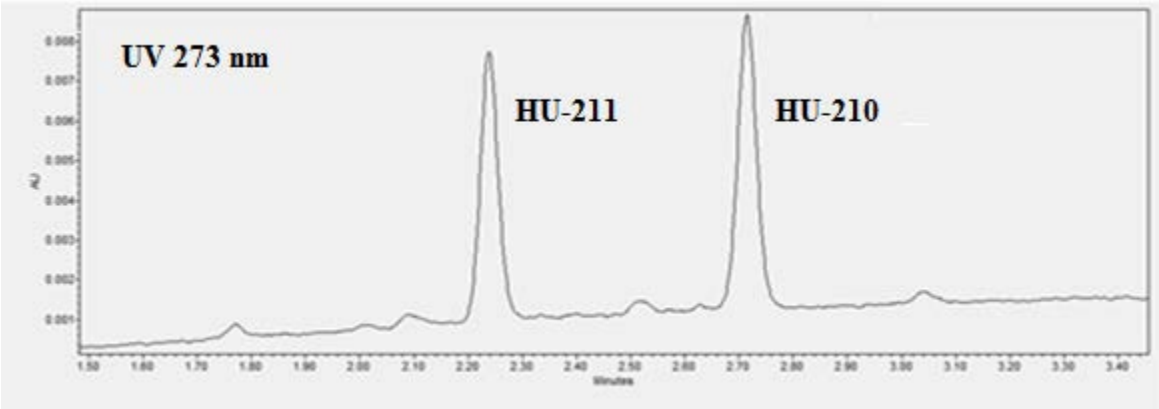
Synthetic Cannabinoids Enantiomers



AM1
APBR 2200 PSI T = 45°C
15%-65% methanol 5 min.
hold 1 min flow 1.25 ml/min.

Figure 8

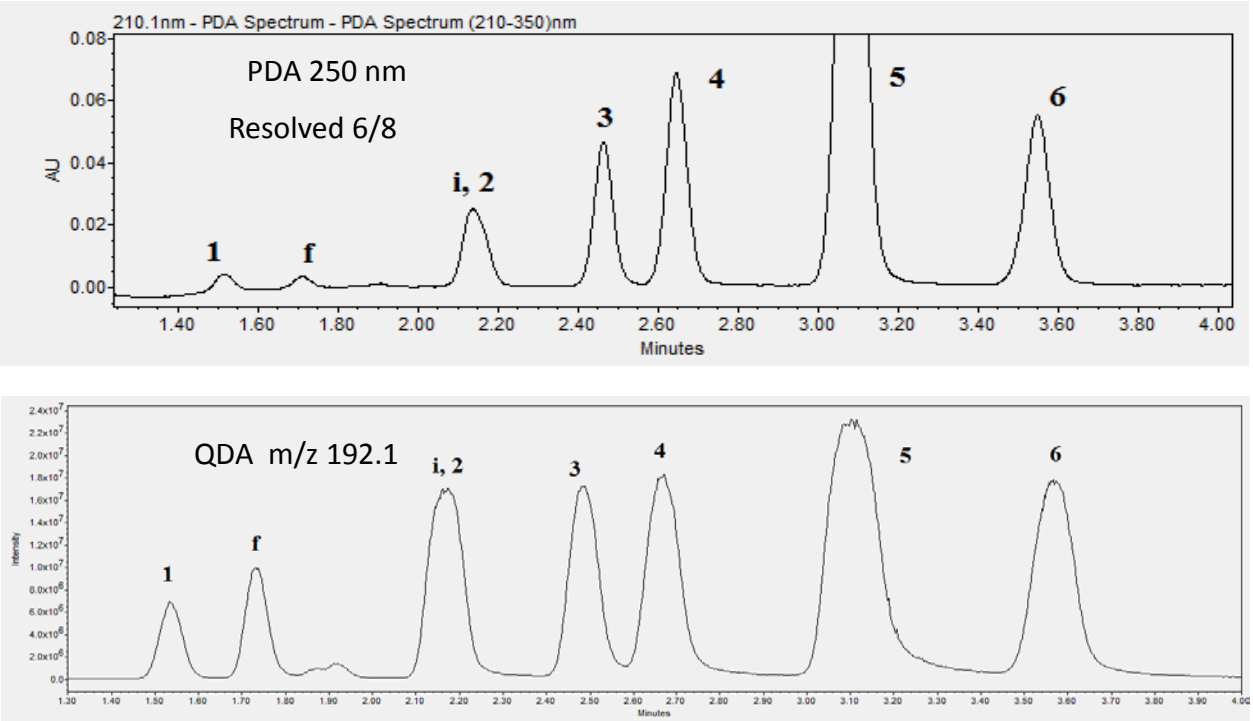
Synthetic Cannabinoids Diasteroisomers



AMY1
APBR 2200 PSI T = 45°C
18%-53% isopropanol 5 min.
hold 1 min flow 1.25 ml/min.

Figure 9

UHPSFC Pentedrone, 4-Methylethcathinone and Six Positional Isomers



f) Pentedrone i) 4-methylethcathinone 1) Isopentedrone 2) 4-methylbuphedrone
3) 2-ethylmethcathinone 4) 2,3-dimethylmethcathinone
5) 2,4-dimethylmethcathinone 6) 3,4-dimethylmethcathinone

DIOL
APBR 2200 PSI T = 40°C
3% methanol, 10mM ammonium formate
flow 1.25 ml/min.

Figure 10

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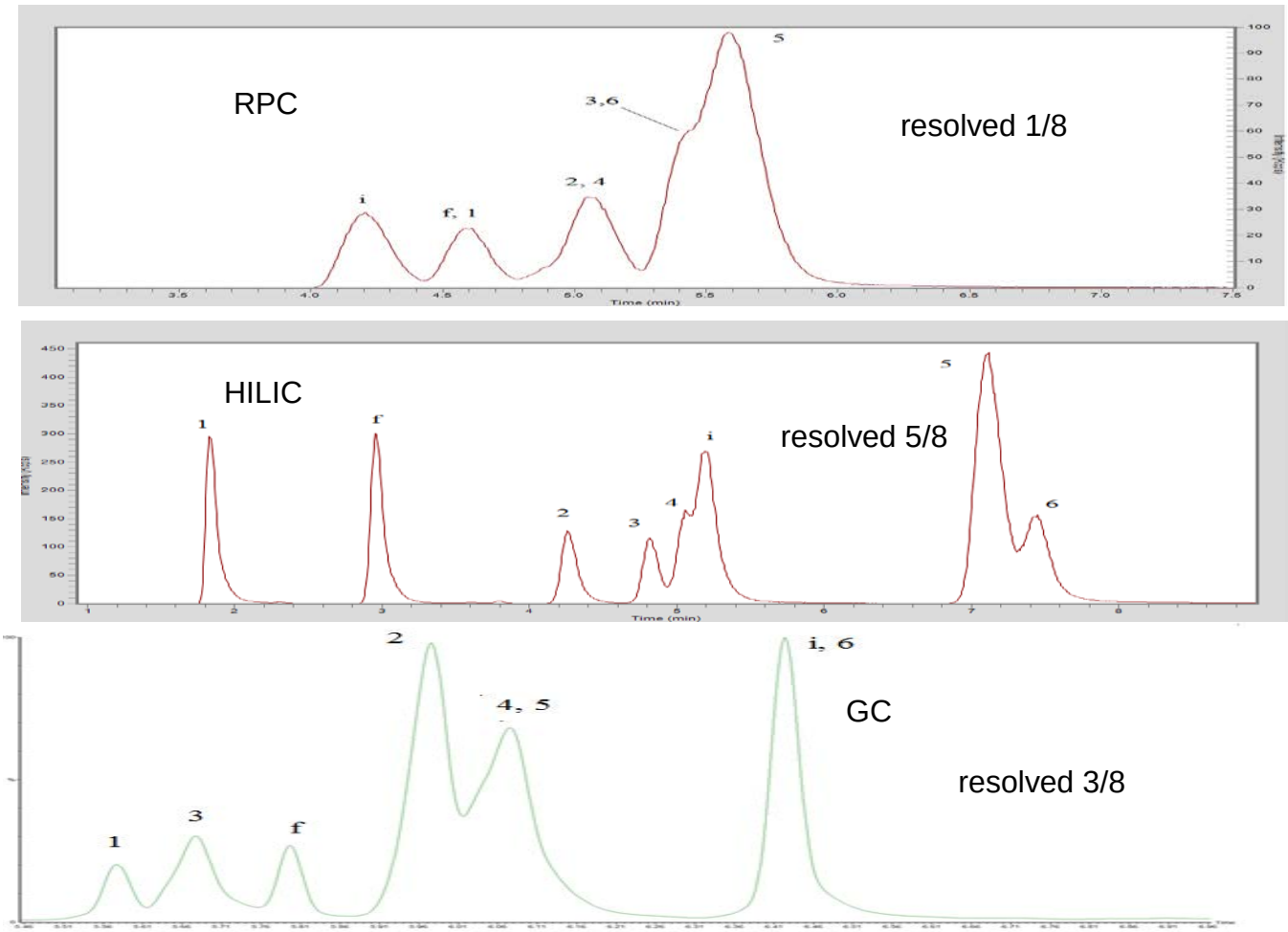
Bath Salts Pentedrone, 4-methylethcathinone and Six Positional Isomers



f) Pentedrone i) 4-methylethcathinone 1) Isopentedrone 2) 4-methylbuphedrone
3) 2-ethylmethcathinone 4) 2,3-dimethylmethcathinone
5) 2,4-dimethylmethcathinone 6) 3,4-dimethylmethcathinone

DIOL
APBR 2200 PSI T = 40°C
3% methanol, 10mM ammonium formate
flow 1.25 ml/min.

Figure 11

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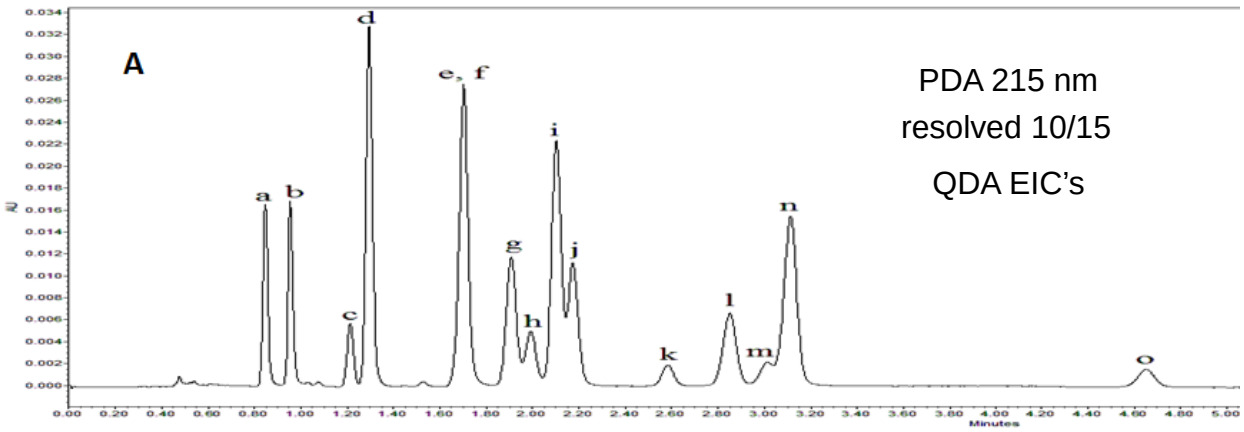
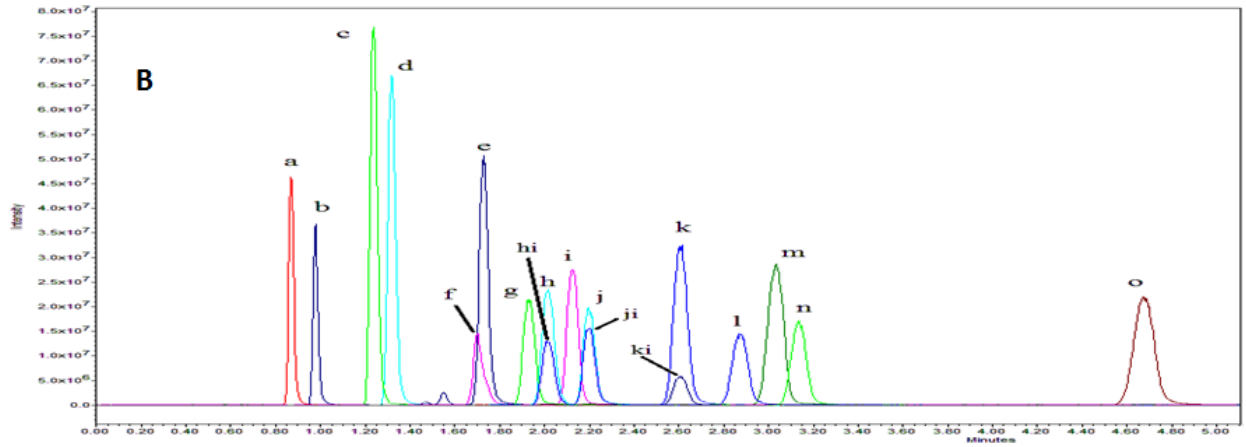
UHPSFC Bath Salts



DIOL
APBR 2200 PSI T = 40°C
3% methanol, 10mM ammonium formate
flow 1.25 ml/min.



a) α- PVP b) α- PBP c) MDPV d) Naphyrone e) 4-MePPP f) Pentedrone
g) Buphedrone h) 3-fluoromethcathinone hi) 3-fluoromethcathinone
i) 4-methylethcathinone j) 4-fluoromethcathinone ji) 4-fluoromethcathinone
k) Pentylone ki) Pentylone l) Methcathinone m) Butylone n) Mephedrone o) Methylone

Figure 12

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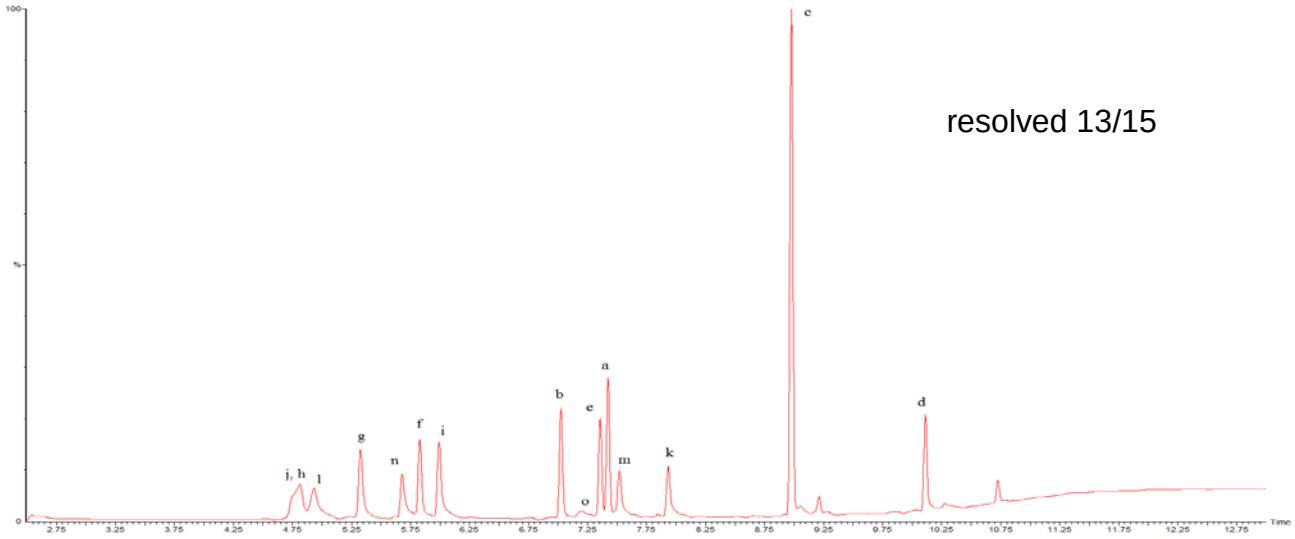
GC Bath Salts

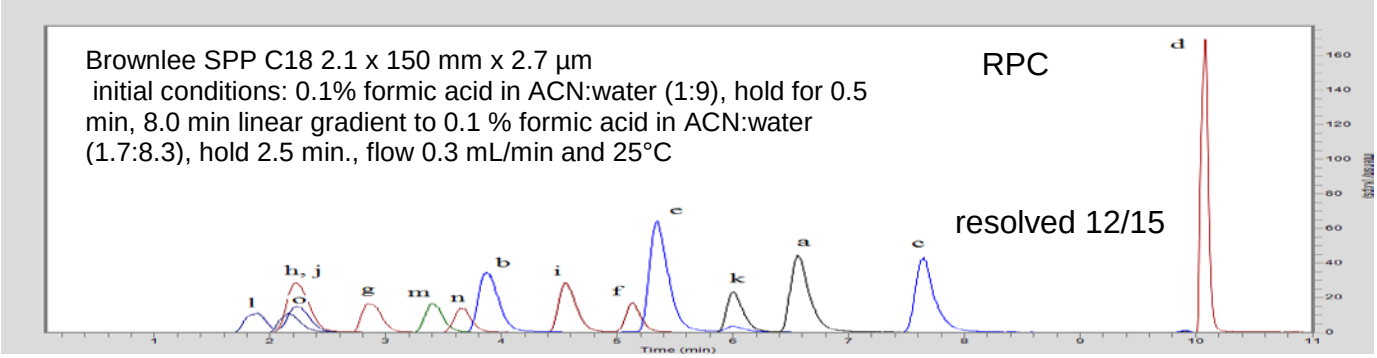


a) α- PVP b) α- PBP c) MDPV d) Naphyrone e) 4-MePPP f) Pentedrone
g) Buphedrone h) 3-fluoromethcathinone hi) 3-fluoromethcathinone
i) 4-methylethcathinone j) 4-fluoromethcathinone ji) 4-fluoromethcathinone
k) Pentylone ki) Pentylone l) Methcathinone m) Butylone n) Mephedrone o) Methylone

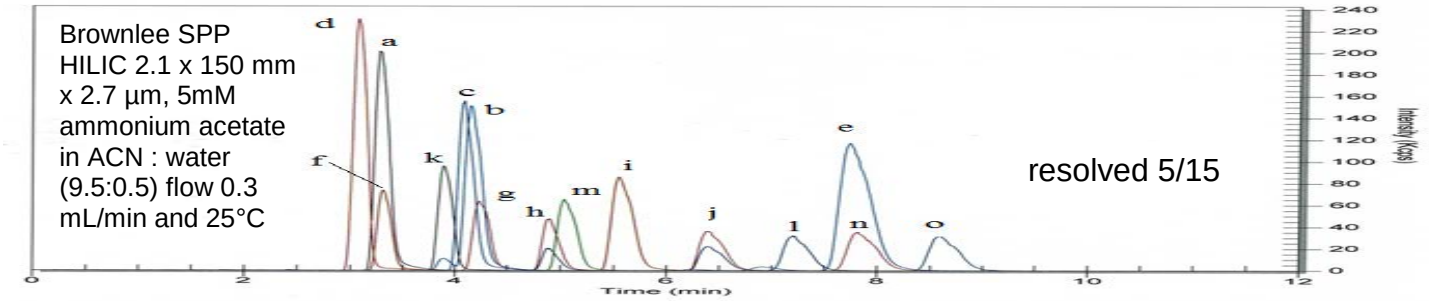
Elite 5MS 30m x 250 x 0.25 µm.
Initial temp. 100°C for 1.0 min.,
ramp to 300°C at 20°C/min,
hold final temp. for 2.0 min.

