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The right analytical technique helps crime and forensic labs confidently identify samples from opioids to paints chips to counterfeit paper. Whether your lab is consumed by a drug crisis or busy identifying a backlog of crime scene evidence, the right instruments help move cases from investigate to solve.

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APPLICATION NOTE

No. AN53164

Distinguishing isomers of fentanyl analogs using FT-Raman

Authors

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Key words

FT-Raman spectroscopy, fentanyl, fentanyl analogs, constitutional isomers, geometric isomers, library search

Thermo Fisher solutions

Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer, Thermo Scientific™ Nicolet™ iS50 FT-Raman module, Thermo Scientific™ OMNIC™ Software.

Abstract

A pair of constitutional fentanyl analog (FA) isomers: butyryl and isobutyryl fentanyl, and a pair of geometric FA isomers: *cis*- and *trans*-3-methyl fentanyl were analyzed by FT-Raman. Upon examination of the FT-Raman spectra of fentanyl and the isomers, it is found that the fingerprint region is dominated by the features from the vibration modes of the two terminal benzene rings. These features are less discriminating for the FA isomers where the structural variations reside in the amide and piperidine moieties of the fentanyl structural scaffold. Instead, the spectral features in the low frequency region (250–400 cm^{-1}) from the δ(C-C) of aliphatic chains are the key differentiators between the isomers. The minute structural variations between isomers results in significant differences in the Raman spectra allowing for positive identification and effective discrimination of the FA isomers using FT-Raman.

Introduction

Illicitly manufactured fentanyl and fentanyl analogs (FAs) is becoming a major driver of opioid overdose. Since 2013, an unprecedented surge in fentanyl overdose deaths has been caused by heroin laced with illicitly produced fentanyl and/or FAs sold as heroin. The US Drug Enforcement Agency's National Forensic Laboratory Information System reported a >300% increase in fentanyl.[1-2] In addition to the high potency of fentanyl and many FAs, the law enforcement agencies are also constantly on their toes to keep up with the rapid and continued emergence of new FAs. Minor modifications to the fentanyl's structural scaffold by illicit drug manufacturers could yield novel and potent FAs that outpace the list of sanctioned compounds.

The challenges in the screening, detection, and identification of synthetic opioids warrant the use of multiple analytical techniques. For example, The CDC advises two-tiered testing, an enzyme-linked immunosorbent assay (ELISA) screen followed by gas chromatography/mass spectrometry (GC/MS) to identify the fentanyl compound in blood and urine in the cases of suspected overdose. Liquid chromatography-mass spectrometry (LC-MS), liquid chromatography tandem mass spectrometry (LC-MS/MS), and ion mobility spectrometry (IMS) have also been used for screening and confirmation of fentanyl and FAs in case samples.[3] However, unequivocal identification of the suspected FA can be complicated by isomeric compounds. Due to their structural similarity, some FA isomers have identical accurate mass, electron impact (EI), and electrospray ionization (ESI) fragmentation patterns, as well as close retention times on most gas and liquid chromatography methods.[1]

Previously, we have demonstrated that FT-Raman spectroscopy is a safe, fast, and complementary technique for the detection and identification of powders and other apprehended materials including fentanyl.[4] Raman analysis requires little to no sample preparation and allows for direct measurements through glass vials and evidence bags, greatly reducing the risk of lab personnel's accidental exposure to high potency fentanyl and FAs. It is nondestructive and allows the custody chain to be maintained from its seizure until the sample is placed in the evidence file.[4]

In this note, two examples of distinguishing FA isomers using FT-Raman spectroscopy are described. A pair of constitutional isomers: butyryl fentanyl and isobutyryl fentanyl, and a pair of geometric isomers: *cis*- and *trans*-3-methyl fentanyl were analyzed by FT-Raman. It is demonstrated that in addition to the fingerprint region, the Raman peaks in the low frequency region (250–400 cm^{-1}) resulting from the δ(C-C) aliphatic chains are the key differentiators between the isomers.

Materials and methods

Fentanyl and FA standards were procured from Cayman Chemicals (Ann Arbor, Michigan) and analyzed as received. All fentanyl and regulated materials used in this study were analyzed at the Albuquerque Police Department Crime Laboratory. Samples were analyzed in double bags or glass vials and handled in compliance with the Scheduled I chemical handling protocols.

A Nicolet iS50 FTIR Spectrometer equipped with a calcium fluoride beam splitter and a Nicolet iS50 Raman module was used for all analyses. A 1064 nm laser with the power set at 0.5 W was used as the excitation source. A total of 128 scans were co-added for each spectrum at 4 cm^{-1} resolution, with a total acquisition time of ~130s. The spectra were analyzed using OMNIC Software.

Results and discussion

Fentanyl and FAs are in the phenylpiperidine class of synthetic opioids. Fentanyl, specifically, has a phenyl propanamide group attached to the phenylpiperidine (Figure 1). Figure 2 shows the FT-Raman spectrum of fentanyl, with the major peaks annotated by their respective wavenumbers. Detailed Raman peak assignments of fentanyl using density function theory (DFT) calculation were reported by Leonard *et al.*[5] In the fingerprint region (400–1600 cm^{-1}), a majority of the spectral features (e.g., 622, 743, 829, 1001, 1075, 1160, 1455, 1585, 1598 cm^{-1}) originate from the vibrations of the two terminal benzene rings. While strong in intensity, those peaks are undiscriminating for the FA isomers in the current study. It is noted that δ(C-C) aliphatic chains produce strong Raman signals in the spectral region of 250–400 cm^{-1} (e.g. 267 cm^{-1} and 308 cm^{-1} in Figure 2).

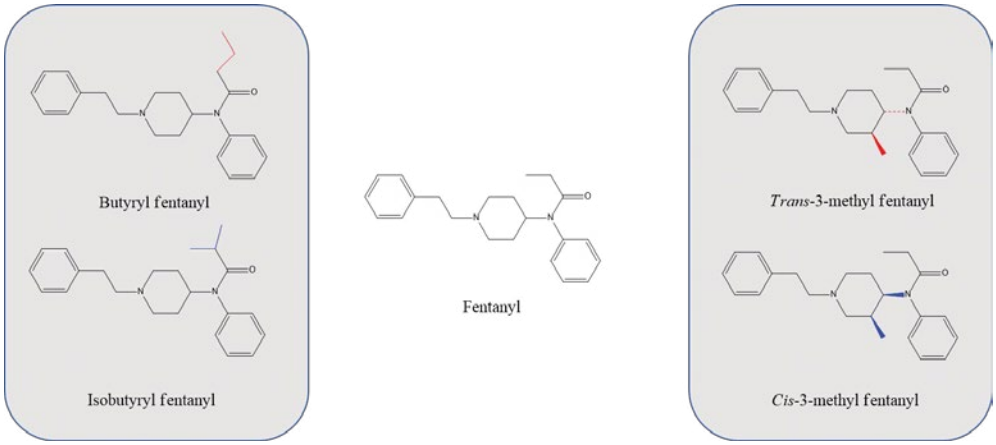

Figure 1. Structures of fentanyl and two pairs of FA isomers in this study.

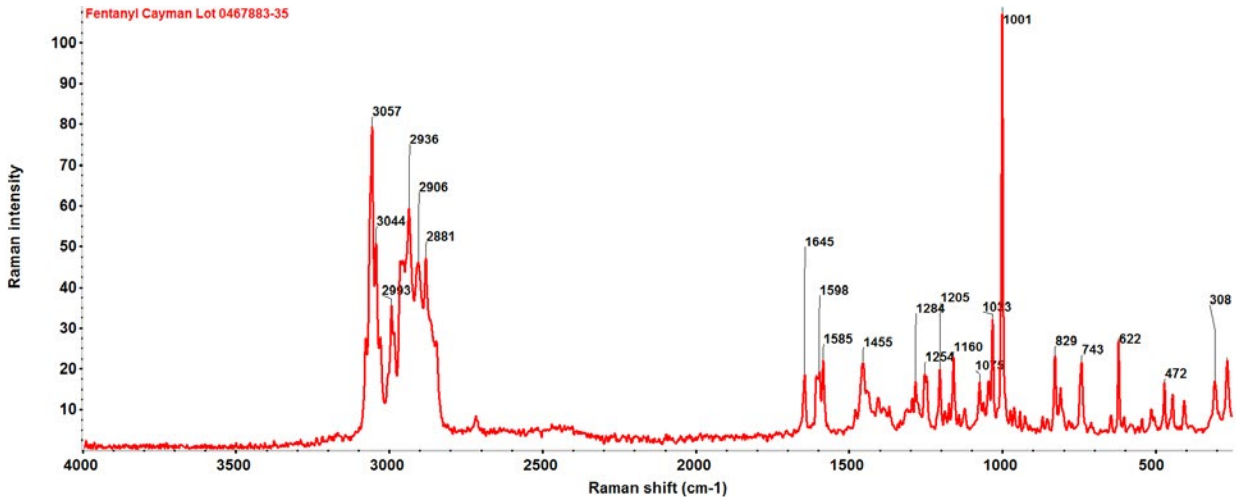

Figure 2. FT Raman spectrum of fentanyl.

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Butyryl fentanyl and isobutyryl fentanyl are a pair of constitutional isomers that differ in the terminal groups attached to the amide moiety of the fentanyl scaffold (Figure 1). They are short-acting FAs approximately 30 times less potent than fentanyl. However, at least 40 confirmed overdose deaths involving butyryl fentanyl abuse were reported by the US DEA.[1] Butyryl fentanyl and isobutyryl fentanyl were classified as Schedule I narcotics in 2016 and 2018, respectively. Figure 3 shows the FT-Raman spectra of butyryl fentanyl and isobutyryl fentanyl. The peak at 735 cm^{-1} resulting from the C-H symmetric bend is unique to isobutyryl fentanyl. Due to the different terminal groups attached to the carbonyl carbon, the positions of the carbonyl C-O stretch peaks are different between the isomers: 1655 cm^{-1} for isobutyryl fentanyl and 1646 cm^{-1} for butyryl fentanyl. In the low frequency region of 250–400 cm^{-1}, there are unique peaks at 323 and 372 cm^{-1} for isobutyryl fentanyl, and 282 cm^{-1} for butyryl fentanyl, although the exact assignments of these low frequency peaks are difficult to ascertain. The Raman spectra of butyryl fentanyl and isobutyryl fentanyl nonetheless exhibit sufficient variance to distinguish this pair of constitutional isomers.

Classified as Schedule I narcotics, 3-methylfentanyl (3-MF) emerged in 1984 in Allegheny County, Pennsylvania and was responsible for 16 fatal overdose cases.[2] 3-MF exists as a diastereomeric species: (±)-*cis*-3-MF and (±)-*trans*-3-MF. The *cis*- and *trans*- isomers contrast in the orientation of the methyl group attached to the piperidine ring (Figure 1). The two isomeric species has markedly different potencies: (±)-*trans*-3-MF has a similar potency to fentanyl and (±)-*cis*-3-MF is 8 times more potent than fentanyl.[6] Figure 4 shows the FT-Raman spectra of *cis*-3-MF and *trans*-3-MF in the spectral range of 250–1800 cm^{-1}. While there are subtle differences between the isomers in the spectral region of 1200–1300 cm^{-1}, where the peaks are mainly from the C-N stretch and C-H twist of piperidine, more pronounced differences are observed in the low frequency region of 250–500 cm^{-1}. There are a group of peaks (265, 307, 409, 478 cm^{-1}) that are unique to *cis*-3-MF but noticeably missing for *trans*-3-MF.

