APPLICATION NOTE

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Bath salts and cannabinoids analyzed by GC-IR

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GC-IR, FTIR, bath salts, cannabinoids, cathinones, methcathinone, forensics

Introduction

Drug case criminal prosecution relies upon laws specifying what is and what is not legal. Underground chemists try to avoid prosecution by modifying illegal materials to produce synthetic "designer drugs" which may slip through legal loopholes. Recent designer drug targets include cathinones and cannabinoids. Cathinones and related drugs are found on the street labeled as "Bath Salts" (due to a resemblance to commercial bath salts, though completely unrelated; methcathinone is a common example). Synthetic cannabinoids have an affinity for the cannabinoid receptor in the brain, providing a "high" similar to marijuana. Marijuana itself contains over 50 different cannabinoids. The Tennessee Bureau of Investigation (TBI) laboratory has considerable experience analyzing street samples of both cathinones and cannabinoids.

Crystalline cathinones tend to be sold in single-dose capsules, labeled either as bath salts or plant food (though never used in either capacity) and bearing a disclaimer of "not intended for human consumption." The capsules often contain relatively pure cathinones in amounts above an effective dosage, leading to toxicity effects ranging from headaches and nausea to death.

Cannabinoids commonly appear in small packages filled with dried plant matter, similar to potpourri. Outlets like gas stations or small cigar shops provide users with easy access; co-location of pipes and potpourri can be a trigger for suspicion. To make the product, the cannabinoid dissolved in a solvent is sprayed or soaked on to the plant matter, which is then dried. For example, one production facility filled a small swimming pool with the mixture and stirred with a wooden paddle. The pool and paddle were not cleaned between batches, so the resulting product contained multiple cannabinoids.

The synthesis of these compounds began through a legitimate search for therapeutic drugs in the 1940s. Street sources of both bath salts and synthetic cannabinoids have become more prevalent since 2009 as the skills and sophistication of the producers have improved, making them a current hot topic in law enforcement circles. Unfortunately, media attention increased the visibility of the drugs and created interest in experimentation. With these driving forces and the current legal landscape, forensic analysts require rapid, efficient analysis leading to chemical identification.

Figure 1 shows the chemical structure of some synthetic cathinones and cannabinoids, including those to be discussed below. The subtle differences highlight the legal issue – by moving one chemical group on the regulated "A" compound to another location, the chemist may produce unregulated "B" which maintains or increases the potency yet avoids prosecution through a legal loophole. Some regions try to fill this hole with broad statements such as "A and analogs," but this is not always successful: what defines an analog?

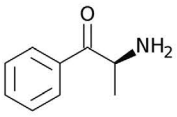
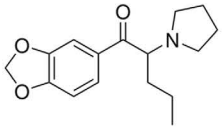
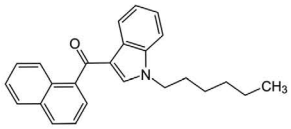
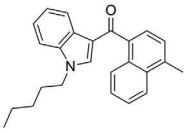
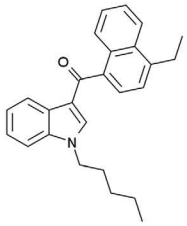
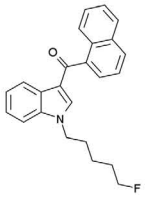
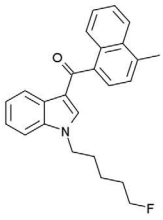
Compounds	Structures
Cathinone (Bath Salt)	
MDPV (Bath Salt)	
JWH-019	
JWH-122	
JWH-210	
AM-2201	
MAM-2201	

Figure 1: Structures of some of the Bath Salts and Cannabinoids seen periodically by the TBI Laboratory

This legal landscape has led to a surge of interest in gas chromatography-infrared (GC-IR) analysis. In GC-MS (mass spectrometry), the molecule is broken down into component pieces for mass analysis, giving excellent sensitivity. However, with the molecule "shattered," the isomeric information is lost ("A" and "B" look the same). GC-IR investigates the molecule while still intact, enabling "A" and "B" to be distinguished. This paper focuses on separation of the compounds and the subsequent analysis including aspects like overlapped peaks and isomeric synthetic drugs.

