

# Why the Sample Holder and Preparation Matter in XRD: Preferred Orientation of Pharmaceutical Samples

Relevant for: pharmaceuticals, texture, sample preparation, preferred orientation

Aspirin and paracetamol are two of the most popular painkillers. X-ray diffraction, a common characterization technique in research, development and quality control of such pharmaceuticals, regularly encounters problems with preferred orientation. It is shown that these issues can be avoided with optimized measurement setups, sample holder selection and sample preparation.



## 1 Introduction

Proper sample preparation is one of the most crucial steps to guarantee high-quality data in powder X-ray diffraction (XRD). The choice of the right sample holder, preparation technique, measurement geometry and measurement parameters all have a significant influence on the data quality.

One of the most regularly observed problems stemming from the sample preparation is preferred orientation or texture effects. These can occur in most samples and can be caused by a number of different sample properties. The easiest way to visualize the phenomenon is by imagining a powder sample that does not consist of spherical grains, but of elongated needles, or plates. The probability that one of these needles or plates orients on a thin edge is small compared to the probability that it orients aligned with neighboring particles. Texture effects can also have other origins, such as crystal properties or crystallization behavior, and can also be observed in samples that only contain spherical particles<sup>1</sup>.

Texture leads to the overexposure of some lattice planes while others are rarely in the correct position to diffract the X-ray beam which in turn results in large

errors in the relative intensities of different peaks. These differences become clearly visible when comparing the measured diffraction pattern to the theoretical (ideal) pattern calculated from the single crystal structure solution. When working with new substances or phase mixtures, texture effects can seriously complicate any quantitative phase analysis or the determination of the crystal structure.

For this application report, preferred orientation effects were investigated for two different pharmaceutical samples using the Automated Multipurpose Powder X-Ray Diffractometer XRDynamic 500 by Anton Paar. Two types of commercially available painkiller tablets, one containing aspirin (acetylsalicylic acid) and the other paracetamol (acetaminophen), were analyzed and compared to their pure active pharmaceutical ingredients (API). The large variety of sample holder types available for XRDynamic 500 was employed to identify the best way to avoid texture effects, which were primarily observed for the pure APIs.

## 2 Experimental setup

### 2.1 Sample properties and preparation

Aspirin and paracetamol are two of the most common commercial painkillers worldwide that both feature on the World Health Organization's List of Essential Medicines<sup>2</sup> and consistently rank highly in the list of the most prescribed drugs in the United States of America.<sup>3</sup>

Paracetamol and aspirin are both available in the form of tablets; these include not only the APIs, but also other components such as excipients and binders. To prepare samples for powder XRD measurements, the tablets were ground by hand in an agate mortar. Additionally, one as-received paracetamol tablet was measured to ensure that the grinding process did not lead to changes in the crystallographic properties of the material.

Pure acetaminophen is commercially available as a fine white powder and was used without any further treatment. The as-received pure acetylsalicylic acid used in this study is a coarse powder that consists of particles of up to 1 mm in diameter. The particles can easily be ground to yield a fine white powder similar to the pure acetaminophen. Diffraction data was collected for the as-received acetylsalicylic acid powder and after grinding to again ensure that the grinding process does not have any influence on the material's crystallographic properties.

For the rest of this application report, the ground drug tablets will be referred to by paracetamol and aspirin, and the pure APIs will be referred to by their chemical names acetaminophen and acetylsalicylic acid.

## 2.2 Diffraction measurement

The powder samples were first investigated in Bragg-Brentano geometry with Cu-K $\alpha$  radiation from a Cu sealed-tube X-ray source with a Ni/C divergent beam multilayer primary monochromator. The samples from the ground tablets were measured on a fixed sample stage with no sample rotation. The APIs were measured on a fixed sample stage first, and measurements were repeated with sample holders of different depths using a sample spinner stage and with a capillary spinner stage in 1 mm capillaries. Measurements using backloading sample holders were also performed for the APIs on both fixed and spinning sample stages. For the capillary measurements, the primary optics were automatically switched from a divergent to a focusing beam geometry with an elliptical focusing beam mirror. The as-received paracetamol tablet was investigated in Bragg-Brentano geometry. All measurements were performed on the Automated Multipurpose Powder X-Ray Diffractometer XRDynamic 500 by Anton Paar.