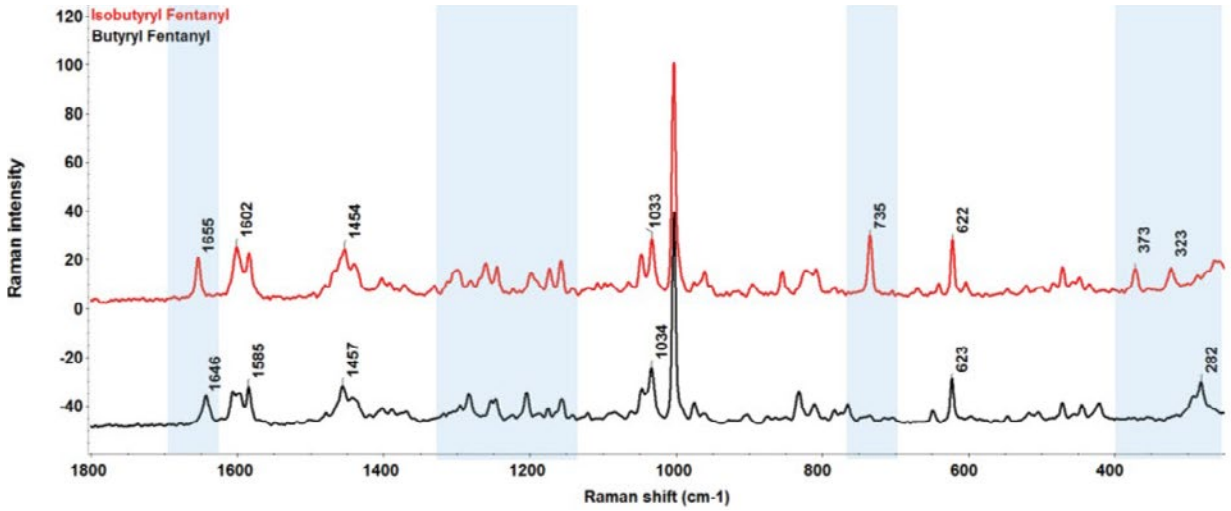

Figure 3. Raman spectra of butryl fentanyl and isobutryl fentanyl in the spectral range of 250–1800 cm^{-1}. The shaded region in blue color highlights the spectral difference between the two compounds.

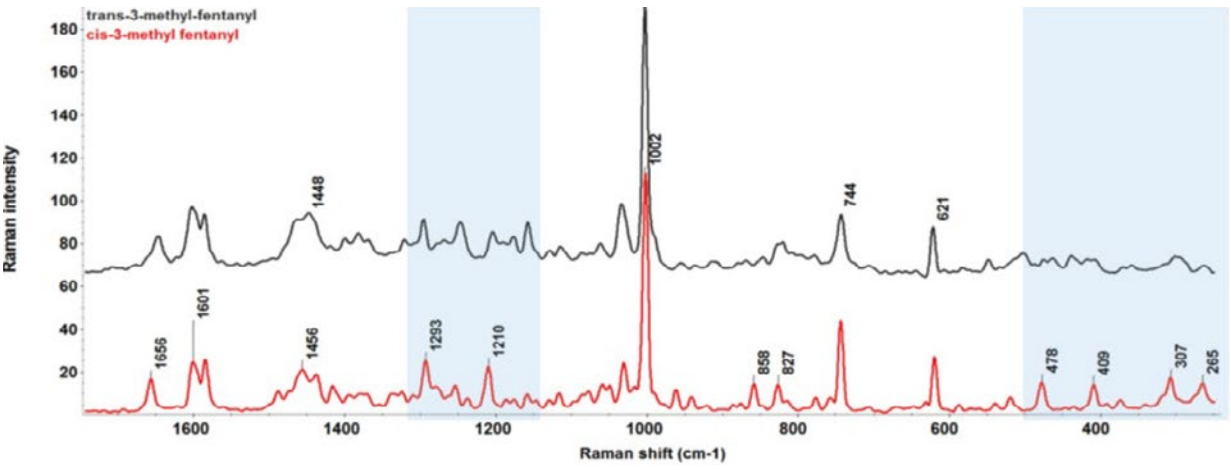

Figure 4. Raman spectra of *cis*-3-MF and *trans*-3-MF in the spectral range of 250–1800 cm^{-1}. The shaded region in blue color highlights the spectral difference between the two compounds.

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The Raman spectra of fentanyl and the isomeric pairs were searched against a fentanyl library which contains fentanyl and 13 FAs. Details of this library will be published elsewhere. The search results are summarized in Table 1. As expected, all compounds were correctly identified. Interestingly, for each isomer, the 2nd best match is not its isomeric counterpart. For example, the 2nd best match for both butyryl fentanyl and isobutyryl fentanyl is cyclopentyl fentanyl. These results suggest that while the structural difference between isomers is minute, the spectral difference is significant enough for an effective discrimination of the isomers.

Conclusions

In this application note, fentanyl, a pair of constitutional FA isomers (butyryl and isobutyryl fentanyl), and a pair of geometric FA isomers: (*cis*- and *trans*-3-methyl fentanyl) were analyzed by FT-Raman. The fingerprint region of the Raman spectra is dominated by the features from the vibration modes of the two terminal benzene rings. These features, however, are less discriminating for the isomeric pairs. The spectral features in the low frequency region (250–400 cm^{-1}) from the δ(C-C) of aliphatic chains, on the other hand, are the key differentiators between the isomers. Library searches against a fentanyl library provided strong correlations for all compounds. More importantly, the 2nd best match for each isomer from the library search is not its isomeric counterpart. The minute structural variations between isomers result in significant differences in the Raman spectra, allowing for positive identification and effective discrimination of the isomers using FT-Raman. FT-Raman spectroscopy is a safe, fast, and discriminating analytical technique for the detection and identification of fentanyl and fentanyl analogs.

Table 1. Search results against the fentanyl library.

Compound	Top match	2nd best match
Butyryl fentanyl	Butyryl fentanyl	Cyclopentyl fentanyl
Isobutyryl fentanyl	Isobutyryl fentanyl	Cyclopentyl fentanyl
Trans-3-methyl fentanyl	Trans-3-methyl fentanyl	Butyryl fentanyl
Cis-3-methyl fentanyl	Cis-3-methyl fentanyl	Fentanyl
Fentanyl	Fentanyl	Acetyl fentanyl

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APPLICATION NOTE

No. AN52418

Bath salts and cannabinoids analyzed by GC-IR

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Michael Bradley, Ph.D., Thermo Fisher Scientific, Madison, WI, USA

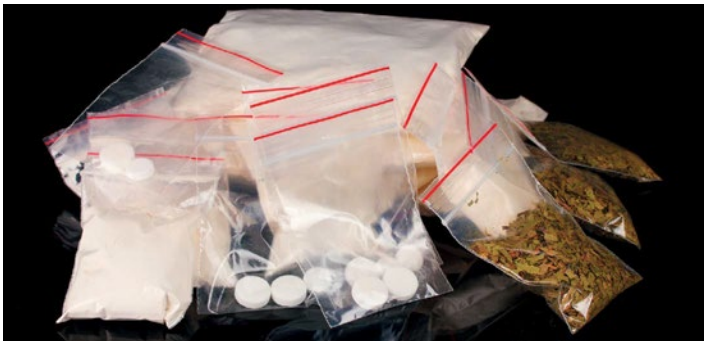
Key words
GC-IR, FTIR, bath salts, cannabinoids, cathinones, methcathinone, forensics

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.

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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?

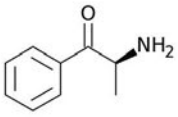
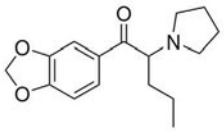
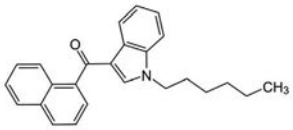
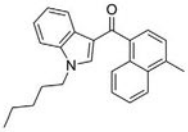
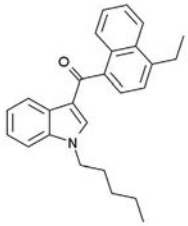
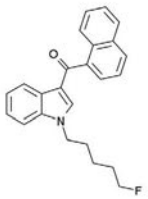
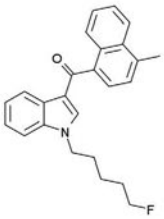
Compounds	Structures
Cathinone (Bath Salt)	
MDPV (Bath Salt)	
JWH-019	
JWH-122	
JWH-210	
AM-2201	
MAM-2201	

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

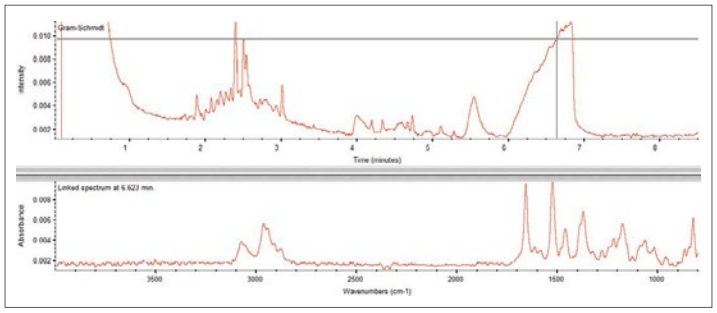


Figure 3: GC-IR data from a typical cannabinoid sample. The multiple peaks at short retention time are impurities from the synthesis and sample preparation; the two longest retention time peaks are the drugs.

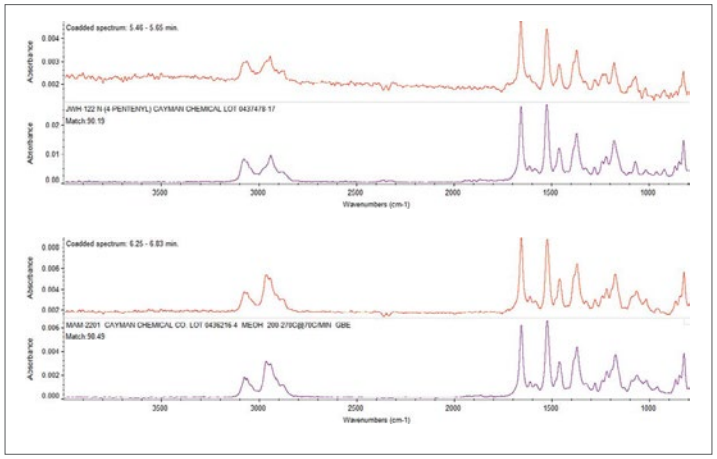


Figure 4: Simple search results for the two cannabinoid peaks in Figure 3.

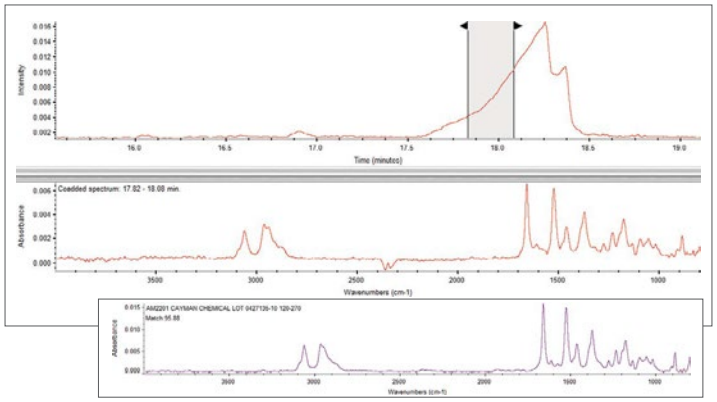


Figure 5: GS Profile for a less well separated sample, showing the co-add region for the first component. The inset shows the search result for this component.

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.

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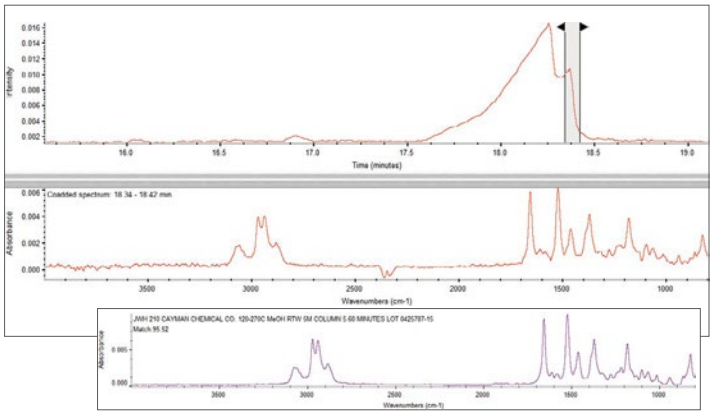

Figure 6: Same GS profile as in Figure 5, but focusing on the second peak. The inset again shows the search result for this component.

