Idaho State Police
Forensic Laboratory Training Manual
Infared Spectrophotometry

1.0.0 Background

Infrared spectrophotometry (IR) is one of the most reliable instrumental techniques used for the identification of drugs. Almost every chemical compound, regardless of its phase (i.e., liquid, solid, gas), produces a different infrared spectrum. Consequently, an infrared spectrum can generally be assumed to be specific for a particular compound. Whereas ultraviolet spectrophotometric (UV) analysis is widely accepted as a quantitative tool, IR is regarded as an indispensable tool for qualitative analysis. However, similar to UV analysis, if a drug sample contains more than one constituent, preliminary treatment of the sample is usually necessary in order to isolate each constituent in a fairly pure state for IR analysis. Fortunately, with the advent of computer data stations for data manipulation, the spectral contributions of one or more components in a mixture can sometimes be digitally subtracted leaving an acceptable spectrum of a single component.

The unit of wavelength in the infrared region of the electromagnetic spectrum is most commonly expressed in either wavelength (given in microns or micrometers) or wave numbers, with wave numbers being the most popular. One micron (μ) = one micrometer (μm) = 10^{-6} meters = 10^{4} angstroms (Å) = 10^{-4} cm. Wave numbers are defined as the number of waves per centimeter and have the units of reciprocal centimeters (cm^{-1}).

The overall infrared region extends for 0.78 to 1000 μm (12,800 to 10 cm^{-1}). This region is subdivided into three categories: near, middle and far. Different literature references give different values for the wavelength ranges each of these regions cover. For our purposes, we will use the ranges given in the following table.

Region	Wavelength Range, □	Wave number Range, cm^{-1}	Frequency Range, Hz
Near	0.78 to 2.5	12,800 to 4000	3.8×10^{14} to 1.2×10^{14}
Middle	2.5 to 25	4,000 to 400	1.2×10^{14} to 1.2×10^{13}
Far	25 to 1000	400 to 10	1.20×10^{13} to 3.0×10^{11}

In forensic chemistry, we are chiefly interested in the mid-infrared region from 2.5 – 25μ or 4000 - 400 cm^{-1}. This region is divided into the "group frequency" region of 4000 – 1300 cm^{-1} (2.5 – 8 μ), and the "fingerprint" region of 1300 – 650 cm^{-1} (8 – 15.4μ). In the group frequency region the principal absorption bands may be assigned to vibration units consisting of only two atoms of a molecule; that is, units which are more or less dependent only on the functional group giving the absorption and not on the complete molecular structure.

2.0.0 IR Instrumentation

There are two types of instrumental designs for obtaining IR spectra: dispersive instruments and interferometer instruments. Dispersive spectrometers have been in existence for decades. In the traditional dispersive spectrometer, radiation from the source passes through the sample and is dispersed by some optical element, usually a grating. The amount of radiation transmitted through the sample is measured 1 wavelength at a time. Dispersive instruments still work reasonably well. However, as current applications continue to require ever-greater speed, sensitivity and accuracy, dispersive instruments are being replaced by instruments based on interferometers, many of which are of the "Michelson" type.

With interferometer instruments, repeated measurements are taken of many wavelengths more or less at the same time and an interferogram (i.e., an output of intensity versus mirror displacement) is generated which is converted to an IR spectrum via a mathematical (Fourier) transformation. This type of instrument is generally known as a Fourier Transform Infrared (FTIR) Spectrophotometer.
There have been major advances in the development of Fourier Transform Infrared (FTIR) spectrophotometers over the past decade. Present FTIR instruments produce an IR spectrum on a computer monitor in a few seconds. Current trends in instrument development are toward smaller, more powerful instruments with the newest generation of instruments adding Raman capabilities to the FTIR bench. Due to the higher energy throughput and increased detector sensitivities of the current generation of instruments a broad spectrum of sampling devices have been introduced.

