

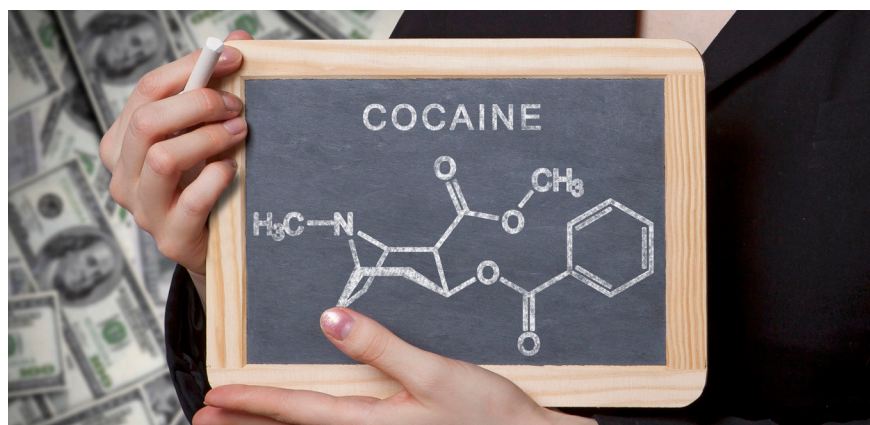
Automated FT-IR screening method for cocaine identification in seized drug samples

Application note

Forensics

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Introduction

Quick and presumptive identification of seized drug samples without destroying evidence is necessary for law enforcement officials to control the trafficking and abuse of drugs.

In this study an automated screening method was developed with Agilent's MicroLab software and was used to detect the presence of cocaine in by analyzing the spectra of seized samples that had been measured using an Agilent 630 or 4500 FTIR spectrometer.

The method used the well-established "center of gravity" peak picking mathematical algorithm [1], combined with a conditional reporting feature in the MicroLab software. This automated method could be successfully used by analysts with varying experience levels. The method detected cocaine in a range of chemical mixtures at concentrations as low as 6 wt %.

Unlike library search and chemometric methods that are either limited to a spectral library database or models built for specific adulterants or diluents, the method presented in this work is independent of the adulterants or diluents present in the mixture.

Methods and Materials

Agilent 4500 Portable series FT-IR and Cary 630 FT-IR spectrometers, equipped with Deuterated Triglycine Sulfate (DTGS) detectors and controlled by Agilent's MicroLab software were used to collect the spectra of seized cocaine samples.

The data collection parameters for two FT-IR instruments are shown in Table 1.

Table 1. Data collection parameters in this study

Instrument	Resolution (cm ⁻¹)	No. scans	Total scan time (s)	Spectral range (cm ⁻¹)
Agilent 4500	4	64	26	4000-650
Cary 630	2	52	128	4000-500

The systems were equipped with Attenuated Total Reflectance (ATR) sample technology employing a 45° single reflection diamond crystal. The ATR sampling method needs little or no sample preparation and is non-destructive.

Agilent's MicroLab software was used to create a method that automatically finds the spectral bands associated with cocaine. A series of conditional statements, using Boolean logic, was then used to assess how many of those peaks were present and thus report a result.

The performance of the cocaine identification method was evaluated by analyzing spectra of seized cocaine samples obtained from two locations: the United Kingdom (where the spectra had been obtained with a Cary 630 FT-IR) and Italy (where the spectra had been obtained with a 4500 portable FT-IR instrument). The concentration of cocaine and identification of adulterants in the samples from the United Kingdom was determined by the South Wales Police, in the UK, using an Agilent 1260 Infinity HPLC system with a Zorbax column and an Agilent 7830A GC-MS system with DB-5MS column following an in-house method, accredited to UKAS 17025 standards. Cocaine concentration in those samples ranged from 6 – 72 wt %. The concentration of cocaine in the Italian samples was provided with the spectra.

Results and Discussion

The ATR spectra of a standard cocaine salt and a cocaine base sample exhibit strong characteristic IR band frequencies (expected peak position), as shown in Table 2.

Table 2. Spectral band positions and threshold values for each characteristic peak of cocaine used in the center of gravity peak calculation.