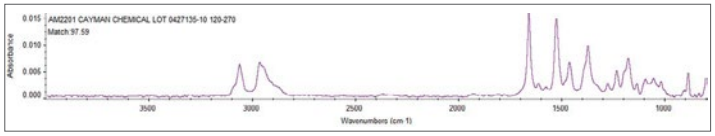

Figure 7: Search result for the spectrum derived by subtracting the co-add from Figure 6 from that in Figure 5. The improvement in the search metric is apparent.

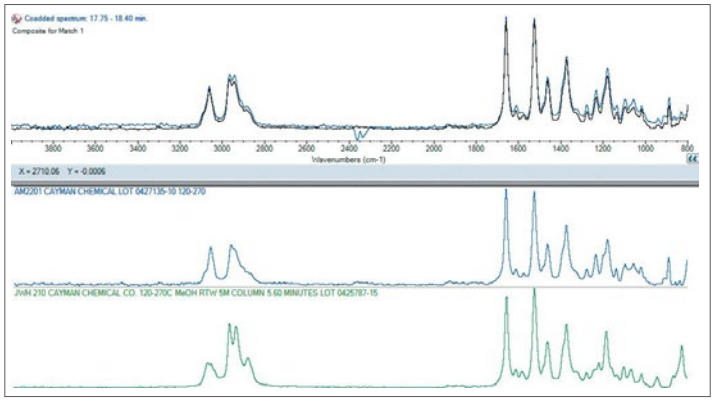

Figure 8: Output from OMNIC Specta's Multi-component Analysis routine operating on the co-added spectrum from the entire peak profile shown in Figures 5 and 6.

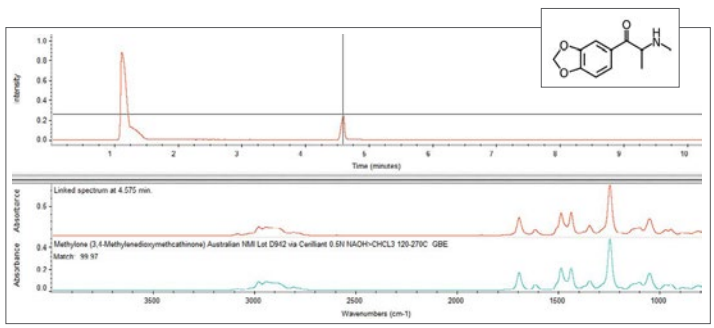

Figure 9: Output from the OMNIC Mercury GC analysis of the entire GS profile for a methylone sample. This bath salt sample was run using a longer (30 meter) column.

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.

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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.

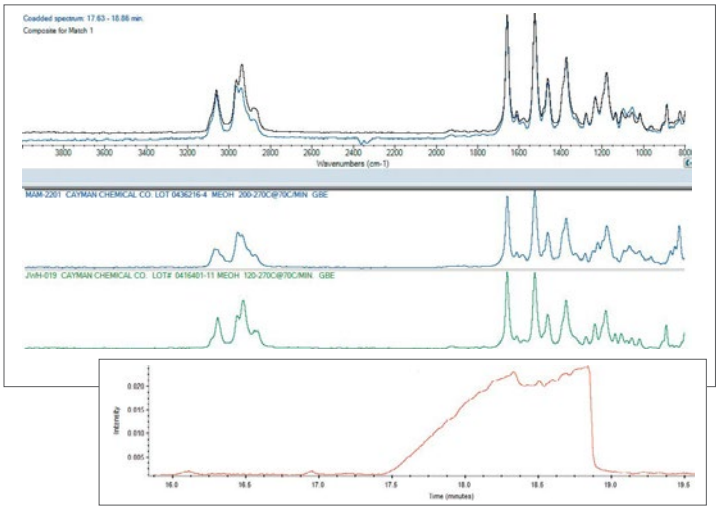

Figure 10: Another example of OMNIC Specta’s MCS routing applied to the data from the heavily overlapped GS profile shown in the inset. The clear identification and high level of confidence in the result is apparent.

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.

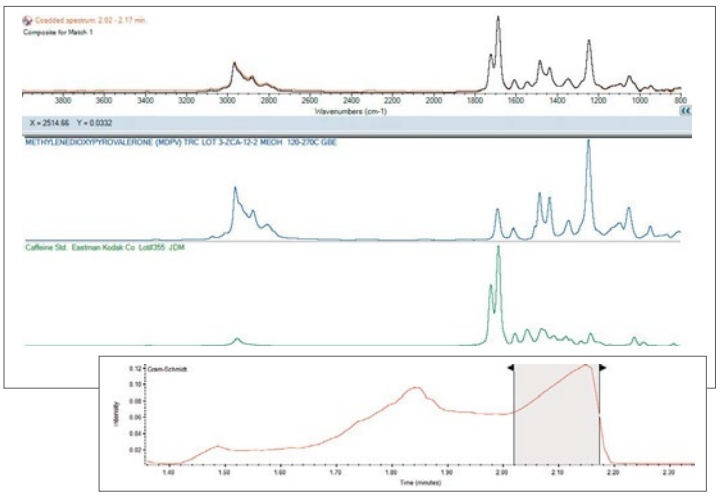

Figure 11: Analysis of a Bath Salt (MDPV) heavily dosed with caffeine. The inset shows the severity of the overlap caused by the short GC column; OMNIC Specta handles this with no difficulty. The resulting composite profile is stunningly identical to the co-added spectrum from OMNIC Series.

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.

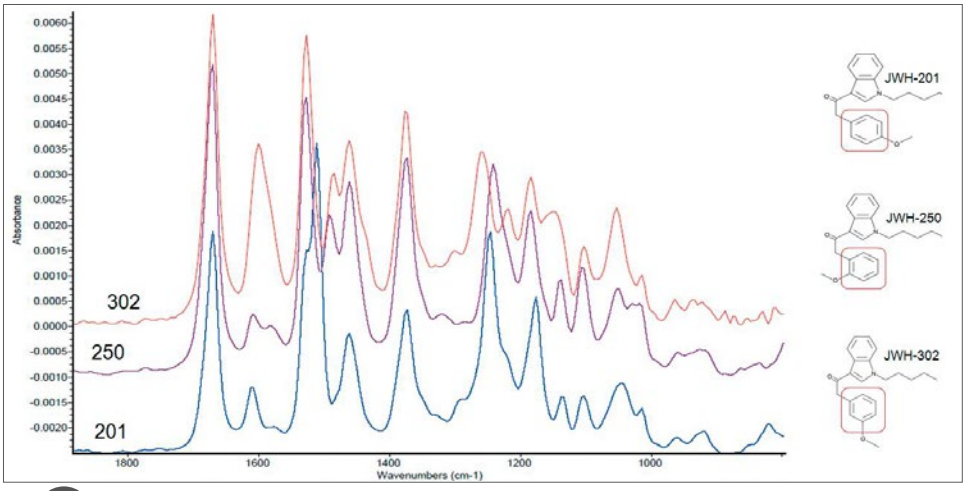

Figure 12: Comparison of the spectra obtained for several closely related cannabinoid compounds; the structures are also shown.

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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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APPLICATION NOTE AN52304

FT-Raman: an invaluable addition to the forensic arsenal to combat the opioid epidemic

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Introduction

Illicitly manufactured fentanyl is becoming a major driver of opioid overdose. According to the Centers for Disease Control and Prevention (CDC), illegal, lab-made fentanyl was involved in more than 50% of opioid overdose deaths in 2016.[1] The growing opioid epidemic presents multifaceted challenges for law enforcement, first responders, and forensic lab personnel. In particular, the high potency of fentanyl and fentanyl analogues makes accidental exposure life-threatening, with ingestion, inhalation, and absorption through the skin as possible exposure routes. With a lethal dose of only a few milligrams, fentanyl is considered 50-100 times more potent than morphine. Carfentanil, a fentanyl analogue, is approximately 100 times more potent than fentanyl. Consequently, the CDC has issued a health alert on the rise of unintentional overdoses of clandestinely produced and trafficked fentanyl in the form of counterfeit pills and heroin adulterants.[1-2] The situation is further exacerbated by the waves of new synthetic opiates. Through chemical modification, new potent fentanyl analogues are created at a fast pace in underground labs, keeping authorities constantly on their toes to identify these emerging chemical entities and to understand their pharmacology and toxicology.

The challenges in the detection, identification, and screening of synthetic opioids mandate the use of multiple analytical techniques and instrumentation, both field-deployable and laboratory-based, in a concerted and holistic manner. For example, The CDC advises two-tiered testing, an enzyme-linked immunosorbent assay (ELISA) screen followed by gas chromatography/mass spectrometry (GC/MS) to identify the fentanyl compound in blood and urine in the cases of suspected overdose. In the meantime, liquid chromatography / mass spectrometry (LC/MS), LC/MS/MS, ion mobility spectrometry (IMS), and thermal desorption direct analysis in real time mass spectrometry (TD-DART-MS) are being explored to meet the demand on low detection limit for many case samples where fentanyl is present with other drugs and cutting agents at low concentration.[2]

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Raman spectroscopy has long been used for the detection and identification of illicit drugs and adulterants, and offers a valuable addition to the forensic toolbox for the analysis of fentanyl and fentanyl analogues. FT-Raman utilizes a long-wavelength laser (1064 nm) which greatly reduces fluorescence and produces high signal-to-noise spectra, making it well suited for many narcotic samples that fluoresce. It also enables sampling through glass vials, polymer blister packs, and plastic evidence bags; hence, often requires little to no sample preparation. It is nondestructive and allows the custody chain to be maintained, given the possibility of qualitative and quantitative evaluation of the sample to confirm its integrity from its seizure until the sample is placed in the evidence file.[3]

In this application note, analyses of fentanyl as well as other illicit drugs using FT-Raman spectroscopy are presented. The advantages of FT-Raman for the detection and identification of illicit drugs are also discussed.

Materials and methods

All samples analyzed in this study were prepared at Albuquerque Police department. Samples were analyzed in double bags, blister packs or glass vials, and handled in compliance with Schedule I controlled substance handling protocols. A Thermo Scientific™ Nicolet™ iS50 FT-IR spectrometer equipped with a calcium fluoride beam splitter and an FT-Raman module with a 1064 nm laser was used for all analyses (Figure 1). A total of 64 scans were co-added for each spectrum at 8 cm-1 resolution, with a total acquisition time of ~75s. The laser power was set at 0.5 mW. The spectra were searched against Thermo Scientific Law Enforcement and Security (LEnS) Raman and DEA Raman libraries using OMNIC library search and Specta multicomponent search options.

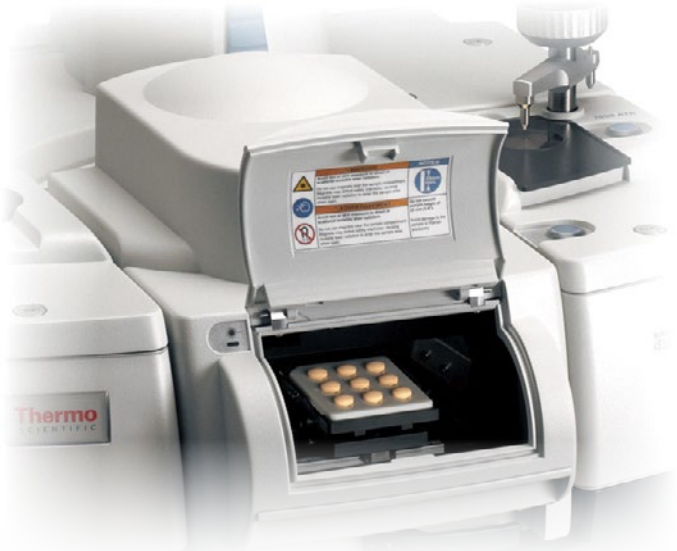

Figure 1: An iS50 Raman module for the Thermo Scientific Nicolet iS50 FT-IR spectrometer.