3.0.0 Sample Preparation Techniques

The type of sample preparation required depends on the nature of the sample. Most samples submitted to forensic laboratories are usually powdered material. Many options are available for powdered samples including:

1. Dispersion in potassium bromide (KBr) and pressing into a transparent KBr disc;
2. Dispersion in KBr and analyzing via a Diffuse Reflectance cell;
3. Mulling in a viscous liquid and dispersing between two IR transparent windows or smearing on an IR transparent plate;
4. Dispersing a film or melt between two IR transparent windows;
5. Dissolving in one or more solvents in a liquid cell;
6. Direct analysis via a FTIR with a ATR attachment

Of the above options, KBr dispersion is the sample preparation technique most commonly utilized. When the KBr pellet or the mull technique is utilized, the sample must be finely ground to prevent radiation scattering. Some compounds absorb more strongly than others and the optimum sample concentration may vary from 0.1% to 3%. A general recommendation for preparation of a 1.2 cm KBr pellet is 1 mg of sample to 200 mg KBr, however the proper concentration is best determined by trial. The mull technique provides an acceptable alternative to the pressed KBr disc. The mulling agent is usually Nujol (mineral oil). Halogenated hydrocarbons (e.g., hexachlorbutadiene, perfluorokerosene, or

chlorofluorocarbon greases) can be used to obtain a useable 3000 cm^{-1} region. Solution cells are useful for both solid and liquid samples. The use of solution cells usually requires a sample concentration of 5% to 10%. In order to cover the main spectral range of 4000 to 400 cm^{-1}, a combination of the solvents carbon tetrachloride and carbon disulfide is usually satisfactory. Solvent and solute combinations that react must be avoided; for example, carbon disulfide cannot be used as a solvent for primary or secondary amines.

Liquids and opaque materials (such as plastic and rubber) can be analyzed via Attenuated Total Reflectance (ATR). The ATR technique depends upon the fact that a beam of light that is internally reflected from the crystal surface passes a short distance beyond the reflecting boundary and then returns to the crystal as part of the process of reflection. (See Figure 1) If a sample of lower refraction index than the transmitting medium is brought in contact with the reflecting surface, the light passes through the material to a depth of a few microns producing an absorption spectrum. This technique is also referred to as Multiple Internal Reflectance (MIR).

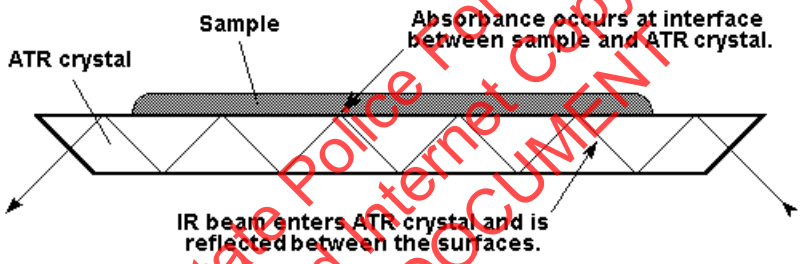

Figure 1

Liquids may also be examined neat by pressing them between IR transparent plates without a spacer, which leaves a thin film of 0.01 mm or less in thickness. Viscous liquids may be smeared on an IR transparent plate.

Gas IR cells can be used for volatile liquids as well as gases. Gas cells are available in lengths of a few centimeters to 40 meters. In general, 10 cm cells are the largest gas cells used without some type of multiple reflection optics. The gas cell is evacuated and filled through a stopcock or needle valve. Fine rotational structure can often be resolved when IR spectra are obtained by use of a gas cell.

4.0.0 Unknown Sample identification

With a little experience, most scientists will be able to recognize the infrared spectra of commonly encountered substances. Beyond the familiar substances however, identifying a compound from its spectrum usually requires comparing the spectrum to a group of reference spectra. Most IR operating systems are capable of comparing an unknown spectrum to reference spectra in libraries. Many libraries of infrared spectra are available. Library searching is not always a perfect process. Many factors can affect the success of a library search and even cause differences between a sample and a known standard ran on the same instrument.

The quality of the unknown spectrum greatly affects how well that spectrum will match a reference spectrum.

Too much sample results in complete absorbance of the radiation at some wavelengths and leads to broad flat absorbance bands that cause the peak locations to not be accurate. Too little sample leads to a weak spectrum where noise and the absorbance's of, H_2O and CO_2 are large in relation to the absorbance from the sample. A good rule of thumb is to try to dilute the sample so that the most intense band gives ~10% transmittance.

