Development and Evaluation of a Synthetic Cathinone Targeted Gas Chromatography Mass Spectrometry (GC-MS) Method

To address challenges associated with the increased prevalence of novel psychoactive substances (NPSs), laboratories often adopt new techniques or new methods with the goal of obtaining more detailed chemical information with a higher level of confidence. To demonstrate how new methods applied to existing techniques can be a viable approach, a targeted gas chromatography mass spectrometry (GC-MS) method for synthetic cathinones was developed. To create the method, a range of GC-MS parameters were first investigated using a seven-component test solution with the goal of maximizing chromatographic separation within a reasonable runtime. Once developed, the targeted method was evaluated through several studies and was compared to a general GC-MS confirmatory method. The targeted method produced a two-fold increase in chromatographic separation of the test solution compounds with a 3.83 min shorter runtime than the general method. Limitations of the targeted method were also studied by analyzing an additional forty-eight cathinones to identify instances where definitive compound identification may not be possible due to similar retention times and mass spectra. Thirty-five pairs of compounds had retention times differences between one another of less than 2 % and of those thirty-five two pairs were found to have indistinguishable mass spectra. A set of case samples were also analyzed using the targeted method to evaluate suitability for casework. An increase in split ratio was required to obtain acceptable sensitivity. The development of this method is part of a larger project to measure benefits and drawbacks of different drug chemistry workflows.

Keywords

Seized Drug Analysis; GC-MS; Cathinones; Targeted Methods; Optimization; Retention Index

Highlights

- A targeted method for the analysis of synthetic cathinones was created and evaluated.
- The method provided twice the chromatographic separation with reduced run time.
- After development with a 7-component solution, the method was expanded with 48 additional cathinones.
- Using retention time and mass spectra, all but two pairs of compounds were distinguishable.

Changing chemical structures and the creation of isomeric analogs pose analytical challenges for drug chemists, especially when using generic methods on instruments like gas chromatography mass spectrometry (GC-MS). While there are numerous approaches to address these challenges, many require new instrumentation or modifications to existing instrumentation. One tactic that does not require new instruments is the development of class-specific GC-MS methods that are developed to maximize chromatographic separation of a selection of target compounds thereby enabling identification of a larger suite of analytes than may be afforded by generic methods.

Phenethylamines, of which cathinones are a subclass, accounted for nearly 30 % of all drug reports in 2019(1). While most (~92 %) of these cases were attributed to methamphetamine, the prevalence of cathinones is rising, as evidenced by the spread of eutylone across the country between 2017 and 2019(1). A significant body of research has demonstrated promising methods for screening synthetic cathinones including electrochemical detection(2), ion mobility spectrometry(3), Raman spectroscopy(4), color tests(5), and direct analysis in real time mass spectrometry(6). However, confirmatory identification of synthetic cathinones is difficult due to their simplistic, and highly similar, electron ionization (EI) mass spectra(7). This challenge is echoed by the Organization of Scientific Area Committees (OSAC) Seized Drug Subcommittee, whose “GC-MS Analysis Consideration” document lists at least ten instances where similar mass spectra of cathinones may hinder identification(8). Approaches for more confident identification such as cold-EI(9), derivatization(10), GC-vacuum ultraviolet (GC-VUV)(11,12), or tandem mass spectrometry(13,14) have been proposed but not widely adopted. Chemometric analysis of EI mass spectra for confirmation has also been suggested(15,16). GC-infrared spectroscopy (GC-IR) has been demonstrated(17–19), and adopted by some laboratories, and for pure samples, benchtop infrared spectroscopy can assist in isomer differentiation(20,21). Even with these options many laboratories rely on differentiation of spectrally similar cathinones using differences in GC retention times. Therefore, development of a GC-MS method specifically designed to maximize separation of synthetic cathinones could assist drug chemists by providing increased confidence in their results while aligning with other recent efforts focused on the increasing repeatability of synthetic cathinone mass spectra(22) and establishment of optimal retention time windows(23).