Figure 13

TOF-MS EIC's



HILIC



a) α- PVP b) α- PBP c) MDPV d) Naphyrone e) 4-MePPP f) Pentedrone
g) Buphedrone h) 3-fluoromethcathinone hi) 3-fluoromethcathinone
i) 4-methylethcathinone j) 4-fluoromethcathinone ji) 4-fluoromethcathinone
k) Pentylone ki) Pentylone l) Methcathinone m) Butylone n) Mephedrone o) Methylone

Figure 14

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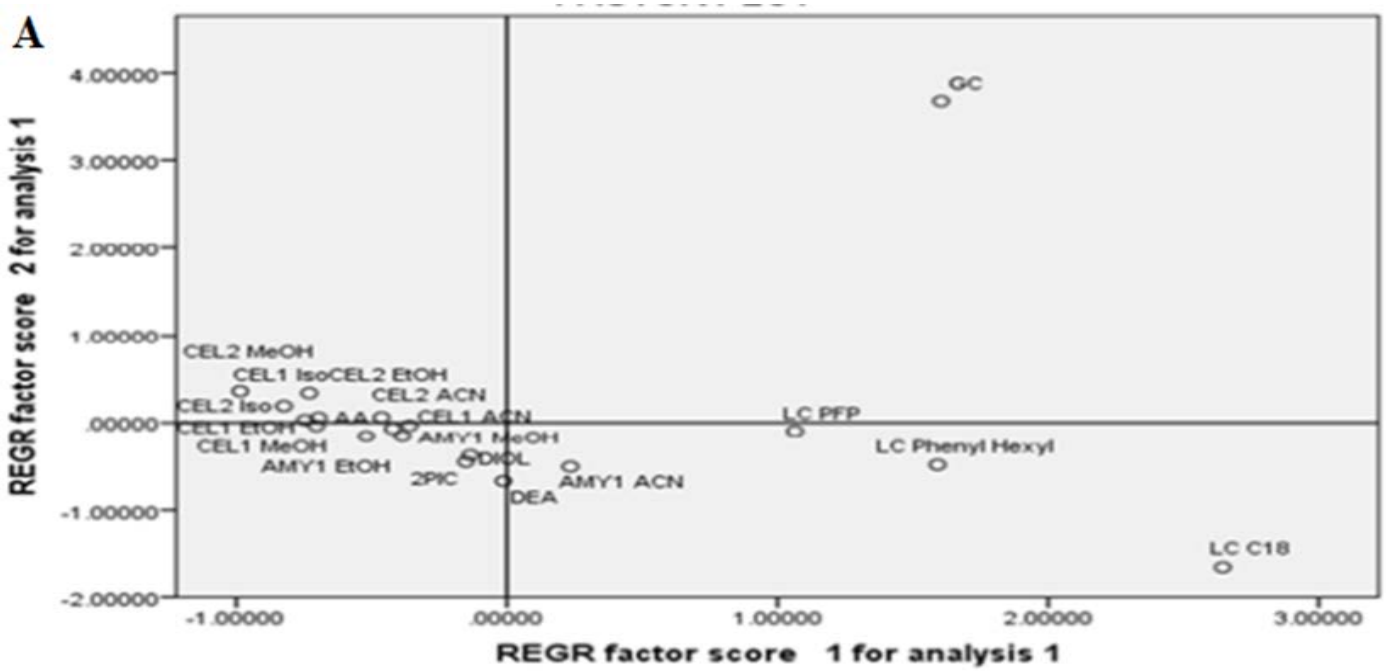
Principal Component Analysis for Various Separation Techniques Synthetic Cannabinoids



The first factor accounts for 90.1% of the variance in the data while the second factor accounts for 7.9% of the variance

Figure 15

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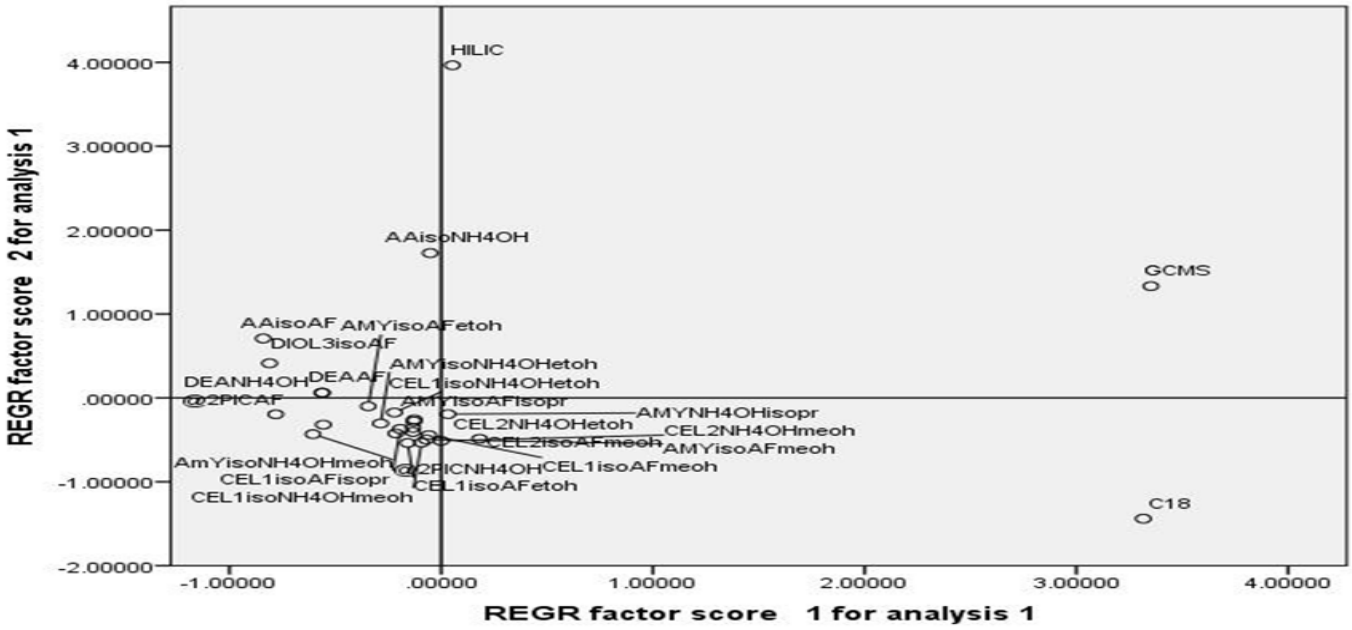
Principal Component Analysis for Various Separation Techniques Synthetic Cathinones



The first factor accounts for 78.3% of the variance in the data while the second factor accounts for 12.1% of the variance

Figure 16

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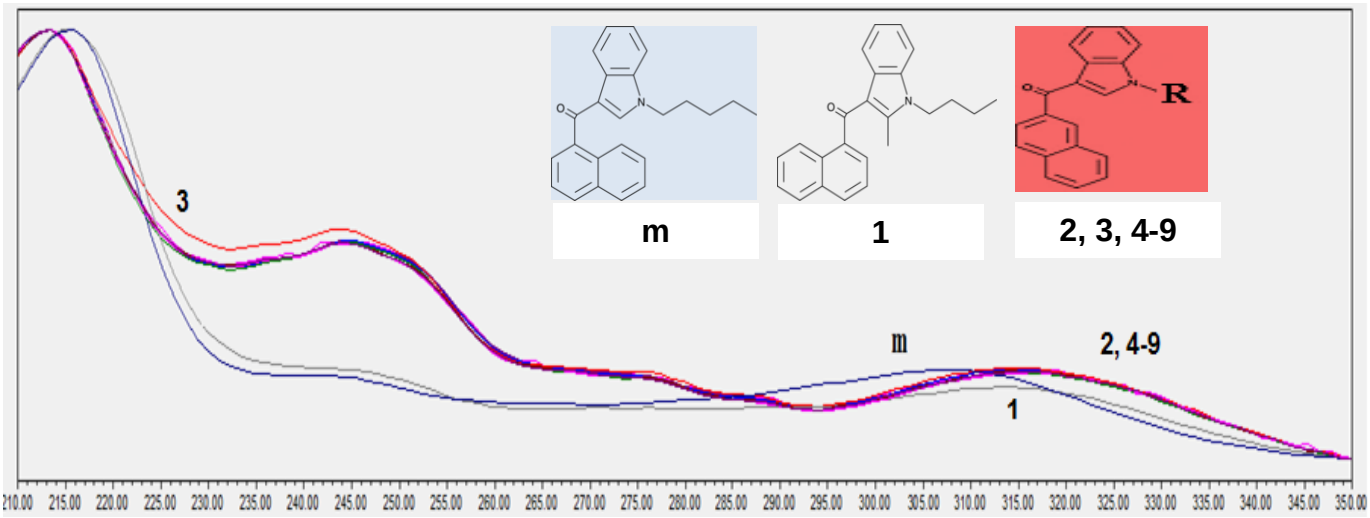
Normalized UV Spectra
JWH018 Positional Isomers



1) JWH-016 m) JWH-018 2) 2'-napthyl isomer 3) 2'-napthyl-N-(1,1-dimethylpropyl) isomer
4) 2'-napthyl-N-(1,2-dimethylpropyl) isomer 5) 2'-napthyl-N-(2,2-dimethylpropyl) isomer
6) 2'-napthyl-N-(1 ethylpropyl) isomer 7) 2'-napthyl-N-(1 methylbutyl) isomer
8) 2'-napthyl-N-(2 methylbutyl) isomer 9) 2'-napthyl-N-(3 methylbutyl) isomer

Figure 17

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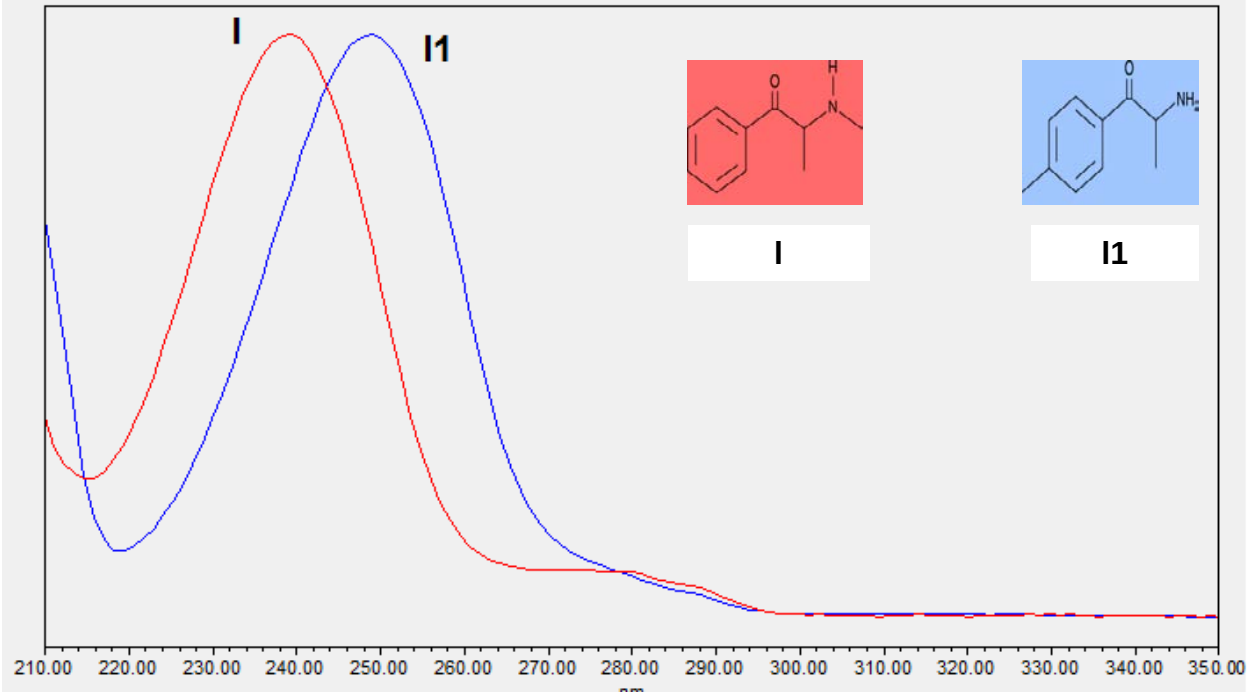
Normalized UV Spectra Methcathinone and Positional Isomer



I) Methcathinone

I1) Nor-methcathinone

Figure 18

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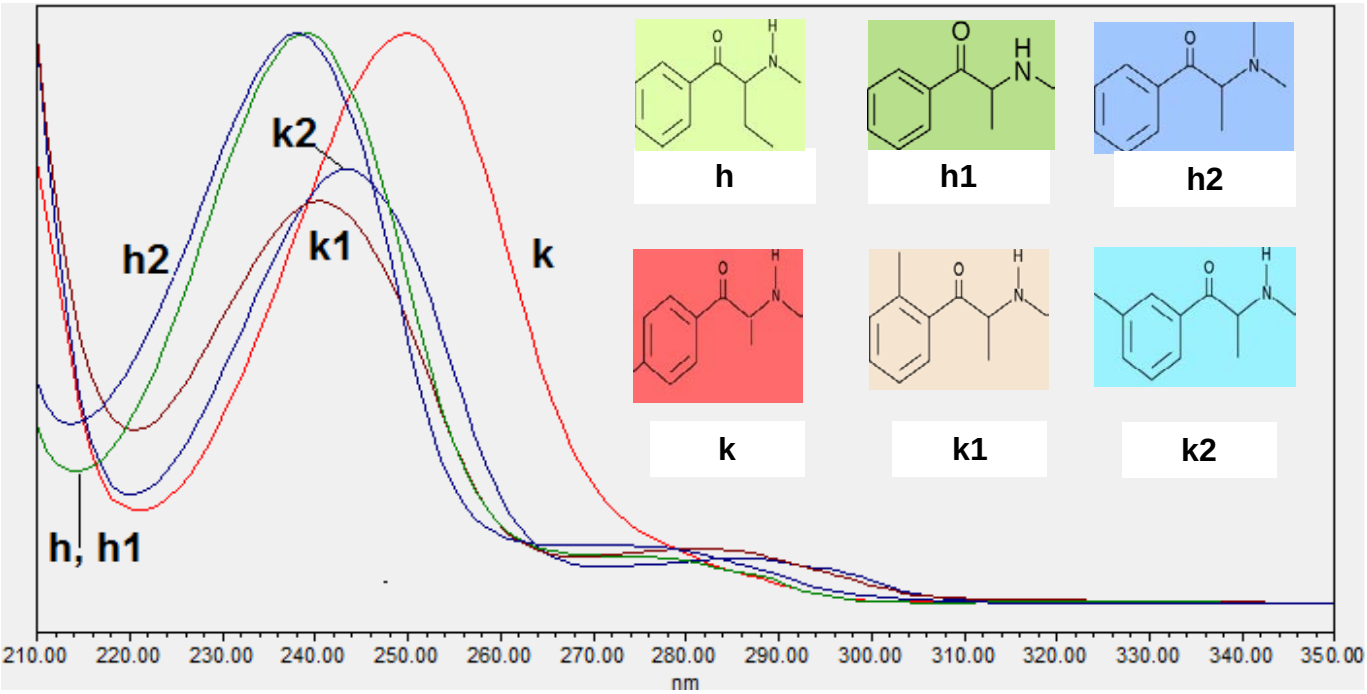
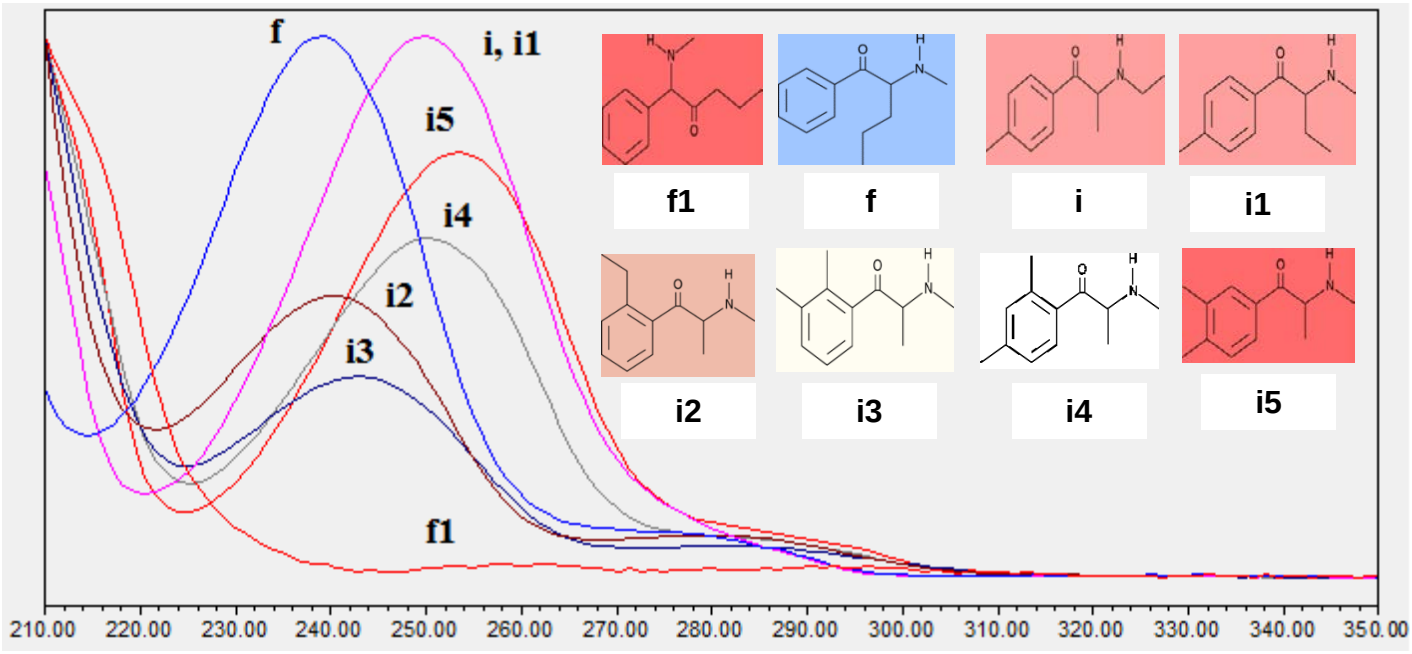
Normalized UV Spectra Buphedrone, Mephedrone and Positional Isomers



h) Buphedrone h1) Ethcathione h2) N, N-dimethylmethcathione
k) Mephedrone k1) 2-methylmethcathinone k2) 3-methylmethcathinone

Figure 19

Normalized UV Spectra Pentedrone, 4-Methylethcathinone and Positional Isomers



f) Pentedrone i) 4-methylethcathinone f1) Isopentedrone
i1) 4-methylbuphedrone i2) 2-ethylmethcathinone i3) 2,3-dimethylmethcathinone
i4) 2,4-dimethylmethcathinone
i5) 3,4-dimethylmethcathinone

