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^(901) DETECTION OF ASBESTOS IN PHARMACEUTICAL TALC

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This chapter provides procedures to detect the presence of asbestos in pharmaceutical talc using the analytical procedures of X-ray diffraction (XRD)—also termed X-ray powder diffraction (XRPD)—coupled with polarized light microscopy (PLM). Both [Procedure 1: X-Ray Diffraction](#) and [Procedure 2: Polarized Light Microscopy](#) are mandatory. For supporting information on the best practices and principles of measurements, see [Theory and Practice of Asbestos Detection in Pharmaceutical Talc \(1901\)](#).

Procedure 1: X-Ray Diffraction

INTRODUCTION

XRD is used to determine whether amphibole and/or serpentine minerals are present in a talc powder matrix and, when applicable, to estimate the percentage present. It is intended to be used in combination with a microscopy technique capable of determining whether asbestos is present. [NOTE—For additional information, see [Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction \(XRPD\) \(941\)](#).]

With regard to sampling and subsampling, see [Bulk Powder Sampling Procedures \(1097\)](#).

SAMPLE PREPARATION

Using the sample holders provided by the XRD manufacturer, fill the cavity using back-, front-, or side-filling techniques depending on the specific holder used. Be sure to use the same sample filling technique for reference standards (if used) and the samples being tested.

SAMPLE ANALYSIS (QUALITATIVE AND QUANTITATIVE SCANS)

Qualitative scans are used to assess overall mineralogy (see [\(1901\)](#) for a list of peak positions for common minerals that may occur with talc). Quantitative scans are used to determine the presence of trace levels of amphibole and serpentine minerals (above minimum detection limits).

The following conventional XRD instrument conditions and parameters can be used to determine the detection limits outlined below.

- X-ray tube voltage: 45–50 kilovolts
- X-ray tube current: 30–45 milliamperes (consult tube manufacturer recommendations; do not exceed tube rating)
- Target: copper
- Monochromator: graphite or multilayer (tungsten/silicon, nickel/carbon, or similar); curved or flat diffracted or primary beam
- Detector: scintillation counter or alternative high-speed detector
- Receiving slit: 0.6 or 0.3 mm, none if using a high-speed detector
- Scintillation or anti-scatter slit: 1° or matched to the size of the high-speed detector
- Divergent height-limiting slit or beam mask: 10 mm
- Divergent slit: 1°, or smaller to ensure no sample overshoot
- Scan mode: fixed time (FT)
- Single-stage or multi-position sample changer attachment
- Spin speed of sample: 30 or 60 rpm

Any variation due to vendor limitations should be noted and used for samples, references, and standards. Additionally, use of high-speed detectors can permit much less analysis time for qualitative and quantitative testing. For example, a full qualitative scan from 3°–85° 2-theta (2θ) can be done in fewer than 35–40 min, and a quantitative evaluation for amphibole and serpentine can be accomplished within 2–3 h. [Table 1](#) outlines the target peak positions and scan ranges for XRD scans.

Table 1. Target Peak Positions and Scan Ranges for Qualitative and Quantitative XRD Scans

Scan Type	Target Phase(s)	Target Peak(s) (2-Theta)	Start Angle (2-Theta)	End Angle (2-Theta)	Step Size (2-Theta)	Count Time per Step(s)
Qualitative scan	All (full scan)	All	3	65 (minimum)	0.02	2

Scan Type	Target Phase(s)	Target Peak(s) (2-Theta)	Start Angle (2-Theta)	End Angle (2-Theta)	Step Size (2-Theta)	Count Time per Step(s)
Quantitative scans	Amphibole ^a	10.5 10.7	9.8	11.2	0.02	100
	Serpentine Chlorite	12.1 12.5	11	13.2	0.02	100
	Serpentine Chlorite	24.4 25.1	23.5	25.6	0.02	100
	Orthorhombic amphibole (optional)	29.3	28.8	29.8	0.02	100

^a Both monoclinic (e.g., tremolite) and orthorhombic (e.g., anthophyllite) amphiboles are included in this scan and can be distinguished from each other. Monoclinic amphibole species may not necessarily be distinguished from each other at the trace level.

