Microanalytical Methods

Sleuthing for contaminants in the drug manufacturing process

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PHARMACEUTICAL COMPANIES HAVE stringent quality control procedures in place to ensure that their final product is free of contaminants and defects. Trained QC inspectors can see product defects as small as 50 µm in size, typically considered to be the lower limit for visual detection. If at this stage the contaminants can be identified, steps can immediately be taken to correct the problem in the manufacturing process.

Sometimes, however, visual inspection isn't sufficient to identify the contaminants. Often, isolating and analyzing these types of contaminants may require specialized technical skills and analytical instrumentation that an in-house QC laboratory does not possess. In these cases, many pharmaceutical companies look to independent analytical/microanalysis laboratories that have the experience, skill and necessary sophisticated instrumentation to identify the contamination and its source.

Our purpose in this article is to demonstrate current techniques and technologies available to ferret out these impurities (Figure 1) using a hypothetical example of a pharmaceutical tablet embedded with dark inclusions (Figure 2).

Scoping It Out

Upon sample receipt, microanalytical laboratories typically first examine the sample visually, and then use a stereomicroscope. The laboratory scientists can often make an immediate assessment of the contaminant, possibly identify it, or at least make an educated guess after viewing the sample in the microscope. Ideally, all sample manipulations take place in a cleanroom to eliminate any possible cross-contamination.

In our case study, the tablet's surface appears granular, composed of compressed fine grains, as opposed to a single solid particle or inclusion. This eliminates the possibility that the contaminant is a chunk of plastic or metal. The grains are heterogeneous, a sign that more than one type of contamination is present, and multiple analytical methods will probably be required.

Observation of the depth of the defect may provide some insight into the point at which the contamination occurred during the manufacturing process. For instance, a surface defect could indicate that the tablet-forming machine is the culprit, whereas contamination that penetrates the core might suggest a problem in the raw material. In the case of our hypothetical tablet, the contamination is at the surface, about 1 mm deep.

Using a tungsten needle, the cleanroom microscopists remove a portion of the dark inclusion material to a clean glass slide, break the granules apart and spread them out for continued inspection using the stereomicroscope. Increasing the magnification three-fold, we observe the following:

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- The contaminant is granular, crumbly, and non-homogeneous.
- The contaminant has a greasy appearance.
- There is a mixture of colorless granules and darker granules, some of which have a shiny, reflective appearance.
- There is some colorless flaky material mixed with the granules.

The Greasy-Looking Contaminant

The greasy appearance of the included material suggests that the tablet may have absorbed machine oil or other lubricants during manufacture.

Confirming the presence of the oil or grease requires a simple, fast microextraction method for isolating soluble material. To begin this analysis a portion of the dark contaminant material is transferred, using a micro-scalpel or tungsten needle, to an aluminum-coated slide that has a mirror finish. A few micro-drops of hexane solvent are applied to the contaminant sample, washing any soluble oil or grease out of the solids and onto the mirror surface, and leaving the oil-free solids for further analysis.

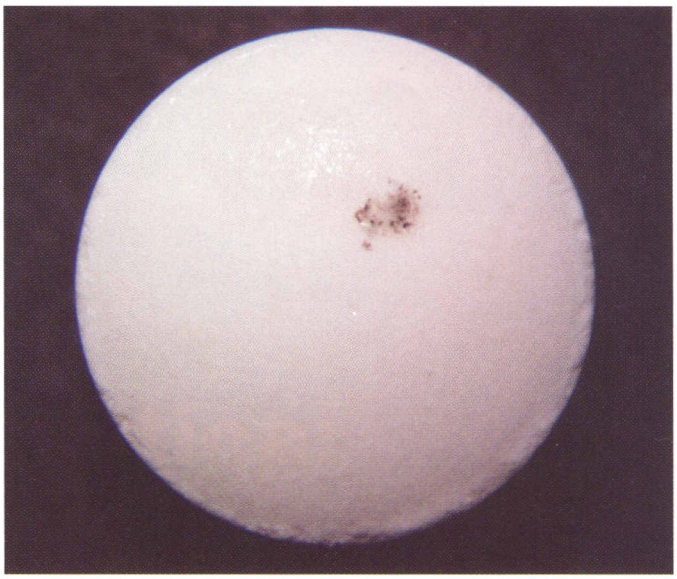
Figure 1: Common contaminants

Scientists at our company have analyzed thousands of different products for the pharmaceutical industry. Here are some of the more common contamination and defect culprits encountered:

- Fibers from cleanroom wipes
- Production machinery oil
- Latex glove fragments
- Metal wear particles from machinery
- Glass particles
- Charred contaminants such as hair, rubber from the stopper, or the active pharmaceutical ingredient (API)
- Streaks or surface irregularities can be a result of excipients or the API not being sufficiently dispersed during the drug formulation mixing process.

When the hexane solvent evaporates, if an oil or grease was present in the contaminant, an oily residue would remain on the mirror slide. The residue can be concentrated into a small spot, with the addition of a few additional micro-drops of hexane, and analyzed directly on the mirror surface by infrared spectroscopy (micro-FTIR) in reflectance mode. FTIR is used to identify organic materials and some inorganic materials. The micro-FTIR system shines a beam of infrared radiation through the sample and records the different frequencies at which the sample absorbs the light, producing a unique infrared spectrum, which is a chemical fingerprint of the material. By comparing the spectrum of the contaminant with spectra of known compounds from a reference library through an automated computer search, scientists can identify the contaminant.