Figure 20

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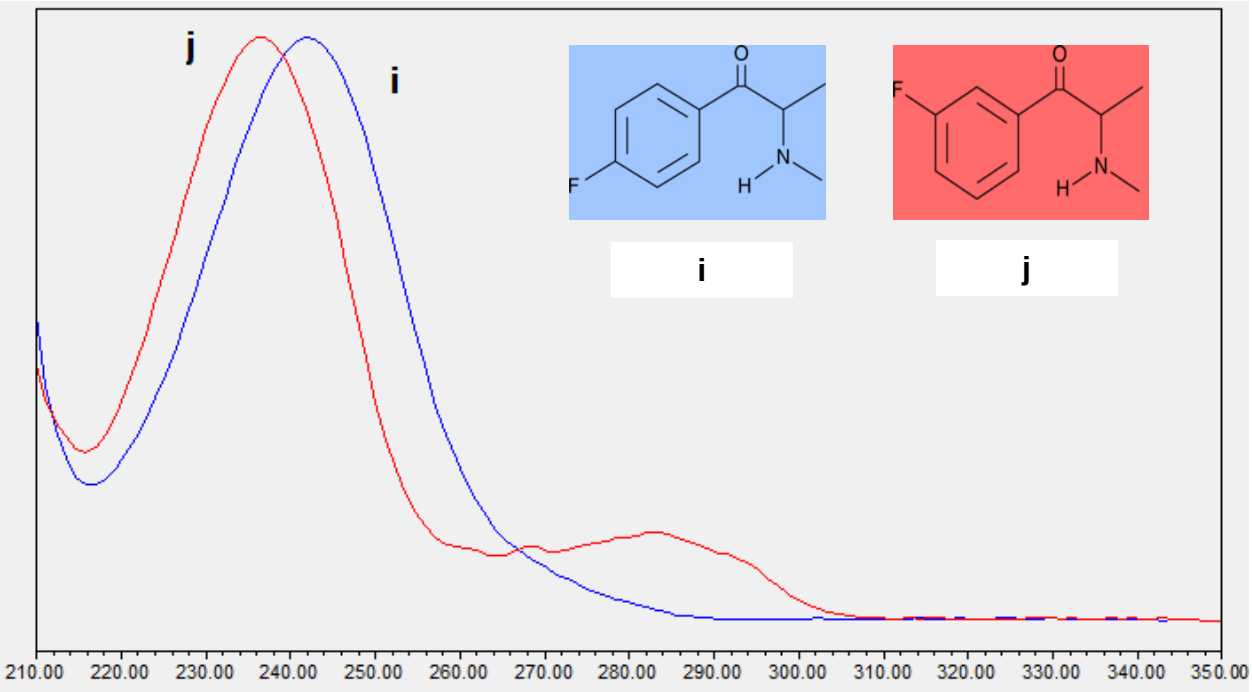
Normalized UV Spectra Fluoromethcathinone Positional Isomers

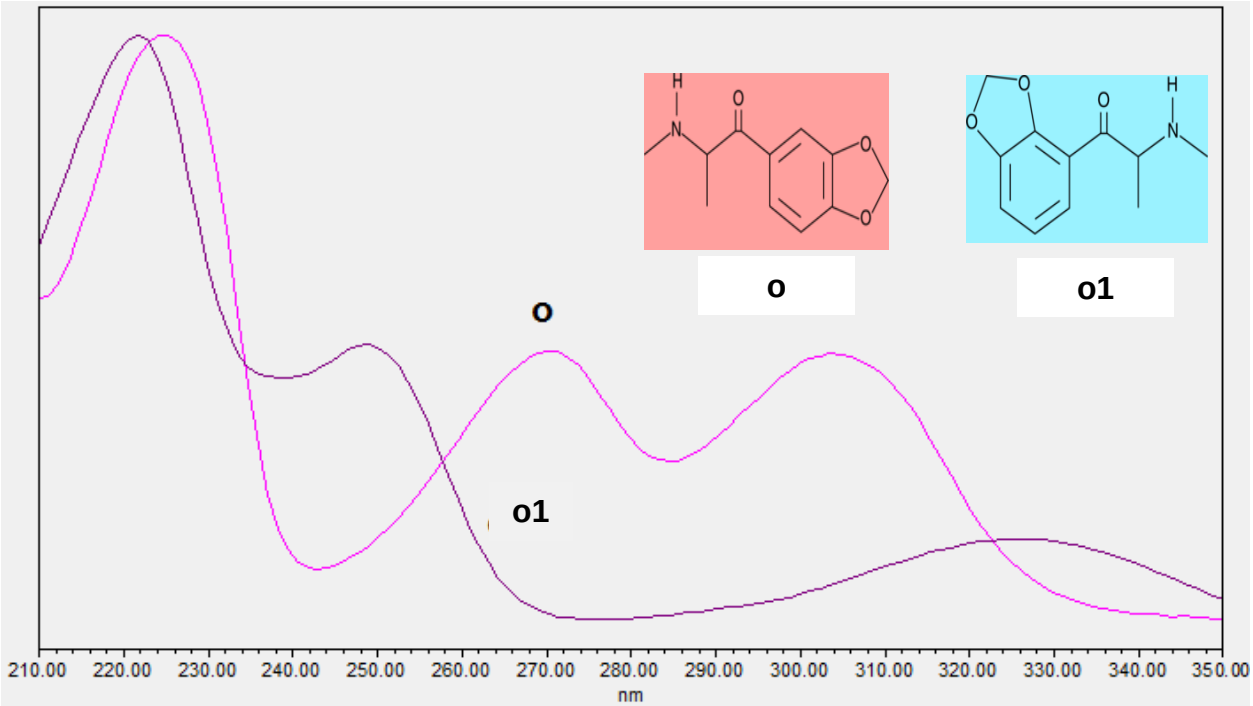


i) 4-fluoromethcathinone

j) 3-fluoromethcathinone

Figure 21

Normalized UV Spectra Methylone Positional Isomers



o) Methylone

o1) 2,3-methylenedioxymethcathinone

Figure 22

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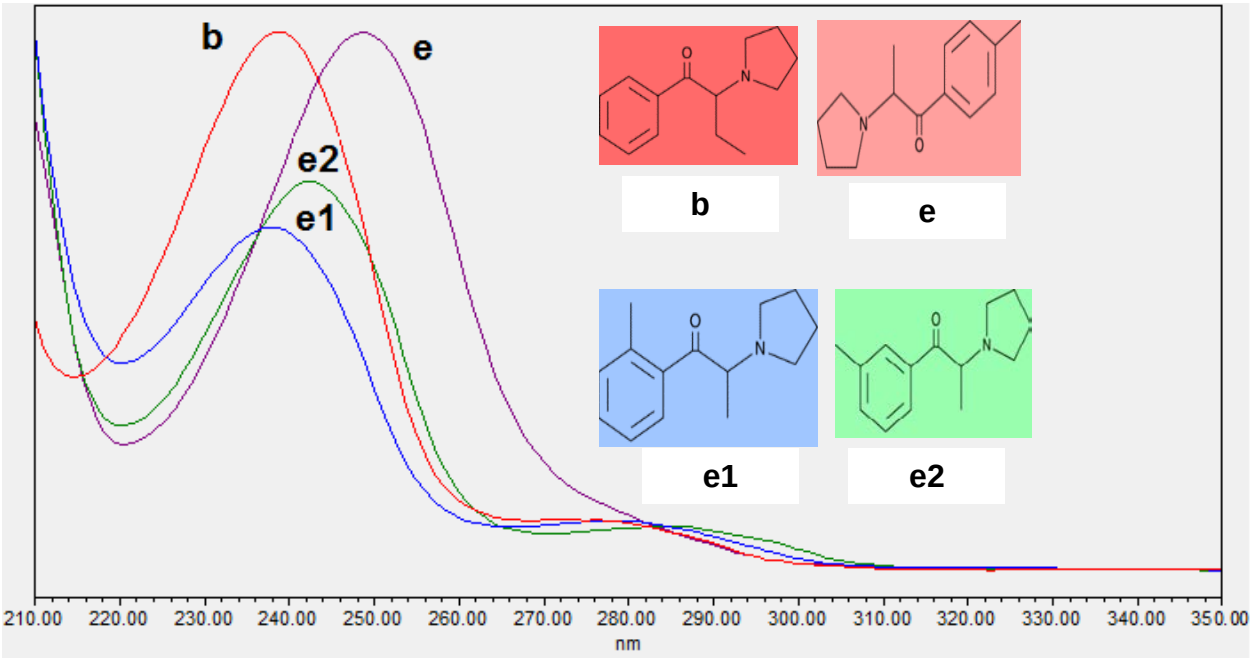
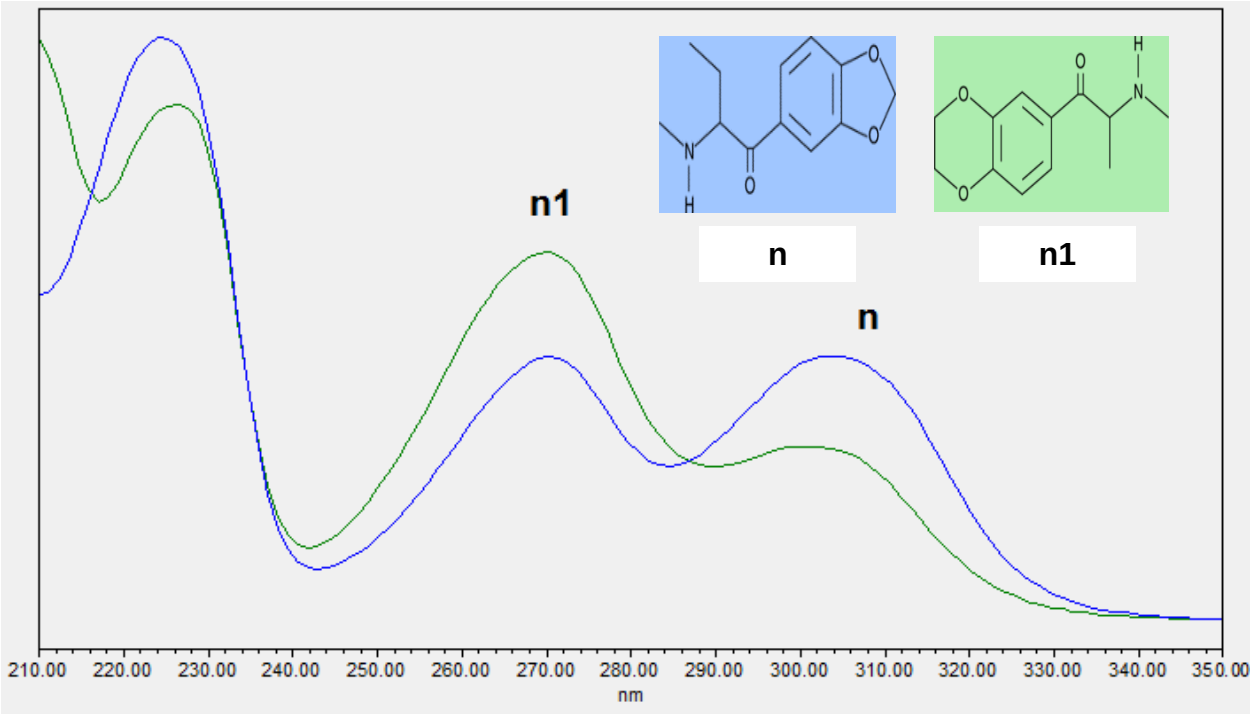
Normalized UV Spectra Alpha PBP, 4 MePPP and Positional Isomers



b) alpha-PBP e) 4-MePPP e1) 2-MePPP e2) 3-MePPP

Figure 23

Normalized UV Spectra Butylone Positional Isomers



n) Butylone n1) 3,4-EDMC

Figure 24

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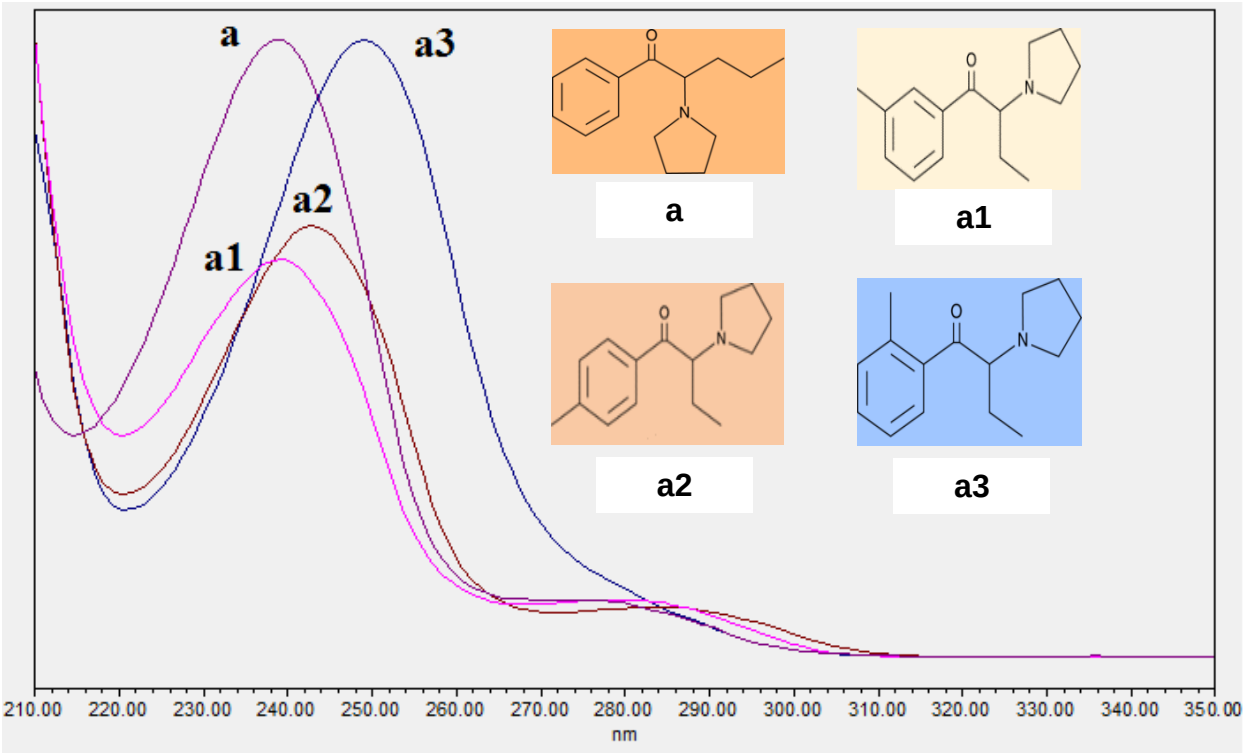
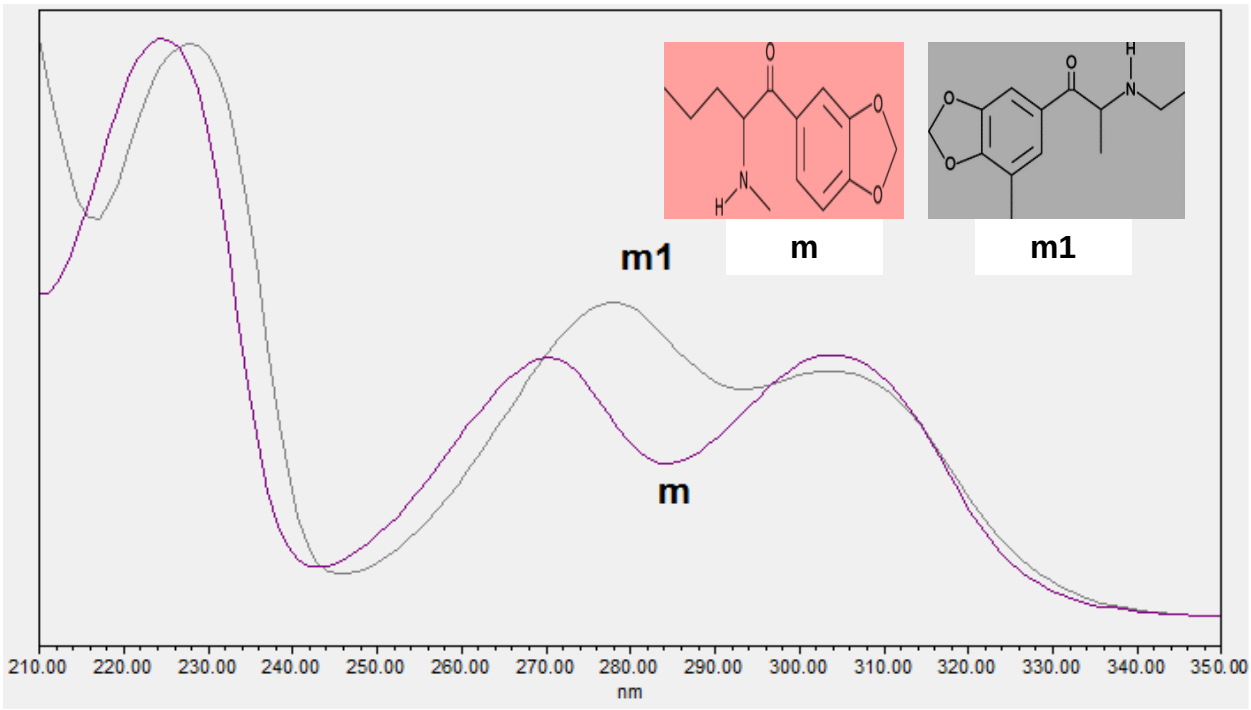
Normalized UV Spectra alpha PVP Positional Isomers



a) alpha-PVP a1) 2-MePBP a2) 3-MePBP a3) 4-MePBP

Figure 25

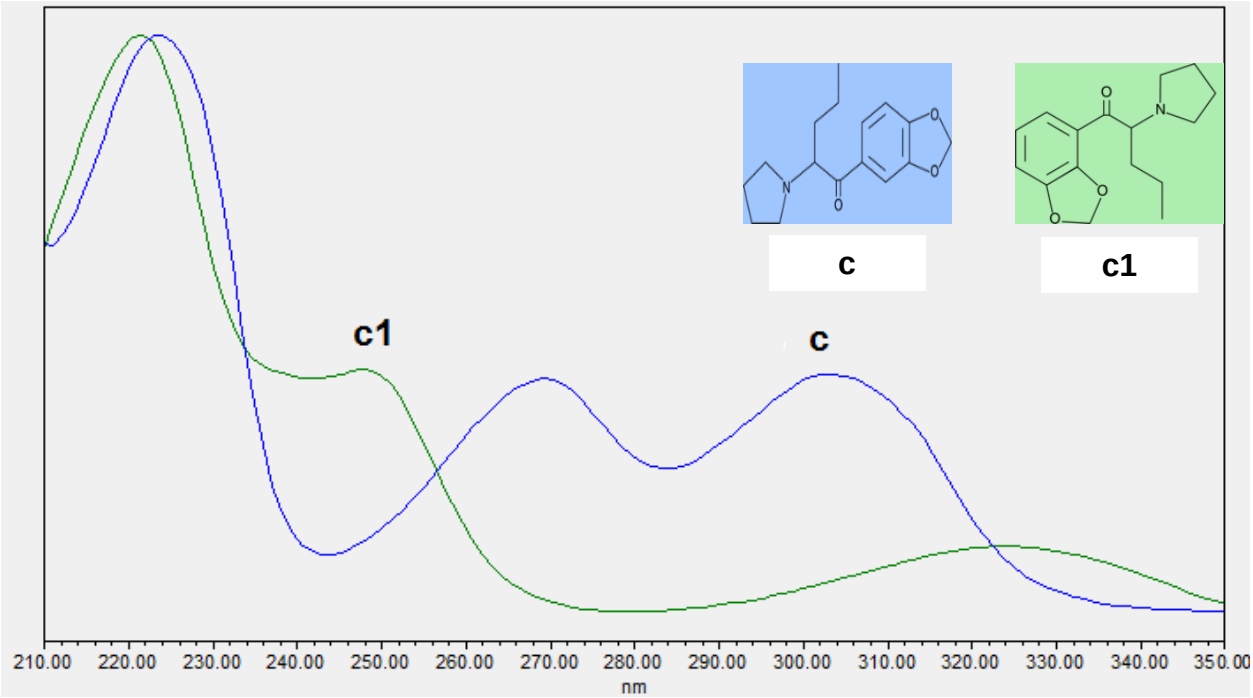
Normalized UV Spectra Pentylone Positional Isomers



m) Pentylone m1) R-MMC

Figure 26

Normalized UV Spectra MDPV Positional Isomers

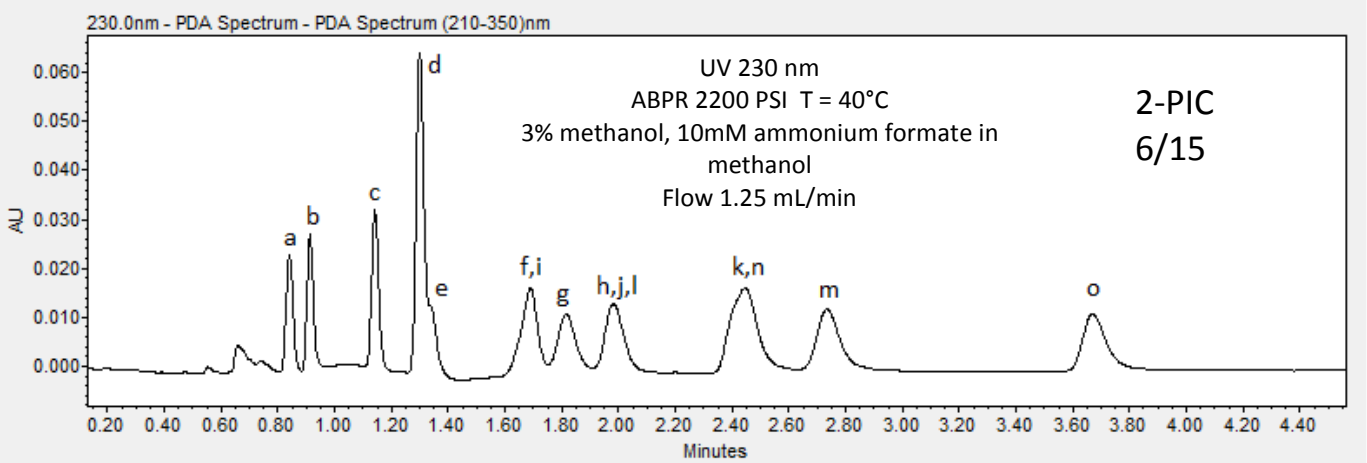


c) MDPV

c1) 2, 3-MDPV

Figure 27

Mix of 15 controlled cathinones with 2-PIC column



a) α- PVP b) α- PBP c) MDPV d) Naphyrone e) 4-MePPP f) Pentedrone
g) Buphedrone h) 3-fluoromethcathinone i) 4- methylethcathinone j) 4-fluoromethcathinone
k) Pentylone l) Methcathinone m) Butylone n) Mephedrone o) Methylone