Results and discussion

Figure 2A shows the Raman spectrum of bulk fentanyl in a double bag, directly acquired without any sample manipulation. Since the laser spot size is approximately 60 µm, small quantity of samples, such as a few granules, can be analyzed with ease. An example is shown in Figure 2B. While Figure 2B exhibits a slightly higher noise level compared to Figure 2A, both samples were nonetheless positively identified as fentanyl citrate through library search.

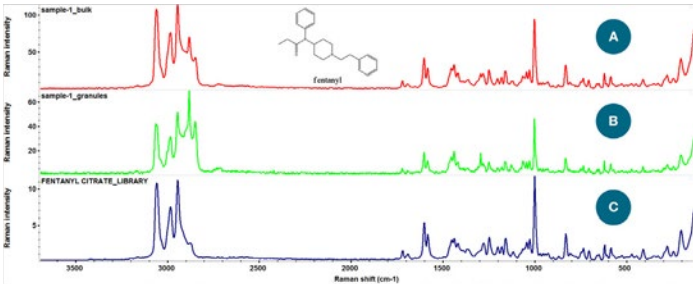

Figure 2: Raman spectra of (A) seized bulk fentanyl in a double-bag; (B) a few fentanyl granules in a double-bag; and (C) fentanyl citrate from DEA Raman library.

Raman spectroscopy is sensitive to both chemical and physical properties. Its unique selection rules generate a molecular fingerprint that is well suited to the differentiation between many illicit drug compounds and their analogues. Figure 3 shows the Raman spectra of a tablet as well as the 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) standards from the library. Despite the minute structural difference between MDA and MDMA (inset of Figure 3), Raman spectroscopy can unambiguously distinguish the two. In this case, the tablet was identified as MDA with a match score of 83, as opposed to a match score of 66 for MDMA.

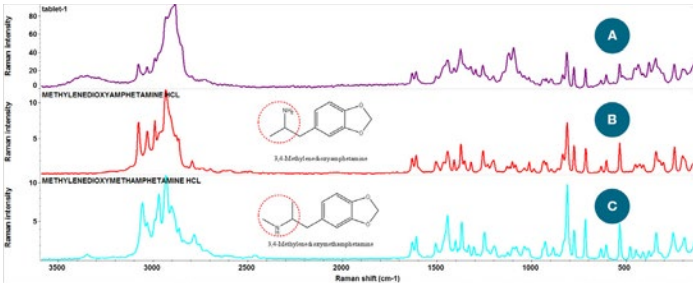

Figure 3: Raman spectra of (A) a tablet; (B) 3,4-methylenedioxyamphetamine (MDA) standard from the library; and (C) 3,4-methylenedioxymethamphetamine (MDMA) standard from the library.

Seized street drugs are often present in the form of mixtures. Figure 4 shows the Raman spectrum of an off-white powder sample in an evidence bag. Initial library search showed similar match score for lactose and cocaine, suggesting that the sample contains multiple components. The spectrum was then subjected to the OMNIC Specta multi-component search, and the sample was identified as a mixture of cocaine and β-D-lactose, a commonly used cutting agent.

Conclusions

The Nicolet iS50 FT-IR spectrometer equipped with the iS50 Raman module offers an invaluable tool for the forensics labs to combat the growing opioid epidemic. Raman analysis requires little to no sample preparation and allows for direct measurements through glass vials and evidence bags, greatly reducing the risk of lab personnel's accidental exposure to high potency drugs such as fentanyl and fentanyl analogues. While the measurement of bulk narcotic samples is fast and straightforward, with a laser spot size of 60 µm, acquisition of high quality Raman spectra from as little as few granules is also possible. Combined with DEA and LEnS Raman libraries, OMNIC Specta enables identification of multi-component illicit drugs with confidence. Raman spectroscopy using the iS50 Raman module is a safe, fast, and complementary technique for the screening, detection and identification of illicit drugs.

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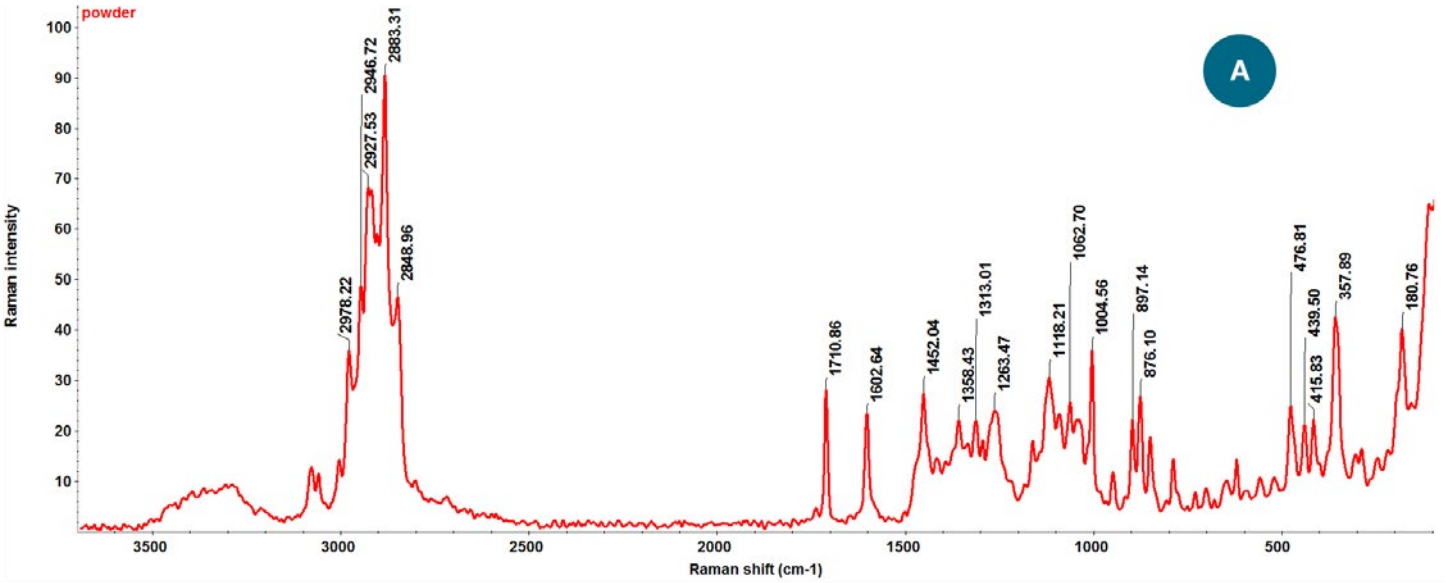

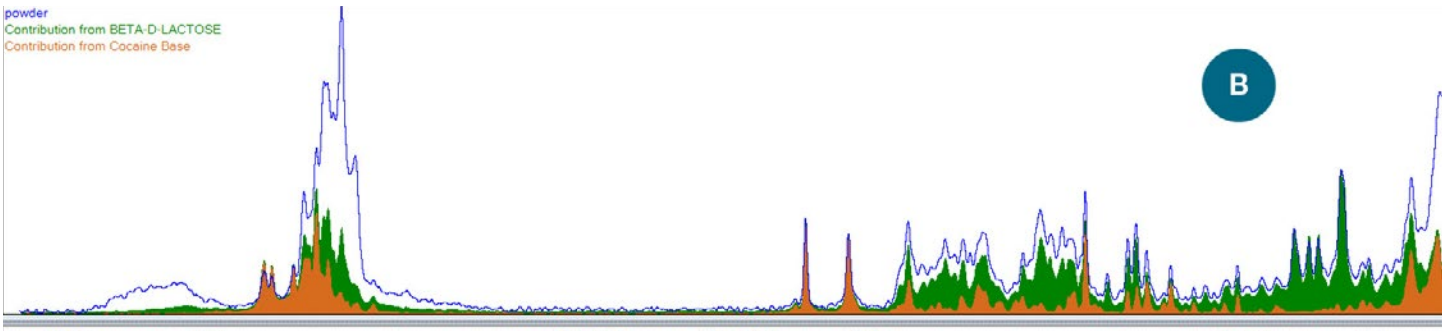

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Figure 4: Raman spectra of a seized street drug sample containing cocaine and β-D-lactose.

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APPLICATION NOTE

No. AN52410

Analyzing automotive paints with extended range ATR: 1800–100 cm^{-1}

Key words
Automotive paint, far-infrared, forensic science, infrared spectroscopy, inorganics, paint analysis

Infrared (IR) spectroscopy is used extensively to characterize the chemical composition of trace evidence such as paints, fibers and adhesives as well as seized drugs and related chemicals. Attenuated Total Reflectance (ATR) has become the sampling technique of choice for many of these measurements. The advent of diamond ATR crystals has allowed spectral data to be collected over an extended range. The Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer offers a novel approach to ATR sampling with the Thermo Scientific™ Nicolet™ iS50 ATR Module that is integrated into the spectrometer. This ensures that the ATR module is always available for rapid analysis of unknown materials even with another accessory mounted in the sample compartment. The built-in ATR combines an optimized optical design with a diamond ATR crystal and a broad range DTGS detector to provide high-sensitivity infrared spectroscopy from 4000 cm^{-1} to 100 cm^{-1}. The enhanced stability, resulting from mounting the ATR within the sealed section of the spectrometer, makes it possible to acquire spectra down to 200 cm^{-1} even with a desiccated system.

Infrared spectroscopy is one of the few analytical techniques considered by the forensic science community to have the highest discriminating power required to provide confirmatory evidence about the chemical composition of a material. FTIR is used extensively in most forensics laboratories to identify materials such as seized drugs and possible evidence from a crime scene. One area where FTIR has proven particularly valuable is analyzing automotive paint chips. A number of forensics laboratories have reported the significance of the infrared spectral range below 400 cm^{-1} in helping to identify specific inorganic pigments or minerals in a paint sample.[1-5]

Traditionally these measurements have been performed with a diamond compression cell or diffuse reflectance accessory on an FTIR system that uses Cesium Iodide (CsI) optics to acquire spectra down to 225 cm^{-1}. Suzuki et al. have published a paper in the *Journal of Forensic Science* describing the application of extended range FTIR to the analysis of pigments in automotive paint.[4] Another excellent resource for analyzing automotive paints and pigments is the *International Forensics Automotive Paint Data Query (PDQ) database* developed by the Royal Canadian Mounted Police Forensics Laboratory Services. Much of the infrared spectral data in this database contains peak information down to 225 cm^{-1}.[6]

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While the advantages of extended range FTIR may be clear, obtaining high-quality transmission spectra requires a great deal of experience and careful sample preparation. The CsI optics typically used to scan this range are delicate and hydroscopic, so great care must be taken to maintain a low humidity environment within the instrument and handle the beamsplitter with extreme caution. Also, preparing a paint sample that is sufficiently thin and fringe-free to work in a diamond anvil cell form can be challenging to an inexperienced examiner. The diamond windows not only compress the sample but are transparent in both the mid- and far-IR spectral region.

In this application note, we will discuss a novel feature of the Nicolet iS50 FTIR Spectrometer that provides a rapid, easy way to acquire spectra down to 100 cm^{-1}. The system that we used was configured with the built-in iS50 ATR module and an Thermo Scientific™ Nicolet™ iS50 ABX Automated Beamsplitter exchanger. With this configuration, both a mid-IR and far-IR spectrum can be acquired from a sample with a single operation. In this report, extended range far-IR spectra were acquired from 1800 cm^{-1} to 100 cm^{-1} with the solid substrate beamsplitter and the integral diamond ATR shown in Figure 1 below. High-quality spectra were acquired at 4 cm^{-1} resolution in a couple of minutes.