Experimental

Typical samples of cathinones arrive at the TBI laboratory as capsules or loose powder. The drug is converted to a base by mixing with 0.5 M NaOH to improve the chromatography. The solution is then separated with chloroform for injection. Cannabinoids arrive in bags containing plant matter and visibly resembling potpourri (flaked leaves). A portion of the sample is soaked in methanol. Minimal methanol is added, just wetting the plant material and leaving a small amount – a drop, ideally – of extra liquid. If an excessive amount of methanol is present, the sample may need to be dried down to concentrate the drug. A GC syringe is used to uptake 2 microliters of the liquid; no other preparation is needed.

Standards of the cannabinoids and bath salts (Cayman Chemical®) were mixed with methanol to obtain 1 mg/mL solutions. These were injected in the same manner as the evidence samples. The resulting reference spectra were stored in the TBI Gas Phase Library, which can be obtained at no charge by qualified Forensics Laboratories through Thermo Fisher Scientific™.

The Thermo Scientific Nicolet™ iS50 FTIR Spectrometer equipped with the iS50 GC-IR module is ideally suited for this analysis. Figure 2 shows the system using a Thermo Scientific TRACE™ 1310 Gas Chromatograph coupled via a heated transfer line to the spectrometer. The GC module contains a liquid nitrogen cooled MCT-A detector for high sensitivity. For this work, the Thermo Scientific OMNIC™ Series Software collected more than one spectrum per second consisting of 4 scans at 8 cm^{-1} resolution (0.7 second acquisition time). As seen below, this yielded excellent signal-to-noise. Further signal-to-noise improvement resulted from co-addition of spectra around the peak maximum.

Figure 2: The Nicolet iS50 FTIR Spectrometer with iS50 GC-IR module. Also shown is the TRACE 1310 GC and the iS50 ATR and iS50 Raman modules in the main spectrometer.

The column used in this work was a 5 meter silica capillary of 0.30 mm cross section and coated with bonded poly (1% diphenyl / 99% dimethylsiloxane). Columns with 5% diphenyl and several others would be suitable as well. The short column (5 meters) is necessary as the cannabinoids have a very low volatility leading to prohibitively long retention times otherwise. The separation for cathinones can sometimes be improved with longer columns (30 meter); all figures here use the 5 meter column except as noted.

Two microliters of sample were injected, with a 3:1 to 5:1 split ratio. The temperature program held 90 °C for 1 minute (driving off the methanol), ramped 70 °C per minute to a final temperature of 270 °C and held there for 20 minutes (different ramps are applied as needed to effect optimal separations). This combination of conditions yielded retention times between 5 and 20 minutes for most of the compounds investigated and provided adequate separation for courtroom-ready identification. The transfer line and heated cell of the GC module were set to 270 °C and held steady throughout. Implementing GC-FID-IR is straightforward with the iS50 GC-IR module, though not done here.

Figure 3 shows the output from a typical cannabinoid sample GC-IR run. The Gram-Schmidt (GS) profile in the top pane reports the total IR signal change over the run – the IR representation of the chromatogram. The lower pane shows the spectrum at the time point indicated by the cross hairs. The complexity of this sample, from a real case in the TBI laboratory, is clear from the number of peaks. The largest peak, at short time, is the solvent elution. The rising edge of that peak occurs around 5 seconds after the injection – a result of the short column used. The last fraction, due to a cannabinoid, fully elutes in under 7 minutes.

Discussion

GC-IR has been used at the TBI laboratory for some time to analyze cannabinoids and bath salts. The laboratory aggressively seeks out new examples, often adding them into the TBI Gas Phase Library before they appear on the street. This experience enables the analysts to direct their attention to the pertinent peaks in working with data such as shown in Figure 3. Much of the content is immaterial, due to poor quality control by the producers (cleaning between batches) or variance in the synthesis and the plant matter.