Correction of badly sloping baselines is sometimes necessary to obtain good library search results.

Most reference (standard) spectra and libraries are of pure compounds. Trying to library search a spectrum from a multi-component sample may not give a good match. However the search results may identify one of the components, which can then be digitally subtracted. The subtraction result can then be searched to identify other components in the sample.

Standard spectra or the spectra in a library may have been collected at a different resolution than the sample spectrum. To assure good matches, the unknown spectrum may have to be acquired at higher or lower resolutions (or the resolution digitally changed) to correlate better with the reference spectra.

Many compounds exhibit polymorphism. That is, as they solidify they may form into different shapes of crystals. These different crystal formations will put different strains on some bonds in the molecule and cause a shifting of peak locations. Diazepam and some of the barbiturates are examples of compounds that exhibit polymorphism.

When comparing a spectrum to reference spectra, it is important to know how the reference spectra were collected. Most forensic chemists obtain IR spectra primarily from samples dispersed in KBr. The KBr-crystal matrix may affect the position of the observed group frequencies of a sample. This could make the correlation of published group frequencies and observed group frequencies difficult, if the spectra in the reference were collected as a mull. In these instances, it may be necessary to use solution cells or mulls to facilitate the identification of an unknown. Most of the newer libraries are collected in KBr-crystal matrix but the spectra in many older references were collected as a mull.

As discussed above, there are occasions where a compound does not match the reference spectrum of a known standard of that compound. There are also occasions when an unknown spectrum matches the reference spectrum of a different compound. In general, infrared spectrometry is a very discriminating technique and these occasions are rare. One instance where this does occur is in a homologous series. For example, infrared spectrometry cannot readily distinguish between the n-hydrocarbons tetradecane, pentadecane and hexadecane. Another class of compounds that infrared spectrometry

cannot always distinguish is enantiomers. For example, the IR spectra of d-ephedrine and l-ephedrine are indistinguishable. However, a racemic mixture of d & l-ephedrine recrystalized from a solvent can easily be distinguished from the spectra of the single isomers. Caution should be exercised however, because if dl ephedrine are simply mixed together and not dissolved and recrystalized from a solvent, they will give an IR spectrum indistinguishable from the single isomer spectra.

5.0.0 Infared Interpretation

Having a computer to compare an unknown spectrum to a reference saves a lot of time and greatly reduces the occasions when a chemist must interpret spectra. However it is still desirable to have a basic competency in IR interpretation, as samples are submitted that do not match any of the reference spectra. Because of the array of information that an infrared spectrum provides, it is almost impossible to explain every feature of an infrared spectrum. The interpretation of infrared spectra is a skill, which a forensic chemist can develop after years of practice. Often, the IR spectrum is used to identify certain functional groups in an unknown. This information can be used in conjunction with other instrumental data such as a mass spectrum, to identify a sample. This training program is not designed to make a chemist into an in expert at spectral interpretation. The following paragraphs give a little background on some of the important spectral features that may be of interest to a forensic chemist.

The region of 3700 cm^{-1} to 3100 cm^{-1} is usually associated with NH and OH stretching vibrations. The NH_2 group gives rise to two bands in this region because of symmetric and asymmetric stretch. (For example, anthranillic acid, a methaqualone precursor, demonstrates a typical NH_2 stretching.) Codeine displays a sharp singlet around 3515 cm^{-1}, which is indicative of the OH group at position six in the codeine molecule. A good example of hydrogen-bonded OH groups and non-bonded OH groups are the common sugars such as lactose, glucose, etc. It should be emphasized that the water molecule has absorption bands in this region and that KBr readily absorbs water.

The 3100 to 3000 cm^{-1} region is generally associated with aryl and olefin CH stretch. These bands are usually weak and may be nonexistent; e.g., benzocaine shows little if any absorption in this region while benzphetamine hydrochloride demonstrates a recognizable band between 3100 and 3000 cm^{-1}.

