# Infrared Chemical Imaging: Semi-Quantitative Analysis of Components

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With new image analysis software for infrared spectroscopy, semi-quantitative studies can be performed without the use of standards providing rapid measurements of component concentrations when an approximate value is suitable or when quantitative standards are not available. Infrared spectroscopy has been used for many years to identify the composition of organic and other materials. Single or multi-component samples can be analyzed and their chemical structures determined by comparing their absorbance peaks to known spectral libraries or fundamental molecular transitions. This technique is used throughout the scientific community and provides rich information.

More recently, chemical mapping or imaging has been developed to measure both the compositions and spatial distributions of components in a sample. This gives two-dimensional information of compositions and provides more useful data when relative amounts or positions are important. In order to perform quantitative analysis, standards must be analyzed and the instrument's response characterized to create a calibration curve for each component of interest. This work is tedious as the analyst must spend time preparing the standards, analyzing them in addition to creating the mathematical equations that will be used to model the unknown samples of interest.

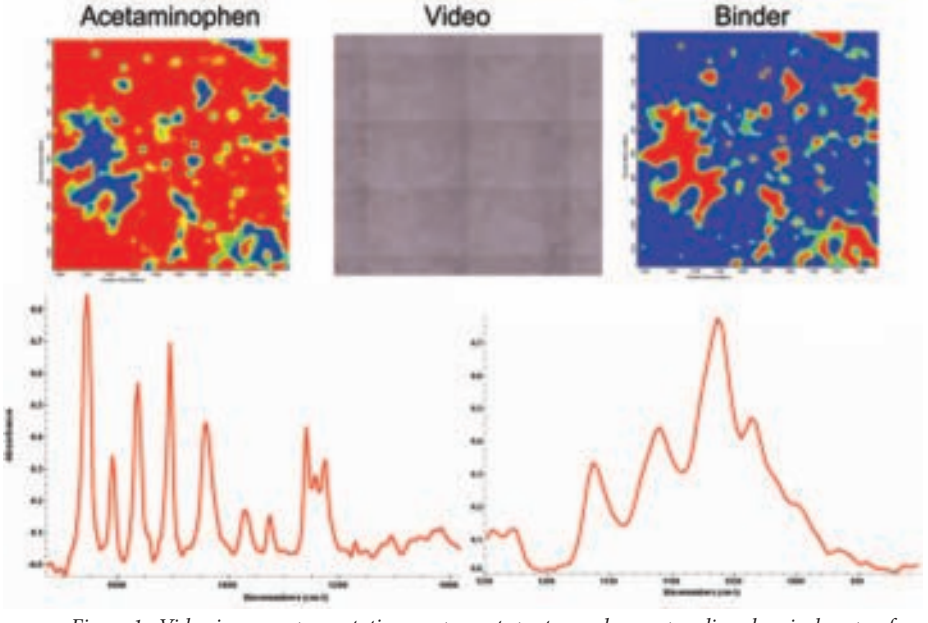
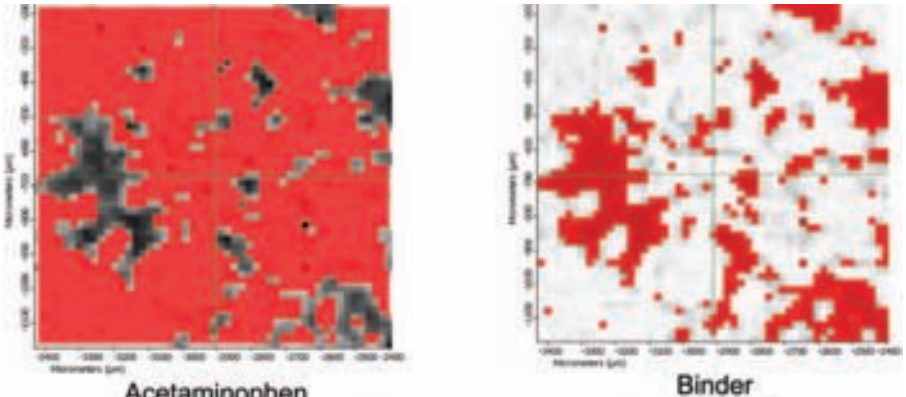