Table 1 gives an overview of the measurements performed and details on the samples and their preparation.

Sample	Sample holder / geometry
Acetylsalicylic acid (ground)	Small (25 mm diameter, 2.5 mm depth), no spinning / reflection
Acetylsalicylic acid (ground)	Large (42 mm diameter, 2.5 mm depth), no spinning / reflection
Acetylsalicylic acid (ground)	Large, spinning / reflection
Acetylsalicylic acid (coarse)	Large, no spinning / reflection
Acetylsalicylic acid (coarse)	Large, spinning / reflection
Acetylsalicylic acid (ground)	Capillary (1 mm diameter), spinning / transmission
Acetylsalicylic acid (ground)	Large, backloading, no spinning / reflection
Acetylsalicylic acid (ground)	Large, backloading, spinning / reflection

Acetaminophen	Large, no spinning / reflection
Acetaminophen	Large, no spinning / reflection
Acetaminophen	Capillary (1 mm diameter), spinning / transmission
Acetaminophen	Large, backloading, no spinning / reflection
Acetaminophen	Large, backloading, spinning / reflection

Table 1: Measurements performed on APIs with different sample preparation and sample stages.

The beam divergence for the different sample holders was varied to ensure the beam did not hit any parts of the sample holder other than the sample. Measurement times for all samples differed according to the measurement setup, with a typical measurement taking 8 – 10 minutes.

## 3 Results

### 3.1 Tablet composition and literature comparison

First, the experimental diffraction patterns of both aspirin and paracetamol were compared to the theoretical patterns of the respective APIs calculated from single crystal data<sup>4</sup> or previously published powder XRD patterns<sup>5</sup> (Figure 1). The APIs are the main component of the tablet, and no other crystalline material can be observed in the diffraction pattern (i.e. there are no additional diffraction peaks). This is reasonable as both tablets weigh roughly 650 mg, containing 500 mg of API, while many of the other ingredients are expected to be amorphous. The presence of amorphous additives in the tablets is indicated by very broad features visible in the diffraction data. Both the peak positions and intensities of the experimental data and of the simulated pattern fit very well for both compounds.

The low signal-to-noise ratio present in the two experimental patterns relates to the relatively weak scattering power of the purely organic samples and can be improved by an increased exposure time, which was done for further investigations.

The diffraction patterns of the paracetamol powder obtained from grinding a tablet and the as-received tablet look almost identical. This proves that the grinding process does not change the crystallographic structure of the material. As the powder was easier to handle and prepare as a sample, it was used for all subsequent experiments.