The last two peaks in Figure 3 contain the information about the drug mixture. The spectra from across each peak are co-added (summing spectra under the chromatographic peak to improve signal to noise) and then searched against the library. The search results are shown in Figure 4. The high match value leads to a

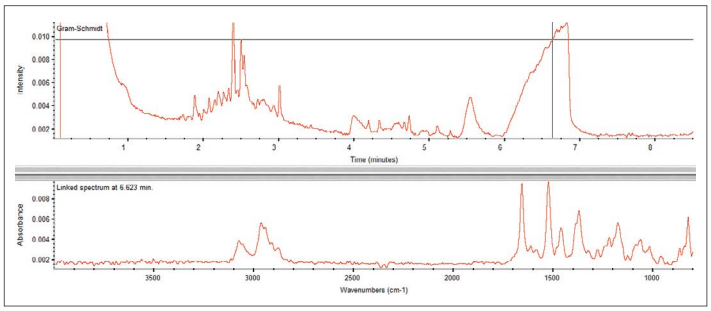


Figure 3: GC-IR data from a typical cannabinoid sample. The multiple peaks at short retention time are impurities from the synthesis and sample preparation; the two longest retention time peaks are the drugs.

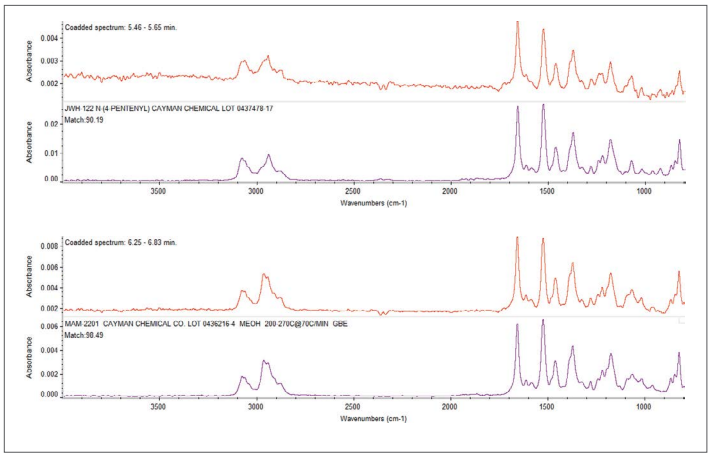


Figure 4: Simple search results for the two cannabinoid peaks in Figure 3.

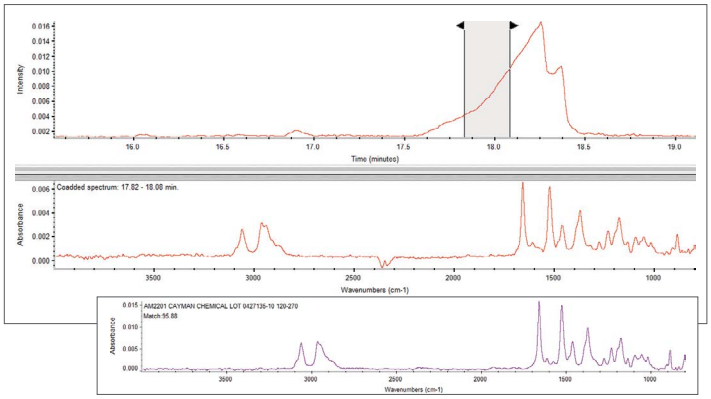


Figure 5: GS Profile for a less well separated sample, showing the co-add region for the first component. The inset shows the search result for this component.

positive identification; visual comparison of the top hits with secondary hits reinforces this.

The two compounds here are JWH-122 and MAM-2201. Close examination of the structures (Figure 1) reveals the only difference is the terminal fluorine on the side chain. MAM-2201 is a modification of the regulated JWH-122; the JWH-122 remaining is likely due to low yield synthesis of the MAM drug.

Figure 5 shows another cannabinoid blend, with the GS profile expanded around the drug peak. The separation does not show two well separated peaks, but there are several options for complete analysis.

