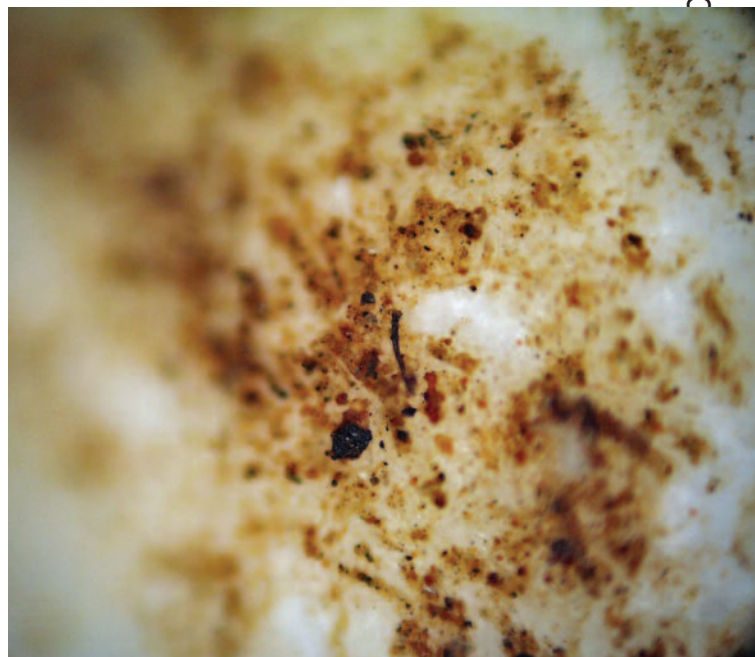


# QA/QC tutorial

## MICRO-EXTRACTION TECHNIQUE FOR ISOLATION OF SOLUBLE CONTAMINANTS IN PHARMACEUTICAL TABLETS

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*This article describes a micro-analytical technique developed to extract and isolate contaminants, such as machinery oil and other lubricants, from pharmaceutical tablets for identification by microscale Fourier transform infrared (micro-FTIR) spectroscopy. FTIR is one of the most common methods used to analyze pharmaceutical contaminants because it can identify most organic materials and some inorganic materials. Quality analysts can easily adopt this fast and inexpensive technique by following the steps laid out in this article and thereby bolster their QA/QC repertoires.*

Pharmaceutical tablets are often rejected due to the presence of "dark spots." These spots often consist of minute amounts of absorbed machine oil or other lubricants, sometimes mixed with metal wear particles or other environmental debris. One source of contamination is material left on manufacturing equipment from previous processing runs. Despite strict cleanliness regulations for all parts of the pharmaceutical development and manufacturing process, cleaning manufacturing equipment

between runs is sometimes insufficient to remove 100 percent of the debris. Therefore, machinery oils and lubricants, as well as drug product ingredients or clumps of charred materials from previous manufacturing runs, may be inadvertently transferred to new batches.

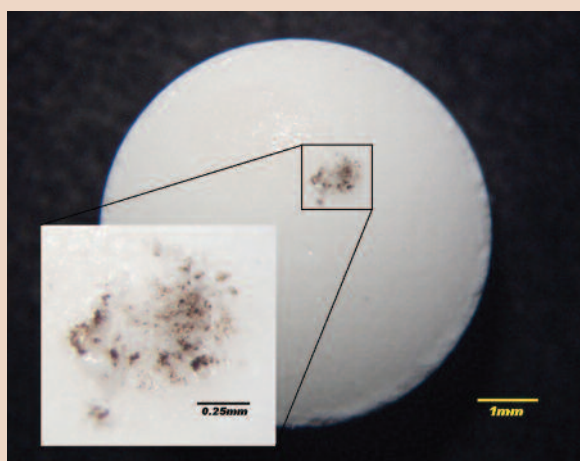
The location of the stain or dark spot on the tablet may also help explain where the contamination occurred. If the dark spot is in the tablet core, contamination likely occurred early in the production process. A dark spot limited to the tablet surface points to the tablet press as the source. The photos above and in Figure 1 show typical dark spots on tablets.

### Micro-extraction for isolating soluble contaminants

When you're working with soluble contaminants, the following micro-extraction technique is a simple, fast, and inexpensive method that can be used in a variety of laboratory settings. This technique is used to isolate oily residues as small as 1 nanoliter from samples of tablet material as small as 50 microns. The technique is also highly adaptable, as it can be used for both large and small samples, and the solvent can be varied according to the type of tablet and the suspected identity of the contaminant.