The goal of this work was to develop and evaluate a GC-MS method specifically for the identification of synthetic cathinones using a previously defined framework for the development of a synthetic cannabinoid method(24). The development and evaluation of targeted methods such as this one are part of a larger effort to develop tools and potential solutions to some of the challenges currently faced by drug chemists.

Materials and Methods

Chemicals

Development of the method was completed using a custom-made test solution, consisting of methamphetamine, phentermine, dimethylone, ethylone, butylone, dibutylone, pentylone, dimethylpentylone, and ethylenepenthylone was created (Cayman Chemical, Ann Arbor, MI, USA) with

each compound present at a nominal concentration of 100 µg mL^{-1} in methanol. Methamphetamine and phentermine were included in this mixture to evaluate whether the development of a combined cathinone and amphetamine method would be beneficial. Compounds were chosen based on cost, availability, and the need to have multiple instances of closely eluting compounds. Standards for the expanded list of cathinones (Table 4) incorporated into this method were also obtained from Cayman Chemical.

Instrument and Consumables

The study was completed on an Agilent 7890/5977B GC-MS (Agilent Technologies, Santa Clara, CA, USA) system using ultra-pure helium as the carrier gas. The instrumental methods varied through the study as discussed below. Six columns with stationary phases of DB-1UI, DB-5, DB-5UI, DB-35, DB-200, and VF-1701ms were purchased from Agilent, all with dimensions of 30 m x 0.25 mm x 0.25 µm. The mass spectrometer was tuned daily using Standard Spectrum Tune (stune), unless otherwise noted. Mass spectrometer settings that were held constant throughout the study included a scan range of *m/z* 50 to *m/z* 450, a threshold of 150 counts, and a scan speed of N = 2.

Approach for Method Development and Data Analysis

Development and evaluation of the targeted method followed the six-step framework that is discussed in detail elsewhere(24). The two main goals of the development process are to maximize chromatographic separation in a reasonable amount of time and understand the relationship between sensitivity and reproducibility for different instrument parameters using the test solution. In Step 1, different column types are investigated while holding other parameters constant to determine the stationary phase that offers the best level of chromatographic separation, sensitivity, and peak purity. In Step 2, the column flow rate and oven temperature program are varied to maximize chromatographic separation of the test solution while maintaining an acceptable run time using the column chosen on Step 1. In Step 3, a design of experiment is used to understand the effect of injection volume, inlet temperature, split ratio, and source temperature have on sensitivity and reproducibility. Tune type is also evaluated in this step. From these results a preliminary targeted method is created which is further evaluated.

Evaluation of the method begins by expanding the list of relevant compounds analyzed (in this case 55 cathinones) to establish locked retention times and retention indices (Step 4). During this step, instances where differentiation of compounds based on retention time or mass spectral similarity is not possible are identified. The method is then compared to a general confirmatory method (Step 5a) after which a set of ten case extracts are run to assess the sensitivity and potential for carryover (Step 5b). Data was analyzed using the methods described in (24) and summarized in the Supplemental Information.

Results and Discussion

Comparing the Performance of Different Columns (Step 1)

Comparison of the six stationary phases was completed by analyzing the test solution on all columns, in triplicate, using the method shown in Supplemental Table 1. Retention time, peak area, peak height, peak width, and mass spectral quality matches (full width half max) for all compounds were then extracted. In addition to these values the percent retention time difference (Eqn. 1) was calculated between sequentially elution pairs of compounds, as a measure of chromatographic separation.

$$\%RTD = \frac{((Retention\ Time\ Compound\ 2) - (Retention\ Time\ Compound\ 1))}{(Retention\ Time\ Compound\ 1)} * 100 \quad \textbf{Eqn.1}$$

Results for the column comparison study are presented in Figure 1 and Table 1, with representative chromatograms of the test solution analyzed on each column displayed in Supplemental Figure 1. Differences in the chromatographic separation (%RTD) between compounds were readily observed (Figure 1A) across the different columns. Use of higher polarity columns (DB-35, DB-200, and VF-1701ms) resulted in smaller %RTDs (poorer separation) compared to the other columns. Resolving two compound pairs – butylone and dibutylone (Figure 1(A.) orange datapoints) as well as pentylone and dimethylpentylone (Figure 1(A.) dark blue datapoints) proved to be particularly difficult on higher polarity columns. Using the DB-200 column, pentylone and dimethylpentylone nearly co-eluted, with a %RTD of 0.03 %.