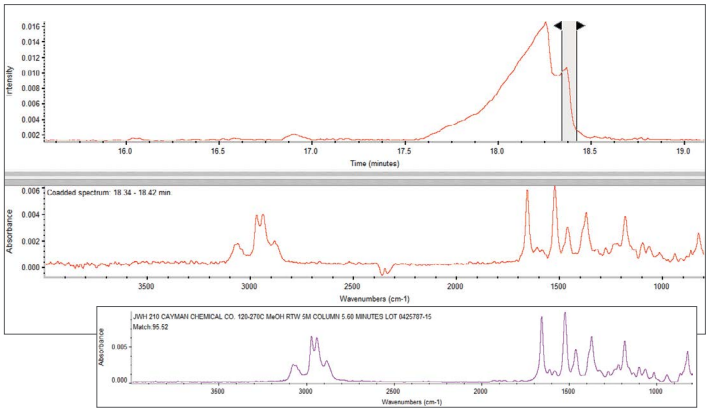


Figure 6: Same GS profile as in Figure 5, but focusing on the second peak. The inset again shows the search result for this component.

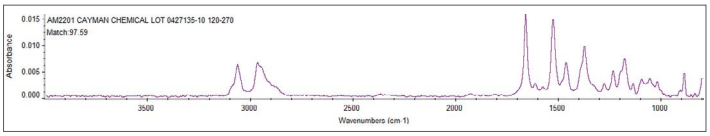


Figure 7: Search result for the spectrum derived by subtracting the co-add from Figure 6 from that in Figure 5. The improvement in the search metric is apparent.

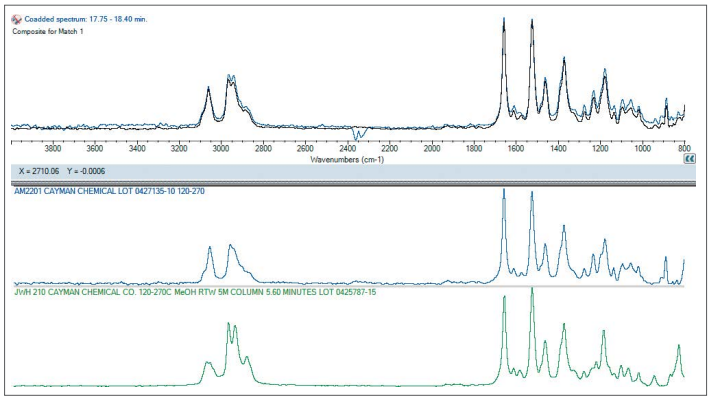


Figure 8: Output from OMNIC Specta's Multi-component Analysis routine operating on the co-added spectrum from the entire peak profile shown in Figures 5 and 6.

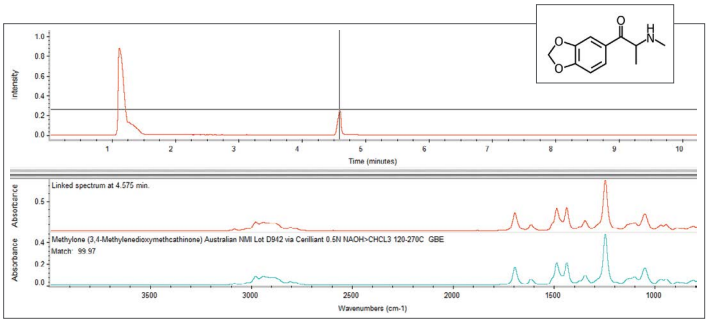


Figure 9: Output from the OMNIC Mercury GC analysis of the entire GS profile for a methylone sample. This bath salt sample was run using a longer (30 meter) column.

First, regions near the opposite edges of the two peaks are co-added for analysis. The shaded region in Figure 5 was co-added and searched. The results appear in the inset. Figure 6 shows the region selected and the results for the second peak. The simplicity of this approach requires some skill to select the regions and to recognize when further processing, such as spectral subtraction, may be needed.

This manual analysis can be slightly improved by removing the residual signals from the second component from the first spectrum via subtraction (and vice versa). Figure 7 shows the search result after the spectra from Figures 5 and 6 were subtracted. This eliminates the small signal from JWH-210 present under the spectrum of the AM-2201. Manual analysis provides maximum control over the results but requires some skill on the part of the user.