Figure 28

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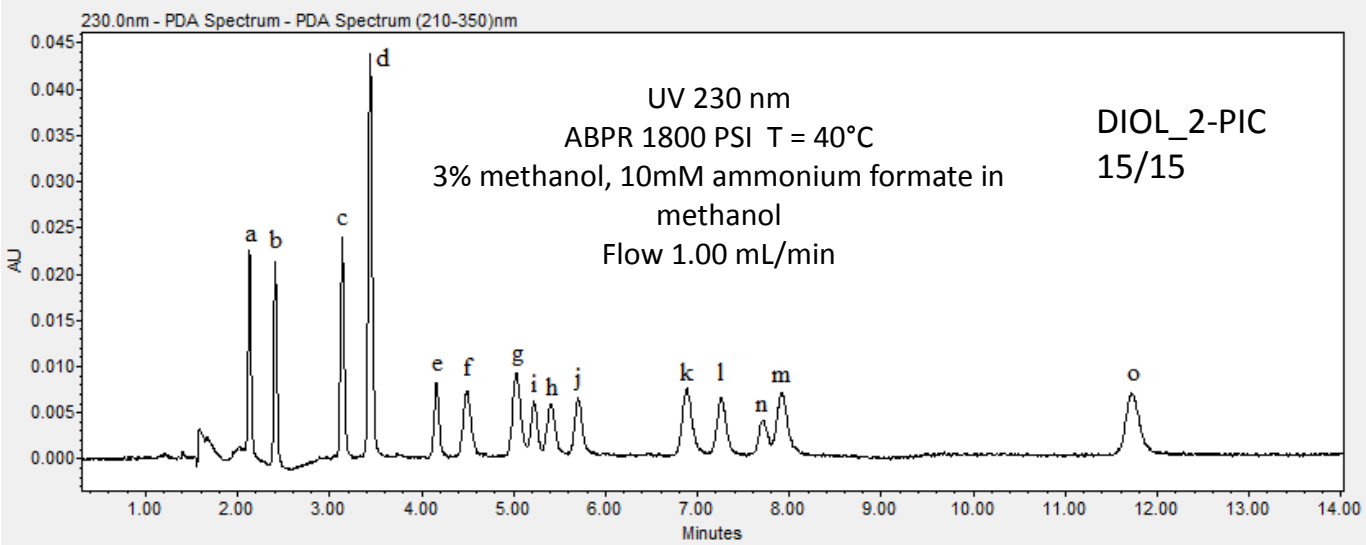
Mix of 15 controlled cathinones with DIOL + 2-PIC column



a) α- PVP b) α- PBP c) MDPV d) Naphyrone e) 4-MePPP f) Pentedrone
g) Buphedrone h) 3-fluoromethcathinone i) 4- methylethcathinone j) 4-fluoromethcathinone
k) Pentylone l) Methcathinone m) Butylone n) Mephedrone o) Methylone

Figure 29

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Supplemental

S1 - Sample Preparation for Synthetic Cannabinoids

The plant material (daminana, bay bean, marshmallow) was ground up into smaller components using sandpaper. The honey goat weed was already in powder form and did not need any further preparation.[1]

Made a secondary stock solution (50 ug/ml in methanol) for each cannabinoid that had a concentration of 1 mg/ml in methanol or acetonitrile. Made a secondary stock solution (100 ug/ml in methanol) for each cannabinoid that had a concentration of 10 mg/ml. For the standard, 800 ul (50 ug/ml) or 400 ul (100 ug/ml) of the secondary stock solution of each drug was added in a 10ml volumetric flask and brought to volume in injection solvent (2:1:1 hexane: ethyl acetate: methanol) (4ug/ml).

For the samples, a 4mg/10ml or 10mg/25ml (plant material to final volume) ratio was used. The samples were prepared in the same way as the standard, but the amounts were adjusted depending on the concentration desired. The samples were brought to volume with injection solvent. The standard and samples were vortexed for 1 minute, filtered into LC vials, and run.

References

1. B. K. Logan, L. E. Reinhold, A. Xu, F. X., Diamond, Identification of synthetic cannabinoids in herbal incense blends in the United States , J. Forensic Sci. 57 (2012) 1168-1180.

S2 - Sample Preparation for the Synthetic Cathinones

A secondary stock solution containing the desired bath salts was prepared at a target concentration (75 µg/mL, 62.5 µg/mL, 50 µg/mL, 37.5 µg/mL, 31.25 µg/mL, 25 µg/mL) with methanol.

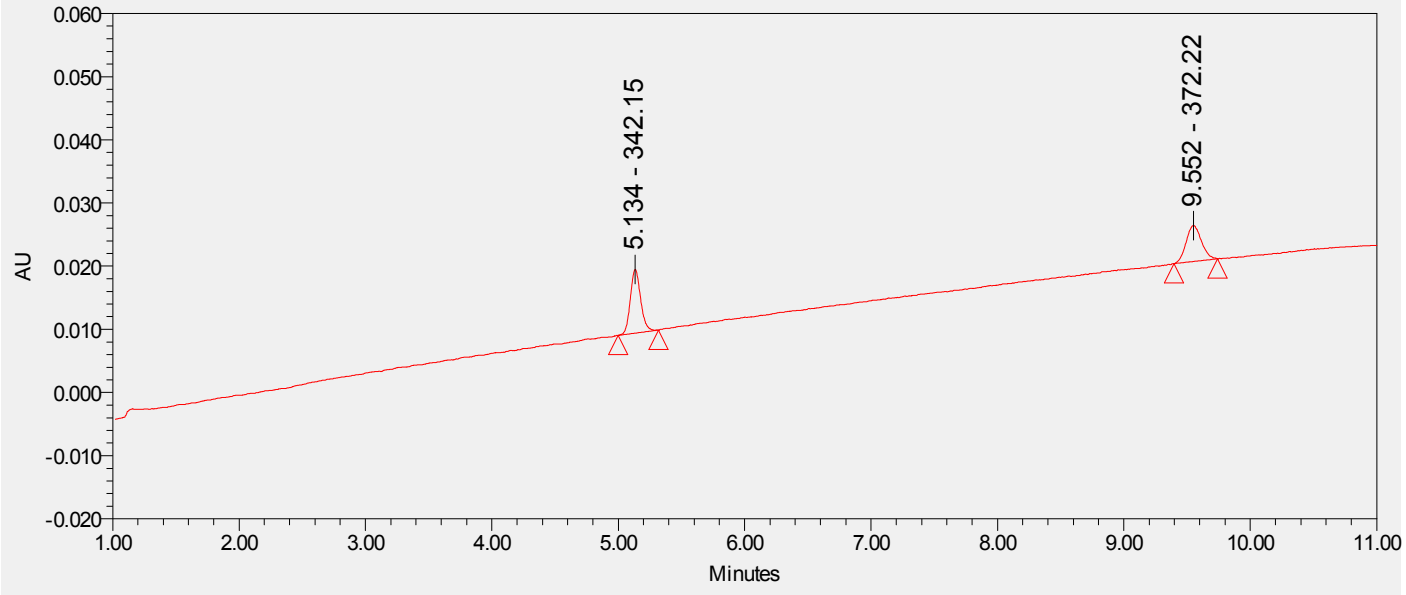
A standard and two duplicates were prepared by taking 1 mL of the secondary stock and placed into corresponding vials. 1 mg of adulterant (lidocaine, benzocaine, caffeine, or pancake mix) was added to the duplicates.

Alternatively, another standard and two duplicates were prepared by adding 250 µL of the above secondary stock solution to corresponding vials. For the standard, 750 µL of methanol was added to get a total volume of 1mL. For the duplicates, 750 µL of methanol spiked with adulterant (2.5mg/10mL or 1mg/10mL) was added to each to get a total volume of 1mL.

To stay in linear range for the mass spectrometer, a 1:100 dilution was prepared for the standard and samples. All samples were vortexed, filtered into LC vials, and run.

SAMPLE INFORMATION

Sample Name:	mix 2 new std	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	sample 2 reprocess
Vial:	1:A,2	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	lurie syn can screen
Injection Volume:	2.00 ul	Channel Name:	Extract 210.0
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum (210-350)nm
Date Acquired:	2/29/2016 3:44:07 PM EST		
Date Processed:	6/14/2016 11:14:31 AM EDT		



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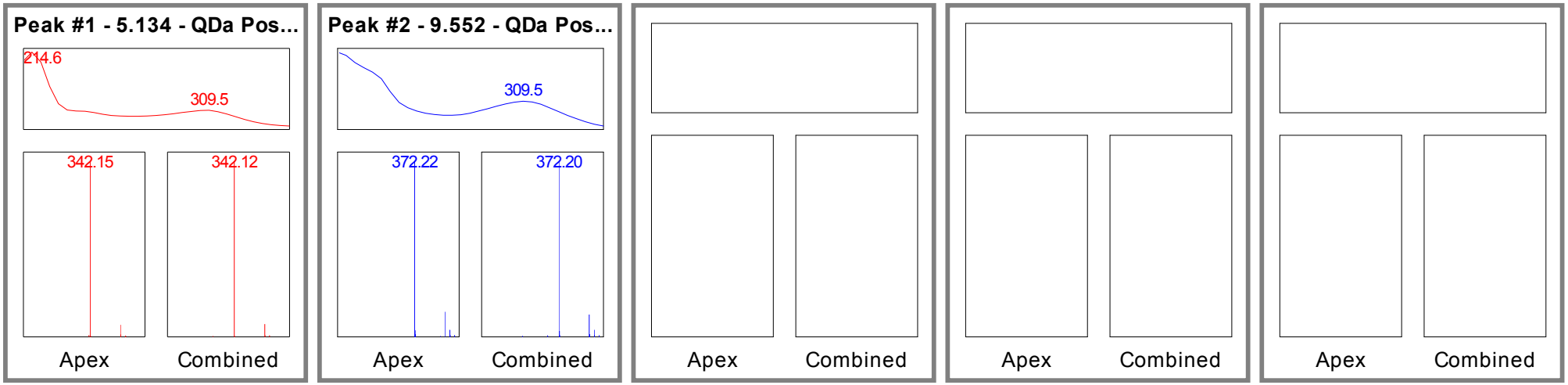

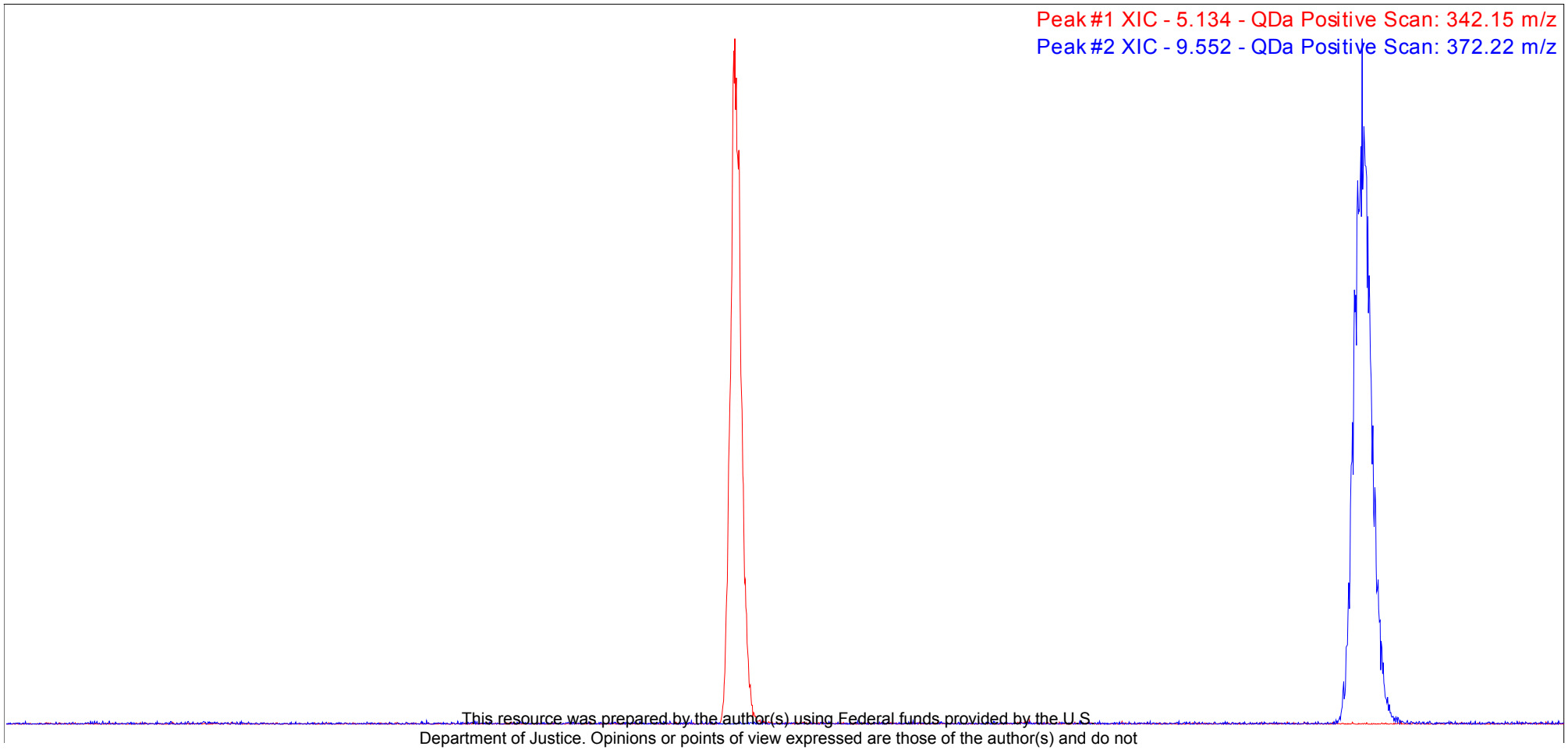

PDA Result Table

	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold	PDA/FLR Match2 Spect. Name	PDA/FLR Match2 Angle	PDA/FLR Match3 Spect. Name	PDA/FLR Match3 Angle	Base Peak (m/z)
1	5.134	0.656	1.442	JWH 18	0.529	1.835	JWH 73	0.534	JWH 19	0.535	342.15
2	9.552	0.681	1.793	JWH 081	0.171	2.249	JWH 200	7.595	AM 694	12.694	372.22

PDA Result Table

	MS Match1 Spect. Name	MS Match2 Spect. Name	MS Match3 Spect. Name
1	JWH 18	JWH 250	RCS 4
2	JWH 81	RCS 4	JWH 250

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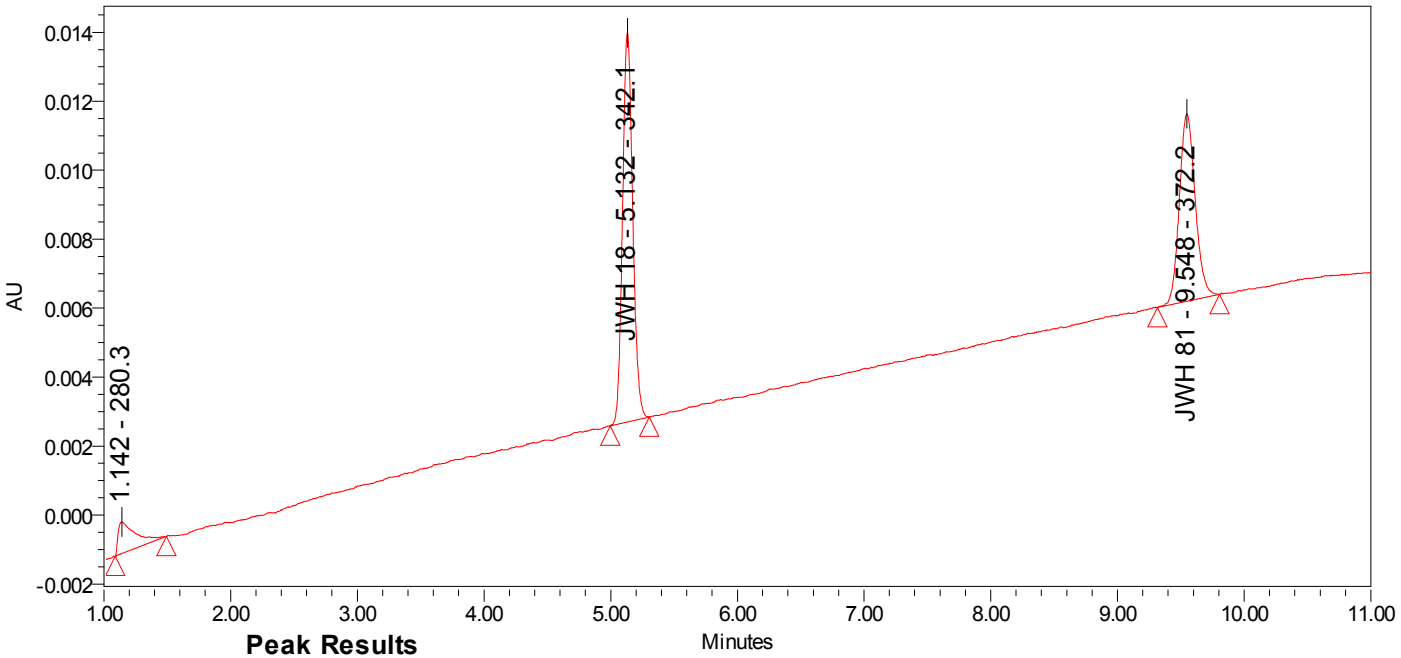
lurie quantitation syn can