Measurement of the peak areas across the different columns showed relatively minor differences (Figure 1(B.)), though the lower polarity columns produced smaller standard deviations across the triplicate measurements. Apart from the DB-200 column, peak purity measurements, which estimate the fraction of peaks in a spectrum attributable to a compound, were above 90 % for all compounds on all columns (Figure 1(C.)). The DB-200 column did have noticeable column bleed which likely caused the lower peak purity measurements. Mass spectral similarity scores (Table 1) which were obtained by comparing the spectra to those present in the SWGDRUG mass spectral library (v3.6), were largly uniform with average scores exceeding 90 for all columns except the DB-200. Peak widths (Table 1) were also similar across the different columns. Based on the results, the DB-5 column was chosen for the targeted method as it is the column currently used in casework and there were no obvious advantages presented by other columns.

Assessment of Temperature and Flow Parameters to Maximize Chromatographic Separation (Step 2)

Once the stationary phase was chosen, the flow rate and oven temperature program were varied to attempt to maximize separation (%RTD) of the components in the test solution in a reasonable amount of time. Both ramped (5 °C min^{-1}, 15 °C min^{-1}, and 30 °C min^{-1}) and isothermal temperature programs were evaluated with flow rates ranging from 0.8 mL min^{-1} to 2.0 mL min^{-1}. The temperature programs that were evaluated, as well as summary results, are shown in Table 2 and representative chromatograms of the different settings are displayed in Supplemental Figure 2.

While completing these experiments, a large difference in elution temperatures and elution times between the amphetamines (methamphetamine and phentermine) and the cathinones was observed (Supplemental Figure 2), leading to the decision to remove the amphetamines from method development efforts and instead focus on the development of an amphetamine specific method in the future. For the cathinones, an isothermal temperature of 250 °C produced non-baseline separation regardless of the flow rate used, while ramped temperature programs produced %RTDs of at least 1 % between all compounds. Ultimately, it was found that a multi-ramp temperature program (190 °C, hold for 0.5 min, ramp 5 °C min^{-1} to 210 °C, ramp 30 °C min^{-1} to 255 °C, hold for 0.5 min) was best, as it provided separation of the necessary cathinones at a slower ramp rate followed by a faster ramp rate to attain elution of less volatile cathinones.

Maximizing Sensitivity and Reproducibility (Step 3)

Once separation was maximized, the effects of instrument parameters on sensitivity and reproducibility were measured using a two-level, 2^{4-1}, design of experiments (DOE). MS source temperature, split ratio, injection volume, and inlet temperature were studied using the levels shown in Figure 2. The DOE required a total of 8 different combinations of settings (Supplemental Table 2), each analyzed in triplicate. Peak height and peak area were used as measures of sensitivity while the percent relative standard deviation (%RSD) for peak height, peak area, retention time, and %RTD across triplicate measurements were used as measures of reproducibility.