*Figure 1. Video image, representative component spectra and corresponding chemical maps of generic pain-relief tablet.*




| | |
|---|---|
| Area of Acetaminophen | = 734500 square microns |
| Area of Binder | = 266900 square microns |
| Total Area | = 1001400 square microns |
| Acetaminophen (calc.) | = 73% a/a |
| Binder (calc.) | = 27% a/a |
| Manufacturer's value: 500 mg/716 mg tablet | = 70% w/w |

*Figure 2. Image analysis of pain-relief tablet showing the binarized chemical maps and quantitative results table.*

In the following studies, semi-quantitative results are obtained using chemical images and powerful image analysis software. The images are first binarized and then the relative areas of components are calculated to give a percent of the total area. This number is in direct correlation to the actual quantitative values, yet no standards are needed. A more accurate value can be obtained by taking into account the densities of the components, yielding a weight percent instead of an area percent concentration. For many purposes, the semi-quantitative result is good enough and is close to the actual value within a few percent. However, in some analyses, quantitative standards are difficult or impossible to obtain. Two case studies are presented: A generic pharmaceutical tablet and a microtomed section of a coffee bean.

## Generic Pain-Relief Tablet

A pharmaceutical tablet containing acetaminophen and binder material was analyzed using a Thermo Electron Corporation Nicolet™ Continuμm™ XL FT-IR imaging microscope. The data was collected in reflectance mode, measuring the absorbance of reflected infrared energy. The tablet was approximately 8×15 mm in size. A representative map measuring approximately 1.5×1.5 mm was collected in the spectral range of 4000-800 cm-1. The chemical images, video image and representative spectra of acetaminophen and the cellulosic binder material are shown in figure 1. The chemical images, or maps, correlate to the spectral intensities of the data at 1655 and 1066 cm-1 for acetaminophen and cellulose, respectfully. Binarized images were created using OMNIC™ Atlus™ image analysis software. The areas in square microns of the two components were calculated by the software. The relative percentages of

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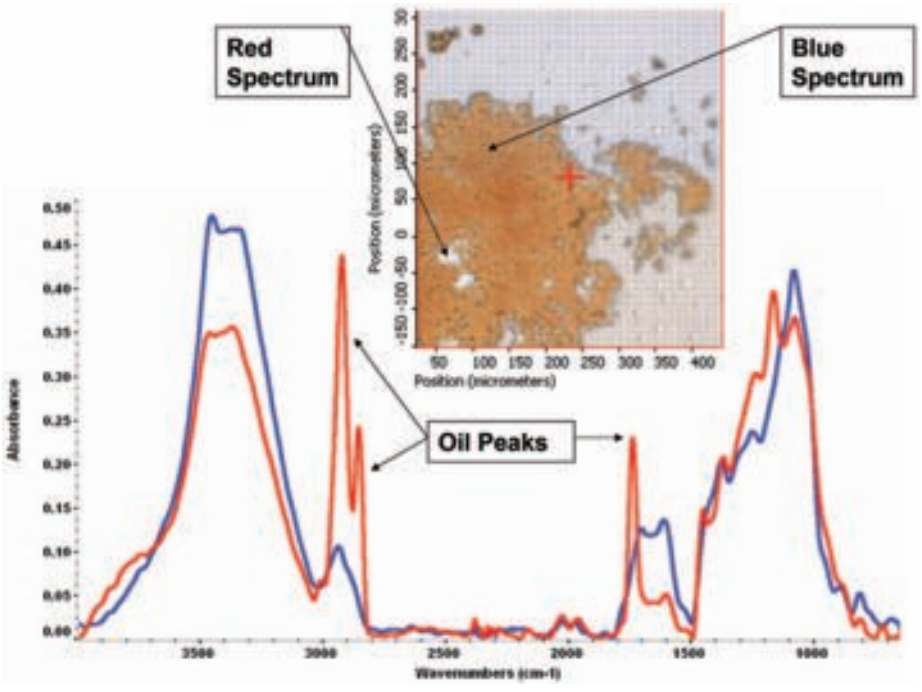


*Figure 3. Video image of coffee bean section and spectra from the two points referenced. The blue spectrum shows a cellulosic structure and the red spectrum shows contributions from cellulose and oils.*