LIMIT OF DETECTION AND QUANTIFICATION

Detection limits were verified using 0.25% spiked standards and the instrument conditions outlined above.

Limit of detection for:

- Amphiboles (tremolite and anthophyllite): 0.2%
- Serpentine with no chlorite interference: 0.2%
- Serpentine with 1% chlorite interference: 0.5%
- Serpentine with >1% to 15%–20% chlorite interference: 1%, with resolution of the two phases confirmed using a 2θ range of 23.5–25.6

A detection limit of 0.1% has also been achieved using this method; however, use of lower limits will need to be verified by individual labs using a 0.1% spiked standard.

[NOTE—See more information in [\(1901\)](#).]

REPORTING RESULTS

- If no detectable peak is present for monoclinic or orthorhombic amphiboles (tremolite or anthophyllite), then report “amphibole not detected (<0.2%)”.
- If no detectable peak is present for serpentine without a chlorite interference, then report “serpentine not detected (<0.2%)”.
- If no detectable peak is present for serpentine with 1%–15% chlorite interference, then report “serpentine not detected (<1%)”.
- If a peak is present for serpentine, then report “serpentine detected”.
- If a peak is present for an amphibole, then report the type, i.e., “monoclinic amphibole detected” or “orthorhombic amphibole detected”.

[NOTE—Alternative detection limits can be determined and reported using operating parameters at individual laboratories.]

Only report the detected and not detected result limit, not the quantitative result.

[NOTE—The limits are based on limits of detection.]

Procedure 2: Polarized Light Microscopy

INTRODUCTION

This procedure uses PLM to identify asbestos particles in a talc powder matrix. It is intended to be used with *Procedure 1* as a complementary microscopy technique. Both *Procedure 1* and *Procedure 2* are mandatory.

CONCENTRATION USING WET SIEVING

Concentration of asbestos using the wet sieving technique has been found to be effective for PLM analysis (see supporting information in [\(1901\)](#)). Use 76-mm (3-inch) sieves, precleaned in a beaker filled with detergent and water in a sonic bath for 15 min. Rinse thoroughly with deionized water.

1. Measure approximately 2 g of talc powder into a 100-mL plastic bottle with a removable lid.
2. Because talc is hydrophobic, wet the powder completely with isopropyl alcohol (IPA), then fill three quarters full of deionized water (approximately 75 mL total); shake vigorously for 30 s.
3. Slowly pour the suspension onto a 400-mesh (37-μm) sieve. The passing fraction should be collected and disposed of properly according to local regulations. Work in batches, alternately rinsing with water or IPA (if foaming) to avoid screen blinding. Optimal rinsing is accomplished by using a standard kitchen sink sprayer or by squeezing the end of a flexible hose attached to the faucet. In either case, water should be set to deliver a gentle spray to avoid splash back.
4. Repeatedly fill the plastic suspension bottle with water and IPA (if foaming) and replace the lid. Shake and pour onto the sieve until the bottle is washed clean.

5. Continue rinsing the sieve under gentle pressure until the water passing through appears clear. [NOTE—A very small amount of residue should be visible by eye, but continuously sprayed water should pass through the screen unimpeded. The optimum amount of residue retained on the screen can be determined by eye with experience.]
6. Rinse the residue from the screen using a squirt bottle with particle-free water into a tared small aluminum pan or glass dish for drying. Dry the sample by air or use an oven at 105°–110°.
7. Once dry, weigh the residue. The objective is to obtain 0.05–0.1 g of residue for subsequent sampling by PLM.
8. If less than 0.05 g is collected from the 400-mesh (37- μm) sieve using 2 g of starting material, one of the following procedures should be used:
 - A. Repeat the above until at least 0.05 g is collected for analysis.
 - B. Repeat the above using a greater amount of starting material. [NOTE—A 254-mm (10-inch) sieve could be used with up to 100 g of starting material weighed into a 1000-mL bottle.]
 - C. Repeat the above using a finer mesh sieve.
9. Sieves should be cleaned and maintained in accordance with manufacturer's recommendations and/or instructions, or disposable sieves can be used. Use of any sieve size other than aforementioned should be validated following [General Notices 6.30. Alternative and Harmonized Methods and Procedures](#).