The 3000-2700 cm^{-1} IR region is associated with aliphatic CH stretch; however, many compounds common to the forensic laboratory are amine salts, which tend to obscure the information in this region. Stearic acid and many of the sugars show representative CH stretching in this area.

Primary amine salts, e.g., amphetamine HCl, show strong absorption between 3200 and 2800 cm^{-1}. O-toluidine HCl, a primary aromatic amine salt, displays characteristic bands around 2800 cm^{-1} and 2600 cm^{-1}. Secondary amine hydrochlorides, such as amphetamine hydrochloride, exhibit strong multiple absorption bands between 3000 and 2700 cm^{-1}. At

still smaller wave numbers, tertiary amine hydrochlorides absorb between 2700 and 2330 cm^{-1}, as demonstrated by diphenhydramine hydrochloride.

The IR region 2300-1900 cm^{-1} is associated with triple bonds. The C≡N stretch of 1-piperidinocyclohexanecarbonitrile (PCC) readily demonstrates the usefulness of this region.

Carbonyl compounds absorb strongly within the 1900-1550 cm^{-1} region. Compounds of forensic interest include amides, esters, and a few anhydrides. Ketones have CO stretch around 1715 cm^{-1} (e.g., methadone and tropinone). Acid carbonyls usually absorb in the 1720 to 1680 cm^{-1} region, but they have a tendency to be somewhat unreliable. For example, aspirin's acid carbonyl occurs around 1750 cm^{-1}, N-acetylanthranillic acid has a 1700 cm^{-1} band, and ecogonine's carbonyl is found around 1690 cm^{-1}. Resonance weakens the C-O bond and thus lowers the absorbing frequency.

The 1695 to 1630 cm^{-1} region is normally assigned to amide carbonyls; meprobamate and acetaminophen (both N-substituted amides), and methaqualone (a di-substituted amide) shows carbonyl absorbances of 1695, 1650 and 1670 cm^{-1} respectively.

Esters absorb strongly near 1740 cm^{-1} and 1200 cm^{-1} because of the C=O and C-O stretch, respectively. Acetyl codeine, having a single ester group, shows strong 1735 cm^{-1} and 1240 cm^{-1} bands. On the other hand, heroin and cocaine show two strong bands in the 1700 cm^{-1} region, indicative of two carbonyl absorptions, and both also exhibit the 1200 cm^{-1} band.

Beyond the 1600 cm^{-1} region, assignment of absorptions becomes more difficult due to the skeletal bending and deformations occurring within the molecule. Nonetheless, there are a few regions where assignments may be made with some consistency. The 1400 cm^{-1} region is usually characterized by CH_2 and asymmetric CH_3 deformations. Absorptions in the middle to high 1300 cm^{-1} region usually arise from CH_3 symmetric deformations. The doublet caused by two methyl groups on the same carbon is well exemplified by phentermine, which exhibits two strong peaks around 1390 and 1370 cm^{-1}, respectively.

Although the 1200 cm^{-1} region has been discussed in conjunction with esters, this region is also common to most C-O stretch; i.e., a strong band in the 1200 cm^{-1} region usually indicates a C-O bond of some type within the molecule; for example the IR spectrum of *p*-dimethoxyamphetamine exhibits a strong absorption around 1250 cm^{-1}, while that of amphetamine shows an absence of bands in this region. MDA may also be compared to amphetamine to demonstrate the effect of the C-O stretch.

The 900-700 cm^{-1} region may be useful in determining the substitution on aromatic rings such as benzene. A single substitution on a benzene ring leaves five adjacent hydrogen atoms, which give rise to two strong bands, one around 750 cm^{-1} and the other around 690 cm^{-1}. PCP and cocaine are typical mono-substituted compounds. Ortho substitution, as characterized by N-acetylanthranilic acid, shows a band around 750 cm^{-1}. The presence of

two bands around 690 cm^{-1} and 780 cm^{-1}, respectively, usually indicates meta substitution, while para substituted compounds demonstrate a band around 820 cm^{-1}.