As a semi-automated alternative, the entire peak shown can be co-added, resulting in a single spectrum combining the two components. Exporting this to the Thermo Scientific OMNIC Specta™ Software permits use of the multi-component search algorithm, with the result shown in Figure 8. The bottom pane shows two spectra identified as comprising the co-added spectrum, while the upper pane compares the co-added spectrum to a composite made with those spectra. Excellent agreement was obtained with no subtraction or other processing, and the results align with the previous work. The big advantage of this approach lies with consistency, since the MCS algorithm provides the same results to any user, regardless of skill. Further, OMNIC Specta works even with 100% co-eluting signals, unlike any of the other methods outlined here.

The two drugs found in this sample were AM-2201 and JWH-210, which have several distinct features. The ethylene on naphthyl ring and the difference in the long side chain are apparent. It is likely this resulted from poor quality control in the cleaning process between batches.

The Thermo Scientific™ Mercury GC Analysis Software permits one click to produce a full report. Figure 9 shows the analysis of a methylone bath salt sample (run on a 30-meter column) using Mercury GC. The algorithm identifies the peaks in the GC profile, co-adds spectra around the peak to improve the signal-to-noise and then performs a search against the chosen libraries. The advantage of this fully automated process is the removal of subjectivity with a complete analysis of all peaks in the GS-profile. Low match results may indicate the presence of mixtures requiring deeper analysis.

The inset shows the structure of methylone; comparison to the MDPV structure shows the similarity and relationship to cathinone. Originally sold under the name “Explosion”, methylone is now a Schedule 1 drug in many states.

Another example of an overlapped elution is shown in Figure 10. The spectra were co-added and then fed into OMNIC Specta. Once again, the clear resolution of the two components and excellent agreement of the composite with the original spectrum reveals the power of this analytical tool. This sample contained MAM-2201 (as did the first sample) and JWH-019.

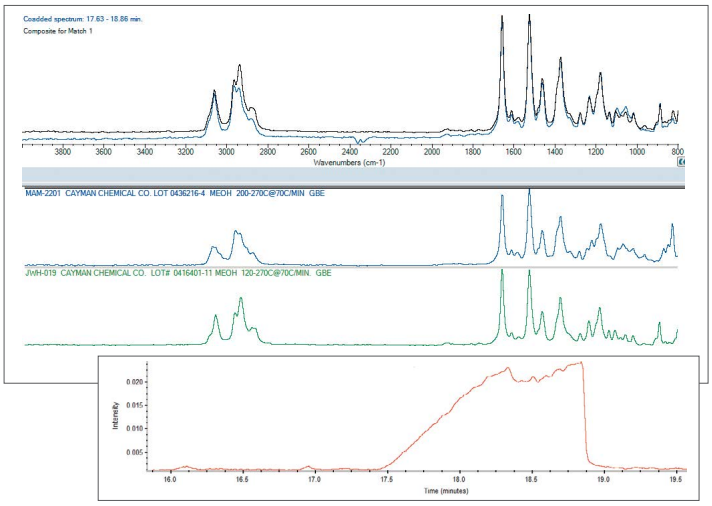


Figure 10: Another example of OMNIC Specta’s MCS routing applied to the data from the heavily overlapped GS profile shown in the inset. The clear identification and high level of confidence in the result is apparent.

The example in Figure 11 shows an extreme chromatographic overlap case for a bath salt sample, where there is only a small region of the GS profile where the drug elutes clear of interference (inset). A simple search of a co-added region around the third hump shows moderate quality matches to caffeine (match value of 80) and MDPV (the second hit listed at 78). The latter is the suspected ingredient, but the results would not be satisfactory in court. Co-adding a wide region and then searching with OMNIC Specta yields a perfect match and clear identification. MDPV (Methylenedioxypyrovalerone) is a heavily modified cathinone (bath salt) which is a stimulant, an effect increased through the addition of the caffeine.