The results from the DOE are shown in Figure 2. Changes in source temperature (2A. and 2E.) and inlet temperature (2D. and 2H.) had minimal effect on the measured sensitivity and reproducibility. The change in split ratio did affect measured quantities (2B. and 2F.), but the observed differences were not statistically significant at the 95 % confidence level. As expected, increased injection volume resulted increased sensitivity (2C.). However, injection volume had minimal effects on reproducibility measures (2G.). Due to the relative purity of encountered cathinone samples combined with the high likelihood of backflash when using a 2 µL injection, a mid-range injection volume of 1 µL was chosen. A source temperature of 280 °C and an inlet temperature of 300 °C were chosen, to minimize potential build up on the source, along with a split ratio of 10:1, to increase sensitivity – though these parameters could be altered to meet the needs of other laboratories. It should be noted that, while not observed in this work, higher inlet temperature have been attributed to breakdown of cathinones in the GC inlet and therefore care should be taken when choosing an appropriate setting(25,26).

Tune type, specifically autotune (atune) and standard spectrum tune (stune), was also evaluated through triplicate analysis of the test solution. Regardless of tune type, mass spectral similarity to reference spectra contained in the SWGDRUG library was greater than 93/100 a.u. as estimated by AMDIS(27) for all compounds. Calculated peak areas were also shown to be approximately equal between tune types. Stune was therefore chosen for the targeted method as it is the tune currently used in the laboratory and no

obvious advantage was gained from using atune. The final settings for the targeted method can be found in Table 3 while the method file and other auxiliary data can be found here(28).

Analysis of Additional Cathinones (Step 4)

To evaluate the method, all cathinone standards available at Maryland State Police Forensic Sciences Division (MSP-FSD) (total of 55 cathinones listed in Table 4) were analyzed. In analyzing the standards, it was determined that the method needed to be extended for an additional minute to allow for elution of late-eluting cathinones 2-methyl-4'-(methylthio)-2-morpholinopropiophenone (MTMP), naphyrone, and 3,4-methylenedioxy-N-benzylcathinone (BMDP). Once extended, the method was retention time locked using butylone (3.182 min) as the lock compound. Mixtures containing well-separated subsets of the 55 cathinones were then analyzed ten times each to established locked retention times and an even-numbered alkane ladder was included in the sequence so retention indices (RIs) could be calculated. The average retention times and RIs are shown in Table 4. Retention times ranged from 1.410 min to 7.229 min, with retention indices between 1376 a.u. and 2395 a.u. The %RTDs between subsequently eluting compounds ranged from 0 % (co-eluting) to 19.24 % (1.072 min), with an average %RTD of 3.19 % (0.110 min) and a median %RTD of 1.96 % (0.046 min). Retention times across the ten replicate injections were highly repeatable, with an average %RSD of 0.04 % and a max %RSD of 0.17 %.

To understand the ability of the method to distinguish cathinones, the locked retention time data was combined with the mass spectral data. To identify compound pairs that may not be differentiable using the targeted method all compound pairs that had a %RTD of less than 2 % were identified (38 pairs). The min-max test, described in the Supplemental Information and elsewhere(28), was then used to compare the replicate mass spectra of the three pairs to determine if the mass spectra of the co-eluting compounds could be differentiated (indicated by a positive min-max index). Two pairs of compounds, 2-chloroethcathinone | 3-chloro-N,N-dimethylcathinone (min-max index of -24 a.u. and %RTD of 0 %) and 3-chloroethcathinone | 4-chloroethacathinone (min-max index of -19 a.u. and %RTD of 1.96 %) were found to have negative min-max indices indicated that they could not be differentiated by their mass spectra. Min-max results and %RTDs for all 38 pairs can be found in Supplemental Table

Limits of Detection and Comparison to the General Method (Step 5a)

The method was then compared to one of the general confirmatory methods used at MSP-FSD – the parameters of which are provided in the Supplemental Table 4. First, approximate limits of detection (LODs) were established for both methods (experimental procedures provided in the Supplemental Information). An approximate LOD of 5 µg mL^{-1} was found for all cathinones using the general confirmatory method (Supplemental Table 5) while the targeted method produced an approximate LOD of 1 µg mL^{-1} for all compounds using an injection volume of 1 µL and a split ratio of 10:1. This was expected given the five-fold decrease in split ratio used in the targeted method. An approximate LOD of 5 µg mL^{-1} was obtained when the split ratio of the targeted method was increased from 10:1 to 30:1, which was necessary for case sample analysis (described below). The undiluted test solution was then analyzed on both the targeted