Chlorphentermine hydrochloride isomers can have the chlorine in the ortho, meta, or para position, and the IR of each of these compounds clearly parallels the above discussions. The 745 cm^{-1} band in each chlorphentermine arises from the C-Cl stretch and illustrates the caution that must be applied to the use of the 900-700 cm^{-1} region. In fact, it is strongly recommended that the 2000-1700 cm^{-1} region be used only in conjunction with the 900-700 cm^{-1} region. Overtones of aryl ring vibrations occur in the 2000-1700 cm^{-1} area; however, because of the relatively low concentration of sample, these bands may not be seen whenever the KBr pressed-disc technique is used. Therefore, it may be necessary to use the mull technique or solution cells whenever unknowns are being identified.

6.0.0 Required Reading

1. Silverstein, R.M., Bassler, C.G., and Morrill, T.C., Spectrometric Identification of Organic Compounds, chapter on Infrared Spectrophotometry
2. Clarke, E.G.C., Isolation and Identification of Drugs, 3rd edition, vol. 1, pg 328-345.

7.0.0 Exercises

1 Read the applicable ISP SOP's for Infrared Analysis and discuss with training instructor.

2 Demonstrate to your training instructor the proper calibration and operation procedures for the Infrared Spectrophotometer.

3 In layman's terms, explain to your training instructor the theory and operation of IR. Defend the specificity of the IR as a tool for identification.

4 Prepare an IR spectrum of each of the following substances:

A) Procaine hydrochloride (using a KBr pellet)
B) Pseudoephedrine hydrochloride
C) Pseudoephedrine Base
D)

Perform the tutorial on software operation that came your IR instrument. Be able to demonstrate the "hot key" commands (keyboard commands) for "collect sample", "delete spectrum", "library search", "convert to absorbance", "convert to %transmittance", "toggle between stacked and overlaid spectra". Be able to display two spectra in stacked mode and have all of the comments displayed (or at least printed out) for both spectra.

5 Display and Print the same spectrum in both %T and Absorbance modes at the same time.

The following exercises are dependent on whether you have the appropriate equipment.

6 Install the Horizontal ATR cell in the instrument. Set up an ATR method and explain to your training instructor the rational for each setting in the method.

7 Install the diffuse reflectance cell in the instrument. Explain to your training instructor the rational for each setting in the method. Collect and print a cocaine spectrum.

8.0.0 Questions

1 Over what wavelength and wave number range is infrared data typically collected in this laboratory?
2 Is the procaine ring substitution verified in the 900 cm^{-1} to 700 cm^{-1} regions? If so, then by what absorption value(s).
3 Which of the three mounting techniques do you consider the best for unknown identification? Why?
4 Give one example of a potential interaction between KBr and sample.
5 Forensic chemists often refer to an infrared spectrum as being analogous to a fingerprint of a compound. In Saferstein's Forensic Scientist Handbook, Vol. II (pp. 84-85) it is stated, "This is an unfortunate use of the term, however, because it ascribes a degree of reproducibility and reliability to an instrumental technique that is hardly deserved." How would you respond to this if it were presented to you in court?
6 List at least three factors that may contribute to an unknown spectra not matching a reference spectra.
7 Cite the main difference between NaCl and KBr salts used as mounting media.
8 What is considered to be the "fingerprint" region of the IR spectrum?
9 Approximately how strong should a sample be made to obtain a good quality IR?
10 Is IR suitable for quantitative work? Explain.
11 What is an interferogram?
12 Would a dispersive IR spectrometer be a good choice to monitor the output from a Gas Chromatograph?
13 What is the relationship between resolution and Signal to Noise?
14 Give a simple explanation of the theory of how an ATR allows the collection of an IR spectrum.
15 Is particle size (sample and diluent) a consideration in Diffuse Reflectance Spectroscopy?
16 What are the ISP requirements for making an identification via IR?
17 What compound is used to calibrate the Infrared Spectrometer?
18 When is a performance verification check run on the IR?
19 When is a blank run on the IR and where is the hardcopy of the blank spectrum stored?

9.0.0 HISTORY

Prior to revision 3 modules in the training manual did not have individual history pages.

Revision #	Issue or review date	History	Author or Reviewer
3	7/08/2011	Updated 3.0.0-6, added 9.0.0, updated 6.0.0-2	David Sincerbeaux