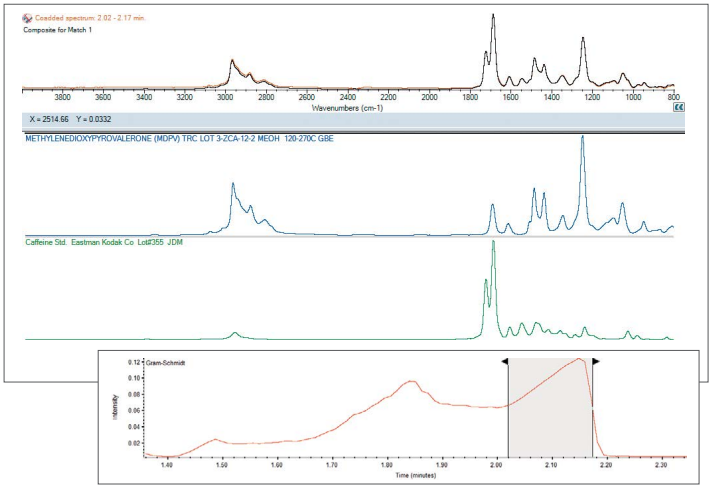


Figure 11: Analysis of a Bath Salt (MDPV) heavily dosed with caffeine. The inset shows the severity of the overlap caused by the short GC column; OMNIC Specta handles this with no difficulty. The resulting composite profile is stunningly identical to the co-added spectrum from OMNIC Series.

Figure 12 compares three closely analogous compounds to illustrate the ability to distinguish the materials. There are significant differences between the three spectra, sufficient for the searching routine to select out the correct isomer from data sets. The three drugs, differing only in the location of the side chain on the ring (small boxes) show both the complexity of the problem with these drugs and the power of GC-IR. The drugs could slip through the legal net unless the legislation was specific, and the isomer-specific identification would be essential to prosecution of the case. The combination of proper laws and specific tools for the identification is required.

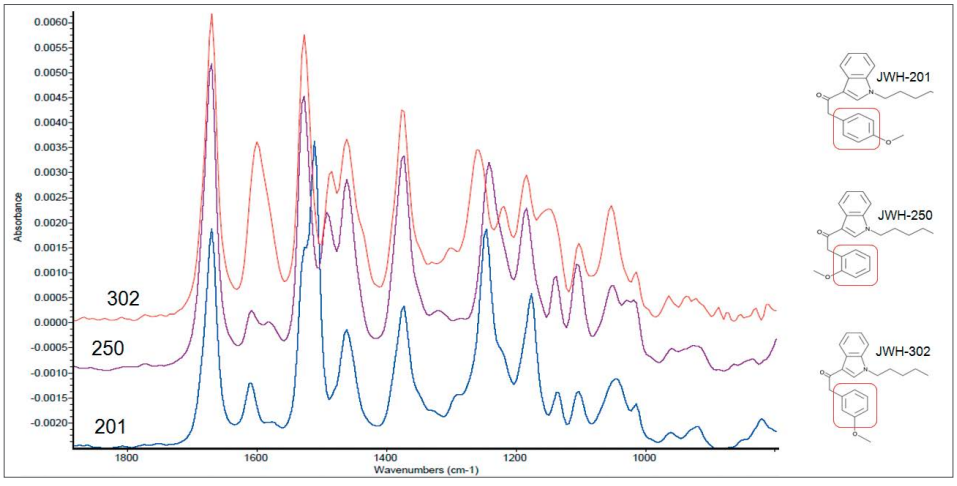


Figure 12: Comparison of the spectra obtained for several closely related cannabinoid compounds; the structures are also shown.

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Conclusion

Synthetic cannabinoids and bath salts are increasingly important to law enforcement. The subtle changes imposed by underground chemists provide a nightmare scenario to the analyst, requiring a simple, fast and specific solution. GC-IR provides that solution, with GC separating the materials for analysis and the FTIR probing the intact molecules leading to definitive identification. The long retention times necessitate a short column, leading to incomplete separation. The OMNIC Suite of tools permits the disentangling of this information, either using subtraction or the automated OMNIC Specta multi-component analysis routine. The latter adds consistency regardless of skill level to the mix, greatly improving the chances for correct, court-room ready identification.

Further, the Nicolet iS50 Spectrometer enables the user to add FT-Raman and ATR spectroscopy to their tool set, rather than depending upon a dedicated system. Both Raman and infrared are listed as Class A methods in the SWGDRUG guidelines, so the fully outfitted system is a powerful tool for use in the forensics laboratory.

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