Figure 1: The Nicolet iS50 FTIR Spectrometer configured with the iS50 ABX Automated Beamsplitter exchanger, the built-in iS50 ATR module, and the iS50 Raman module

Infrared spectroscopic analysis of inorganic pigments: ATR spectra from 1800 to 100 cm^{-1}

As mentioned, one of the important advantages of extended range FTIR is the ability to detect the peaks from inorganic pigments and fillers found in many paints, coatings, and plastics. The first sample analyzed was a small piece of white plastic. The best match found with the spectral search was the spectrum of rutile, which is a TiO_2 compound used as a whitener. Figure 2 shows a comparison of the ATR spectrum and the transmittance reference spectrum from the Washington State Crime Laboratory (WSCL) library. The two reference peaks described in Dr. Suzuki's paper for rutile are clearly present in this sample.

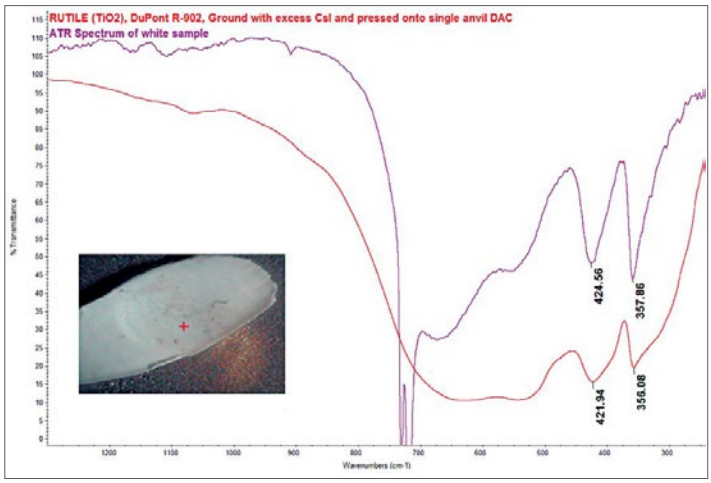

Figure 2: Extended range ATR spectrum compared to reference rutile spectrum acquired in transmittance with a diamond anvil cell

A second example is actually a bright yellow plastic material with two large peaks below 400 cm^{-1}. While it may be possible to identify the calcite with the peaks above 400 cm^{-1}, as in Figure 3, the only peak in the reference spectrum of cadmium yellow is near 250 cm^{-1} and matches nicely with the strong feature in our extended range ATR spectrum.

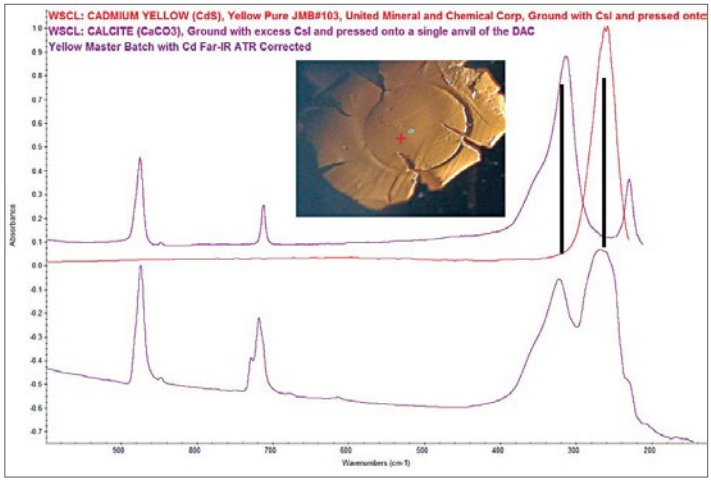

Figure 3: Comparing the spectrum from a yellow material to reference spectra of calcite and cadmium yellow

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The analysis of automotive paint with extended range FTIR: ATR spectra from 1800–100 cm^{-1}

One unique feature of ATR spectroscopy is the limited depth of penetration into the sample. The depth of penetration is dependent on the wavelength of the infrared light and the refractive index of the sample. While the depth of penetration in the far-IR region may be several microns, this may still be smaller than the thickness of the paint layers. Figure 4 shows the spectra acquired from both sides of a paint sample. The spectra from the two sides are clearly different, indicating that we have at least two paint layers present in the sample. Spectral subtraction can often be employed to sort out the different spectral features and avoid having to manually separate the paint layers. Because all of the components in the built-in ATR module were optimized specifically for the Nicolet iS50 Spectrometer, the sensitivity is excellent. Although the Nicolet iS50 instrument is not designed as a microspectrometer, excellent infrared spectra can be rapidly obtained from samples smaller than 1 mm. This sensitivity is shown in the ATR spectra from a small sliver of paint in Figure 5.

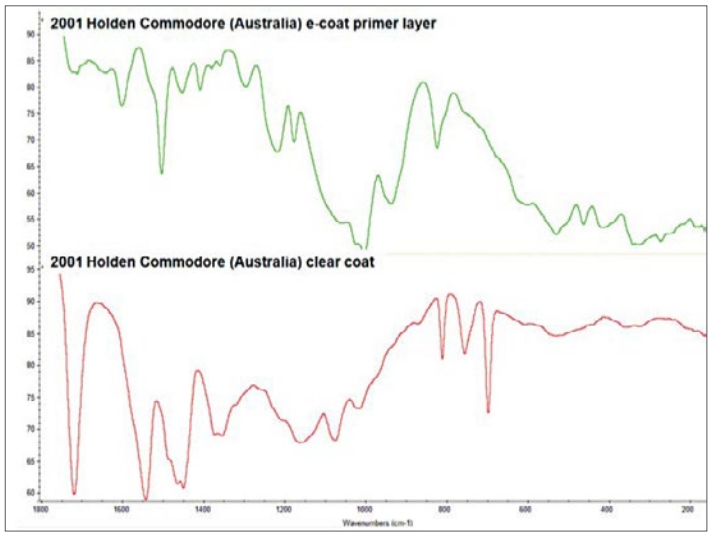

Figure 4: Spectra acquired from two sides of a paint chip showing obvious differences

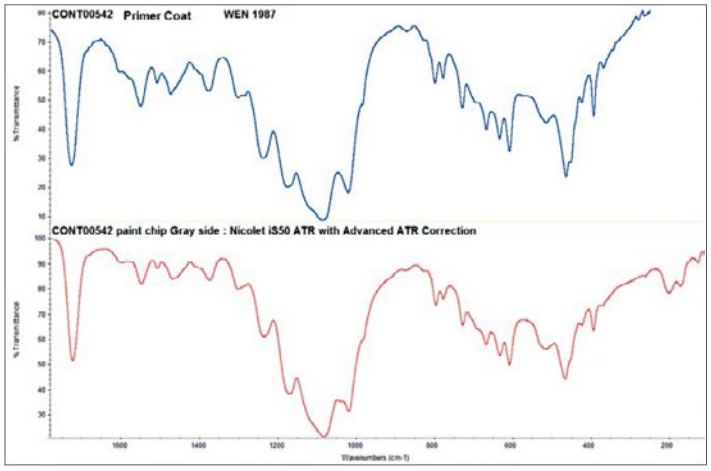

Figure 5: A comparison of a transmittance spectrum acquired with CsI optics to a spectrum acquired with the built-in Nicolet iS50 ATR and the solid substrate beamsplitter

Comparing ATR and transmission spectra

A spectrum acquired with the extended range, built-in ATR from a second paint chip is compared to the corresponding transmission spectrum. Although the sample preparation methods were different as well as the measurement techniques, the spectra are quite similar after applying the Thermo Scientific™ OMNIC™ Software advanced ATR-correction function. In addition, the ATR spectrum shows a few significant features below the 220 cm^{-1} cutoff of the transmittance spectrum acquired with CsI optics.

Conclusion

In this report, we have shown a number of spectra acquired with the Nicolet iS50 Spectrometer and the new extended range, built-in ATR module. The integrated ATR module makes it easy to rapidly obtain infrared spectra all the way down to 100 cm^{-1} from small amounts of sample. This report demonstrates that a high-sensitivity extended range, built-in ATR can play a significant role in the forensics laboratory.[7] We believe that with proper validation the extended range ATR can provide a complementary method to characterize materials of interest to the forensic scientist. Building the Nicolet iS50 Spectrometer with the integrated iS50 ATR module offers the user an instrument with two sampling stations. The sample compartment remains available for transmission analysis or for inserting other accessories and modules. Combining the built-in ATR with the Nicolet iS50 Raman module with an infrared microscope creates a strong foundation for molecular spectroscopy in the forensics laboratory.[8]

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Acknowledgment

We would like to acknowledge Dr. Mark Sandercock, Manager of Trace Evidence Operations Support, National Centre for Forensic Services, Royal Canadian Mounted Police for helpful discussions and providing the paint samples with corresponding spectra.

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APPLICATION NOTE

No. AN51517

FTIR microspectroscopy in forensic and crime lab analysis

Key words
Counterfeit, FTIR microspectroscopy, hair, ink, paint, tablet

Introduction

Forensic and crime lab samples can range from drugs to fibers. Some of these samples can be quite small and light microscopes are often used to help examine evidence collected from the crime scene. The visual aid of an optical microscope can provide investigators with a clearer picture of the evidence, especially at the microscopic level. However, sometimes more information is necessary in order to prove beyond a reasonable doubt that a suspect is guilty or innocent. Therefore, a reliable and flexible analytical technique is necessary to provide both visual and chemical information.

Fourier transform infrared spectroscopy (FTIR) has proven to be a valuable tool for the forensic scientist on the macroscopic level. FTIR microspectroscopy extends the use of traditional FTIR by allowing for quick, non-destructive analysis of samples approaching 10 microns. The new Thermo Scientific™ Nicolet™ iN10 Infrared Microscope is a powerful combination of an optical microscope with an integrated FTIR. The Nicolet iN10 provides the forensic scientist with an analytical tool to visually and chemically analyze illicit tablets, hair, fibers, inks, and paints. The integrated design does not require an external spectrometer, making the Nicolet iN10 a powerful, compact FTIR microscope.

Evidence is an essential part of any court case. For the first time, the unique ability to verify microscope performance through software provides the investigator, and the jury, with confidence that the data is reliable. The Nicolet iN10 can be operated without the need for liquid nitrogen, allowing the lab to quickly examine evidence in any location. The Thermo Scientific™ OMNIC™ Picta™ Software makes operation simple and quick for even the untrained microscopist. Powerful wizards help guide the user through reflection, transmission, and ATR analysis.

Ink on paper

Counterfeiting money is one of the oldest criminal activities known. Criminals no longer have to rely on highly skilled offset printing techniques to produce counterfeit notes. Technological advances allow for the unskilled person, with access to a photographic copier or scanner, to produce high-quality counterfeit currency. However, there are distinct characteristics present in the paper and ink used in the printing process, which can identify counterfeit bills and even trace their origin.

Typically, ink is analyzed by elemental analysis, X-ray and mass spectroscopy. These methods allow for complete characterization but are destructive and time-consuming. Infrared spectroscopy has not been

fully utilized in the identification of ink and contaminants found on paper due to the strong infrared absorbance from cellulose between 1200-950 cm^{-1}. However, the quick and non-destructive nature of infrared imaging and Attenuate Total Reflectance (ATR) FTIR microscopy and has emerged as a key benefit when analyzing criminal evidence. The Thermo Scientific Nicolet iN10 Microscope can now play an important role in fraudulent document analysis.

Analysis of suspect inks can help reveal the type of ink and how it was applied to the paper. Ink applied by photostatic or inkjet methods can often be distinguished from offset printing techniques by visual inspection. However, with advanced printing technology, this is becoming more difficult. FTIR microscopy allows for quick chemical imaging of both the ink and paper material. This provides unambiguous information that can be directly compared to genuine documents.