SAMPLE INFORMATION

Sample Name:	mix 2 new std	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	sample 2 reprocess
Vial:	1:A,2	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	uv quant 215 comp
Injection Volume:	2.00 ul	Channel Name:	PDA Ch2 215nm@4.8nm -Compens.
Run Time:	11.0 Minutes		
Date Acquired:	2/29/2016 3:44:07 PM EST		
Date Processed:	6/14/2016 11:28:48 AM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.142	8512	916		
2	JWH 18	5.132	62918	11288	4.000	ug/mL
3	JWH 81	9.548	47131	5439	4.000	ug/mL

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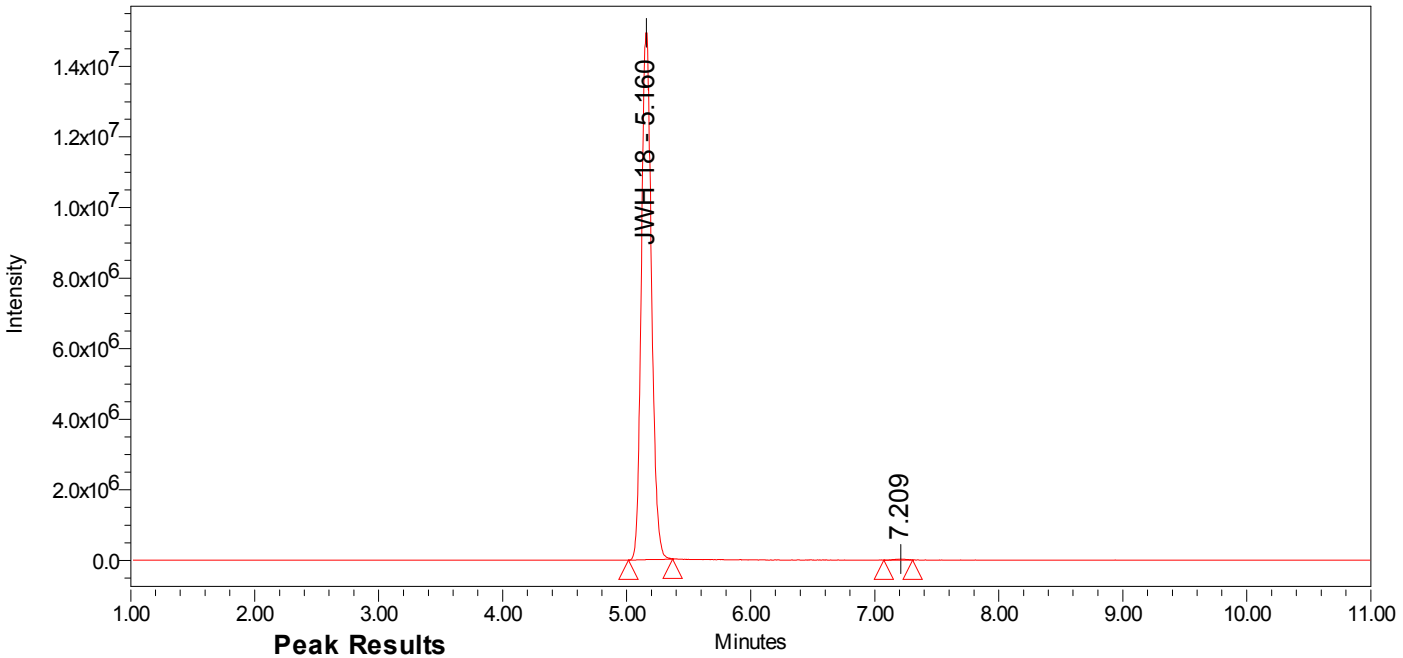
SAMPLE INFORMATION

Sample Name:	mix 2 new std	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	sample 2 reprocess
Vial:	1:A,2	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	ms quant 342
Injection Volume:	2.00 ul	Channel Name:	QDa Ch2 342.26 Da
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch2 342.26 Da, CV=10
Date Acquired:	2/29/2016 3:44:07 PM EST		
Date Processed:	6/14/2016 11:28:49 AM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	JWH 18	5.160	84760953	15077144	4.000	ug/mL
2		7.209	184456	26566		

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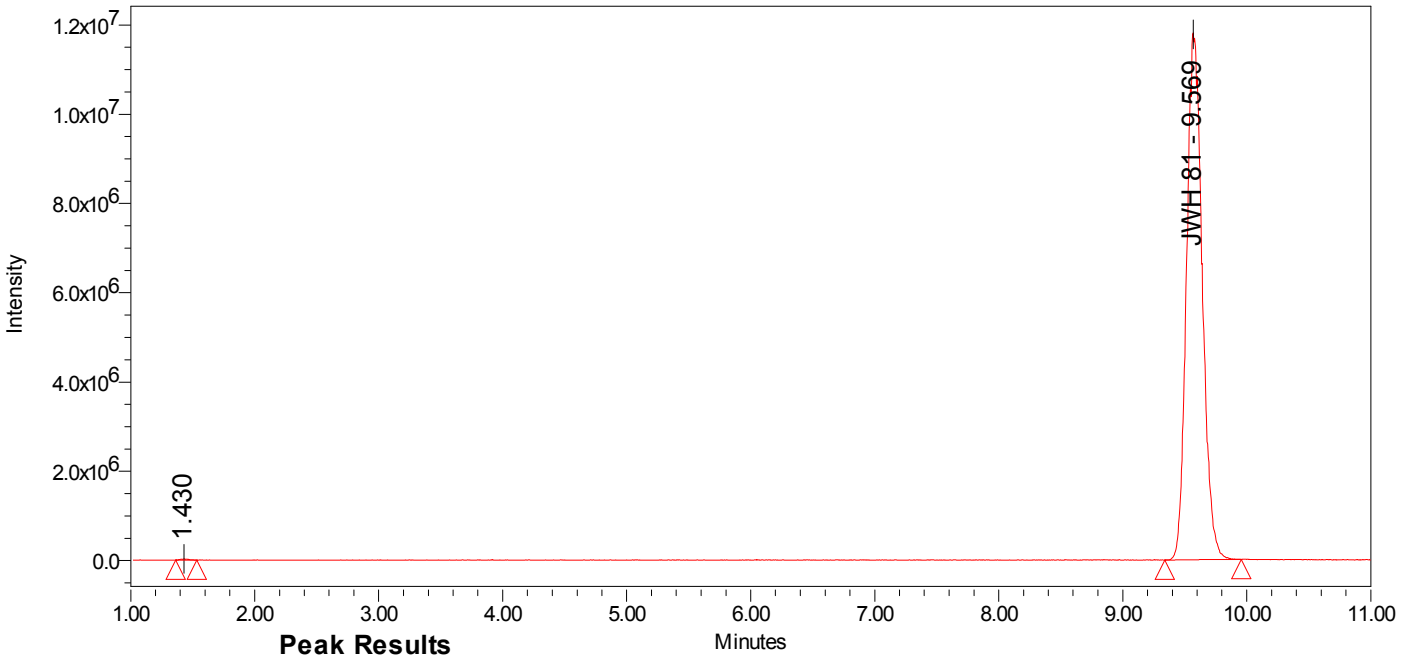
SAMPLE INFORMATION

Sample Name:	mix 2 new std	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	sample 2 reprocess
Vial:	1:A,2	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	ms quant 372
Injection Volume:	2.00 ul	Channel Name:	QDa Ch14 372.20 Da
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch14 372.20 Da, CV=10
Date Acquired:	2/29/2016 3:44:07 PM EST		
Date Processed:	6/14/2016 11:28:49 AM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.430	113179	26141		
2	JWH 81	9.569	104146282	11761033	4.000	ug/mL

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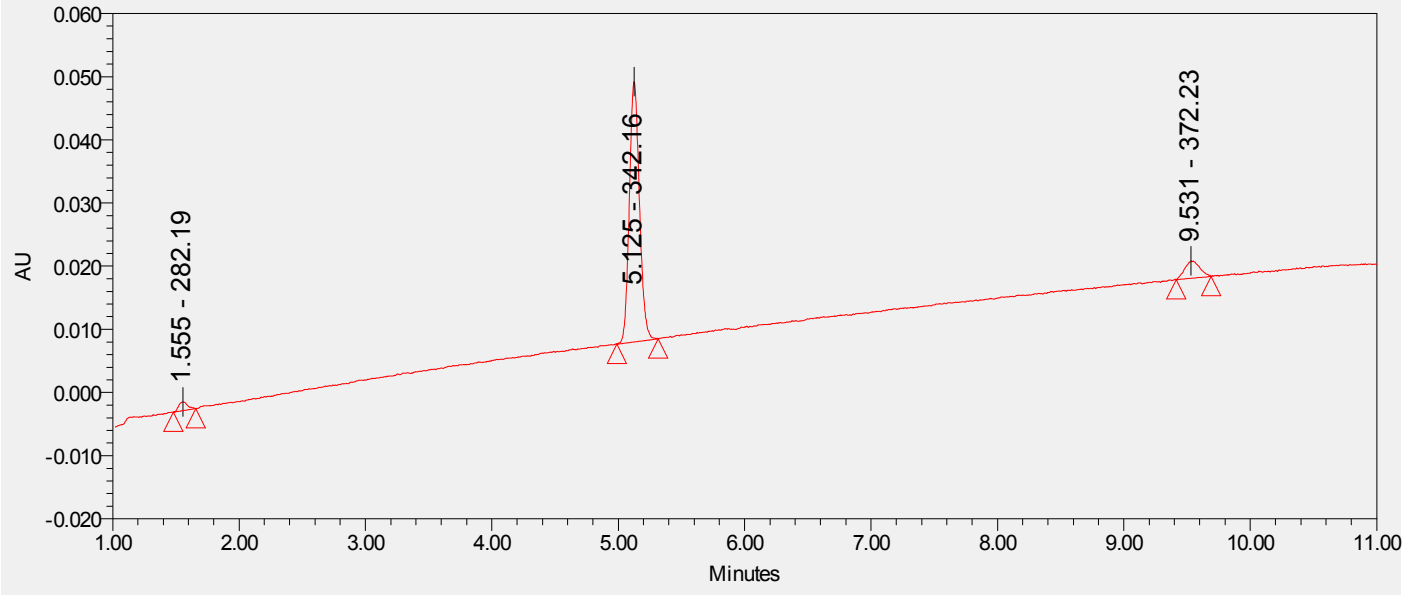
Empower 3 SOFTWARE

screen cannabinoids

SAMPLE INFORMATION

Sample Name:	mix 2 new sample 1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	sample 2 reprocess
Vial:	1:A,3	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	lurie syn can screen
Injection Volume:	2.00 ul	Channel Name:	Extract 210.0
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum (210-350)nm
Date Acquired:	2/29/2016 4:10:19 PM EST		
Date Processed:	6/14/2016 11:14:37 AM EDT		



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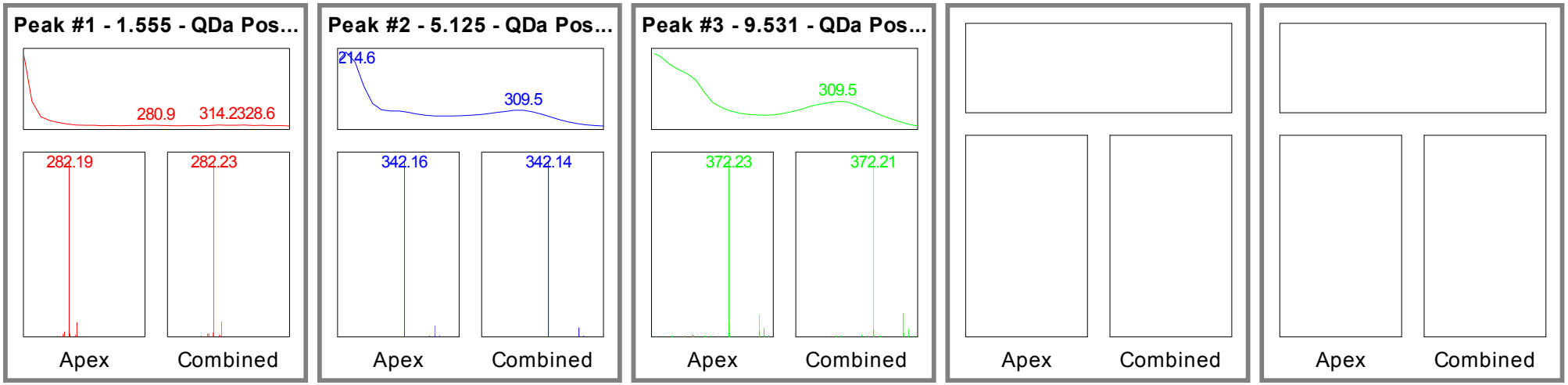

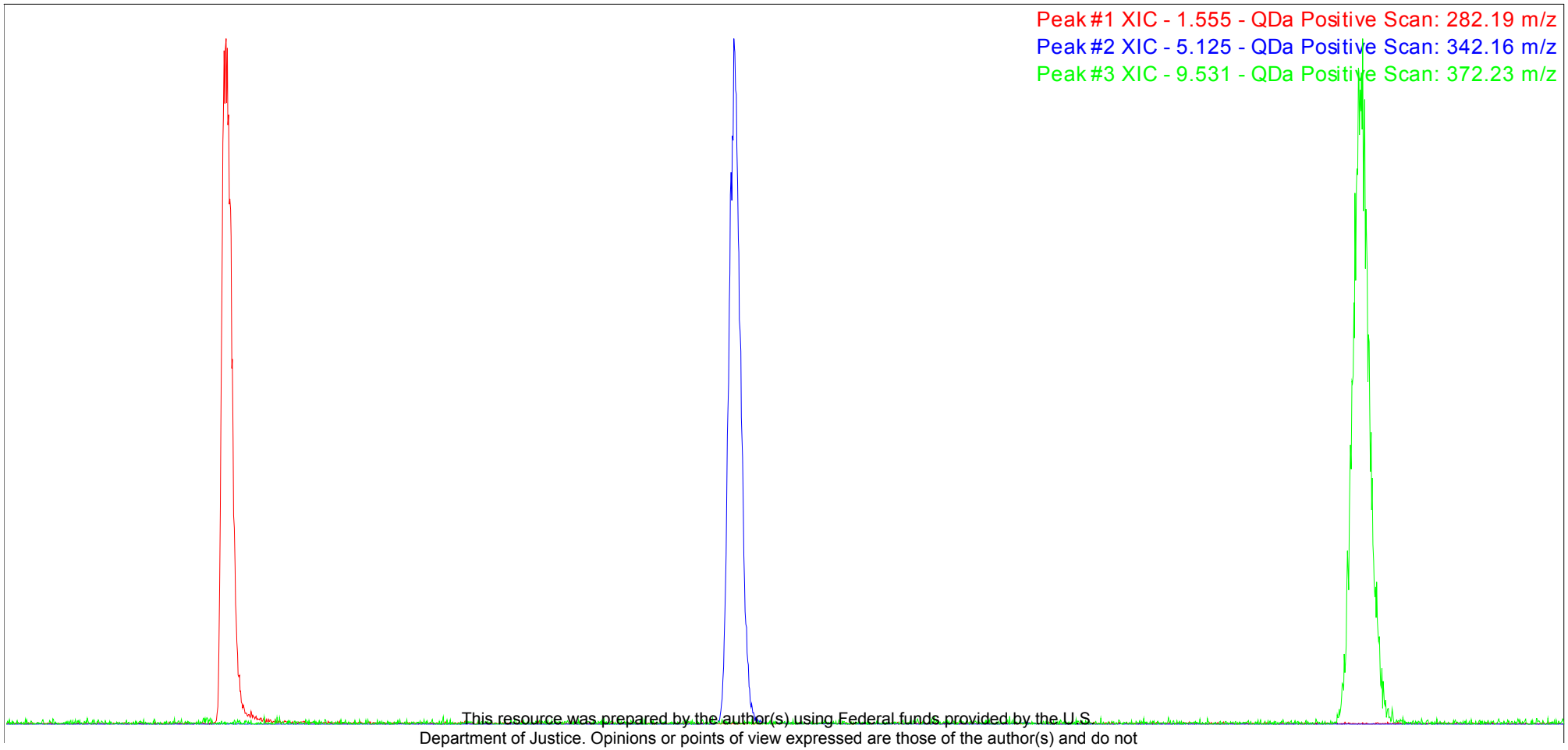

PDA Result Table

	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold	PDA/FLR Match2 Spect. Name	PDA/FLR Match2 Angle	PDA/FLR Match3 Spect. Name	PDA/FLR Match3 Angle	Base Peak (m/z)
1	1.555	22.106	21.733								282.19
2	5.125	0.304	0.381	JWH 19	0.403	1.181	JWH 18	0.408	JWH 73	0.411	342.16
3	9.531	2.516	1.924	JWH 081	3.585	2.506	JWH 200	9.573	AM 694	12.613	372.23

PDA Result Table

	MS Match1 Spect. Name	MS Match2 Spect. Name	MS Match3 Spect. Name
1	RCS 4	UR 144	XLR 11
2	JWH 18	RCS 4	UR 144
3	JWH 81	CP 47, 497	JWH 250

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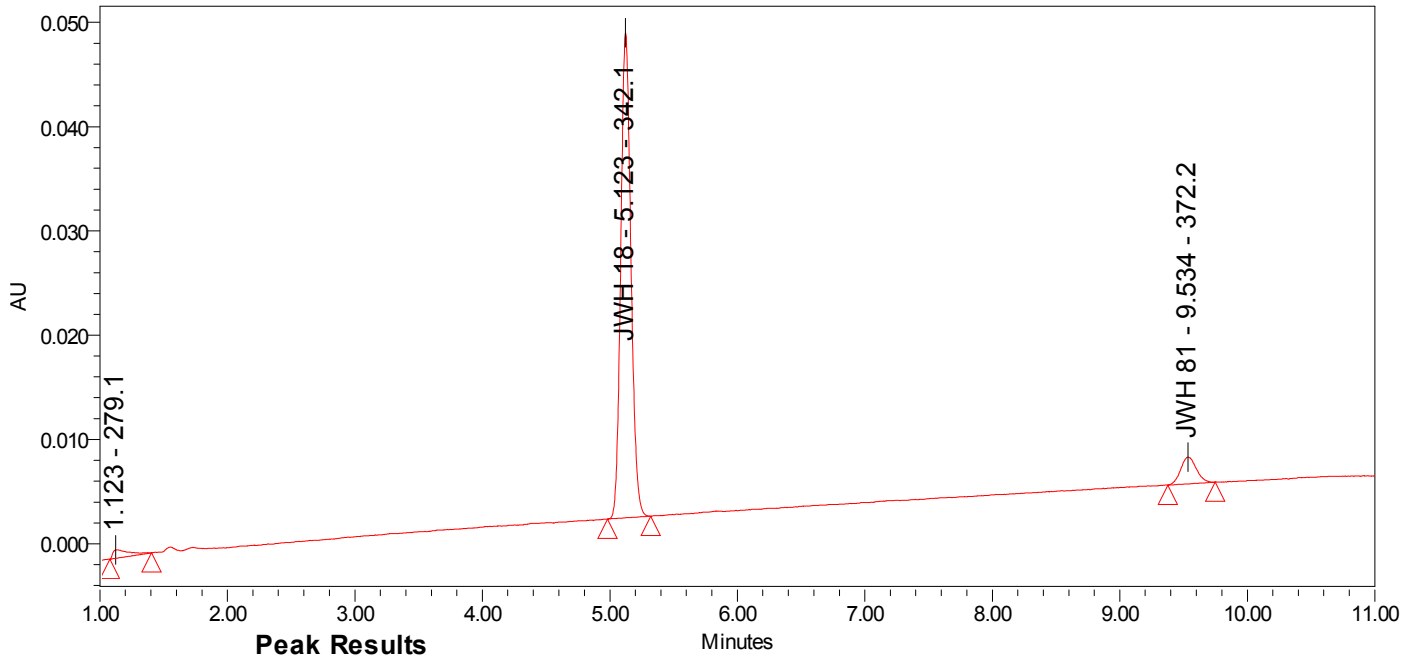
lurie quantitation syn can