**FIGURE 1**

Example of "dark spots" on a tablet

**Step-by-step technique**

This micro-extraction technique requires only a few simple materials: a 5-milliliter glass vial with a Teflon-lined cap, a tungsten needle with needle holder, an aluminum-coated slide, a plastic slide, several GELoader pipette tips (Eppendorf, Westbury, NY), 5/8-inch-long disposable Pasteur pipettes, a few beakers, and a suitable solvent. The solvent is chosen based on the type of tablet and the type of contaminant. For example, hexane is a good solvent for hydrocarbons and silicone oil. The solvent also needs to be the cleanest grade (usually a chromatographic grade) so that it leaves very little residue on evaporation.

Using a razor blade, cut the ends of the pipette tips to make "micropipettes" to handle the extraction solvent. The cuts should be made at a 45-degree angle and on a plastic slide to avoid crushing the tips. By placing the cuts at different locations along the length of the micro-

pipettes, you can craft tips with different diameters to produce different sized solvent drops. Next, fill the micropipette via capillary action by inserting the tip into a container of solvent. To dispense the solvent, hold the solvent-filled micropipette between your thumb and forefinger at an angle and dab the tip repeatedly against the surface of the aluminum slide, allowing drops of solvent to flow onto the slide. Alternately, the solvent can be dabbed from the micropipette onto a KimWipe tissue (Kimberly-Clark, Dallas, TX) in order to empty the micropipette for cleaning purposes.

In this micro-extraction technique, episcopic illumination (reflected light) is used to view the aluminum-coated surface of the slide. Use the tungsten needle to scratch numbers or other identifying marks into the aluminum-coated surface of the slide. These marks allow you to quickly locate the sample in the microscope.

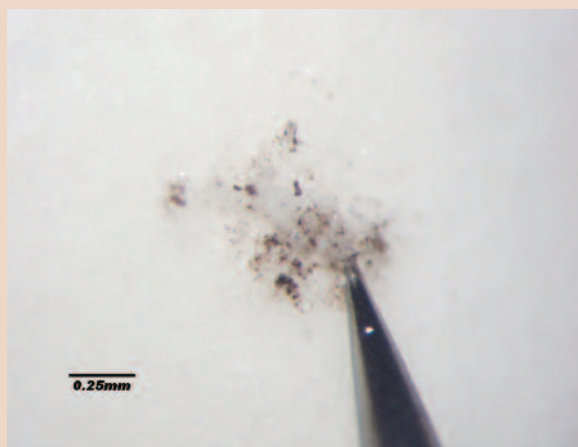
In order to achieve a very low detection limit for this method, all of the surfaces that will contact the solvent and sample must be cleaned to prevent the introduction of additional contaminants. The sidebar below describes how to clean these surfaces.

**Perform a blank test**

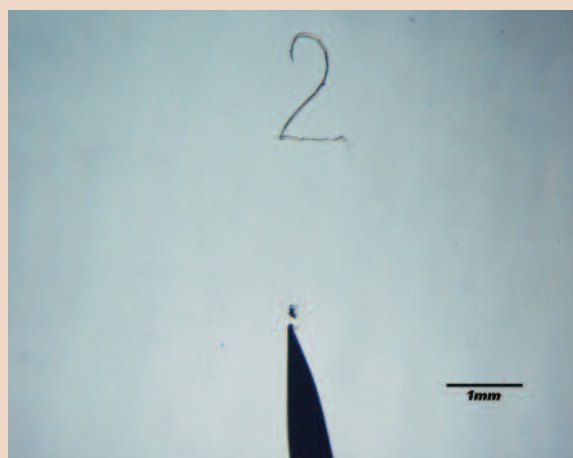
Once all materials are thoroughly cleaned, perform a "blank" test to verify that no contamination is present on any of the surfaces. Select a numbered location on the slide for this test. Fill the micropipette, and apply several drops of solvent by repeatedly dabbing the tip of the micropipette against the surface of the slide in the same location each time. When the solvent evaporates, you should observe little or no residue on the slide. If a substantial amount of residue is present, the cleaning process must be repeated. If a very small amount of residue is present in the blank, and you expect the sample will produce a larger amount of residue, you may decide to proceed with the extraction of the sample, as long as the amount of residue from the blank

**FIGURE 2**

Using a fine tungsten needle, remove a small portion of the stained area from the tablet.