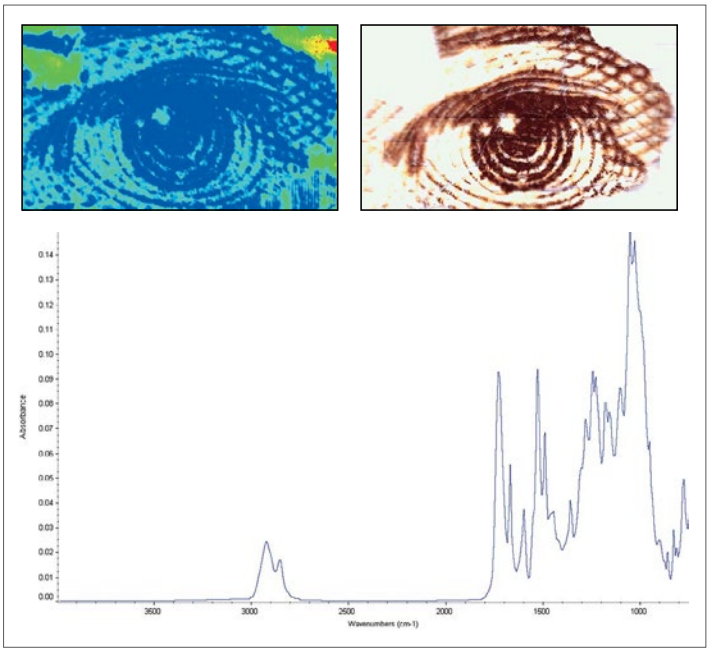

Figure 1: (Upper left) Chemical image of Andrew Jackson's eye on $20 U.S. currency (Upper right) Video mosaic of Jackson's eye (Lower) Black ink spectrum collected by Tip ATR. Chemical imaging highlights the distribution, while ATR analysis provides detailed spectral information of the ink.

Figure 1 shows a chemical image of a U.S. twenty-dollar bill. The black ink can be chemically distinguished from the paper and the surrounding background ink. The chemical and visual images can be compared, showing the high resolution of the infrared microscopic information. Once the desired information is located by rapid chemical imaging, ATR analysis can provide detailed spectral information with little interference from the cellulose contribution.

Fiber and hair analysis

Many kinds of fibers are often found at crime scenes and can provide valuable or even crucial information. For instance, forensic scientists are trained to identify and correlate physical hair features and appearances to a particular ethnic group. This information may be useful in identifying potential suspects but may not isolate one suspect from another. FTIR microscopy can combine visual microscopic hair fiber analysis with valuable and discriminating infrared chemical information. Hair fiber chemical information can reveal residual hair styling products (such as hairspray and conditioners) and protein structure changes due to chemical treatments (such as bleaching). This additional information may prove essential in identifying a suspect.

Oxidation of hair can occur chemically or by natural sunlight. Chemical oxidizers such as hydrogen peroxide and persulfates are often found in bleaching products. Oxidation of the amino acid cystine to cysteic acid can occur in hair, resulting in an increase of the S=O stretching absorbance. Hair fiber analyzed by reflection absorption and Ge Tip ATR clearly show the difference between untreated and chemically treated hair. Figure 2b shows the region between 1400 and 900 cm^{-1} revealing the spectral differences due to the oxidation of cystine to cysteic acid. The top spectrum shows the increase of the S=O symmetric cysteic acid stretch located at ~1040 cm^{-1} and the asymmetric S=O stretch at 1175cm^{-1} due to the bleaching process.

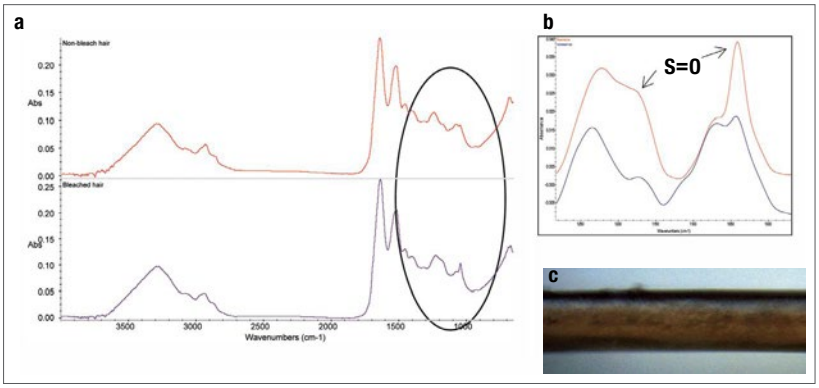

Figure 2: (a) Spectra of non-bleached (red) and bleached (purple) hair. (b) Expanded region showing S=O stretching regions (c) Video image of un-bleached hair fiber.

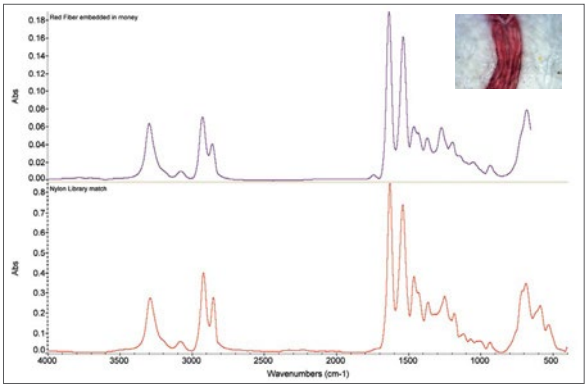

Figure 3: (Upper spectrum) Spectrum of Nylon fiber embedded in currency. (Lower spectrum) Nylon Spectral library match. (Right) Visual image captured by OMNIC Picta software.

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Visual microscopy is also used to identify and compare natural and synthetic fiber evidence. A highly skilled forensic scientist can identify the physical characteristics that distinguish between different generic fiber classes. However, further analysis, including chemical analysis, is needed to determine the chemical subclass.[1] FTIR microscopy has emerged as a powerful analytical tool that can quickly determine a fiber's subclass in a non-destructive manner and little sample preparation. All of which is important in the forensic community where preserving the evidence is critical.

Recently, federal currency mints have embedded special fibers into the paper as an added defense against counterfeiting. Utilizing attenuated total reflection (ATR) on the Nicolet iN10, the small security fibers in a circulating bill can be examined as shown in Figure 3. The visual image through the Nicolet iN10 clearly shows the red fiber, and the ATR data identifies it as nylon. ATR microspectroscopy provides high spectral quality with little surrounding cellulose contribution. This allows for exceptional library identification.

Tablets

Rapid analytical techniques that provide chemical composition and distribution of active ingredients for illicit drug tablet analysis are very important in forensic investigations. Sentencing guidelines can be based on both possession and quantity, so both qualitative and quantitative information are needed. Imaging with the Nicolet iN10 MX Infrared Imaging Microscope provides a quick and non-destructive analysis technique well suited for homogenous and heterogeneous tablets. Unlike other macroscopic analytical techniques, FTIR microspectroscopy does not require sample dissolution, which can destroy evidence and cause insoluble or re-crystallized products. The Nicolet iN10 MX, OMNIC Picta Software and Thermo Scientific™ OMNIC™ Specta™ Analysis Tools provide drug composition information and insight to the criminal manufacturing process. Coupled with the system verification tools, this can give the investigator powerful information for use in court.

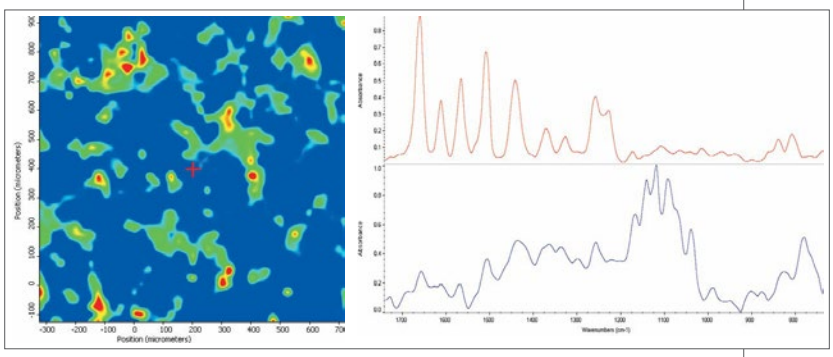

Figure 4: (Left) Chemical image of prescription drug. (Right) Red spectrum is the active ingredient and the blue spectrum is the excipient.

The Nicolet iN10 MX imaging Infrared Microscope is the first system engineered specifically for chemical imaging analysis, while providing the speed, sensitivity, and resolution of traditional infrared microscopy. Figure 4 shows a chemical image of prescription drug tablet collected using rapid imaging mapping on a Nicolet iN10 MX.

A 5 × 5 mm area was selected and the infrared data collected in approximately five minutes. The chemical image indicates the active ingredient in blue; clearly, this is the bulk of the material. However, the green and red contours indicate another component is present. Simply clicking inside one of the green/red contours reveals the spectrum of the other tablet component – in this case, an unregulated excipient.

The OMNIC Picta Software also features automatic collection and analysis wizards. For example, the random mixture wizard can automatically analyze and identify multiple components with a single click. Figure 5 shows the multicomponent wizard screenshot for an over the counter tablet. The wizard automatically creates a list of the main components by cross-correlating the collected map spectra. The wizard calculates the individual component area contribution and provides semi-quantitative distribution information. Each component can then be identified by spectral library information to provide further chemical information.

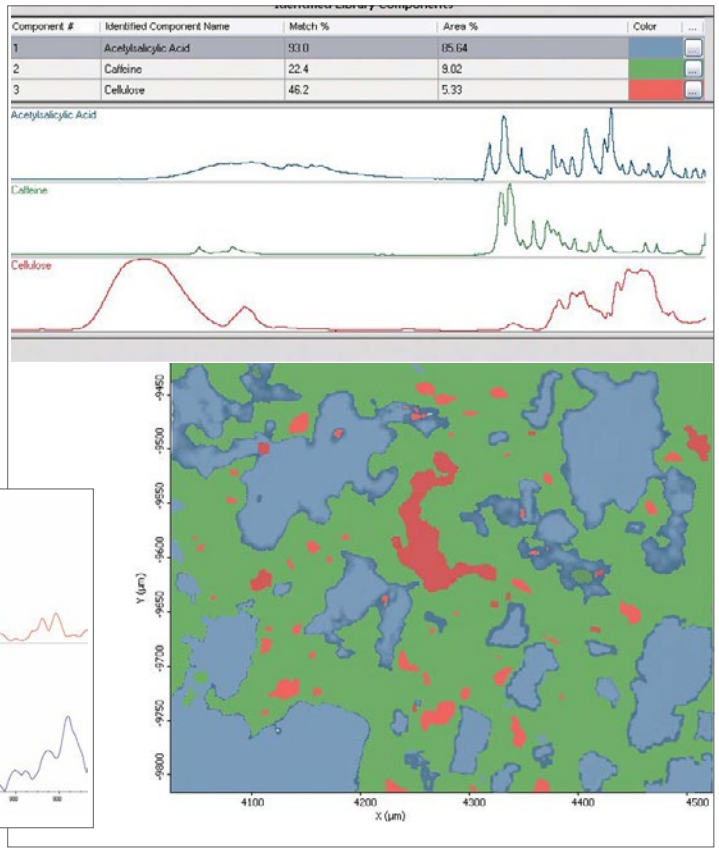

Figure 5: Tablet analysis of over-the-counter tablet by Picta Multicomponent Wizard.

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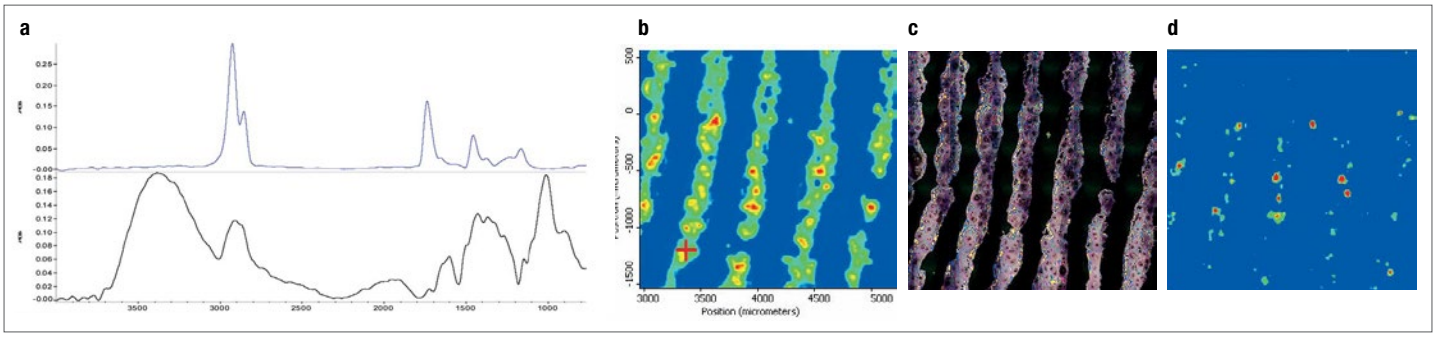

Figure 6: (a) Spectrum of natural triglyceride esters; (b) chemical image of fingerprint; (c) video image of fingerprint; (d) chemical image highlighting the fibrous wood contaminate.