method and the general confirmatory method, in triplicate, to establish the gains in sensitivity (measured by peak area) and chromatographic separation (measured by %RTD) obtained by using the targeted method. The targeted method produced between a 5-fold and 8-fold increase in peak area (Supplemental Table 5) using an injection volume of 1 µL and a split ratio of 10:1. In terms of chromatographic separation, the %RTD between neighboring peaks increased roughly two-fold for all compounds, even with a 3.83 min reduction in runtime. Representative chromatographs of the test solution run on the targeted method and the general confirmatory method are displayed in Figure 3.

Evaluation of the Method with Case Samples (Step 5b)

The final evaluation study involved analyzing case samples with the method as a pre-validation step to identify and address issues that may occur because of increased sensitivity or decreased runtime. Ten adjudicated case sample extracts containing synthetic cathinones were analyzed on the targeted method with a five-minute isothermal (290 °C) methanol blank run between case samples. Initial analyses found that while carryover did not occur, the use of a 10:1 split ratio led to peak saturation for some samples. The split ratio was increased to 30:1 in order to obtain the desired level of sensitivity given the sample preparation methods used (discussed in the Supplemental Information). The resulting chromatograms are shown in Supplemental Figure 3.

Detection of the synthetic cathinone in each of the case extracts was readily achievable and retention times were within 0.008 min (0.17 %) of the standard's average retention time in all instances. Mass spectral similarity scores, computed using AMDIS, were at or above 88/100 a.u., and detector saturation was not observed. Elution of the non-catinone compounds that were less volatile was not observed but mannitol (3.147 min) and caffeine (3.654 min) were found to elute during the run. If the intended use of the method is to only detect synthetic cathinones, a short (5 min to 7 min) high temperature isothermal (290 °C) blank run can be implemented between samples to allow for elution of these compounds and prevent carryover to the next sample. Alternatively, if seeing the entire chemical profile of the sample is desired, an additional ramp and hold can be added to the temperature program of the method (30 °C min^{-1} to 290 °C, hold for 7 min) that will allow for elution of the additional compounds in the run, such as fentanyl or synthetic cannabinoids. This approach increases the run time by 8.33 min but maintains the separation capability for the synthetic cathinones, compared to the general confirmatory method.

Conclusion

From this work, a method for the targeted analysis of synthetic cathinones was created. Compared to a general confirmatory method used at MSP-FSD the targeted method was found to provide a two-fold increase in chromatographic separation, and a reduction in analysis time of up to 3.83 min. When evaluated against a larger panel of 55 cathinones, only two pairs of compounds, 2-Chloroethcathinone | 3-Chloro-N,N-dimethylcathinone (0.01 % %RTD) and 3-Chloroethcathinone | 4-Chloroethcathinone (1.96 % %RTD) were not distinguishable based on a 2 % %RTD and the min-max spectral comparison test. The method was evaluated against case samples and found to perform well even with the presence of additional drugs

and excipients. The development of targeted GC-MS methods is part of a larger project aimed at providing forensic laboratories potential solutions for challenges they commonly face. Current, and future, research efforts are focusing on the validation of this method for casework along with expansion of synthetic cathinones examined, creation of additional targeted methods, and studies to measure the gains and drawbacks from implementation of targeted methods. Updated data incorporating expansion of the method can be found here(28). Further developments regarding the min-max mass spectral similarity test are also underway.

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Tables:

Table 1. Summary results of key metrics for the different stationary phases examined. Uncertainties indicate the standard deviation of averages obtained for each of the nine compounds in the test solution.