SAMPLE INFORMATION

Sample Name:	mix 2 new sample 1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	sample 2 reprocess
Vial:	1:A,3	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	uv quant 215 comp
Injection Volume:	2.00 ul	Channel Name:	PDA Ch2 215nm@4.8nm -Compens.
Run Time:	11.0 Minutes		
Date Acquired:	2/29/2016 4:10:19 PM EST		
Date Processed:	6/14/2016 11:28:51 AM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.123	6870	793		
2	JWH 18	5.123	257227	46507	16.422	ug/mL
3	JWH 81	9.534	22001	2573	1.874	ug/mL

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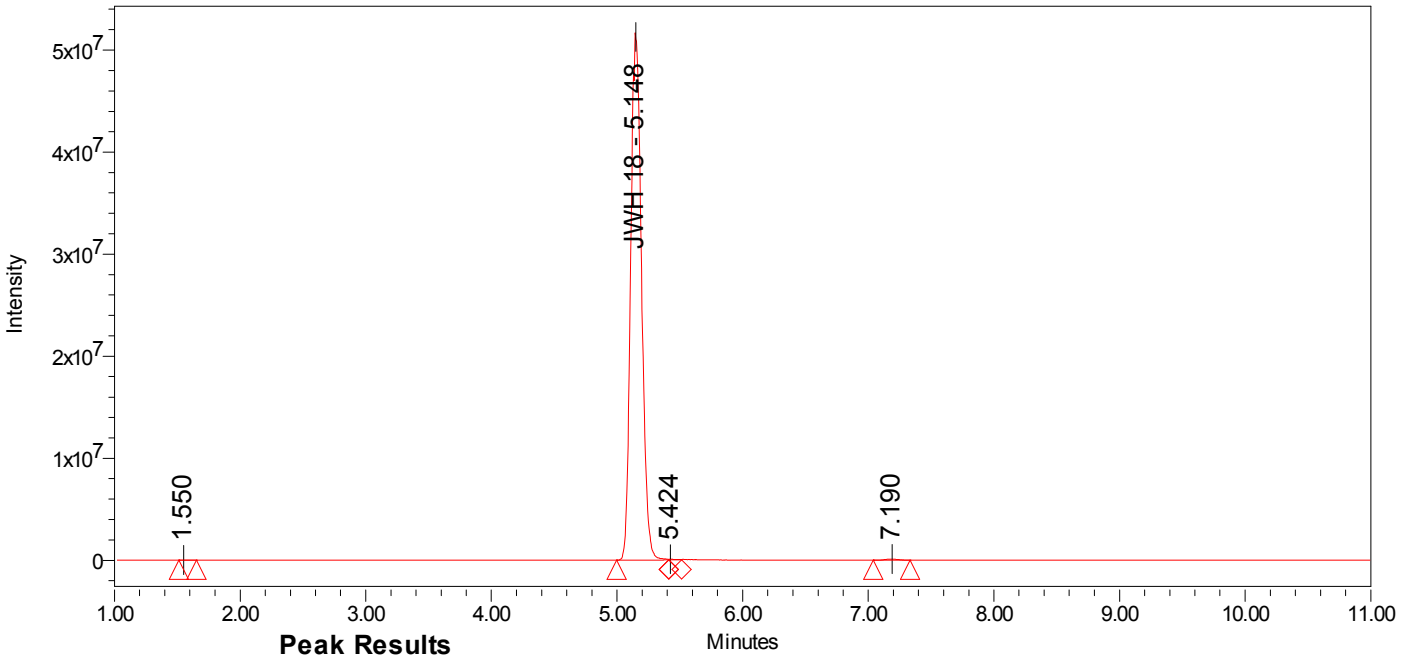
lurie quantitation syn can

SAMPLE INFORMATION

Sample Name:	mix 2 new sample 1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	sample 2 reprocess
Vial:	1:A,3	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	ms quant 342
Injection Volume:	2.00 ul	Channel Name:	QDa Ch2 342.26 Da
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch2 342.26 Da, CV=10
Date Acquired:	2/29/2016 4:10:19 PM EST		
Date Processed:	6/14/2016 11:28:51 AM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.550	102662	22754		
2	JWH 18	5.148	309609450	51803333	14.739	ug/mL
3		5.424	273029	68555		
4		7.190	764638	107543		

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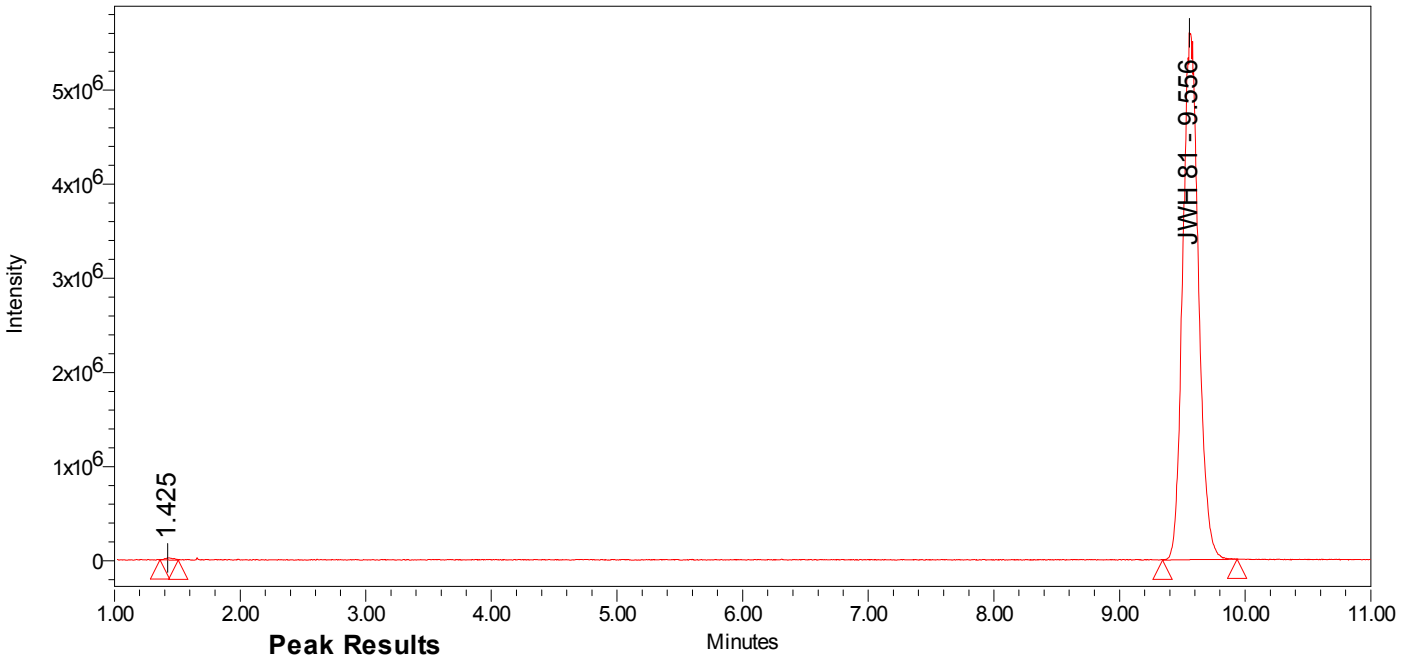
lurie quantitation syn can

SAMPLE INFORMATION

Sample Name:	mix 2 new sample 1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	sample 2 reprocess
Vial:	1:A,3	Acq. Method Set:	screen cannabinoids isopr CEL1
Injection #:	1	Processing Method:	ms quant 372
Injection Volume:	2.00 ul	Channel Name:	QDa Ch14 372.20 Da
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch14 372.20 Da, CV=10
Date Acquired:	2/29/2016 4:10:19 PM EST		
Date Processed:	6/14/2016 11:28:52 AM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.425	101591	21934		
2	JWH 81	9.556	49118736	5634900	1.898	ug/mL

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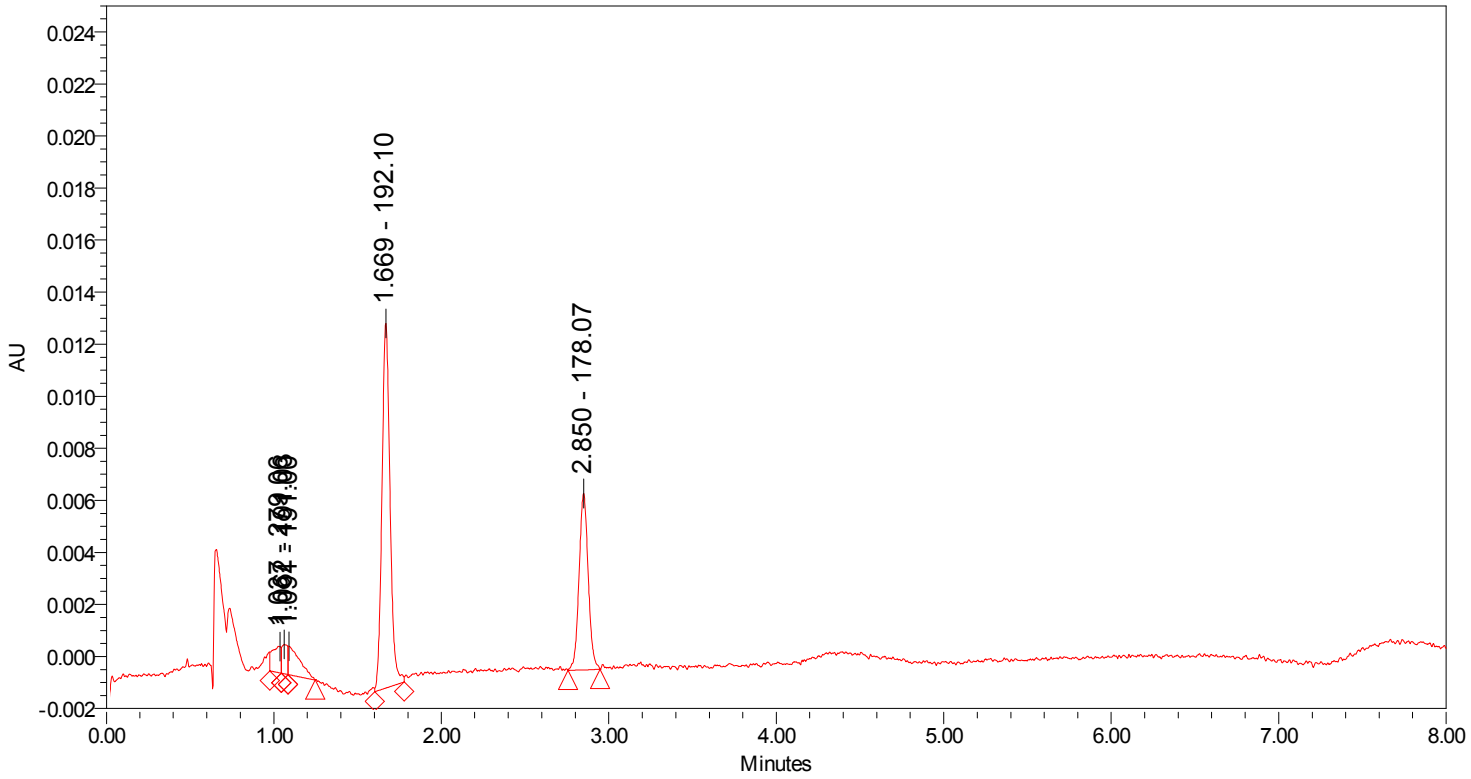
SAMPLE INFORMATION

Sample Name:	mix 14 standard	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	sample 14 UV reprocess
Vial:	2:E,1	Acq. Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts screening
Injection Volume:	0.50 ul	Channel Name:	PDA 230 nm
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum (210-350)nm
Dilution 1.0000			
Date Acquired:	5/12/2016 5:12:49 PM EDT		
Date Processed:	6/14/2016 2:00:36 PM EDT		



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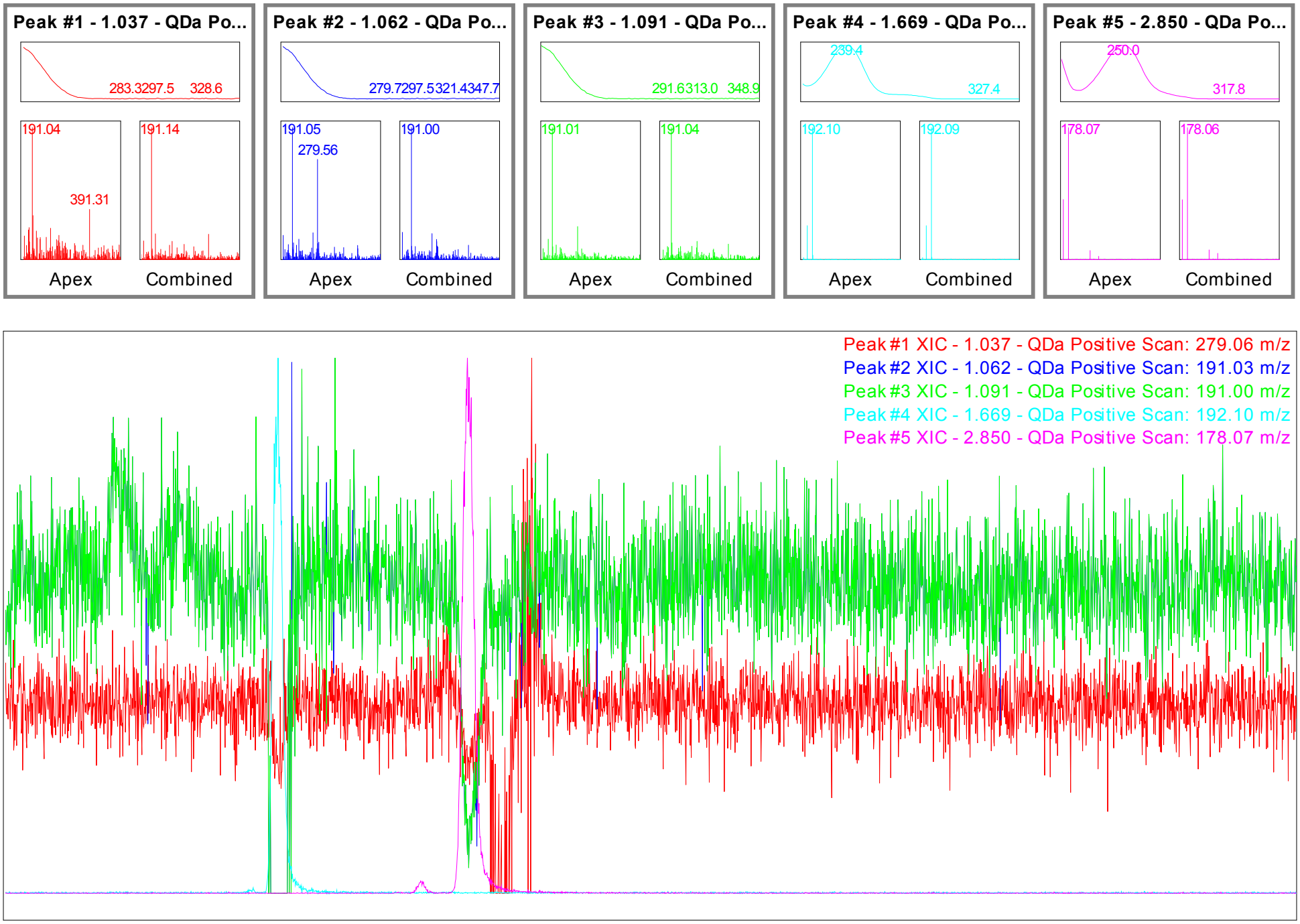

PDA Result Table

	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold	PDA/FLR Match2 Spect. Name	PDA/FLR Match2 Angle	PDA/FLR Match3 Spect. Name	PDA/FLR Match3 Angle	Base Peak (m/z)
1	1.037	9.061	90.000	lidocaine	21.067	90.000					279.06
2	1.062	13.766	90.000	lidocaine	12.163	90.000					191.03
3	1.091	13.046	90.000	lidocaine	11.178	90.000					191.00
4	1.669	0.955	8.614	Buphedrone	0.699	5.686	Pentedrone	1.071	Methcathinone	1.603	192.10
5	2.850	0.520	5.609	Mephedrone	1.064	4.156	4 Methylethcathinone	1.961	4 MePPP	5.121	178.07

PDA Result Table

	MS Match1 Spect. Name	MS Match2 Spect. Name	MS Match3 Spect. Name
1	4-methylethcathinone	lidocaine	MDPV
2	lidocaine	MDPV	Naphyrone
3	4-methylethcathinone	lidocaine	MDPV
4	4-methylethcathinone	Pentedrone	lidocaine
5	Mephedrone	Buphedrone	Naphyrone

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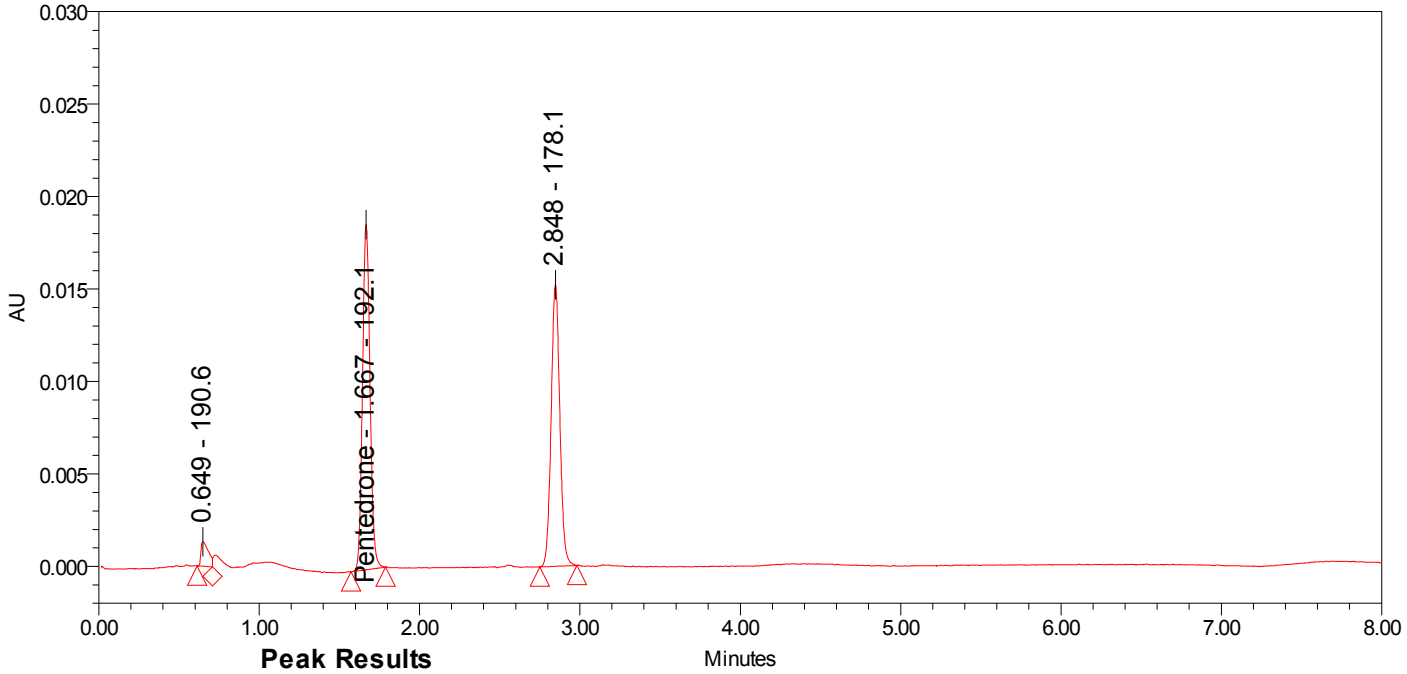
quantitation bath salts