Trace analysis

Fingerprint information can be useful in identifying or confirming a suspect's involvement in a crime. While fingerprints are unique to an individual, there is more information present than just the fingerprint pattern. FTIR microspectroscopic analysis can elicit chemical information left behind with the fingerprint. This chemical information can help trace a suspect's last step before a crime.

Figure 6 shows the chemical image and corresponding video image of a 2 × 2 mm fingerprint impression made on a reflective microscope slide. The main component of the fingerprint is natural sebum oil from the skin (triglyceride esters). However, several small contours outside the fingerprint indicate another component. The lower right chemical image in Figure 6 shows a region of the fingerprint revealing a small amount of fibrous wood material. Chemical imaging quickly provides the unique fingerprint pattern while revealing important and unexpected trace chemical information.

Paint analysis

Paint chip evidence can be found at a crime scene involving an automobile. In most cases, the paint or paint chip is transferred to a victim or object involved in the accident. Automotive paint consists of multiple layers of chemically different materials, including binders, primers, pigments and protective resins, which are applied individually to a car's plastic or metal surface. A chip of paint will usually preserve information about the individual paint layers and can be visually examined through an optical microscope. Normally, the chemical identification of the paint layers requires dissolution and chemical extraction. Using fast mapping FTIR microscopy provides quick chemical identification of each layer. The images in Figure 7 show the analysis of a multi-layer paint sample. Layer 1 is the outer protective polyurethane coating, layer 2 shows the base coat and polypropylene polymer (the main component of the bumper), layer 3 is the paint binder layer.

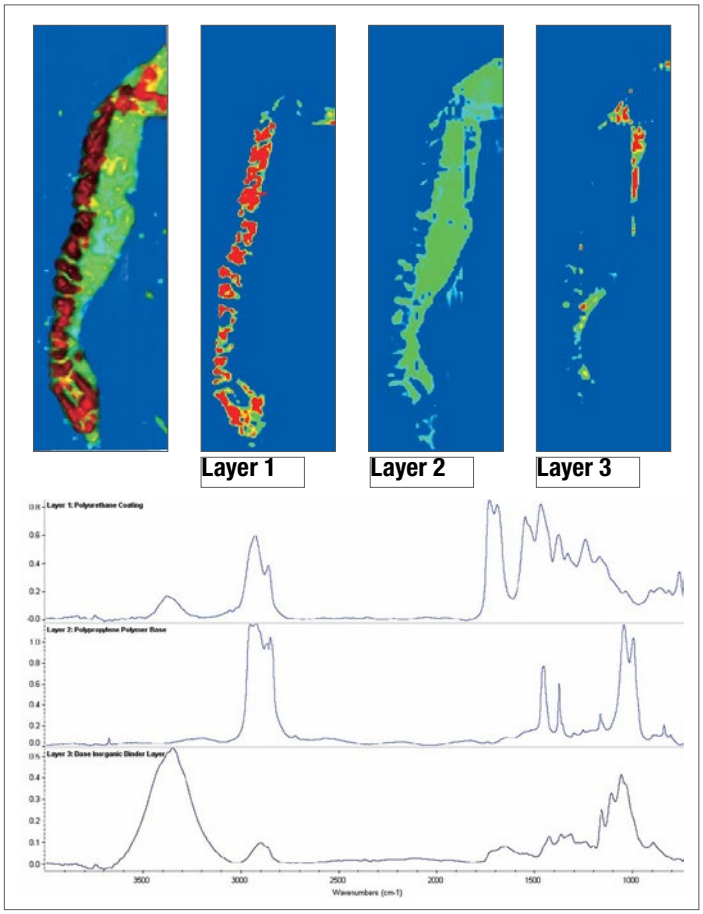

Figure 7: (Upper) Chemical image of a car bumper paint chip layers. (Lower) Spectra of identified layers: Layer 1: protective coating. Layer 2: base coat and polypropylene polymer, Layer 3: binder layer.

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Residues

Chemical residues obtained from a crime scene can provide valuable information and additional clues. Residues are often sensitive to evidence handling and ideally analyzed with little interaction. FTIR microscopy can locate and analyze trace residues without sample preparation or removal. Figure 8 demonstrates the sensitivity of infrared microscopy for this application. A portion of a 10-cent Euro coin was analyzed using the Nicolet iN10 MX Imaging Microscope. The detailed chemical image (upper left) reveals a thin pink outline around the stamped coin markings. The residue spectrum indicates the material is protein-based and is most likely attributed to human skin and oil residue. However, this demonstrates how quickly evidence can be analyzed for trace materials.

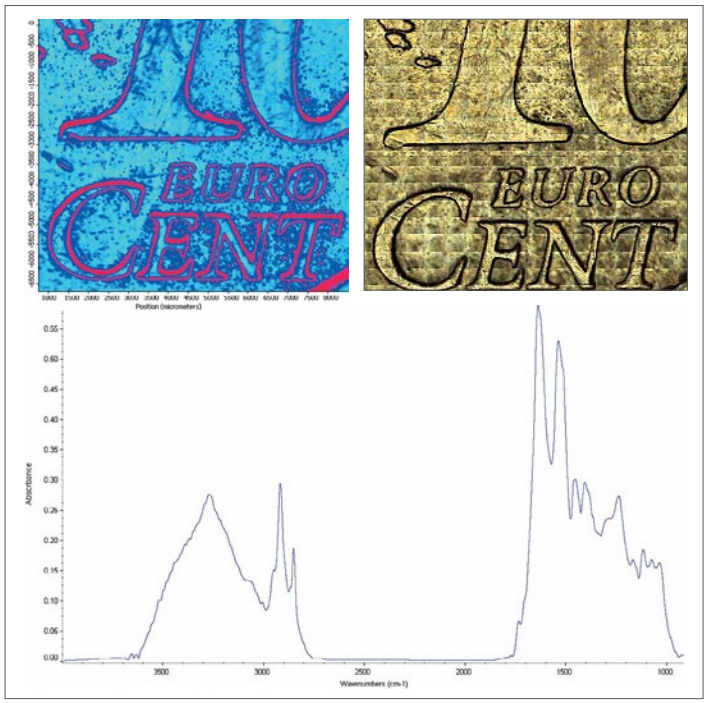

Figure 8: (Upper Left) Chemical image of 10-cent Euro coin (Upper Right) Mosaic video capture of coin sample area (Lower) Spectrum of amide residue.

Summary

The Nicolet iN10 Infrared Microscope and the Nicolet iN10 MX Imaging Microscope provide the forensic scientist with rapid visual and chemical information for many types of samples. The sensitivity and non-destructive nature of infrared ensures accurate interpretation while preserving evidence. The spatial resolution and sensitivity of linear array imaging can quickly reveal the presence of trace materials. OMNIC Picta's performance verification and available validation package provides confidence in the results, which is important when presenting data in court. In addition, we offer innovative OMNIC Specta Software. OMNIC Specta has the most advanced peak and multi-component search feature.

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APPLICATION NOTE

No. AN52531

Forensic analysis of paper currency with FTIR microscopy

Key words
iN10 FTIR microscope, counterfeit, forensic science, FTIR microscopy, questioned documents

Nicolet iN10 FTIR Microscope

Confirming the authenticity of documents is so important to commercial trade, border security and law enforcement groups that these investigations represent a distinct branch of Forensic Science. Questioned document specialists use a wide array of analytical techniques to assist in their investigations ranging from first-line visual inspection tools to advanced chemical-specific analytical instrumentation. Visible light microscopy techniques are well suited for characterizing the printing techniques of individual characters and to look for evidence of alteration. Imaging methods, often using various light sources and filter combinations, complement direct visual inspection to enhance comparisons to genuine articles.

The need for advanced chemical analysis to support forensic casework has become more important because of the complexity and sophistication of the fraudulent items. Counterfeiting money is one of the oldest-known criminal activities and, as police are witnessing, criminals no longer need access to complex offset printing techniques to produce quality counterfeit notes. A reasonably high-quality photographic copier can produce very realistic counterfeit currency. For example, the European Central Bank reported that approximately 600,000 individual Euro notes were detected and removed from circulation in each of the last three years.[1] Not only do merchants and consumers need to stay alert for suspicious bills, but research and development continue to improve detection methods for automated cash handling machines (e.g., vending, ATM – automated teller machine, bill counters, etc.).

High-performance laboratory instruments enable inspection of Questioned Documents, such as banknotes, to progress into the chemical domain, allowing detection and study of anomalies not observable by visual inspection alone. The Fourier Transform Infrared (FTIR) microscope, a mainstay of the forensic laboratory, is exceptionally well suited for study of the inks, toners, and papers of fraudulent documents because it combines standard visible light microscopy with non-destructive molecular spectroscopy analysis. One instrument that combines several forensic-relevant techniques with excellent analysis software is the Thermo Scientific™ Nicolet™ iN10 FTIR Microscope. The Nicolet iN10 Microscope combines standard color video inspection, polarized light, infrared transmission and reflection spectroscopy, and micro-contact-mode sampling using attenuated total reflectance (ATR). Using these various modes of chemical imaging inspection, investigators can undertake in-depth studies of paper currencies.

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The “backbone” of any banknote is, of course, the paper it is printed on. Currency is printed on high cotton-content paper for durability. Figure 1 shows representative spectra of paper specimens. All paper products have a similar carbohydrate chemical structure because of their natural product origins (e.g., wood pulp, cotton or the like), so the main features of the infrared spectra are also similar. However, paper makers use different fiber stock and additives, like mineral or polymeric fills, to provide different properties. These variations affect the patterns seen in the infrared spectra, which can help identify the paper source. In Figure 1, the spectral patterns are similar, but some unique features stand out, generating useful differences for comparisons.

Inks and pigments applied to the paper are generally present at low concentration, just covering the top layer of paper fibers. Using surface-sensitive attenuated total reflectance (ATR), the infrared pattern of the ink alone can be obtained. Figure 2 shows some representative spectra of the inks from printed features on different paper currencies. Because the ink layer is thin, spectral features of the underlying paper often remain (note the carbohydrate signal towards the left end of some spectra in Figure 2), but there are also distinct patterns between 1800 and 700 wavenumbers representing “fingerprints” of the ink. The spectrum in plot (A) is significantly different than the other three due to this particular print containing a grade of calcium carbonate as shown by the strong peaks at 1420 and 875 cm^{-1}. This gives the ink bulk.

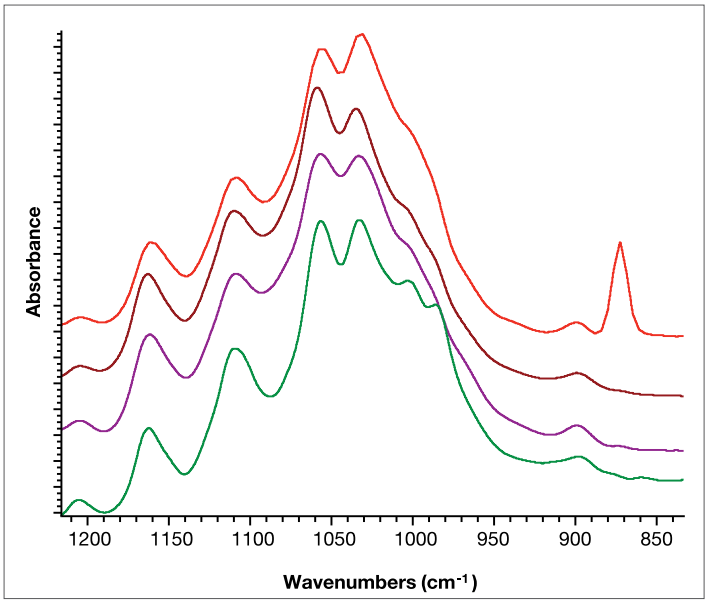

Figure 1: Characteristic spectra of paper products. From top: bright white copy paper; linen business paper; 25% cotton bond paper; natural color cotton paper (€20 note).