Cocaine salt (cm ⁻¹)			Cocaine Base (cm ⁻¹)		
Expected peak position	Threshold		Expected peak position	Threshold	
	Critical Low	Critical High		Critical Low	Critical High
1728	1725.6	1729.6	1734	1731.8	1735.8
1712	1708.3	1712.8	1707	1701	1708.3
1265	1261.8	1266.8	1273	1271	1275
1230	1227.2	1232.2	1227	1225.3	1229.3
1105	1102	1108.5	1107	1105.3	1111.3
1071	1068.2	1072.2	1068	1066.4	1070.4
1026	1023.9	1027.9	1035	1033.9	1037.9
729	725	731.5	712	710.1	714.1

ATR spectra of cocaine salt measured on the 4500 FT-IR and Cary 630 instruments are shown in Figure 1.

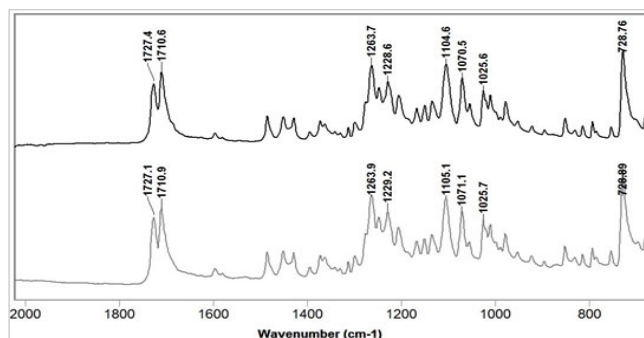


Figure 1. ATR spectra of a 70% cocaine salt sample collected with a bench top Cary 630 instrument (upper) and a 4500 portable instrument (lower). Note the similar peak positions of cocaine salt sample between two instruments.

The peak picking method

The center of gravity (CG) algorithm is useful in the determination of peaks because it is unaffected by variations in intensity, line shape or line width, or resolution. The thresholds for each of the expected peak positions, as shown in Table 2, were defined in the software such that if a peak falls outside the given threshold range, then that peak is not considered for cocaine identification. The critical thresholds were chosen empirically by analyzing multiple spectra. They were set to be narrow enough not to capture other peaks, but wide enough to not miss the target peak. This allowed the identification of the representative peaks of cocaine, even when the spectra underwent subtle shifts due to external stimuli, such as pressure and temperature.

Table 3. Cocaine peaks that were grouped for cocaine identification in sample mixture. ● indicates the peak included in the combination

Cocaine salt peaks (cm ⁻¹)	1728	1712	1265	1230	1105	1071	1026	729	No. of peaks identified by the method	Likelihood of cocaine in seized sample
	●	●	●	●	●	●	●	●	8 peaks	High Confidence
	●	●	●	●	●	●		●	7 peaks	High Confidence
	●	●	●		●	●	●	●	7 peaks	High Confidence
	●	●	●	●	●	●	●		7 peaks	High Confidence
	●		●	●	●	●	●	●	7 peaks	High Confidence
	●		●		●	●	●	●	6 peaks	Certain
	●	●	●		●	●		●	6 peaks	Certain
	●	●			●	●		●	5 peaks	Most Likely
	●	●	●		●			●	5 peaks	Most Likely
	●	●			●			●	4 peaks	Likely

Cocaine base peaks (cm ⁻¹)	1734	1701	1273	1227	1107	1068	1035	712	No. of peaks identified by the method	Likelihood of cocaine in seized sample
	●	●	●	●	●	●	●	●	8 peaks	High Confidence
	●	●	●		●	●	●	●	7 peaks	High Confidence
	●	●		●	●	●	●	●	7 peaks	High Confidence
	●	●			●	●	●	●	6 peaks	Certain
	●	●			●	●		●	5 peaks	Most Likely
	●	●			●		●	●	5 peaks	Most Likely
	●	●			●			●	4 peaks	Likely

Eight characteristic ATR peaks of the spectra (Table 2) were used for cocaine identification. Based on the number of peaks identified in the analyzed spectrum, a likelihood level was assigned for the presence of cocaine in the mixture. The peak combinations and the likelihood level assigned for each group are shown in Table 3. The minimum number of peak combinations required for assigning a reasonable confidence level was set to four. If seven or eight characteristics peaks were correctly identified in the analyzed spectrum, then there was “high confidence” that the sample contained cocaine. Similarly, if 6, 5, or 4 peaks were correctly identified, then cocaine presence in the sample was given the likelihood of “certain”, “most likely”, and “likely” respectively.