SAMPLE PREPARATION OF SIEVED RESIDUE

1. Place a known amount of talc from the sieved residue on a glass slide so that particles cover 30%–50% of the area under the slide. This can be accomplished by taring a lab scale with the glass slide before adding an appropriate amount of sample.
2. Add 1 or 2 drops of refractive index (RI) liquid to the powder (see *Sample Analysis* for use of RI liquids). Swirl with a cleaned metal probe to distribute particles.
3. Place a coverslip on the sample, and, if needed, use a pencil eraser (or equivalent soft-tipped tool) to press gently on the coverslip to remove air bubbles and better disperse the particles.

[NOTE—It may take some trial and error to obtain the optimal loading and starting sample weight. A minimum of 5 mg should be analyzed in total for each RI liquid used. This typically equates to 3–5 slide preparations with 1–2 mg each, using 22-mm \times 22-mm square coverslips. Adjust as necessary.]

SAMPLE ANALYSIS

Screen particles using the dispersion staining technique

1. Configure the microscope in dispersion staining mode [i.e., plane-polarized light (PPL), no condenser lens, centered dispersion staining objective].
 - Confirm that the microscope is set up and aligned properly by obtaining dispersion staining colors on prepared standards of amphibole and chrysotile asbestos before analyzing unknown material.
2. Prepare microscope slides (number determined by the weight of sample on each) in 1.550 and 1.605 RI liquids.
3. Scan each slide in its entirety in an orthogonal fashion in dispersion staining mode, characterizing any suspect particles (see explanation below).
 - Use a mechanical stage to aid in the scanning process.
 - If particles are brought to the center of the field of view for characterization or the stage is rotated during the course of documentation, be sure to return the particle to its original location and orientation before resuming the scan.
4. Observe any suspect particles of amphibole and chrysotile, which will display low relief and dispersion staining colors, as referenced in the International Organization for Standardization's ISO 22262-1 (2012), when immersed in 1.605 and 1.550 RI liquids, respectively (summarized below and in [Table 2](#)). The appropriate dispersion staining colors are determined when the axis of elongation of the particle is oriented parallel to the lower polarizer (*n*-parallel) and perpendicular to the lower polarizer (*n*-perpendicular) in a standard PLM configuration.
 - 1.550 RI liquid
 - Talc plates lying flat (with their plate surface parallel to the microscope slide) will appear pale yellow during the full rotation of the stage.
 - Chrysotile particles will appear magenta (*n*-parallel) and blue (*n*-perpendicular).
 - Elongate talc particles can appear yellow (*n*-parallel) to blue (*n*-perpendicular). Rotating the stage to observe the yellow color in 1.550 RI liquid will distinguish elongate talc from chrysotile.
 - 1.605 RI liquid
 - Talc plates lying flat (with their plate surface parallel to the microscope slide) will appear pale blue during the full rotation of the stage.
 - Elongate amphibole particles will appear characteristically yellow to pale yellow (*n*-parallel).
 - For *n*-perpendicular, amphiboles can range in color from blue or magenta (tremolite) to golden yellow or yellow (actinolite or anthophyllite).

- Amphibole particles have a lower relief in the n -perpendicular orientation, and the darker dispersion staining colors may be more difficult to see. If this is the case, rotating the stage should reveal the characteristic yellow color (n -parallel).
- Carbonate particles in 1.605 RI liquid can also appear yellow in certain orientations. These particles can be distinguished by their high birefringence. Alternatively, pretreating the sample with 2 M hydrochloric acid will remove carbonate particles with interfering yellow color (refer to ISO 22262-2 for details).