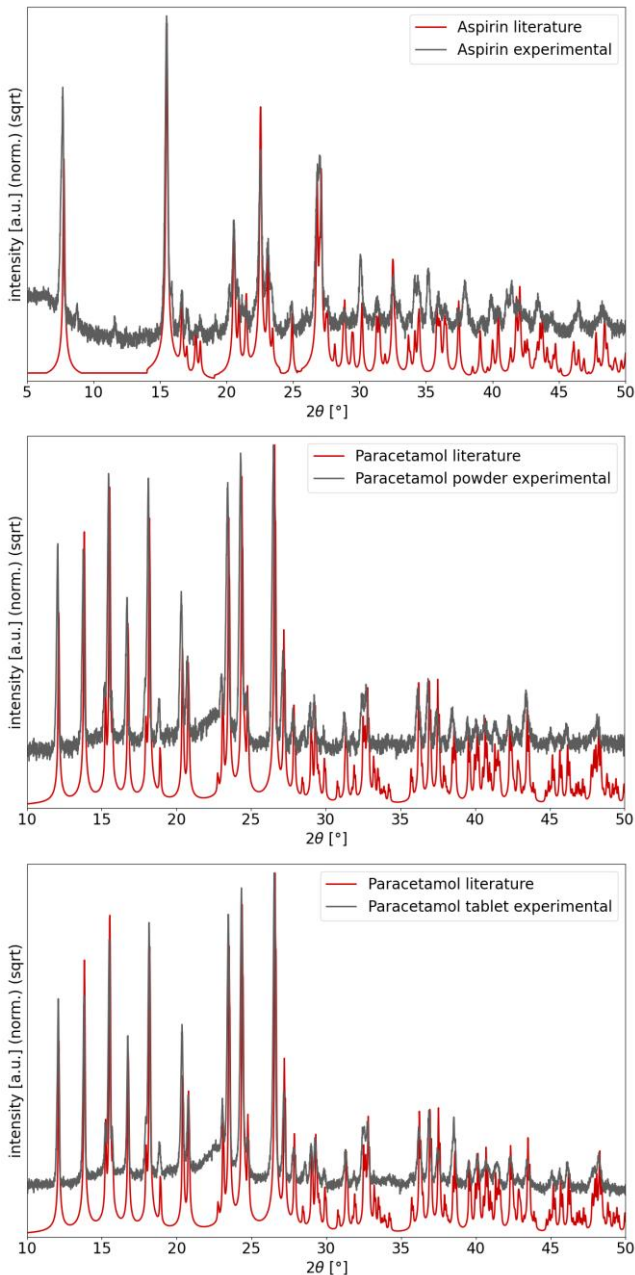


Figure 1: Comparison of diffraction patterns of aspirin and paracetamol (powder and tablet) to diffraction patterns simulated from literature data for acetylsalicylic acid<sup>4</sup> and acetaminophen<sup>5</sup>.

### 3.2 Preferred orientation effects

While the diffraction patterns of the drug tablets showed the expected results when compared to the literature data, the powder patterns of the APIs differed significantly. While the peak positions were correct, significant differences in the relative peak intensities were observed, indicating preferred orientation effects. Some peaks were so weak that they no longer appeared to be present in the diffraction pattern, while other peaks, that are barely visible in the literature data, show some of the highest intensities in the experimental pattern (Figure 2). This phenomenon is present for both APIs, though the effect is far more pronounced for acetylsalicylic acid.

For most diffraction patterns of acetylsalicylic acid obtained in reflection geometry, the reflections at  $7.7^\circ$  and  $15.5^\circ$   $2\theta$  showed the highest intensity (see Figure 2, top). The two strong reflections show much more intensity than the other peaks with most peaks only reaching about 10 % of the strong peaks' intensity.

In the simulated powder pattern, the reflections at  $7.7^\circ$ ,  $26.8^\circ$  and  $27.1^\circ$   $2\theta$  all have similar intensities, the peak at  $22.5^\circ$   $2\theta$  is slightly higher in intensity than these three, and the reflection with the highest intensity is visible at  $15.5^\circ$   $2\theta$ . The peak at  $15.5^\circ$   $2\theta$  shows roughly double the intensity of the three peaks at  $7.7^\circ$ ,  $26.8^\circ$  and  $27.1^\circ$   $2\theta$  and the intensities of strong and weak reflections are much closer together than in the measured patterns.

As mentioned above, the texture effects are less prominent in the acetaminophen measurements, but clear differences between the measured and the simulated pattern are still visible (see Figure 2, bottom). In the measured pattern, the reflections at  $12.0^\circ$  and  $13.6^\circ$   $2\theta$  (\*) have similar intensities, and the same is true for the reflections at  $16.7^\circ$  and  $18.8^\circ$   $2\theta$  (x). However, in the literature pattern, there is a clear difference in intensity visible for both of these pairs of reflections. The peak at  $13.6^\circ$   $2\theta$  has higher intensity than the one at  $12.0^\circ$   $2\theta$  and the peak at  $16.7^\circ$   $2\theta$  is much higher in intensity than the one at  $18.8^\circ$   $2\theta$ . The peak at  $24.2^\circ$   $2\theta$  shows the highest intensity in the measured sample, while it should be lower than the peak at  $26.6^\circ$   $2\theta$  (o) according to the literature data. Finally, the two reflections at  $20.4^\circ$  and  $20.8^\circ$   $2\theta$  (+) show very different intensities in the measured patterns, with the peak at  $20.4^\circ$  being almost ten times as intense as the neighboring peak. In the simulated pattern, the peak at  $20.4^\circ$   $2\theta$  is slightly more intense than the one at  $20.8^\circ$   $2\theta$ , but the weaker peak shows at least 80 % of the neighboring peaks' intensity.