Column Type	Max RT (min)	Min %RTD	Avg. MS Match Score	Avg. Peak Width (min)
DB-1 UI	5.12	1.04 (±0.01	95.7 (±3.0)	0.06 (±0.01)
DB-5	5.16	1.02 (±0.00)	93.3 (±6.5)	0.07 (±0.02)
DB-5 UI	5.28	1.17 (±0.07)	92.2 (±6.2)	0.07 (±0.01)
DB-35	5.73	0.51 (±0.00)	92.1 (±6.6)	0.05 (±0.01)
DB-200	4.76	0.03 (±0.00)	89.3 (±10.5)	0.04 (±0.01)
VF-1701ms	5.76	0.17 (±0.06)	92.7 (±6.5)	0.06 (±0.02)

Table 2. Summary results from the different temperature and flow programs investigated in Step 2. The notation "CEP" denotes instances where there were co-eluting peaks. Results do not include data for methamphetamine and phentermine. Uncertainties are the standard deviation of triplicate measurements.

Temperature Program	Flow Rate (mL min^{-1})	Minimum %RTD (%)	Median %RTD (%)	Maximum Retention Time (min)
100 °C, hold for 0 min, 5 °C min^{-1} to 300 °C	0.8	1.48 (±0.02)	2.49 (±0.01)	21.11
100 °C, hold for 0 min, 5 °C min^{-1} to 300 °C	2.0	1.64 (±0.00)	2.89 (±0.00)	18.61
100 °C, hold for 0 min, 15 °C min^{-1} to 300 °C	0.8	1.21 (±0.01)	1.72 (±0.00)	9.63
100 °C, hold for 0 min, 15 °C min^{-1} to 300 °C	2.0	1.33 (±0.01)	1.98 (±0.00)	8.46
100 °C, hold for 0 min, 30 °C min^{-1} to 300 °C	0.8	0.97 (±0.00)	1.34 (±0.01)	6.02
100 °C, hold for 0 min, 30 °C min^{-1} to 300 °C	2.0	1.19 (±0.07)	1.56 (±0.01)	5.21
Isothermal 250 ºC	0.8	CEP	2.28 (±0.01)	2.64
Isothermal 250 ºC	2.0	CEP	2.25 (±0.05)	1.70
190 °C, hold for 0.5 min, 5 °C min^{-1} to 210 °C, then 30 °C min^{-1} to 255 °C, hold for 0.5 min	2.0	3.29 (±0.11)	4.78 (±0.05)	4.47

Table 3. Settings for the targeted method. Split ratio and injection volume could be altered, as necessary, to achieve the desired method sensitivity. Settings in parenthesis represent those chosen after the analysis of representative case samples, which required a decrease in instrument sensitivity. Inlet temperature and source temperature can be varied without effecting reproducibility.

Column	DB-5 30 m x 0.25 mm x 0.25 µm
Temperature Program	1) 190 °C for 0.5 min 2) Ramp 5 °C min^{-1} to 210 °C 3) Ramp at 30 °C min^{-1} to 255 °C 4) Hold 1.5 min
Flow Rate	1.9 mL min^{-1}
Injection Volume	1.0 µL
Inlet Temperature	300 °C
Split Ratio	10:1 (30:1)
Transfer Line	300 °C
Quad Temperature	150 °C
Source Temperature	280 °C
Tune Mode	stune
Solvent Delay	1.15 min
Mass Scan Range	*m/z* 40 – *m/z* 550
Threshold	150
Scan Speed	N = 2 [≈4 scans s^{-1}]
Total Run Time	7.5 min

Table 4. Expanded list of compounds analyzed by the targeted method. Retention times and indices are the averages of ten replicates from the approximate 100 µg mL^{-1} solution. The uncertainties represent the standard deviation of ten replicates.