Typical central stop dispersion staining colors with corresponding λ_0 in nanometers are observed for talc and amphibole minerals in various Cargille Series E liquids for both parallel and perpendicular directions. The range of colors and λ_0 are a function of compositional variation for amphiboles in the compositional space for the stated amphibole (e.g., anthophyllite) taken from reported ranges of refractive indices. See [\(1901\)](#).

Table 2. Typical Color of Particles in Dispersion Staining Mode

Name	RI Liquid for Screening	Dispersion Color ^a	
		n -parallel (approximate wavelengths, nm)	n -perpendicular (approximate wavelengths, nm)
Talc plates	1.550	Pale yellow (≤ 400)	Pale yellow (≤ 400)
Talc plates (edge view)	1.550	Pale yellow (≤ 400)	Blue-green to light blue-green (≥ 620)
Chrysotile	1.550	Orange to blue-green (480–620)	Blue to light blue-green (580–680)
Talc plates	1.605	Pale blue (≥ 800)	Pale blue (≥ 800)
Talc plates (edge view)	1.605	Pale blue (≥ 800)	White (≥ 1000)
Tremolite	1.605	Pale yellow to yellow (≤ 400)	Magenta to blue-green (520–620)
Actinolite	1.605	White to pale yellow (≤ 400)	Pale yellow to yellow (≤ 400 –450)
Anthophyllite	1.605	White to orange-magenta (≤ 300 –500)	White to light blue-green (≥ 640)
Tremolite	1.615	Pale yellow to golden yellow (400–460)	Blue-green to pale blue-green (620–780)
Actinolite	1.615	White to pale yellow (≤ 400)	Pale yellow to magenta-purple (400–540)
Anthophyllite	1.615	White to blue-green (≤ 620)	Yellow to white (≥ 420)
Tremolite	1.620	Yellow to orange-magenta (440–500)	Pale blue-green (≥ 700)
Actinolite	1.620	Pale yellow to yellow (≤ 420)	Yellow to blue (420–590)

Name	RI Liquid for Screening	Dispersion Color ^a	
		<i>n</i> -parallel (approximate wavelengths, nm)	<i>n</i> -perpendicular (approximate wavelengths, nm)
Anthophyllite	1.620	Pale yellow to blue-green (≤620)	Pale yellow to pale blue-green (≤400 to ≥800)
Tremolite	1.635	Magenta-purple to light blue-green (540-660)	Pale blue-green to white (≥800)
Actinolite	1.635	Pale yellow to magenta (≤540)	Magenta to pale blue-green (520-800)
Anthophyllite	1.635	Pale yellow to pale blue-green (≤400 to ≥1000)	Pale yellow to white (≤400 to ≥1000)
Tremolite	1.640	Blue to pale blue-green (580-760)	White (≥1000)
Actinolite	1.640	Pale yellow to blue-green (400-600)	Blue to pale blue-green (580 to ≥800)
Anthophyllite	1.640	Pale yellow to pale blue-green (≤400 to ≥800)	Yellow to pale blue-green (420 to ≥800)

^a Dispersion colors may vary between microscopes and microscopists. Colors given in printed lists may not correspond to those seen in a particular microscope system. Use of specific matching liquids with freshly mounted standards by the analyst should be used for direct color reference.