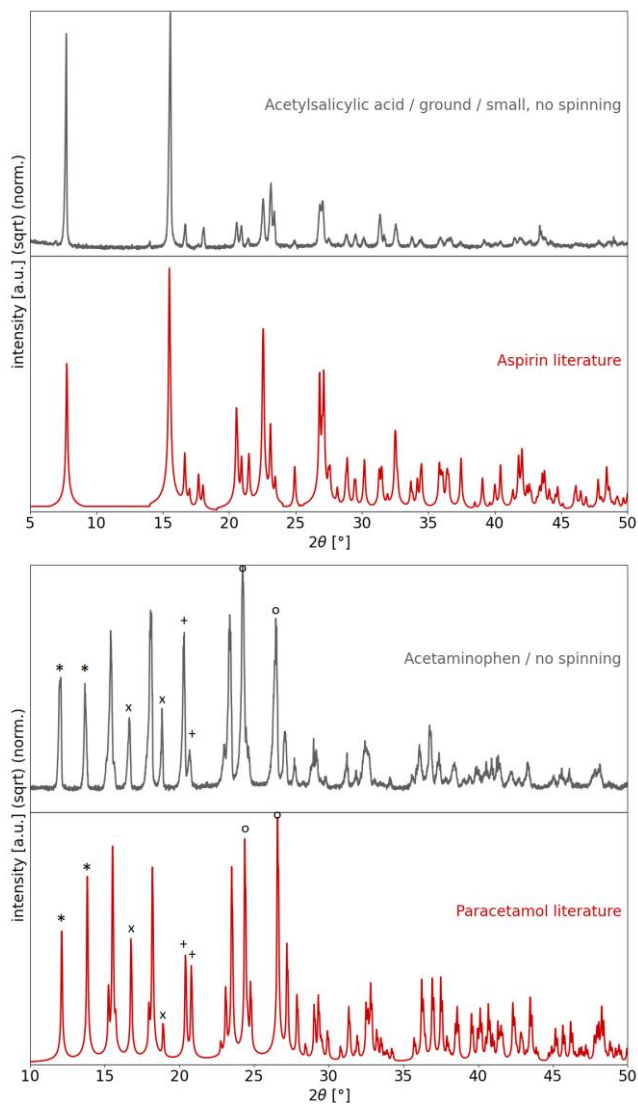


Figure 2: Comparison of diffraction patterns of APIs compared to diffraction patterns simulated from literature data.

### 3.3 Ways to reduce preferred orientation

Several ways to reduce the preferred orientation effects were investigated. First, acetaminophen was prepared in a sample holder with a larger capacity (see Table 1) to reduce the influence of surface effects. The sample was measured in reflection geometry as before, but this time on a spinning sample stage (Figure 3). This did not lead to an improvement of the relative intensities, and for some crystallographic planes the observed texture effects even appear to increase. One reason why the sample spinning stage does not reduce texture effects for certain samples is because the sample material only rotates around the axis perpendicular to the sample surface. When the powder consists of plate like particles that orient themselves parallel to the sample surface, perpendicular rotation will not reduce the texture effects as the prominent crystal planes will not change their tilt angle.

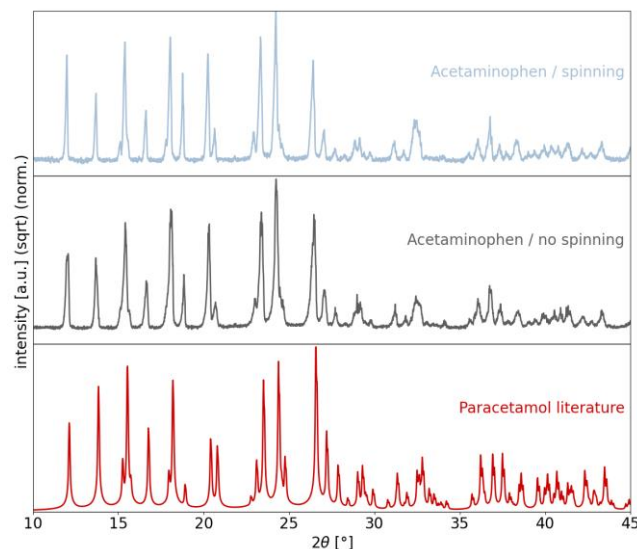


Figure 3: Comparison of diffraction patterns of acetaminophen measured with and without sample spinning and a powder XRD pattern simulated from literature data.