Figure 2: Photograph of hypothetical tablet with inclusions



In the case of our hypothetical tablet, the IR spectrum confirms that the oily residue is hydrocarbon oil.

The Colorless, Flaky Material

Once any extractable material has been removed using the microextraction method described above, the insoluble portion of the contaminated material can then be more easily examined and identified by further analysis.

A 10-µm or larger portion of the flaky material is isolated by hand using a tungsten needle, mounted on a potassium bromide crystal, pressed into a thin film, and analyzed by micro-FTIR in transmission mode.

The IR spectrum reveals the presence of polytetrafluoroethylene, also known as Teflon. The Teflon may have come from an o-ring, valve or tubing from the process line.

The Colorless Granules

In our initial observation, the contaminant contained a mixture of colorless granules and darker granules, with some having a shiny appearance. A sample of the mixed color granules is flattened by pressing them under a glass cover slip. The flattened granules are pressed onto a potassium bromide crystal, and the sample is analyzed by micro-FTIR.

The IR spectrum indicates lactose, a typical excipient ingredient, and a small amount of Teflon, likely due to small flakes that could not be

manually removed from the granule mixture. The lack of any other IR bands suggests that the dark granules are either inorganic material, severely charred organic material, or carbonaceous material such as graphite.

The Darker Granules That Have a Shiny Appearance

The dark, reflective granules may be metal wear particles—small particles of steel that arise out of the friction created when pumps and compressors rub against each other, like pistons in a car, during the tablet-making process. Metal wear particles often occur in conjunction with machine oil contamination in pharmaceutical tablets. A portion of the hexane-washed granules is mounted on a beryllium stub for analysis by scanning electron microscopy (SEM) combined with an energy dispersive X-ray spectrometer (EDS) detector. Using the SEM-EDS method, two types of information can be obtained. The SEM provides images of the sample, while the EDS identifies the elements that are present. In the case of our hypothetical tablet, the image reveals that some of the dark particles are consistent with metal flakes, and their composition—including iron and chromium as major elements—are consistent with stainless steel. Oxidation of the metal explains the darker color. The SEM image also indicates that some of the dark particles are not metallic, but are instead rich in carbon, suggesting possible graphite or amorphous organic char. EDS analysis of the light-colored granules and particles also identifies carbon and oxygen, attributed to the lactose, and a small amount of fluorine due to the Teflon flakes.

To confirm the presence or absence of graphitic carbon, the beryllium stub preparation that was used for the SEM/EDS analysis is submitted for analysis by Raman spectroscopy, a complementary technique to infrared analysis that provides "fingerprints" of many inorganic materials and other opaque samples that are not suitable for IR. For instance, while IR cannot distinguish between different crystalline forms of carbon, Raman can. In the case of our hypothetical tablet, we observe a sharp peak due to graphitic carbon and some weak bands due to lactose.

The darker granules are composed of two components: oxidized stainless steel from metal wear particles and graphitic carbon, possibly from pump bushings or a system lubricant.

Figure 3: Three Other Useful Microanalytical Techniques

An order of magnitude more sensitive than EDS, **X-ray photoelectron spectroscopy** (XPS, also known as ESCA [electron spectroscopy for chemical analysis]) is the method of choice for detecting thin layers of surface contamination present at trace levels. XPS has been used successfully to detect low levels of silicon, which might originate from siliconized rubber stoppers on liquid-containing vials that otherwise would not be detectable by IR or EDS analysis.

Chromatographic methods such as **gas chromatography** or **liquid chromatography** can be used to verify the identity of the API, or identify certain absorbed volatile or liquid contaminants in pharmaceutical products. A **mass spectrometer** detector used in combination with GC or LC analyzes the fragmentation pattern of each peak and provides a fingerprint of the unknown compound. The resultant mass spectrum is compared to reference spectra of known compounds in order to identify the unknown sample. The GC-MS method has been used successfully to identify plasticizers from vinyl materials and other plastic additives from packaging materials that have been absorbed by pharmaceutical products.

Micro X-ray diffraction (micro-XRD) is useful for identification of inorganic materials. The resulting diffraction patterns are compared to a database of known compounds. In addition to identifying contaminants, micro-XRD can also distinguish between crystalline phases (polymorphs) of the API.

Project Completion

Upon completing the analysis and identification of a contaminant or defect, the laboratory provides the customer with a detailed report including a summary of the analyses performed, photographs taken during the analysis, raw data (spectra), and a summary of the data evaluation. The report includes the identity of the contaminant and some possible sources of the contaminant based upon its composition and the information gathered from discussions with the client. If the identification of the contaminant leads to changes in the client's production process, the client should retest the product to ensure that the problem has been eliminated.

For our hypothetical tablet, a summary of the identity of the contaminants follows:

Observation	Detection Method	Contaminant Identity
The contaminant has a greasy appearance	microextraction followed by IR analysis	Hydrocarbon oil
There is a mixture of colorless granules and darker granules, some of which have a shiny, reflective appearance	IR, SEM-EDS, and Raman analyses	*Darker granules:* oxidized stainless steel and graphitic carbon *Colorless granules:* lactose
There is some colorless flaky material	IR analysis	Teflon