**FIGURE 3**

The sample to be extracted has been transferred to an aluminum slide.



test will be negligible compared to the sample residue. If that is the case, record an infrared spectrum of the residue from the blank test. This spectrum can be compared to the spectrum of the extracted material from the tablet, enabling you to verify that the spectrum of the blank residue does not contribute to the spectrum of the extracted sample.

### Extract the sample

Once the cleaning and blank tests are completed, use a fine tungsten needle to remove a small portion of the stained area from the tablet (Figure 2). If possible, extract only a portion of the dark material so that some of it remains for potential further tests. Place the sample to be extracted on the blank aluminum slide in the same location that you conducted the blank test (Figure 3).

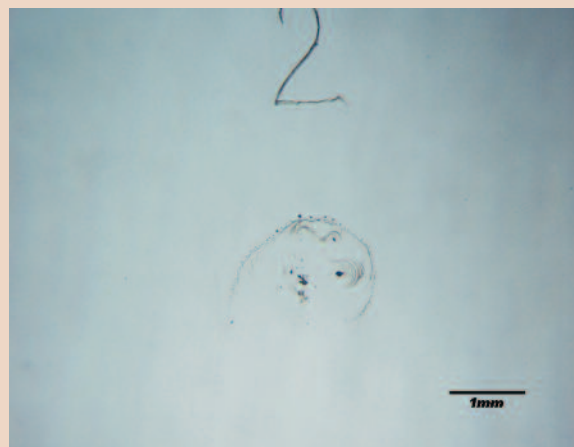
With the sample on the aluminum slide, fill a cleaned micropipette with solvent and apply several drops next to the sample by repeatedly dabbing the micropipette against the slide surface. The solvent is applied next to the sample, not on top of it. In this way, solvent can flow across and through the sample, carrying the extractable, soluble material with it. When the solvent drops evaporate, a ring of residue will be seen around the particle if the sample contained extractable materials (Figure 4).

Re-apply the solvent until it appears that no more residue can be removed from the particle. If only a very small amount of residue was obtained from the particle, a

larger portion of the stained material can be removed from the tablet, added to the sample on the slide, and extracted again. Ideally, the total amount of solvent used for extraction will be similar in volume to the amount used for the blank test. This is particularly important if the blank test contained a small amount of residue.

FIGURE 4

A ring of residue forms around the particle if the sample contains extractable materials.



## The importance of being clean

Cleanliness of the solvent, slide, and pipette are critical to the success of this method, since the analyst is typically working with microgram quantities of contaminant. The following method, although time-consuming, will result in a clean blank test, and allows for the isolation of minute quantities of soluble contaminant from the tablet.

**Solvent.** Select a clean 50-milliliter beaker and fill it half-full with solvent from the original bottle. Swirl to rinse all interior surfaces of the beaker, and discard. Repeat twice. Fill the beaker with solvent. This will be your source of clean solvent for all further washings in the cleaning process.

**Solvent storage vial.** Pour solvent from the beaker into the 5-milliliter vial with Teflon-lined cap. Cap the vial, shake, and discard. Repeat nine times. Fill the vial and cap it.

**Pasteur pipette.** Pour clean hexane from the beaker down the outside of the pipette, rinsing the bottom half of the pipette's outer surface. Place a dropper bulb on the pipette and,

inserting only the tip into the beaker of clean solvent, draw solvent at least halfway into the pipette. Remove the pipette from the beaker and expel and discard the solvent. Repeat twice. Discard the solvent in the beaker, rinse the beaker twice, refill the beaker, and repeat the procedure for washing the pipette's interior and exterior surfaces three times. Store the clean Pasteur pipette in such a way that the tip will not contact anything.

**Aluminum slide.** Holding the slide at one end and tilting the other end downward, use the clean Pasteur pipette to flush the surface of the slide (avoiding the end where your finger is), allowing the solvent to flow away from your finger and off of the slide surface. While the slide is still wet with solvent, quickly wipe the surface with a KimWipe tissue, again avoiding the end where your finger is. The slide surface scratches easily, but minor scratches will not affect the end results. Fill the Pasteur pipette again, and flush the surface of the slide. Allow the solvent to evapo-

rate. When dry, no residue should be seen on the cleaned portion of the slide. The end of the slide where your finger held it will be coated with finger oils, and this area of the slide should never be used for micro-extractions. Store the clean slide in a slide box or Petri dish when not in use.