For acetylsalicylic acid, the same experimental changes were employed. In addition, to make sure that the grinding does not increase the texture effects, the coarse, as-received, powder was also measured (Figure 4). Like acetaminophen, neither the larger sample holder nor using the sample spinner led to a significant improvement of the data quality. In contrast, every newly prepared sample showed different relative peak intensities without a clear correlation to the measurement conditions. One notable outlier is the measurement of the coarse powder measured without sample spinning, where the strongest reflection of the pattern is located at  $27.2^\circ$   $2\theta$ .

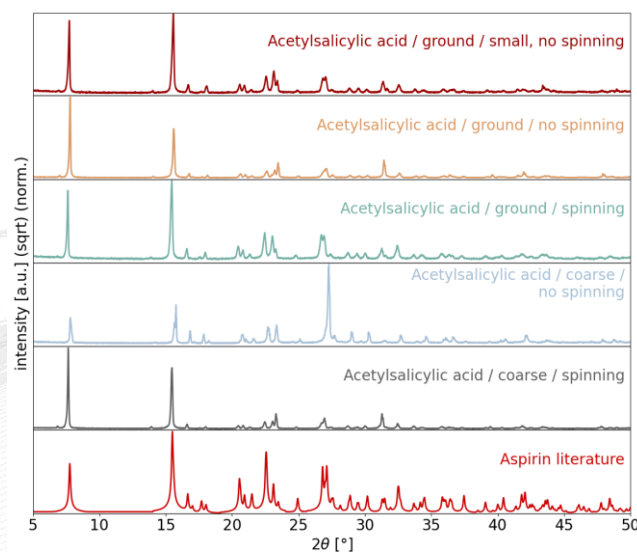


Figure 4: Comparison of powder XRD patterns of different acetylsalicylic acid samples in different sample holders and on different sample stages with a powder XRD pattern of acetylsalicylic acid simulated from literature data.

Both APIs were also measured in backloading sample holders. These sample holders can reduce preferred orientation effects caused by the sample preparation because there is no need to apply pressure or motion to the sample to create a smooth and even sample surface. Backloading sample holders were used both on a fixed and a spinning sample stage. The differences in peak intensities that were observed with the frontloading sample holders were still visible in the data measured with backloading sample holders for both APIs (Figure 5). Using backloading sample holders on a spinning sample stage also did not reduce the texture effects. This proves that the texture effects are not caused by the preparation method, but by the material properties.

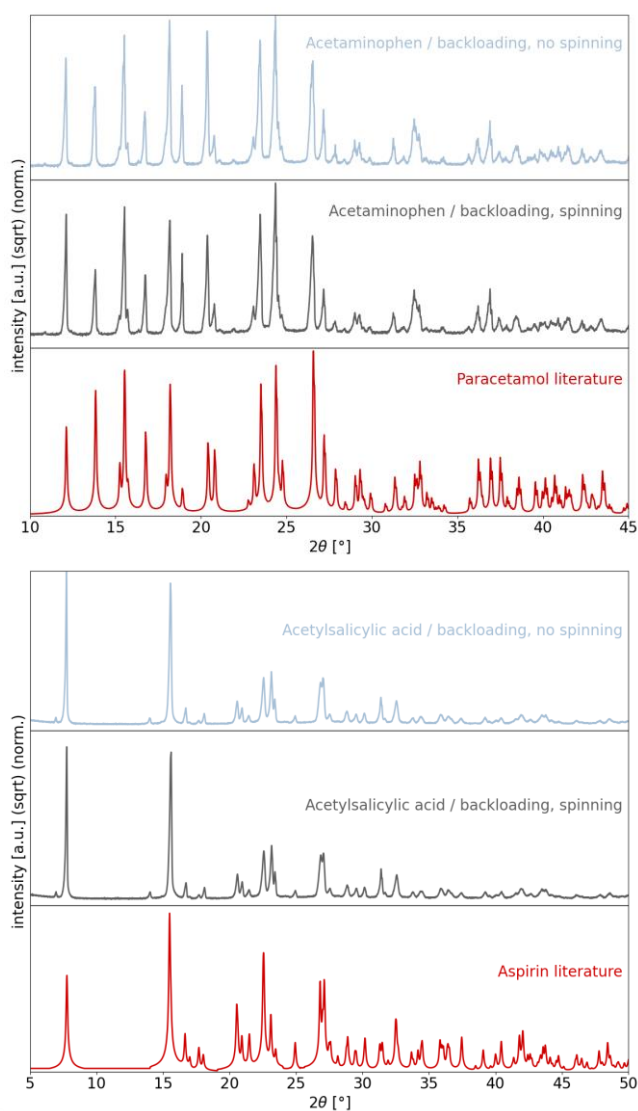


Figure 5: Comparison of powder XRD patterns of acetylsalicylic acid (top) and acetaminophen (bottom) measured with backloading sample holders and simulated from literature data.