At least four consistent strong peaks are frequently observed in the FT-IR spectra of cocaine samples: two strong bands due to two carbonyl group stretching (1728 cm⁻¹, 1712 cm⁻¹ for cocaine salt, and 1734 cm⁻¹, 1707 cm⁻¹ for cocaine base), the C-O and C-N stretching bands (1105 cm⁻¹ for cocaine salt and 1107 cm⁻¹ for

cocaine base), and also the out-of-plane bending vibration (729 cm⁻¹ for cocaine salt and 712 cm⁻¹ for cocaine base). These peaks were included in almost all the combination groups of the method.

The MicroLab software’s component reporting feature was used to set the conditions for the peak combinations as shown in Figure 2. For each group of peaks shown in Table 3, the method reports the cocaine likelihood only when all the peaks for that group falls within the threshold values (defined in Table 1). In the condition box (Figure 2), the test state of peak 1728 is set to “good”, meaning that the peak falls in between the low critical threshold (1725.6 cm⁻¹) and high critical threshold (1729.6 cm⁻¹). Similarly, all other 7 peaks should fall within the defined threshold value for the method to report that the analyzed sample contains cocaine with a likelihood defined as “high confidence”.

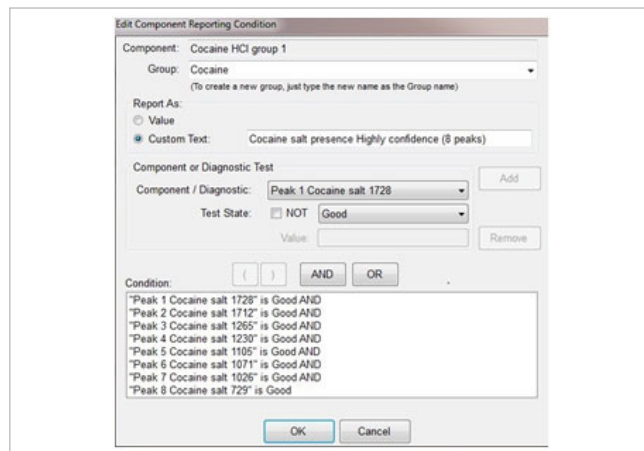


Figure 2. MicroLab software’s component reporting feature was used to set the conditions for peak combinations to indicate the likelihood of the presence of cocaine. For each peak to satisfy the “Good” condition, the peak should be present in the spectrum and also be within the threshold value as defined in Table 2.

Table 4 shows the results of applying the peaking picking method to the spectra of 22 seized cocaine samples, each containing different concentrations of cocaine (as determined by the standard forensic laboratory techniques i.e. chromatography).

The lowest concentration of cocaine in a sample was 6%.

The data set in Table 4 was used to fine tune the high and low critical thresholds for peak locations while creating the method. Subsequently, a separate set of spectra of 5 more samples, with differing additional constituents, was used to test the final peak picking method. Those results are show in Table 5.

Table 4. Comparison of the measured concentration of seized cocaine samples, versus the likelihood that they contain cocaine, as determined by the peak picking method during development of the method.

Spectra collected on Cary 630 ^a FT-IR instrument					
Sample	Sample Constituents	Cocaine Form	Cocaine conc. ^b	No. of peaks	Cocaine Presence Likelihood (via the peak picking FT-IR method)
1	Cocaine + related compounds ^c , phenacetin and tetramisole	Base	17%	5	Most Likely
2	Cocaine + related compounds, phenacetin and tetramisole	Base	27%	6	Certain
3	Cocaine + related compounds, phenacetin and tetramisole	Salt	26%	6	Certain
4	Cocaine + related compounds	Base	38%	7	High Confidence
5	Cocaine + related compounds and phenacetin	Base	6%	4	Likely
6	Cocaine + related compounds and phenacetin	Base	34%	6	Certain
7	Cocaine + related compounds, phenacetin and tetramisole	Base	17%	5	Most Likely
8	Cocaine + related compounds, phenacetin and tetramisole	Salt	26%	6	Certain
9	Cocaine + related compounds and tetramisole	Salt	70%	8	High Confidence
10	Cocaine + related compounds, benzocaine and tetramisole	Salt	72%	7	High Confidence