Compound	RT (min)	RI (a.u.)	%RTD	RTD (min)	Resolution (a.u.)
3-Fluoromethcathinone	1.410 (±0.000)	1376 (±0)	0.78	0.011	
4-Fluoromethcathinone	1.421 (±0.002)	1380 (±2)	8.59	0.121	
Cathinone	1.543 (±0.003)	1424 (±2)	5.83	0.090	
2-Chloro-N,N-dimethylcathinone	1.633 (±0.002)	1457 (±2)	5.27	0.087	
N-Ethylbuphedrone	1.719 (±0.000)	1487 (±0)	0.00	0.000	
4-Methylmethcathinone	1.719 (±0.001)	1487 (±0)	1.40	0.024	
2-Ethylmethcathinone	1.743 (±0.000)	1496 (±0)	2.98	0.052	
3-Methylethcathinone	1.795 (±0.000)	1514 (±0)	0.67	0.012	
Pentedrone	1.807 (±0.000)	1519 (±0)	0.94	0.017	
4-Methyl-N,N-dimethylcathinone	1.824 (±0.000)	1525 (±0)	0.99	0.018	
3-Methylbuphedrone	1.842 (±0.001)	1532 (±1)	1.25	0.023	
2,4-Dimethylmethcathinone	1.865 (±0.000)	1539 (±0)	0.64	0.012	
4-Chloromethcathinone	1.877 (±0.000)	1544 (±0)	1.17	0.022	
3-Ethylmethcathinone	1.899 (±0.002)	1551 (±2)	1.26	0.023	
4-Methylbuphedrone	1.923 (±0.002)	1560 (±1)	0.94	0.019	
2,3-Dimethylmethcathinone	1.941 (±0.000)	1566 (±0)	0.31	0.006	
2-Chloroethcathinone	1.947 (±0.000)	1569 (±0)	0.00	0.000	
3-Chloro-N,N-dimethylcathinone	1.947 (±0.000)	1569 (±0)	2.36	0.046	
4-Chloro-N,N-dimethylcathinone	1.993 (±0.000)	1585 (±0)	0.90	0.018	
4-Ethylmethcathinone	2.011 (±0.000)	1592 (±0)	0.45	0.009	
3-Chloroethcathinone	2.020 (±0.003)	1595 (±2)	1.98	0.040	
4-Chloroethcathinone	2.060 (±0.003)	1604 (±2)	2.72	0.056	
4-Chloro Buphedrone	2.116 (±0.000)	1616 (±0)	1.09	0.023	
3,4-Dimethylmethcathinone	2.139 (±0.000)	1620 (±0)	7.62	0.163	
Methedrone	2.302 (±0.000)	1651 (±0)	0.17	0.004	
N-Ethyl Hexedrone	2.306 (±0.003)	1652 (±2)	5.16	0.119	