5. Rotate the slide 90° and scan the entire slide again, so that any amphibole particles showing colors that are more difficult to see (and possibly missed) in the perpendicular orientation may be more visible in the opposite orientation.
6. For any suspect amphibole particle encountered, confirm that RIs in both *n*-parallel and *n*-perpendicular orientations are greater than the 1.605 RI liquid using the Becke line technique. With the following exception, all amphibole RIs are higher than 1.605, while all talc RIs are lower than 1.605.
 - For the rare case where a specific orientation of low-iron anthophyllite indicates an RI slightly below 1.605, rotating the microscope stage or “tapping” the coverslip with a probe so that the particle rolls around its longitudinal axis will confirm the presence of RIs higher than 1.605, thereby distinguishing a possible amphibole particle from a talc particle. Also, the difference in the alpha prime (α') RI and the 1.605 liquid will still be close enough to provide a measurable dispersion staining color for determination of n_D (the standard refractive index at the “D” Fraunhofer line of approximately 589 nm).
 - With the Becke line technique, the bright white (or light) Becke line moves into the material (either the particle or the surrounding liquid) that has the higher relative RI during defocusing in the down direction (i.e., when increasing the distance between the objective and the sample). The opposite is true for defocusing in the up direction (i.e., the bright white or lighter colored Becke line moves into the material that has the lower relative RI when decreasing the distance between the objective and the sample). It is often helpful to observe both defocusing conditions to confirm the movement of the Becke lines. Where the RI of the particle and liquid are close enough to observe dispersion, the Becke lines become colored.
7. Document the following optical properties of any confirmed suspect particles (determined using a combination of illumination conditions and magnifications between 100× and 400×):
 - *Morphology and aspect ratio*—observed in all illumination conditions
 - Examples: straight, curved, bundle, wavy, splayed ends, polyfilamentous, etc.
 - *Color and pleochroism*—observed in PPL
 - *Extinction angle and characteristics*—observed in cross-polarized light (XPL)
 - Inclined extinction may be indicative of monoclinic amphibole. Anthophyllite (orthorhombic amphibole) always has parallel extinction; however, asbestiform amphibole can have anomalous parallel extinction for all types.

- *Sign of elongation*—observed in XPL with first-order wave plate (RPL) inserted
 - Typically, positive for amphibole and chrysotile (blue in orientation parallel to the slow direction of the first-order wave plate and yellow in orientation perpendicular to the slow direction of the first-order wave plate). Crocidolite/riebeckite (not commonly associated with talc) has a negative sign of elongation, where these colors occur in opposite directions.
- *Refractive index (RI)*—observed in dispersion staining mode and in PPL using the Becke line method. In 1.550 and 1.605 RI liquids, indicate if the RI of the particle is higher, lower, or a close match in both *n*-parallel and *n*-perpendicular orientations. For a more precise match, use additional RI liquids.

For determination of RIs, proceed as follows:

- A. Use the appropriate RI liquids to determine the RIs of suspect particles encountered and use [Table 2](#) as a guide.
 - B. Qualitatively determine a match of RI using dispersion staining colors and/or Becke lines. (See [\(1901\)](#) for more information about quantitatively determining wavelength of match). The following conditions indicate a close match between particle and liquid: deep blue and magenta dispersion staining colors, colored Becke lines, and low particle relief (particles appear to disappear in plane-polarized light). Light blue and yellow colors in dispersion staining mode can indicate a greater difference between particle and liquid than colored Becke lines. Additional RI liquids may be needed to evaluate a match.
 - C. Correlating RI to wavelength of match can also be completed (see [\(1901\)](#)). [NOTE—Confirm observed colors with known minerals under the operational conditions at the observation temperature.]
 - D. Where monoclinic amphibole is suspected from optical properties, document “monoclinic amphibole”. Where orthorhombic amphibole is probable, document “orthorhombic amphibole”.
 - E. If ambiguity still exists after this analysis, scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDS) or transmission electron microscopy (TEM) with EDS should be used to determine the elemental composition; TEM with selected area electron diffraction can be used to confirm if a phase is amphibole and establish if it is orthorhombic or monoclinic. Optionally, a heating and cooling stage can be used to vary the RI liquid values to more precisely determine matching indices of the phases analyzed. For more information, see [\(1901\)](#).
- *Birefringence*—Report the difference in the maximum and minimum observed RI of a suspect particle type.
8. Use the definition below to evaluate all of the particles documented to determine if presence of asbestiform amphibole or serpentine is indicated. [The definition of “asbestiform” for this method is taken from the Glossary section of EPA 600/R-93/116 (emphasis in original).]