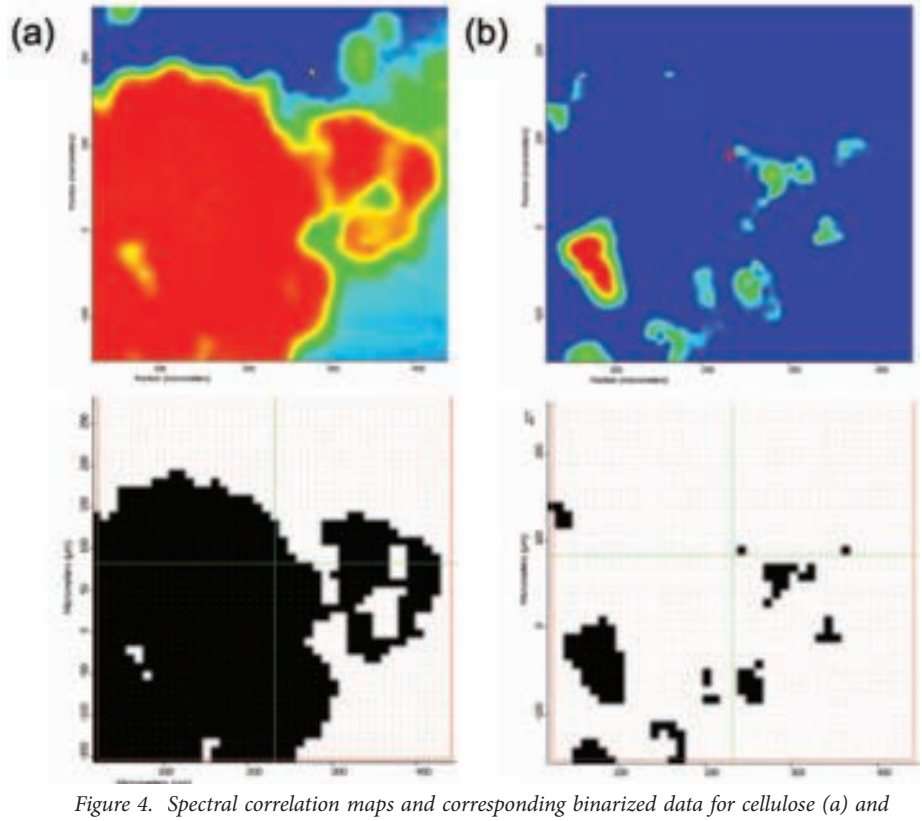


*Figure 4. Spectral correlation maps and corresponding binarized data for cellulose (a) and oil-containing (b) areas.*

| | |
|---|---|
| Area of Cellulose | = 95911 square microns |
| Area of Oils | = 10592 square microns |
| Total Area | = 106503 square microns |
| Cellulose | = 90% a/a |
| Oils | = 10% a/a |

*Figure 5. Quantitative results of image analysis applied to the binarized coffee bean data.*

the total area were then calculated for each component. The tablet was weighed using an analytical balance. The manufacturer listed the amount of acetaminophen in the tablet and the rest of the mass was assumed to be binder material. Using this information, a theoretical weight percent value was determined for the active ingredient and binder. These values were compared to those obtained from the image analysis software. The binarized images and results are given in figure 2. The experimental values obtained are within 3% of the theoretical values.

## Coffee Bean Section

A fresh roasted coffee bean was microtomed into a 20-micron section. The section was placed in a diamond compression cell and analyzed using infrared transmission spectroscopy. The video image and spectra from two points, A and B, on the map are presented in figure 3. The spectrum at point A shows a typical cellulosic structure and the spectrum at point B (the large clear area in the video image) shows both cellulose and vegetable oil components. Spectral correlation maps of the cellulose spectrum and the oily spectrum as well as their respective binarized images are given in figure 4. The two correlation maps were obtained by comparing the two spectra, A and B, to all of the spectra in the data set and mathematically assigning a correlation coefficient to each. The binarized data was analyzed and the total area of each component was calculated as a percentage of the total area. Figure 5 gives the results of the analysis. The area of oils was determined to be 10% of the total area.

## Conclusions

Infrared imaging in combination with image analysis allows the composition of heterogeneous materials to be determined without the need for external standards or calibration. A high-contrast chemical image can be created based on the IR spectral signature that is unique to each component. Area analysis of the image then yields the quantitative composition of the sample. In other words, infrared imaging provides information regarding the identity, quantity and location of components within complex materials. In the case of the pharmaceutical tablet image analysis provided a close estimate of the known concentrations of components. With the coffee bean section, there was no known compositional data for reference. Image analysis provided an estimate of the oil concentration that can be used to predict the flavor content of the coffee. ■

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