4-Methyl-α-ethylaminopentiophenone	2.425 (±0.000)	1674 (±0)	9.98	0.242	
α-Piperidinobutiophenone	2.667 (±0.003)	1721 (±2)	3.41	0.090	
Methylone	2.758 (±0.002)	1739 (±1)	1.02	0.028	
2,3-Ethylone isomer	2.786 (±0.000)	1744 (±0)	6.89	0.192	
Dimethylone	2.978 (±0.000)	1781 (±0)	0.91	0.027	
MePPP	3.005 (±0.003)	1786 (±2)	2.60	0.078	
Ethylone	3.083 (±0.000)	1801 (±0)	0.91	0.028	
α-PVP	3.111 (±0.003)	1804 (±1)	2.28	0.071	
Butylone	3.182 (±0.000)	1812 (±0)	1.10	0.035	
3,4-Methylenedioxy-N-isopropylcathinone	3.217 (±0.002)	1816 (±1)	4.17	0.135	
2,3-Pentylone	3.351 (±0.000)	1833 (±0)	1.13	0.037	
Dibutylone	3.389 (±0.001)	1837(±2)	1.65	0.056	
3,4-Methylenedioxy-α-methylamino-Isovalerophenone	3.445 (±0.000)	1844 (±0)	0.17	0.006	
3',4'-Methylenedioxy-N-tert-butylcathinone	3.451 (±0.001)	1845 (±0)	2.00	0.070	
Eutylone	3.520 (±0.000)	1853 (±0)	6.48	0.228	
α-Pyrrolidinohexanophenone	3.748 (±0.000)	1880 (±0)	0.00	0.000	
3,4-Methylenedioxy-N-propylcathinone	3.748 (±0.000)	1880 (±0)	0.67	0.025	
Pentylone	3.773 (±0.003)	1883 (±1)	6.12	0.231	
N,N-Dimethylpentylone	4.004 (±0.000)	1912 (±0)	3.65	0.146	
N-Ethylpentylone	4.150 (±0.000)	1929 (±0)	14.34	0.595	
MPHP	4.745 (±0.001)	2001 (±0)	3.84	0.182	
N,N-Diethylpentylone	4.927 (±0.003)	2029 (±1)	3.19	0.157	
N-sec-butyl Pentylone	5.084 (±0.002)	2053 (±1)	2.71	0.138	
N-isobuytl Pentylone	5.222 (±0.001)	2074 (±0)	5.92	0.309	
N-propyl Hexylone	5.531 (±0.000)	2100 (±0)	0.72	0.040	
N-Butyl Pentylone	5.571 (±0.002)	2126 (±1)	19.26	1.072	
2-Methyl-4'-(methylthio)-2-Morpholinopropiophenone	6.644 (±0.002)	2297 (±1)	5.72	0.380	
Naphyrone	7.024 (±0.001)	2360 (±0)	2.92	0.206	
BMDP	7.229 (±0.003)	2395 (±1)	N/A	N/A	

Table 5. Results from the analysis of adjudicated and mock case samples. The notation “NEP” denotes instances where other compounds in the mixture did not elute within the run time of the method.

Case #	Case Contents	Cathinone Peak Height (cps)	Cathinone RT (min)	Standard RT (min)	MS Match Score (Weighted)
1	MPHP	$3.9x10^5$	4.753	4.745	96
2	4-Ethylmethcathinone	$1.5x10^6$	2.007	2.011	96
3	Dibutylone Caffeine	$1.6x10^6$	3.392 3.654	3.392	96
4	4-Methyl-α-ethylaminopentiophenone	$3.7x10^6$	2.420	2.425	96
5	α-PVP	$3.7x10^5$	3.107	3.111	97
6	Eutylone	$4.0x10^6$	3.527	3.520	95
7	N-Ethylpentylone	$7.1x10^7$	4.168	4.150	96
8	4-Chloro-N,N-dimethylcathinone Eutylone BMDP	$6.9x10^5$ $6.0x10^4$ $1.6x10^7$	1.988 3.524 7.274	1.993 3.520 7.229	94 88 97
9	α-PBP Mannitol 5F-AKB48	$1.5x10^6$	2.663 3.147 NEP	2.664	97
10	Dibutylone Fentanyl JWH250	$3.6x10^6$	3.398 NEP NEP	3.392	96

Figure Captions:

FIG. 1 – Results of the column comparison studies. Chromatographic separation, defined as the percent difference in retention time (%RTD) between subsequent compounds, labeled 1 through 9, with consistent elution order, is shown in (A.), where points closer to the center of the web indicate a smaller degree of separation. Note that the points in (A.) are plotted on a log scale. The average peak areas (B.) and peak purities (C.) for the each of the compounds are also presented. Uncertainties indicate the standard deviation of triplicate measurements.

FIG. 2 – Results of the DOE study for source temperature (A. and E.), split ratio (B. and F.), injection volume (C. and G.), and inlet temperature (D. and H.). Peak area (blue) and peak height (orange) are shown in A. through D. while the average %RSD of peak area (blue), peak height (orange), retention time (green), and %RTD (yellow) across triplicate injections are shown in E. through H. Boxes with asterisks (*) indicate those settings in which a statistically significant difference was observed at the 95 % confidence level.

FIG. 3 – Comparison of the test solution analyzed using the targeted method (top, red) and the general confirmatory method (bottom, blue).