Finally, the samples were prepared in glass capillaries and measured in transmission geometry with a focused X-ray beam (see Figure 6). The capillary was spun during the measurement. This led to a significant improvement in the data quality for both APIs, as capillary measurements can reduce or even remove certain causes of texture effects. As described above, rotation around an axis perpendicular to the sample surface is not always suitable to improve data quality. In a capillary, it is much more likely that all lattice planes fulfil the diffraction condition equally often. Additionally, the round capillary is filled with a bulk of powder rather than a layer, meaning there is no flat surface for plate-like particles or crystallites to align with.

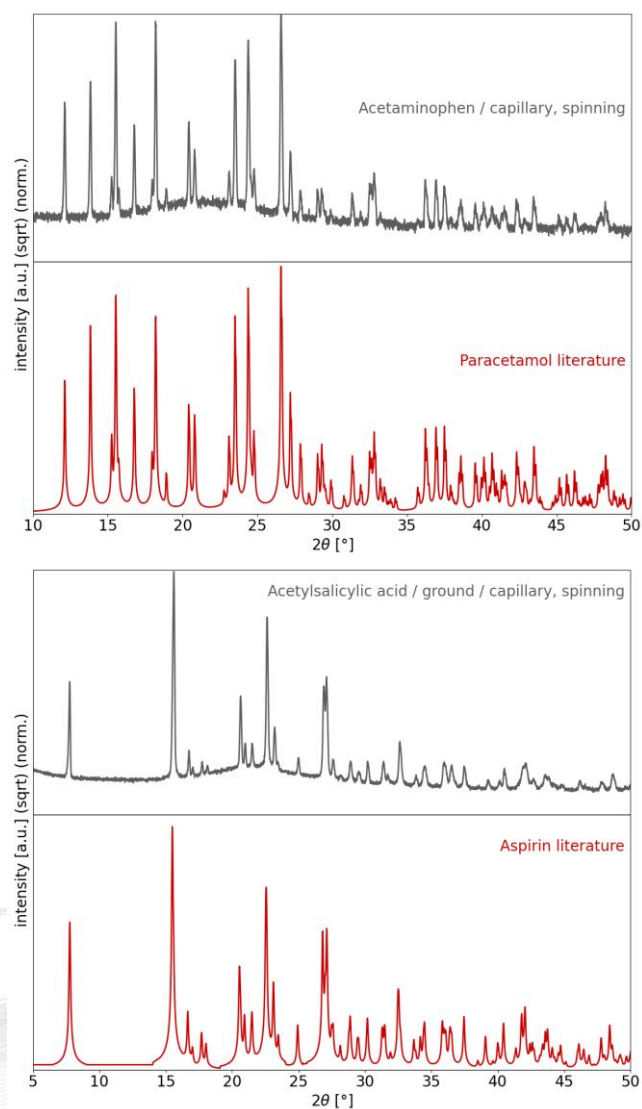


Figure 6: Comparison of powder XRD patterns of both APIs measured in spinning capillaries compared to powder XRD patterns simulated from literature data.

## 4 Conclusion

The importance of good sample preparation and the selection of the right measurement setup to reduce texture effects in XRD measurements of pharmaceutical samples was shown. Preliminary experiments similar to those conducted in this study can be essential to obtain reliable data for subsequent phase or structural analysis of both pure APIs and tablets. To be able to test multiple different setups, beam geometries and powder types quickly and easily, it is of great importance to work on a versatile X-ray powder diffractometer that offers automatic switching of beam geometries, easy exchange of sample holder setups, and an autosampler. Anton Paar's Automated Multi-purpose Powder X-ray Diffractometer XRDynamic 500 includes automated beam optics, automatic sample stage recognition, and automatic instrument and sample alignment routines. Together with a wide variety of sample holders and sample stages including the sample spinner and capillary spinner described here, this flexibility makes XRDynamic 500 the perfect choice for the described application.

## 5 References

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