Counterfeit currency produced using laser printer technology may look like an excellent reproduction to the naked eye, but the toner used with these printers is easily distinguished from printing inks used on authentic bills. Laser printer toner is composed of pigmented polymer particles that are transferred to the paper in the printing process. Figure 3 shows the infrared spectra of color text produced using a laser printer. The patterns show strong polystyrene bands indicating that this is the polymer used in the toner. Polystyrene is the most common toner material in today’s market – it gives the powdery feel anyone who has changed a toner cartridge will remember. The IR features linked to the pigments are also present in the spectrum, as well as bands from the paper base layer.

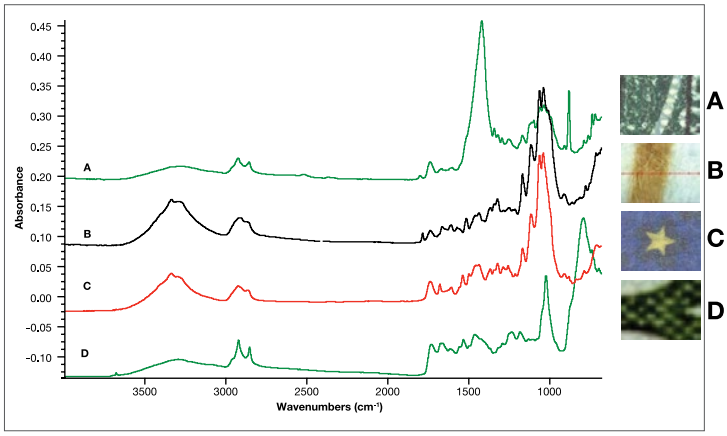

Figure 2: Representative spectra of inks printed on paper currencies. A) Green raised treasury seal (USD); B) Brown printed design (RMB); C) Yellow star (EUR); D) Part of the character “B”, (KRW).

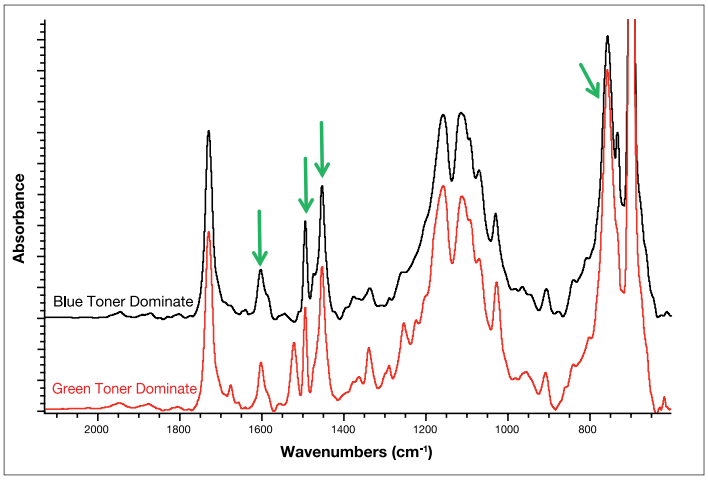

Figure 3: Infrared spectra of text printed with a digital color printer showing strong features of the polystyrene-based toner matrix indicated with arrows.

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Infrared microscopy's key value is its ability to target a very specific location for data collection. For this reason, the technique is very popular for studying defects and trace contamination. Figure 4 shows a dark clump, approximately 100 microns in size, located in an unprinted area of a circulated 20 Euro note. The red spectrum is from this embedded particle and includes a peak (signals) from the paper. Comparing this spectrum of just the base paper (purple spectrum), you can clearly see the peak differences. The spectrum of the particle (upper trace of Figure 4) matches a black raised print feature in Figure 2A. We speculate that small pieces of the raised print have been abraded through handling and stuck to this location. Paper currency also collect particles as they pass from hand to hand, including particles that might be useful as trace evidence in law enforcement cases unrelated to counterfeiting.[2]

Advanced FTIR microscopes, such as the Nicolet iN10 Microscope, have imaging modes for studying distributions of chemical features over wider areas. Figure 5 shows an example of an infrared image examining different attributes of a pair of banknotes (circulated and new) from the Ukraine. The imaging data set contains a full spectrum at each point. To convert this massive information into a 2D image, we select a pattern known to represent one component (ink or contaminant). Colorizing (red where the pattern is strong, blue where it is weak) leads to the imagery shown. Red will mean that pattern – component – is present there; blue that it is absent. Image (B) was created searching for the spectral pattern of cotton fibers. This image demonstrates how the IR microscope can "see" through the print layer (the fibers are seen basically everywhere, even under the ink). Similarly, image (C) is generated from the IR signature associated with a blue/gray pigment. The dark print on the left (A) does not show in this image because it has a different spectral pattern – hence a different chemical composition. Under the microscope, it is easy to see that the circulated bill's surface is rough, making it easy for particles to get trapped on the surface. Image (D) highlights materials with a polyamide spectral pattern – shown by the arrow. The red "hot spots" in image (D) are most likely small skin flakes (which are polyamides) from people handling the bill.

Treasury departments continue to deploy new security features on paper currencies, but it seems criminal attempts to defeat the new features soon follow. Law enforcement is responding by adding new analytical tools, like FTIR microscopy, to study the chemical nature of suspicious articles. Until recently, these tools were found only in central laboratories with expert operators. Fortunately, the easy-to-use Nicolet iN10 Microscope with its intelligent software and sampling options makes FTIR microscopy much more widely accessible. As the skills of criminals improve, placing powerful tools like the Nicolet iN10 Microscope in the hands of more investigators will help keep law enforcement a few steps ahead.

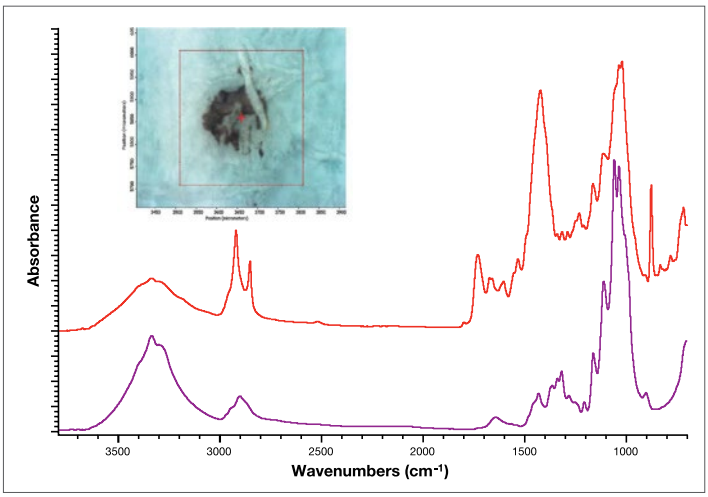

Figure 4: Using micro-ATR to investigate possible contaminants and particles in a unprinted area of 20 Euro note. Upper trace is the spectrum of an embedded particle in the paper matrix; lower trace is paper region just adjacent to the particle.

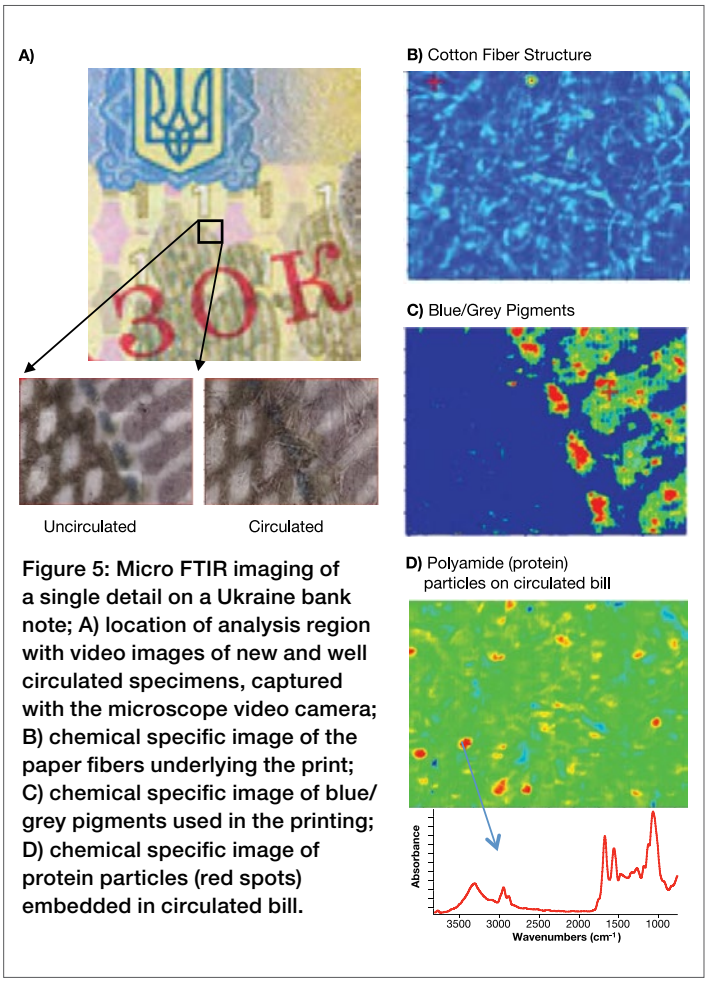

Figure 5: Micro FTIR imaging of a single detail on a Ukraine bank note; A) location of analysis region with video images of new and well circulated specimens, captured with the microscope video camera; B) chemical specific image of the paper fibers underlying the print; C) chemical specific image of blue/grey pigments used in the printing; D) chemical specific image of protein particles (red spots) embedded in circulated bill.

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Nicolet iS50 FTIR Spectrometer

Make complete and confident identification of seized materials and trace evidence from narcotics to paint chips. The Nicolet iS50 FTIR Spectrometer provides two SWGDRUG/SWNAT Class A Instrumental methods in one platform to positively identify physical evidence. Automated tools optimize system performance measurements utilizing traceable standards, giving assurance for use of the data in courtrooms.

The Nicolet iS50 has many modules that provide correlated analysis from 1 measurement and includes a wealth of spectral library information for identification of unknowns. These modules include Raman, ATR and GC.

- **FT-Raman Module:** FT-Raman spectroscopy is a fast and safe complementary technique for detecting and identifying powders and other unknown materials. Raman analysis requires little to no sample preparation and allows direct measurements through glass vials and evidence bags, reducing any risk of accidental exposure by lab personnel to materials such as Fentanyl. It is also non-destructive.
- **GC-IR Module:** GC-IR provides highly selective identification for isomers in cannabinoids and bath salts, by examining the intact molecule spectroscopically. This provides confidence in identification of modified synthetic drugs, especially when immersed in a messy mixture.
- **ATR Module:** An integrated ATR module makes it easy to rapidly obtain infrared spectra all the way down to 100 cm from small amounts of sample. The far-IR provides tools for examining paint chips while the speed and ease provides screening for drugs in seconds.

Nicolet iN10 Infrared Microscope

The Nicolet iN10 Infrared Microscopes is well suited for analyzing inks, toners, and suspicious documents, along with hair, paint and tablets, because it combines visible light microscopy with non-destructive molecular spectroscopy. Forensic scientists can quickly see the presence of trace materials with rapid visual and chemical information all while preserving evidence with high confidence.

Find out more at **thermofisher.com/specforensics**

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