**Micropipette.** Holding the micropipette at the top, use the clean Pasteur pipette to flush the exterior of the micropipette several times. To clean the interior, insert only the tip of the micropipette into the beaker of clean solvent, filling the micropipette about halfway. Expel the solvent by dabbing the tip on a clean KimWipe tissue or cleanroom wipe. Repeat the rinse of the interior of the micropipette nine times. Store the clean micropipette in such a way that the tip will not contact anything. The autoclave rack that is supplied with the GELoader tips is useful for this. Cover the top of the autoclave rack with plastic film so that dust particles cannot fall into the micropipette.

—M.S.

### Remove debris for further analysis

Using the tungsten needle, remove as much of the solid particulate from the slide as possible, so that it will not interfere with the infrared analysis of the extracted materials. The solids can be submitted for other analyses, such as energy dispersive x-ray spectrometry (EDS), to look for the presence of metal wear particles or other inorganic materials that may contribute to the dark color of the stained area. Analyzing the solids using a scanning electron microscope equipped with an EDS detector provides a high-quality image of the contaminant and a spectrum of the elemental data found in the sample.

### Concentrate the sample for micro-FTIR analysis

The residue from the extraction can be concentrated into a single small spot, so that a more intense infrared spectrum can be obtained from the sample. After filling the micropipette with a small amount of solvent, place the tip of the micropipette against the surface of the slide (next to the residue, not on top of it) and, without lifting the tip from the surface, move the tip across the surface toward the residue, using the solvent front to push the residue from all directions into a single spot.

Next, obtain a reflectance infrared spectrum (via micro-FTIR) of the concentrated spot (Figure 5). The micro-FTIR system, a polarizing light microscope interfaced with an infrared spectrometer, reflects a beam of infrared radiation through the sample. Every substance absorbs light at a different frequency and produces a unique infrared spectrum, which is a chemical fingerprint of the material. At my company, we maintain a reference library of thousands of standards and known materials. Thus we can often identify the contaminant by comparing its spectra with the spectra of different sources using an automated computer search.

The extraction procedure should be repeated on a similar-sized portion of non-stained tablet material in order to verify that the extract of the stained area does not represent ingredients of the normal tablet formulation.

The infrared spectra should be obtained as soon as possible after extracting the sample. Some oils (silicone oil in particular) tend to spread out over the surface of the aluminum slide with time, forming a very thin film that produces a weak spectrum, if it produces one at all.

Remember to clean the micropipette between each test by repeatedly filling the tip of the pipette and dabbing it on a clean KimWipe tissue, followed by a blank test on the aluminum slide. Each time you dip the micropipette into the vial of solvent, a minute amount of residue from the samples will contaminate the solvent in the vial. After repeated extractions, the amount of contaminant in the solvent will be sufficient to produce a residue when the blank test is performed. At this point, the complete cleaning procedure must be repeated, including replacing the solvent in the vial. My colleagues and I can usually perform 15 to 20 sample extractions before all of the components need to be cleaned.

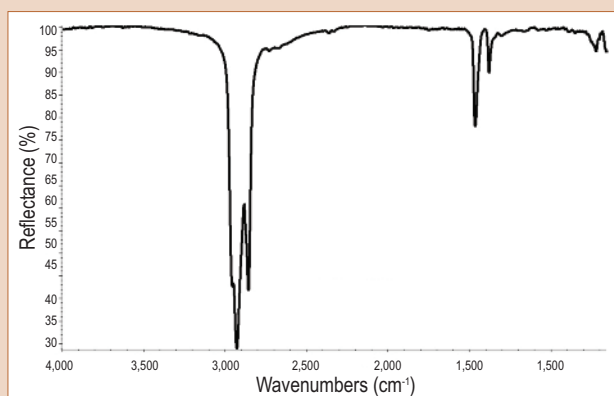
### Discussion

Although the cleaning process is time-consuming, the actual extraction process, from the blank test to concentrating the final residue for infrared analysis, takes less than 5 minutes. Multiple solvents of different polarities can be used to perform a series of extractions on a single sample by moving the solids to a new location on the slide for each new solvent. Minute amounts of soluble oils can be identified from tablet stains as small as 50 microns. Different reflective substrates, such as low-emissivity glass slides, can also be used with this method. The speed and versatility of this test makes it an ideal method for QC laboratories.

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FIGURE 5

Example of an infrared spectrum generated from extracted material



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