“Asbestiform (morphology)—Said of a mineral that is like asbestos, i.e., crystallized with the habit of asbestos. Some asbestiform minerals may lack the properties which make asbestos commercially valuable, such as long fiber length and high tensile strength. With the light microscope, the asbestiform habit is generally recognized by the following characteristics:

- Mean aspect ratios ranging from 20:1 to 100:1 or higher for fibers longer than 5 µm. Aspect ratios should be determined for fibers, not bundles.
- Very thin fibrils, usually less than 0.5 micrometers in width, and
- Two or more of the following:
 - Parallel fibers occurring in bundles,
 - Fiber bundles displaying splayed ends,
 - Matted masses of individual fibers, and/or
 - Fibers showing curvature

These characteristics refer to the population of fibers as observed in a bulk sample. It is not unusual to observe occasional particles having aspect ratios of 10:1 or less, but it is unlikely that the asbestos component(s) would be dominated by particles (individual fibers) having aspect ratios of <20:1 for fibers longer than 5 µm. If a sample contains a fibrous component of which most of the fibers have aspect ratios of <20:1 and that do not display the additional asbestiform characteristics, by definition the component should not be considered asbestos.”

[NOTE—This definition of asbestiform is appropriate for light microscopy, including phase contrast and polarized light microscopy. However, it is not generally extendable to fibrils visible in the transmission electron microscope.]

LIMIT OF DETECTION AND QUANTIFICATION

The working detection limit of this method is 0.01% weight percentage, 100 ppm by weight (µg/g) established via a round robin study of spiked reference materials.

[NOTE—See more information in [\(1901\)](#).]

REPORTING RESULTS

1. All amphibole or serpentine particles must be documented via a digital image capturing system attached to the microscope. The following images should be collected for each particle (if an image cannot be obtained, note this as well):
 - Dispersion staining and/or Becke line color with the fiber oriented parallel to the polarizer

- Dispersion staining and/or Becke line color with the fiber oriented perpendicular to the polarizer
- Sign of elongation with the fiber oriented parallel to the slow vibration direction of the first-order wave plate under crossed polarizers
- Optional: additional images to show morphology (microscope in any configuration)
- Optional: image(s) showing the extinction angle (inclined versus parallel) to supplement the documented angle (a cross-polarized image taken with first-order wave plate at extinction)

2. The final report should include:

- Number of particles found
- Length and width for the individual particles
- Digital images or photographs of each particle (especially those of bundles or other unique features of asbestos)
- Either an assessment of “detected” or “not detected” of asbestos with the detection limit of 0.01% weight percentage or 100 ppm by weight (µg/g)

3. If a single particle is observed in an analysis of 5 mg, analysis of an additional 5 mg of the originally received material is required. If the additional analysis results in 1 or fewer particles, report the result as below the detection limit of <0.01% weight percentage or <100 ppm by weight (µg/g). [NOTE—For this method, the typical number of particles found at 100 ppm is 8–16 particles when approximately 2 mg of material is tested.]

4. A single particle, even if conclusively identified as asbestos, is not sufficient to constitute a positive finding as it cannot be proven to be different from contamination. More than 1 particle with conclusive identification (e.g., 2 particles) is necessary to constitute a positive identification. All results with fewer than 2 confirmed asbestos fibers are reported as below the detection limit of <0.01% weight percentage or <100 ppm by weight (µg/g).

5. Determination of the type of amphibole asbestos (i.e., tremolite, actinolite, or anthophyllite) is not required; results will be reported simply as “chrysotile detected” or “amphibole asbestos detected”. Further clarification of the amphibole asbestos type can be achieved using EDS elemental analysis with an electron microscope.

[NOTE—The limits are based on limits of detection.]▲ (USP 1-DEC-2023)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

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