Spectra collected on a 4500 Portable FT-IR Instrument					
Sample	Sample Constituents	Cocaine Form	Cocaine conc. ^d	No. of peaks	Cocaine Presence Likelihood (via the peak picking FT-IR method)
11	Cocaine + Caffeine	Salt	16%	6	Certain
12	Cocaine + Caffeine	Salt	25%	7	High Confidence
13	Cocaine + Caffeine	Salt	40%	7	High Confidence
14	Cocaine + Caffeine	Salt	50%	8	High Confidence
15	Cocaine + Caffeine	Salt	60%	8	High Confidence
16	Cocaine + Caffeine	Salt	70%	8	High Confidence
17	Cocaine + Lidocaine	Base	16%	8	High Confidence
18	Cocaine + Lidocaine	Salt	25%	8	High Confidence
Spectra collected on a Cary 630 FT-IR Instrument					
19	Cocaine + Lidocaine	Salt	40%	8	High Confidence
20	Cocaine + Lidocaine	Salt	50%	7	High Confidence
21	Cocaine + Lidocaine	Salt	60%	8	High Confidence
22	Cocaine + Lidocaine	Salt	70%	8	High Confidence

a. 5 replicate measurements for each sample measured by Cary 630 have the same result.

b. The % cocaine present was measured by the reference chromatography method.

c. Related compounds = methyl ester ecgonidine, methyl ester ecgonine, tropacocaine, benzoylecgonine, trans-cinnamoylcocaine and norcocaine

d. % cocaine present was supplied with the spectra from Italy

Table 5: The results of applying the finalised peak picking method to the spectra of 5 addition seized cocaine samples, with different cocaine concentrations and differing additional constituents. The % cocaine present was measured by the reference chromatography method.

Sample	Sample Constituents	Cocaine Form	Cocaine Conc.	No. of peaks	Cocaine Presence Likelihood (via the peak picking FT-IR method)
1	Cocaine + Benzocaine, Tetramisole	Salt	27%	8	High Confidence
2	Cocaine + Benzocaine	Salt	12%	4	Likely
3	Cocaine + Phenacetin	Base	33%	6	Certain
4	Cocaine + Tetramisole	Salt	63%	8	High Confidence
5	Cocaine + Tetramisole	Salt	65%	8	High Confidence

As expected, the number of peaks that were identified by the peak picking FT-IR method was influenced by the chemical complexity of the samples and the concentration of cocaine in each. As the sample complexity increases, so does the probability of peak overlapping and shifting due to broadening. More peaks were identified in the spectra of samples with higher concentrations of cocaine.

To assess the robustness of the method, samples such as heroin, methamphetamine, diamorphine, caffeine, sugars, lidocaine, and other common pharmaceutical compounds that do not contain any cocaine were also tested with the method. The peak picking method did not identify the minimum number of peaks (i.e. four peaks) required to identify any of these samples as

containing cocaine, which assured the credibility and specificity of the method.

One of the advantages of this method is that the sensitivity and specificity of FT-IR prevents the masking of peaks of interest. This, together with the high number of bands used to identify cocaine (at least four bands), makes the method robust.

As shown in Tables 4 and 5, the likelihood of cocaine being in a sample, as determined by the FT-IR peak picking method, aligned well with the actual concentration of cocaine in each sample, as determined by the chromatography reference technique.

Conclusion

An automated peak picking method to determine the likelihood of a sample containing cocaine was developed.

The automated FT-IR method was used to analyze the spectra of seized cocaine samples, which had been measured on either an Agilent compact Cary 630 FT-IR or an Agilent 4500 portable FT-IR spectrometer by law enforcement personnel in two European countries.

The peak picking method was able to identify the presence of cocaine, showing good correlation with the measured cocaine concentration of each sample, as provided with the samples. The method was able to identify cocaine in samples with varying chemical compositions and with cocaine concentrations as low as 6% by weight.

The method proved to be suitable as a non-destructive way to pre-screen seized drugs, suspected to contain cocaine.

When coupled with either Agilent's easy to use benchtop FT-IR instrument, the Cary 630, or the portable 4500 FT-IR instrument, the peak picking method would be an ideal tool for law enforcement teams involved in policing illegal drugs.

Acknowledgement

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