SAMPLE INFORMATION

Sample Name:	mix 14 standard	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	sample 14 UV reprocess
Vial:	2:E,1	Acq. Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts quant comp 240nm
Injection Volume:	0.50 ul	Channel Name:	PDA Ch3 240nm@4.8nm - Compens.
Run Time:	8.0 Minutes		
Dilution 1.0000			
Date Acquired:	5/12/2016 5:12:49 PM EDT		
Date Processed:	6/14/2016 2:00:53 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		0.649	4337	1321		
2	Pentedrone	1.667	60683	18677	62.500	ug/mL
3		2.848	57157	15238		

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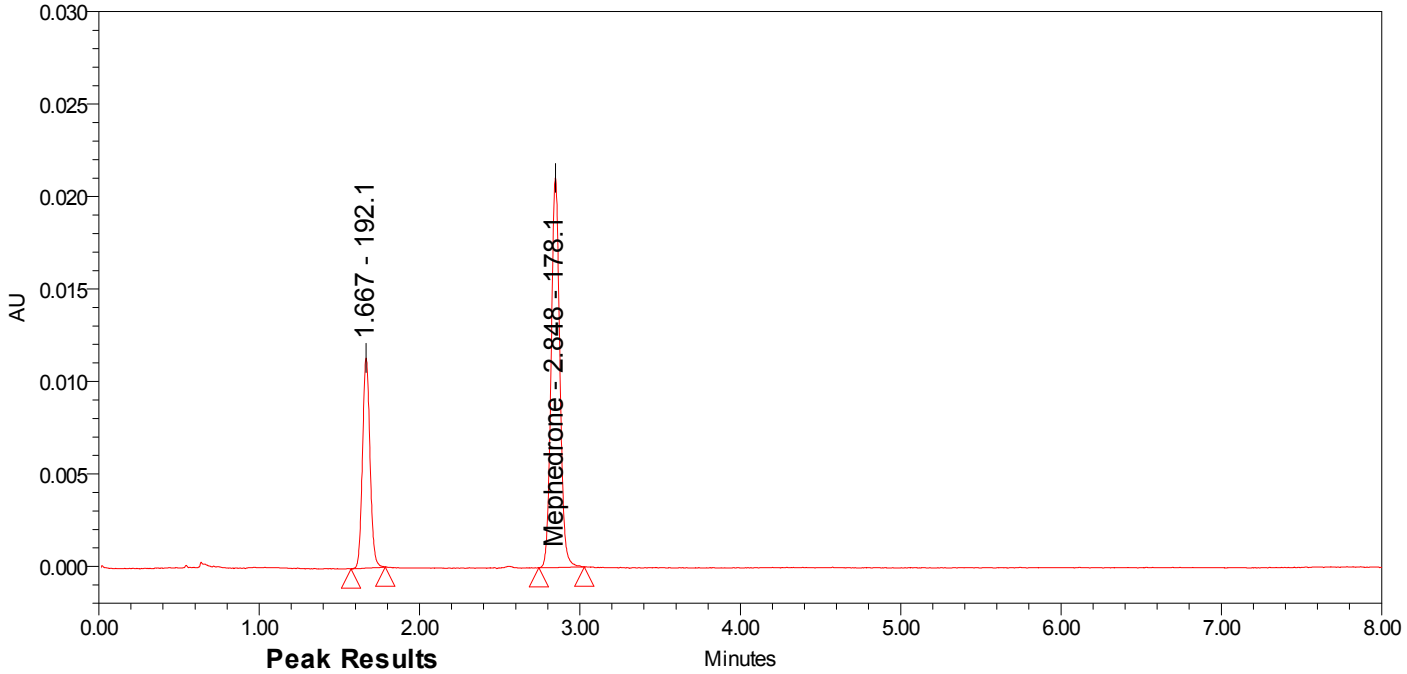
quantitation bath salts

SAMPLE INFORMATION

Sample Name:	mix 14 standard	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	sample 14 UV reprocess Acq.
Vial:	2:E,1	Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts quant comp 250nm
Injection Volume:	0.50 ul	Channel Name:	PDA Ch1 250nm@4.8nm -Compens.
Run Time:	8.0 Minutes		
Dilution 1.0000			
Date Acquired:	5/12/2016 5:12:49 PM EDT		
Date Processed:	6/14/2016 2:00:54 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.667	36970	11374		
2	Mephedrone	2.848	79509	21076	62.500	ug/mL

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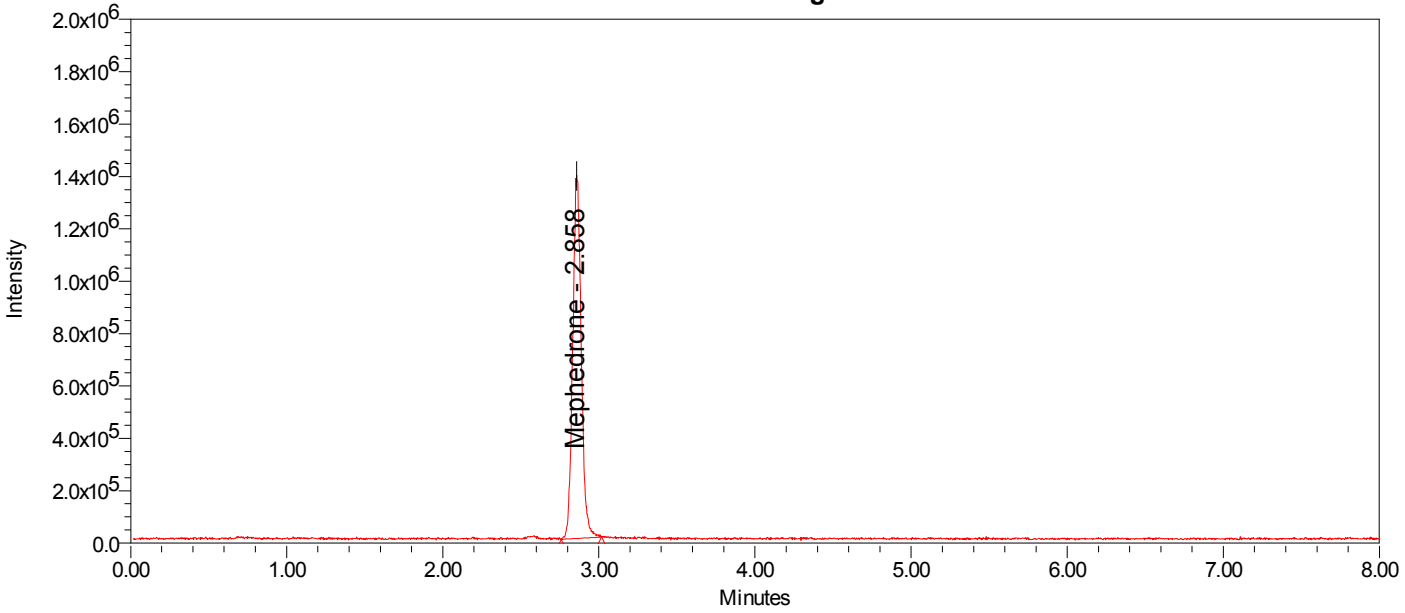
quantitation MS bath salts

SAMPLE INFORMATION

Sample Name:	mix 14 standard dilution	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	mix 14 MS reprocess
Vial:	2:C,5	Acq. Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts 178
Dilution	1.0000	Channel Name:	QDa Ch3 178.12 Da
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch3 178.12 Da, CV=15
Injection Volume:	0.50 ul		
Date Acquired:	5/12/2016 12:02:37 AM EDT		
Date Processed:	6/14/2016 1:58:28 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	Mephedrone	2.858	5248776	1369446	0.625	ug/mL

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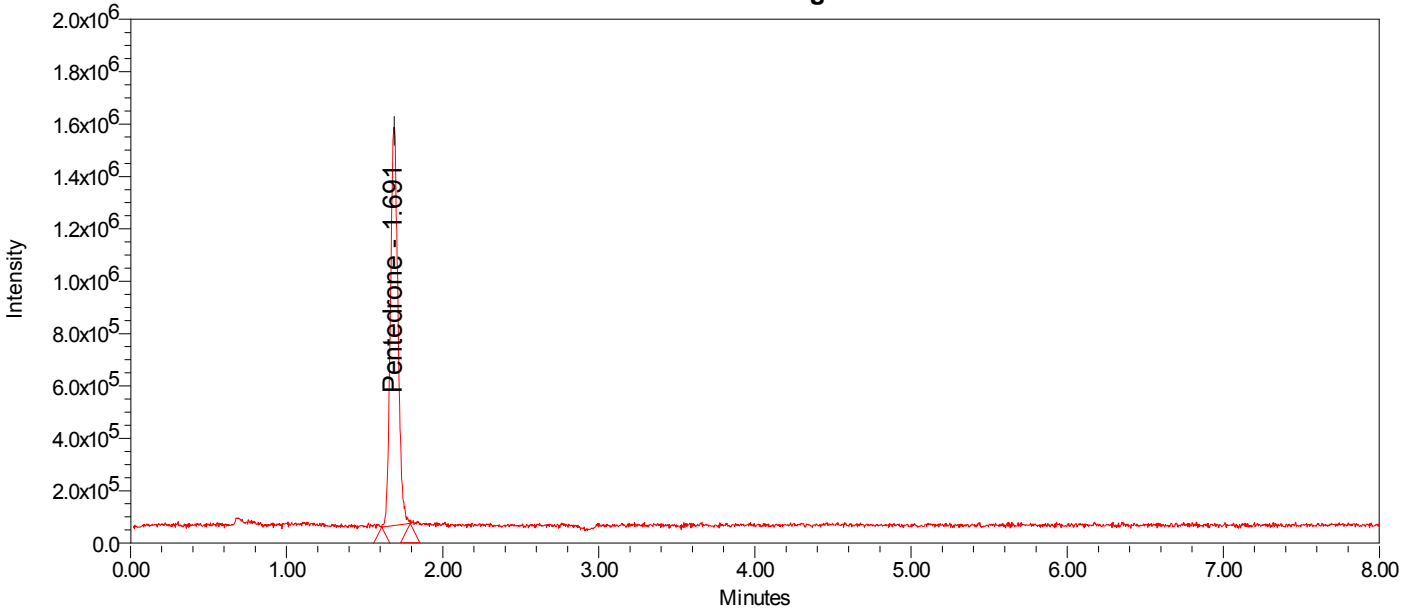
quantitation MS bath salts

SAMPLE INFORMATION

Sample Name:	mix 14 standard dilution	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	mix 14 MS reprocess
Vial:	2:C,5	Acq. Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts 192
Dilution	1.0000	Channel Name:	QDa Ch5 192.14 Da
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch5 192.14 Da, CV=15
Injection Volume:	0.50 ul		
Date Acquired:	5/12/2016 12:02:37 AM EDT		
Date Processed:	6/14/2016 1:58:28 PM EDT		

Auto-Scaled Chromatogram

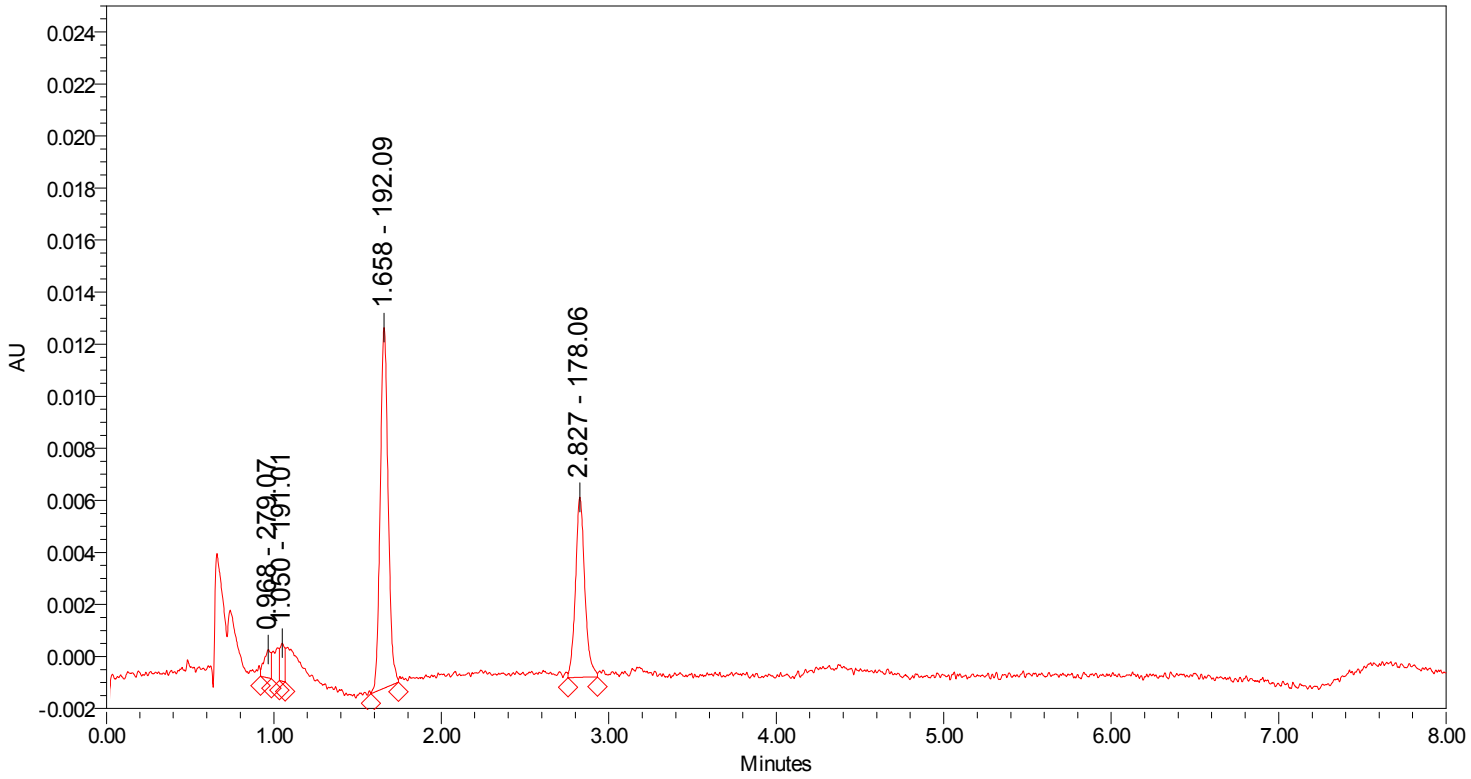


Peak Results

	Name	RT	Area	Height	Amount	Units
1	Pentedrone	1.691	5088924	1513173	0.625	ug/mL

SAMPLE INFORMATION

Sample Name:	mix 14 sample 1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	sample 14 UV reprocess
Vial:	2:E,2	Acq. Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts screening
Injection Volume:	0.50 ul	Channel Name:	PDA 230 nm
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum (210-350)nm
Dilution 1.0000			
Date Acquired:	5/12/2016 5:30:51 PM EDT		
Date Processed:	6/14/2016 2:00:39 PM EDT		



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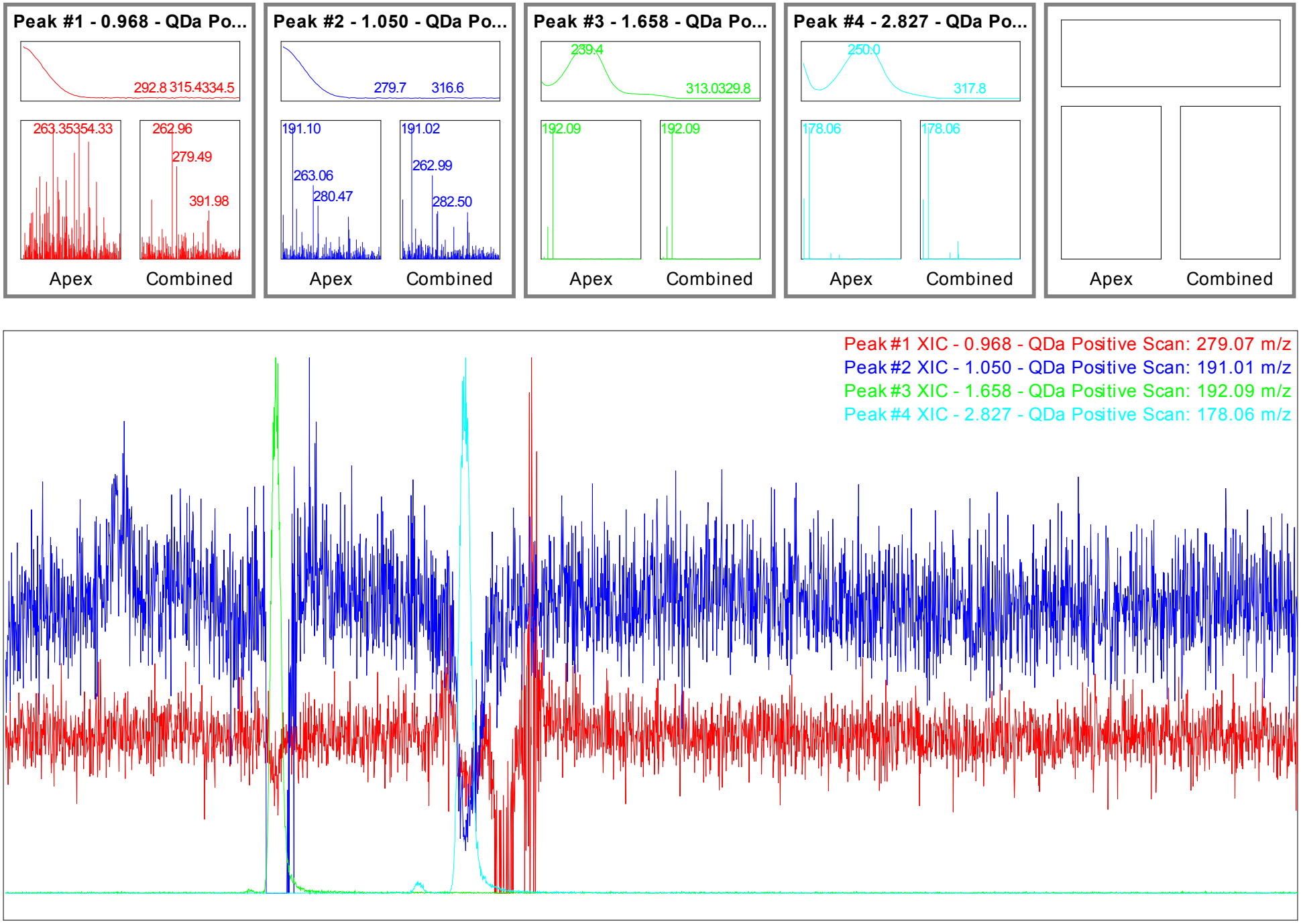

PDA Result Table

	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold	PDA/FLR Match2 Spect. Name	PDA/FLR Match2 Angle	PDA/FLR Match3 Spect. Name	PDA/FLR Match3 Angle	Base Peak (m/z)
1	0.968	33.954	90.000	lidocaine	12.925	90.000					279.07
2	1.050	13.830	90.000	lidocaine	13.606	90.000					191.01
3	1.658	0.929	8.536	Buphedrone	0.565	5.687	Pentedrone	0.801	Methcathinone	1.343	192.09
4	2.827	0.810	5.623	Mephedrone	0.840	4.350	4 Methylethcathinone	1.763	4 MePPP	4.929	178.06

PDA Result Table

	MS Match1 Spect. Name	MS Match2 Spect. Name	MS Match3 Spect. Name
1	Butylone	lidocaine	MDPV
2	lidocaine	MDPV	Naphyrone
3	4-methylethcathinone	Pentedrone	lidocaine
4	Buphedrone	Mephedrone	lidocaine

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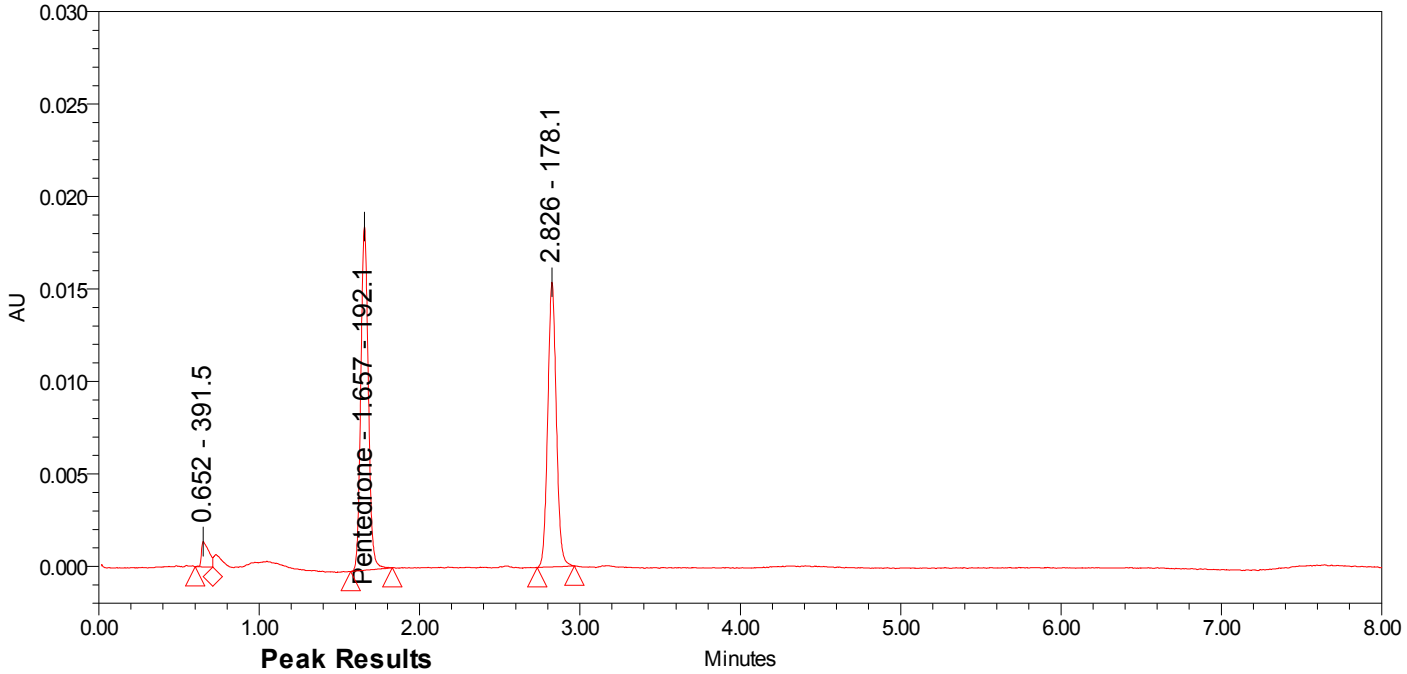
quantitation bath salts

SAMPLE INFORMATION

Sample Name:	mix 14 sample 1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	sample 14 UV reprocess Acq.
Vial:	2:E,2	Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts quant comp 240nm
Injection Volume:	0.50 ul	Channel Name:	PDA Ch3 240nm@4.8nm-Compens.
Run Time:	8.0 Minutes		
Dilution 1.0000			
Date Acquired:	5/12/2016 5:30:51 PM EDT		
Date Processed:	6/14/2016 2:00:57 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		0.652	4376	1368		
2	Pentedrone	1.657	60895	18584	62.399	ug/mL
3		2.826	57279	15395		

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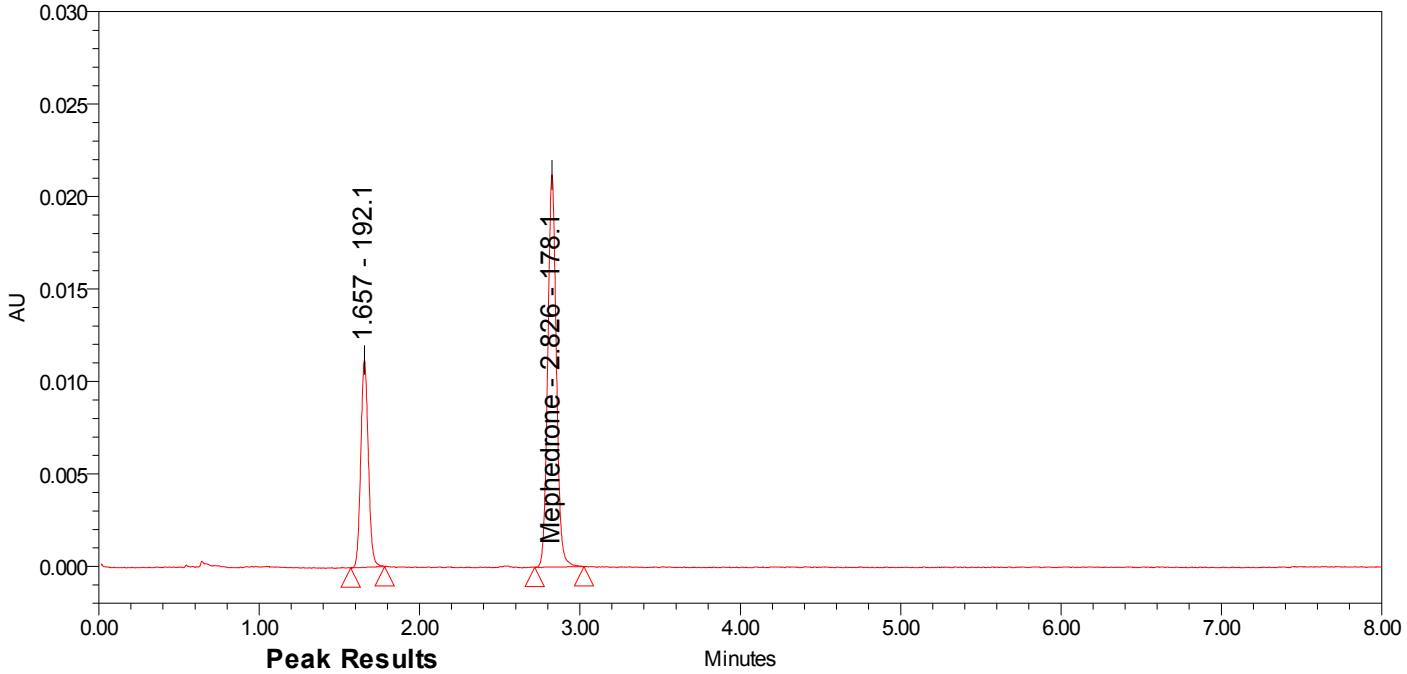
quantitation bath salts

SAMPLE INFORMATION

Sample Name:	mix 14 sample 1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	sample 14 UV reprocess Acq.
Vial:	2:E,2	Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts quant comp 250nm
Injection Volume:	0.50 ul	Channel Name:	PDA Ch1 250nm@4.8nm-Compens.
Run Time:	8.0 Minutes		
Dilution 1.0000			
Date Acquired:	5/12/2016 5:30:51 PM EDT		
Date Processed:	6/14/2016 2:00:59 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.657	36512	11214		
2	Mephedrone	2.826	79490	21220	62.408	ug/mL

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quantitation MS bath salts

SAMPLE INFORMATION

Sample Name:	mix 14 sample 1 dilution	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	mix 14 MS reprocess
Vial:	2:C,6	Acq. Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts 178
Dilution	100.0000	Channel Name:	QDa Ch3 178.12 Da
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch3 178.12 Da, CV=15
Injection Volume:	0.50 ul		
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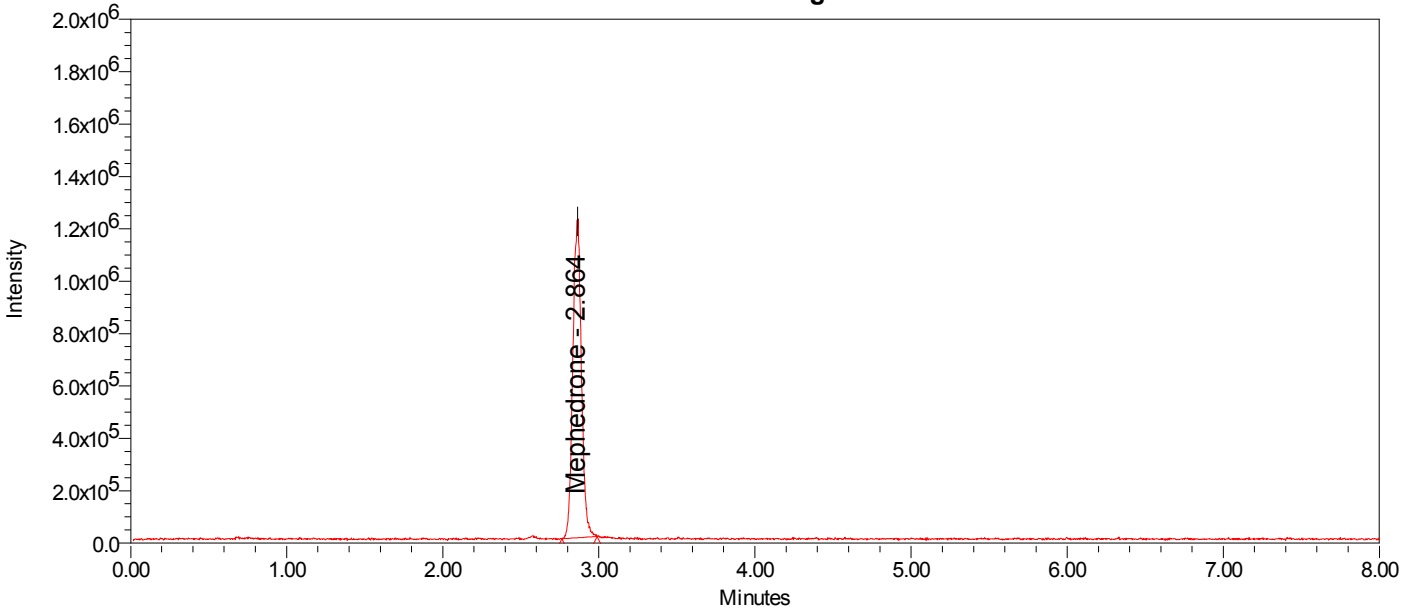
Date Processed:	6/14/2016 1:58:29 PM EDT		

Auto-Scaled Chromatogram



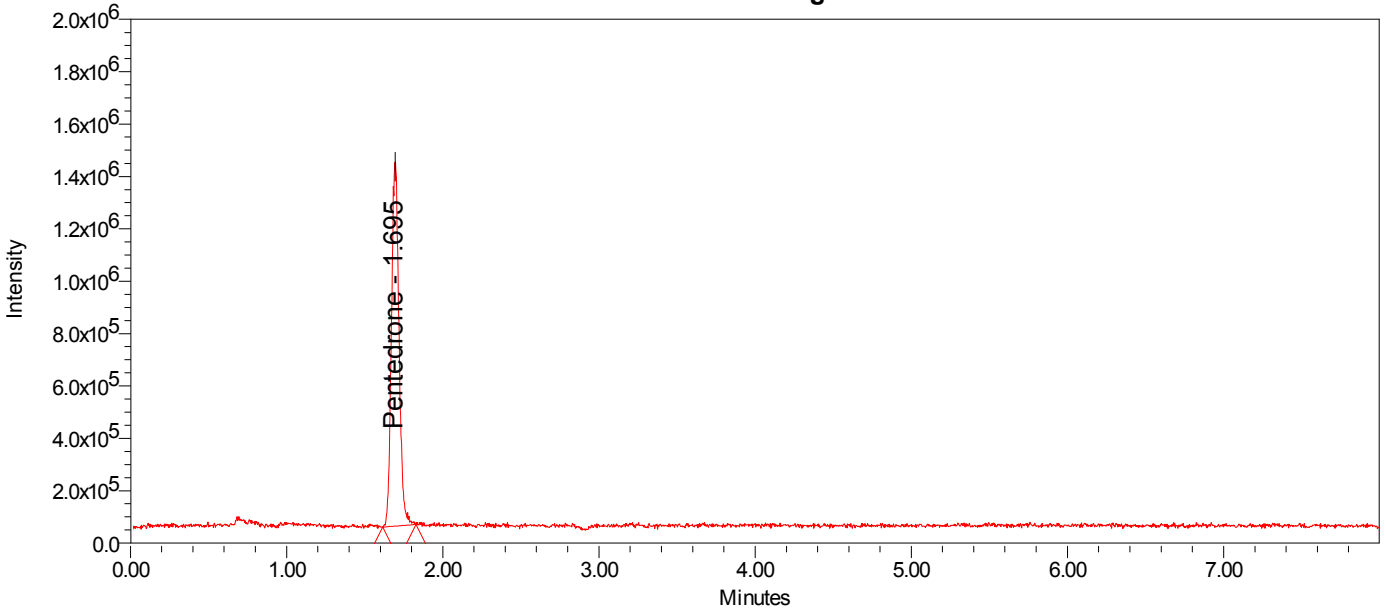
Peak Results

	Name	RT	Area	Height	Amount	Units
1	Mephedrone	2.864	4660802	1211401	56.896	ug/mL

SAMPLE INFORMATION

Sample Name:	mix 14 sample 1 dilution	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	mix 14 MS reprocess
Vial:	2:C,6	Acq. Method Set:	screen bath salts
Injection #:	1	Processing Method:	bath salts 192
Dilution	100.0000	Channel Name:	QDa Ch5 192.14 Da
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	QDa Positive(+) SIR Ch5 192.14 Da, CV=15
Injection Volume:	0.50 ul		
Date Acquired:	5/12/2016 12:20:40 AM EDT		
Date Processed:	6/14/2016 1:58:30 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	Pentedrone	1.695	4684348	1385370	58.849	ug/mL

S5 UHPSFC protocol synthetic cannabinoids

Synthetic cannabinoids drug screening or confirmation and quantitation

Sample preparation- Accurately weigh out 100 mg of ground [1] plant material into a 250 mL volumetric flask. Dilute to volume with solvent A containing heptane/ethyl acetate/methanol (40:20:40). Vortex sample for one minute, filter sample into a 2.0 mL glass vial through a 0.22 µm PTFE syringe filter. For quantitation dilute if necessary with solvent A for sample solution to be in linear range.

Standard preparation- Accurately weigh standard material of target compounds into an appropriate volumetric flask and dilute to volume with solvent A so that final concentration is approximately 4 µg/mL. Ideally, especially for quantitation by MS, the standard concentration should be within 2X of the sample concentration.

UHPSFC conditions[1] [2]- column- Acquity UPC[2] Trefoil CEL2 (2.5 µm 3.0 x 150 mm); mobile phase conditions- injection size, 2µL,; Initial conditions: 20% isopropanol, 80% carbon dioxide. Final conditions: 31% isopropanol, 69% carbon dioxide, 10.3 minute linear gradient, 1.0 min gradient re-equilibration; flow rate 1.25 mL/min; temperature 55°C, ABPR 2200 psi; PDA-UV and single quadropole MS detection. See Figure 1 for chromatogram of controlled synthetic cannabinoids using UV and MS detection.

Linear range and LOD's- See Table 9 for linear range and LOD's for both UV and MS detection.

Data analysis - Empower 3- The following files are available on electronic media upon request of the PI (*optimized methods* and *MS libraries* and *UV libraries* to accomplish both drug screening and quantitation for up to three drugs).

Method Set	Channel Name	Processing Method	Report Method
screen cannabinoid 210	Extract 210.0	lurie syn can screen[a,b]	screen cannabinoids
lurie quant cannabinoids	PDA Chx 215nm@4.8 nm-Compens.	lurie syn cann 031116 215 cmpe	lurie quantitation syn can
	QDA Chx $[M-H]^+$ Da	ms quant $[M-H]^+$	lurie quantitation syn can
	QDA Chx $[M-H]^+$ Da	ms quant $[M-H]^+$	lurie quantitation syn can
	QDA Chx $[M-H]^+$ Da	ms quant $[M-H]^+$	lurie quantitation syn can

[a]UV library - Synthetic Cannabinoids Optimal

[b]MS library- Synthetic Cannabinoids Optimal

For sample reports see S3.

[1] Chromatographic conditions for Water's UHPSFC instrument. These conditions may have to be tweaked for other vendors depending on the dwell volume.

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S6 UHPSFC protocols synthetic cathinones

Bath salt drug screening or confirmation and quantitation

Sample preparation- Weigh out 50 mg of powdered sample into a 500 mL volumetric flask. Dilute to volume with solvent containing methanol. Vortex sample for one minute, filter sample into a 2.0 mL glass vial through a 0.22 µm PTFE syringe filter.

Standard preparation- Accurately weigh standard material of target compounds into an appropriate volumetric flask and dilute to volume with methanol so that final concentration is approximately 62 µg/mL. For MS quantitation dilute 1/100 with methanol. Ideally, especially for quantitation by MS, the standard concentration should be within 2X of the sample concentration.

UHPSFC conditions[1] - column- Acquity UPC[2] Torus DIOL (1.7 µm 3.0 x 100 mm); mobile phase conditions- injection size, 2µL,; Conditions: 3% methanol with ammonium formate, 97% carbon dioxide, 8 minute linear gradient, 1.0 min gradient re-equilibration; flow rate 1.25 mL/min; temperature 40°C, ABPR 2200 psi; PDA-UV and single quadropole MS detection. See Figure 1 for chromatogram of controlled bath salts using UV and MS detection.

Linear range and LOD's- See Table 12 for linear range and LOD's for both UV and MS detection.

Data analysis - Empower 3- The following files are available on electronic media upon request of the PI (*optimized methods* and *MS libraries* and *UV libraries* to accomplish both drug screening and quantitation for up to three drugs).

Method Set	Channel Name	Processing Method	Report Method
screen bath salts	PDA 230 nm	bath salts screening[a,b]	screen bath salts
quantitation bath salts	PDA Chx X nm@4.8 nm-Compens.	bath salts quant com X nm	quantitation bath salts
quantitation MS bath salts	QDA Chx $[M-H]^+$ Da	bath salts $[M-H]^+$	quantitation MS bath salts

[a]UV library – Bath Salts Optimal

[b]MS library- Bath Salts Optimal

For sample reports see S4.

[1] Chromatographic conditions for Water's UHPSFC instrument. These conditions may have to be tweaked for other vendors depending on the dwell volume.

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